201-14860

November 17, 2003

Mr. Mike Leavitt, Administrator
U.S. EPA
P.O. Box 1473
Merrifield, VA 22116

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Attn: Chemical Right-to-Know Program

RE: HPV Chemical Challenge Program, AR-201

Dear Mr. Leavitt:

This letter was drafted is to inform you that the Eastman Chemical Company is planning to move our sponsorship for the below two chemicals into a category that was just recently developed to take advantage of structure activity relationships with other chemicals. The category is called "Alkyl Nitriles" and is being managed by a consortium through PCA Services (Durham, NC).

CAS No. 78-82-0	Isobutyronitrile
CAS No. 109-74-0	Butyronitrile

The HPV registration number for Eastman Chemical is If there are any questions regarding the management of this chemical please call Dr. James Deyo at 423-229-5208 (deyo@eastman.com).

Sincerely,

James A. Deyo D.V.M., Ph.D., D.A.B.T.
Senior Associate Applied Toxicology

CC: Dr. Rod Gerwe
PCA Services

201-14860

PCA Services, Inc.
2704Trail Wood Drive
Durham, NC 27705

November 18, 2003

Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

Dear Administrator:

On behalf of joint sponsors Eastman Chemical Company and Solutia Inc., PCA Services, Inc. is pleased to submit to the Agency dossiers and a test plan for the Alkyl Nitriles Category. This submission is being made under the EPA Chemical RTK HPV Challenge Program for public posting and review.

The category consists of the following members:

Propionitrile (CAS No.107-12-0)
Butyronitrile (CAS No. 109-74-0)
Isobutyronitrile (CAS No. 78-82-0)

Please note that Solutia Inc. originally notified the EPA of its intention to sponsor propionitrile separately, and that Eastman Chemical Company originally notified of its intention to sponsor butyronitrile and isobutyronitrile as individual chemicals. Subsequently both companies jointly determined that these three chemicals were best reviewed together as a category, taking advantage of the structure activity relationships of these close analogs. See letter of November 17, 2003 from Dr. James Deyo, Senior Associate for Applied Toxicology of Eastman Chemical Company (attached), and the letter of February 14, 2003 of Dr. Frederick Johannsen, HPV Coordinator of Solutia Inc. (at http://www.epa.gov/chemrtk/c14258.htm).

If there are any questions regarding the management of this chemical and concerning the test plan submission, please contact Dr. Rod Gerwe at (919) 382-8787 or r.gerwe2@verizon.net.

Yours truly,

Rod Gerwe

Rod Gerwe, Ph.D.
President, PCA Services, Inc. (contractor)

Cc Don A. Lederer, Solutia Inc.
James A. Deyo, Eastman Chemical Company

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ALKYL NITRILES TEST PLAN AND CATEGORY JUSTIFICATION

November 13, 2003

OVERVIEW

Eastman Chemical Company and Solutia Inc. jointly submit the following test plan and category justification for review under the Environmental Protection Agency's High Production Volume Chemical Challenge Program. The category consists of three alkyl nitriles – propionitrile, butyronitrile and isobutyronitrile.

The sponsors conclude that the category approach for these closely related substances is appropriate, and that the existing available data presented in the test plan meet the screening data needs for this category.

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1. Identification of Category Sponsors

The Alkyl Nitriles Category is being sponsored by Eastman Chemical Company and Solutia Inc.

The technical contact for this category is:

Dr. James A. Deyo
Eastman Chemical Company
100 N. Eastman Road
Kingsport, TN 37662
Phone (423) 229-5208
Email: deyo@eastman.com

2. Introduction and Identification of Category Members

The EPA's "Chemical Categories" guidance sets forth a definition of what constitutes a "chemical category, for the purposes of the Challenge Program." Specifically, that definition states that a chemical category under the HPV Challenge Program "is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity." The Alkyl Nitriles Category has been selected with this guidance in mind.

The **category** consists of the following members:

1. Propionitrile (EINECS Propiononitrile), CAS No. 107-12-0, molecular structure: $CH_3CH_2C{\equiv}N$

2. Butyronitrile, CAS No. 109-74-0, molecular structure: $CH_3CH_2CH_2C{\equiv}N$

3. Isobutyronitrile (2-methylpropanenitrile), CAS No. 78-82-0, molecular structure: $(CH_3)_2CHC{\equiv}N$

The generic chemical structure of the category members and surrogates is depicted as:

$$RC{\equiv}N, \text{ where } R = CH_3CH_2\text{-}, CH_3CH_2CH_2\text{-} \text{ or } (CH_3)_2CH\text{-}$$

As can be seen from the molecular structures shown above as well as the generic chemical structure, all three category members have closely related structures and the same functionality. The category members differ in having either two or three carbons in the alkyl chain, and in the case of the butyronitrile and isobutyronitrile members the only difference is in their being isomers.

Given the same functionality and similar structure, one would expect much of the category behavior to be driven by the nitrile function. A putative metabolite of all three materials is cyanide (see section 3.4). As shown in the test plan below, symptoms of acute and repeated dose toxicity are similar to those of cyanide. In addition, the relatively short aliphatic hydrocarbon side chains and lack of functional groups that strongly promote hydrogen bonding is also expected to influence chemical physical and environmental fate properties in a similar manner. These properties will be compared and examined in the appropriate sections below.

Butyronitrile and isobutyronitrile are manufactured by one producer at one site (Eastman Chemical Company). Propionitrile is manufactured solely by Solutia Inc. at one site. All three category members are used as industrial chemicals, primarily as intermediates that are chemically converted to other chemicals. Although they are sold and shipped from the manufacturing sites to other manufacturing companies, these customers chemically convert them to other products or use them as industrial chemicals. There are no reported uses in consumer products or formulations. Any exposures to these chemicals are expected to occur primarily in the workplace. Propionitrile is used to make di-N-propylamine and pharmaceuticals, with reported uses as an industrial solvent and dielectric fluid (Lewis, 1997). Butyronitrile is used as a chemical intermediate for butyric acid, butyramide and pharmaceuticals and other organics, such as ketones and esters. Isobutyric acid is used to manufacture insecticides, as a catalyst in the polymerization of ethylene, and as a gasoline additive (Lewis, 1997)(Clayton, 1994).

Although these products are industrial chemicals with limited exposure potential, the sponsors are not making a claim for reduced testing. The sponsors believe that the existing data and category approach for these chemicals will address all required HPV Chemical screening endpoints.

3. Test Plan

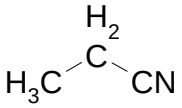
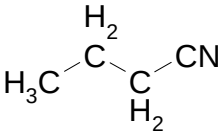
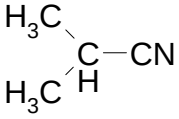
Criteria for Determining Adequacy of Data

All available studies were reviewed and assessed for adequacy according to the standards of Klimisch et al. (1). Studies receiving a Klimisch rating of 1 or 2 are considered to be adequate. Studies from other category members and surrogates were used to support studies assigned a reliability rating of 4 (not assignable).

Test Plan Matrix

The Alkyl Nitriles Category test plan matrix (as shown in Table 1) was constructed after a careful evaluation of all existing data (see Section 3 below). This matrix is arranged by category members (columns) and screening data endpoints (rows), and indicates if data are provided for each end point in the sets of robust summaries.

Table 1. Test Plan Matrix for Aliphatic Nitriles

Endpoint			
	Propionitrile 107-12-0	Butyronitrile 109-74-0	Isobutyronitrile 78-82-0
Physical-Chemical Data			
Melting Point	D	D	D
Boiling Point	D	D	D
Vapor Pressure	D	D	D
Partition Coefficient	D	D	D
Water Solubility	D	D	D
Environmental Fate Endpoints			
Photodegradation	E	E	E
Stability in Water*	E	E	E
Biodegradation	D	D	D
Fugacity	E	E	E
Ecotoxicity			
Acute Toxicity to Fish	D	D	D
Acute Toxicity to Aquatic Invertebrates	D	D	D
Toxicity to Aquatic Plants	C	D	D
Toxicological Data			
Acute Toxicity	D	D	D
Repeated Dose Toxicity	D	C	C
Genetic Toxicity – Mutation	D	D	D
Genetic Toxicity - Chromosomal Aberration	D	D	D
Reproductive Toxicity	D	C	C
Developmental Toxicity	D	D	D
Other Data			
Elimination (NR)	D	NR	NR
Mechanism of Toxicity (NR)	D	NR	NR

D = adequate experimental data; E = Endpoint fulfilled via estimation; T = endpoint to be filled by testing; C = endpoint fulfilled by other category members; NR = not required; *Estimate based on measured data for related material (acetonitrile) and analysis done by EPA personnel.

3.1 Chemical/Physical Properties

The results of chemical/physical property testing are shown in Table 2.

Table 2. Chemical/Physical Properties of Alkyl Nitriles

Chemical	Melting Point (°C)	Boiling Point[a] (°C)	Vapor Press.[b] (hPa)	Density (g/cm^3)	Water Sol.[b] (mg/l)	Log Kow[b]
Propionitrile CAS No. 107-12-0	-92.8[c]	97[c]	52[d]	0.7818[e]	93,380[f] 55,650[g]	0.16[h,i] 0.35[g]
Butyronitrile CAS No. 109-74-0	-112[e]	117.5[e]	26[j]	0.7954[e]	33,000[c] 27,840[g]	0.53[h] 0.837[g]
Isobutyronitrile CAS No. 78-82-0	-71.5[k]	103.8[k]	55.2[l]	0.77[m]	39,000[n] 32,500[g]	0.46[h] 0.76[g]

Refer to Section 2 of dossiers for additional information on category members.
[a] at 1013 or 1016 hPa; [b] at 20-25 °C; [c]Riddick et al. , 1986; [d]Solutia Inc., 2003; [e]Windholz et al. 1983; [f] Yaws, et al., 1992; [g] Estimated using EPIWIN [The EPI (Estimation Programs Interface) Suite™ developed by the Environmental Protection Agency Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC)(2000); [h] Sangster, 1989; [i] Temperature wasn't listed ; [j]Daubert and Danner 1989; [k]Weast, 1979; [l]Eastman Kodak Company, 1986a; [m] Eastman Chemical Company, 2002; [n] Unpublished study, Eastman Chemical Company, 2003.

3.1.1 Melting Point

Measured melting point data, which are available for all category members, indicate that these substances are all liquids with melting points well below -50°C.

3.1.2 Boiling Point

Measured boiling point data, which are available for all category members, indicate that these substances behave similarly, with boiling point ranges between 97 to 117.5 °C at atmospheric pressure.

3.1.3 Vapor Pressure

Consistent with their boiling points, category members have measured vapor pressures falling within a relatively close range of 26 to 55.2 hPa at 20-25°C.

3.1.4 Octanol/Water Partition Coefficient

The estimated octanol/water coefficients of category members fall within a range of 0.35 to 0.837. As one might expect, the log Pows increase somewhat going from propionitrile to the next higher homologs with higher molecular weights (butyronitrile and isobutyronitrile).

3.1.5 Water Solubility

Measured water solubility data are available for all category members. Solubility ranges from 33,000 mg/l for butyronitrile to 99,400 mg/l for propionitrile, the lowest homolog. Carbon branching appears to have little effect on water solubility (i.e. the solubility of isobutyronitrile was similar to straight chain butyronitrile).

3.1.6 Summary/Test Plan for Physical Properties

Measured data are available for melting point, boiling point, vapor pressure and water solubility for all category members. Estimated data for octanol/water partition coefficients are available. These data indicate that the category members behave similarly with respect to physical properties, and serve to support the category approach for these members. The members are all liquids with low melting points and boiling points that fall within a narrow range. Water solubilities range from slight to moderate. Partition coefficients are similar and are consistent with slight to moderate water solubility, and suggest limited potential to bioaccumulate. Existing data are sufficient to characterize physical property endpoints for these chemicals.

3.2 Environmental Fate/Pathways

As shown in Table 3 below, environmental fate parameters are consistent for category members.

Table 3. Environmental Fate Parameters for Alkyl Nitriles

Chemical	Henry's Law Constant (atm-m^3/mole)	Photodeg. OH radical rate constant (cm^3/molecule-sec) ($T_{1/2}$)	Predicted Environmental Distribution (Level III fugacity model)			
			Air (%)	Water (%)	Soil (%)	Sed. (%)
Propionitrile CAS No. 107-12-0	4.06E-5	0.194 x 10^{-12} 55.2 days	14.4	48.7	36.9	0.0821
Butyronitrile CAS No. 109-74-0	5.38E-5	0.498 x 10^{-12} 21.5 days	15	47.9	37	0.0821
Isobutyronitrile CAS No. 78-82-0	5.38E-5	0.703 x 10^{-12} 15.2 days	17.8	47.3	34.7	0.0809

All values are estimated from EPIWIN. Refer to Section 3 of repositories for additional information on category members.

3.2.1 Photodegradation

Photodegradation with hydroxyl radical sensitizer was estimated using EPIWIN/Aop (v1.90). Overall hydroxyl radical rate constants were calculated based on the summation of individual rate constants for each bond fragment in the molecule using the program algorithm. Estimated photodegradation hydroxyl radical rate constants range from 0.194 to 0.703 E-12 cm^3/molecule-sec. Calculated photodegradation half-lives for the three alkyl nitriles are in the range of 15.2 to 55.2 days, assuming a constant concentration of OH radical and pseudo first order kinetics.

3.2.2 Stability in Water

No hydrolysis studies were located for propionitrile, butyronitrile or isobutyronitrile, and EPIWIN Hydrowin cannot derive a water hydrolysis rate constant for nitriles. The theoretical hydrolysis of propionitrile and several other chemicals has been examined by Dr. Lee Wolfe at the USEPA Environmental Research Laboratory in Athens, Georgia. The results of these analyses were published in a report that could not be located by Dr. Wolfe. In a personal communication, Dr. Wolfe stated that propionitrile can hydrolyze (albeit slowly). According to a study cited in the Hazardous Substances Data Bank, the chemical hydrolysis of the related material acetonitrile in water is base-catalyzed (the rate constant for base catalyzed hydrolysis is 5.8X10-3/M-hr), but the half-life at pH 7 is more than 150,000 yrs (Ellington et al., 1988). Acetonitrile ($CH_3C{\equiv}N$)(CAS No. 75-05-8) is the 2-carbon analog of the category members, possessing the same functionality, but having one less carbon than propionitrile. Taken together, these data suggest that hydrolysis of propionitrile, butyronitrile or isobutyronitrile at environmentally relevant pHs will occur too slowly to be a significant means of degradation.

3.2.3 Fugacity

Level III fugacity data were calculated for category members using the EPIWIN program. Measured inputs to the program for each category member were the respective melting points, boiling points, vapor pressures and water solubilities presented in Table 2. The mass percentages are shown in Table 3. All three nitriles are predicted to partition mainly to water and soil (approximately 40 % in each compartment).

3.2.4 Biodegradation

As shown in Table 4 below, biodegradation data are available for all three nitriles.

Table 4. Comparison of Biodegradation Rates of Alkyl Nitriles

Category Member	Biodegradation Rate
Propionitrile CAS No. 107-12-0	40% biodegradable in 10 hours [a] Toxic to activated sludge at 500 mg/l [b]
Butyronitrile CAS No. 109-74-0	BOD5/COD ratio = 0.47 [c] 1.0 – 10.5 % of theoretical BOD at 500 mg/l [a]
Isobutyronitrile CAS No. 78-82-0	BOD5/COD ratio = 0.28 [d] BODs = 53.9 - 66.3% [e]

See Section 3.5 in dossiers for study details; [a]Symons et al., 1960; [b]Lutin, 1960; [c] Eastman Kodak, 1999a,b; [d] Eastman Kodak 1998b,c; [e] HSDB, 1999. Study was given a reliability rating of 4 because primary reference was not available.

Low concentrations of butryonitrile and isobutyronitrile (0.000195 – 0.000592 %) have been tested in BOD/COD studies similar to OECD TG-301C, Modified MITI Test (Eastman Kodak, 1998a,b). Both of these studies were given reliability ratings of 1 (valid without restriction). Results of these studies indicate that biodegradation of both materials occurred; however they were not readily biodegraded over a period of 5 days (both BOD5/COD ratios were < 0.5). Over a period of 20 days, the BODs for both of these materials increased (from 1.0 to 1.9 and 0.53 to 2.6, respectively), suggesting that the

materials can biodegrade over time. Results of a Japanese MITI test that was not available for review showed that a concentration of 100 ppm, isobutyronitrile was readily biodegraded over 14 days (BOD = 53.9 - 66.3%)(HSDB, 1999; Kitano, 1978; Sasaki, 1978). An additional test with results that could not be quantified showed that a mixed microbial culture that had been acclimated to organic cyanides and polychlorinated biphenyls could use propionitrile, n-butyronitrile and isobutyronitrile as a growth substrate (Chapatwala et al., 1992).

The ability of three different activated sludges to degrade propionitrile and butyronitrile has been tested using a Warburg respirator (Lutin, 1970). Results of these studies showed that over a period of 72 hours, a 500 mg/l concentration of butyronitrile was biodegraded to some extent (1.0 and 10.5% of theoretical BOD) by two of the sludges, but was toxic to another. At the same concentration, propionitrile was toxic to all three sludges. In contrast, an additional study employing a Warburg respirator and activated sludge showed that propionitrile (at a concentration of 1000 mg/l related to COD) was biodegraded by 40% after incubation for 10 hours (Symons et al., 1960). In this study, the kinetics of metabolism suggested that propionitrile was sequentially metabolized to propionamide and propionic acid.

3.2.5 Summary/Test Plan for Environmental Fate Parameters

Estimated values are available for the hydroxyl radical induced photolysis rate constant and atmospheric half-life, Henry's Law Constant, and Fugacity Level III environmental transport parameters. These data, which are summarized in Table 3, indicated that category members tend to partition to air, water and soil to a significant extent, and have limited retention in sediment. The Henry's Law Constants indicate that the substances will volatilize from the surface of water to the atmosphere, where they undergo hydroxyl radical induced photodegradation with a half-life of 15.2 to 55.2 days. The Kocs of propionitrile (8.3), butyronitrile (15.3), and isobutyronitrile (12.8) were estimated using the EPIWIN Pckoc Program. These low soil/sediment partition constants indicate that category members display high soil mobility, and can readily volatilize from soil surfaces or enter any adjoining aqueous environment.

Adequate biodegradation studies have been performed on all of the category members. The results suggest that the aliphatic nitriles are not readily biodegradable but will degrade over time, and that high concentrations may be toxic to microbes. The likelihood of obtaining environmentally relevant concentrations is low, based on use of this material as an industrial intermediate. No further testing is planned for any of the environmental fate endpoints.

3.3 Aquatic Toxicity

The acute toxicity of all three nitriles was measured in fish, invertebrates, and algae (with the exception of propionitrile, which has not been tested in algae). Results of the studies are summarized in Table 5.

Table 5. Comparative Aquatic Toxicity of Alkyl Nitriles

Chemical	**Fish LC_{50} (mg/l)[a]**	**Invertebrate EC_{50} (mg/l)[b]**	**Algae EC_{50} (mg/l)[c]**
Propionitrile CAS No. 107-12-0	1520 (fh minnow)[d] 41 (bluegill) 340 (trout)	250	789[e]
Butyronitrile CAS No. 109-74-0	> 107 (fh minnow)	> 110	> 133.4 364.9[e]
Isobutyronitrile CAS No. 78-82-0	> 102.1 (fh minnow)	> 94.3	> 87.8 429.5[e]

LC50 = lethal concentration in 50% of organisms, EC_{50} = concentration required for 50% inhibition of growth, fh= fathead. See Section 4 of dossiers for details.
[a] Static, 96 hours (unless listed otherwise); [b] Static, Daphnia magna (48 hrs); [c] 72 hours; [d] Flow through study; [e] estimated using EPIWIN/ECOSAR.

3.3.1 Acute Toxicity to Fish

The toxicity of propionitrile has been tested in fathead minnows in a flow through study (Geiger et al., 1990) and in bluegill sunfish and rainbow trout in static studies (ABC Laboratories Inc., 1981a,b). The 96-hour LC50 values (with confidence limits) for the respective species were 1520 (1450-1580) mg/l (measured concentration), 41 (28-66) mg/l (nominal concentration), and 340 (180-560) mg/l (nominal concentration). Reliability ratings of 2 (valid with restrictions) were assigned to the static studies since concentrations of material were not analytically confirmed. A rating of 1 was assigned to the flow through study, since it was comparable to a guideline study.

The toxicity of n-butyronitrile and isobutyronitrile to *Pimephales promelas* (fathead minnow) was tested in static, 96-hour limit studies which conformed to OECD test guideline 203 and were given reliability ratings of 1 (valid without restriction)(Eastman Kodak, 1998d; 1999c). Nominal concentrations of 120 mg/l were tested in both studies. The analytically measured concentrations of n-butyronitrile and isobutyronitrile in the studies were 107 and 102.1 mg/l, respectively. In both studies, none of the exposed fish died or exhibited abnormal behavior. Therefore, 107 and 102.1 mg/l were considered to be the no observable effect concentrations (NOECs) for n-butyronitrile and isobutyronitrile, respectively.

3.3.2 Acute Toxicity to Aquatic Invertebrates

A static, 48-hour toxicity test to *Daphnia magna* has been performed with propionitrile (ABC Laboratories, Inc., 1981c). The 96-hour EC_{50} value (with confidence limits) was 250 (210-290) mg/l (nominal concentration). A reliability rating of 2 (valid with restrictions) was assigned since concentrations of material were not analytically confirmed.

The toxicities of n-butyronitrile and isobutyronitrile to *Daphnia magna* were tested in static, 48-hour limit studies which conformed to OECD test guideline 202 and were given reliability ratings of 1 (valid without restriction) (Eastman Kodak, 1998e, 1999d). Nominal concentrations of 120 mg/l were tested in both studies. The analytically measured concentrations of n-butyronitrile and isobutyronitrile in the

studies were 110 and 94.3 mg/l, respectively. No abnormal behavior or immobility was observed in daphnia treated with 94.3 mg/l isobutyronitrile; therefore, this concentration was the NOEC. In the study with n- butyronitrile, 1/20 daphnids was observed to be immobile at 48 hours. This was not considered to be related to treatment, since the incidence was < 10%. Therefore, 100 mg/l was the NOEC for n-butyronitrile. According to the EPA's assessment criteria, the LC50 values correspond to a "low concern level".

3.3.3 Acute Toxicity to Aquatic Plants

The toxicity of n-butyronitrile and isobutyronitrile to *Selenastrum capricornutum* was tested in 72-hour limit studies, which conformed to OECD test guideline 201 and were given reliability ratings of 1 (valid without restriction) (Eastman Kodak, 1999e, 2000). Nominal concentrations of 200 mg/l were tested in both studies. The analytically measured concentrations of n-butyronitrile and isobutyronitrile were 133.4 and 87.8 mg/l (mean), respectively. There was no effect of treatment on growth rate or biomass; therefore, the EC_{50} values were higher than the concentrations that were tested. Both of these concentrations are within an order of magnitude of the EC_{50} concentrations calculated by ECOSAR for these materials (364.9 and 429.5 mg/l, respectively). ECOSAR is an appropriate model to estimate toxicity to algae since the mechanism of toxicity to fish (interruption of cellular utilization of oxygen by cyanide) is not relevant for plants. Since the ECOSAR value estimated for propionitrile is greater than these values, it is expected that the experimental EC_{50} concentration for propionitrile toxicity to algae is at least 87.8 mg/l, which is the experimental value for the nitrile with the lowest ECOSAR-estimated value.

3.3.4 Summary/Test Plan for Ecotoxicity

In conclusion, adequate tests exist for all three nitriles in all three of the required species (with the exception of priopionitrile toxicity to algae). Algal toxicity data for n-butyronitrile and isobutyronitrile (which are similar), along with the EPIWIN estimate for propionitrile toxicity to algae are sufficient to fill the algal toxicity endpoint for propionitrile. The fact that the EC_{50} values estimated by ECOSAR for n-butyronitrile and isobutyronitrile toxicity to algae are within an order of magnitude of experimental values for these materials lends support to this approach. No further testing is necessary.

3.4 Mammalian Toxicity

3.4.1 Acute

Acute toxicity data for the category members are summarized in Table 6. The oral and inhalation LD_{50} values of propionitrile, n-butyronitrile and isobutyronitrile in rats are similar. The oral LD_{50} values in rats for all three nitriles are within the range of 40 – 270 mg/kg (Younger Laboratories Incorporated, 1979, 1980; Eastman Kodak, 1957, 1960, 1961; Smyth et al., 1962). Inhalation LD_{50} values (for 1 or 4 hours of exposure) are from > 1000 to 1465 ppm (Younger Laboratories Incorporated, 1978, Eastman Kodak, 1986b,c,d). Whereas the dermal LD_{50} values of n-butyronitrile and isobutyronitrile in rabbits are similar (389 and 239 mg/kg, respectively), the dermal LD_{50} value of propionitrile in rabbits is lower (40-90 mg/kg depending on purity)(Smyth et al., 1962; Biodynamics Inc., 1981a,b,c).

Table 6. Acute Mammalian Toxicity of Alkyl Nitriles

Category member	Acute Rat Oral LD_{50} (mg/kg)	Acute Rat Inhalation LC_{50} (ppm)	Acute Rabbit Dermal LD_{50} (mg/kg)
Propionitrile CAS No. 107-12-0	40 [a] 75 (M), 270 (F) [b]	1465 (4 hr LC50) [c]	40-90 [d]
Butyronitrile CAS No. 109-74-0	50-100 [e] 111 (male rat) [f]	1848 (1 hr LC10) [g] > 1147 (1 hr LC50) [h]	398 [f]
Isobutyronitrile CAS No. 78-82-0	77 (male rat) [f] 50 [i] 50-100 [j]	1173 (1 hr LC10) [k] > 1000 (1 hr LC50) [l]	239 [f]

LD_{50} = lethal dose in 50% of animals (male and female unless listed otherwise); LC_{50} = 4 hour lethal conc. in 50% of rats (unless listed otherwise). Study details are found in the dossiers (Section 5.1)
[a] Younger Laboratories Inc., 1980; [b] Younger Laboratories Inc., 1979; [c] Younger Laboratories Inc., 1978;
[d] Biodynamics Inc. 1981a,b,c; [e] Eastman Kodak, 1960; [f] Smyth, 1960; [g] Eastman Kodak, 1987; [h] Eastman Kodak, 1986b; [i] Eastman Kodak, 1961; [j] Eastman Kodak, 1957; [k] Eastman Kodak, 1986c; [l] Eastman Kodak, 1986d;

Johnannsen and Leviskas have hypothesized that the acute toxicity of nitriles is due to release of cyanide upon metabolism (Johannsen and Leviskas, 1986). These investigators cite several different findings that support this hypothesis. First, the signs of acute nitrile intoxication (dyspnea, gasping, ataxia, and convulsions) are similar to those seen after acute cyanide poisoning. Second, the time course lasts several hours, suggesting that these agents require metabolism by the liver to be toxic. Third, cyanide and thiocyanate have been found in urine and blood after exposure to aliphatic nitriles. In support of this hypothesis, Willhite and Smith (1981) have shown that the acute toxicities of aliphatic nitriles (including propionitrile and butyronitrile) and formation of thiocyanate in mice are inhibited by antagonists of inorganic cyanide (sodium thiosulfate and sodium nitrite) and a hepatotoxicant (carbon tetrachloride). This study is summarized robustly in the dossiers for propionitrile and butyronitrile, and unequivocally shows that these two materials cause acute toxicity by the same mechanism. Since the symptoms of acute toxicity of isobutyronitrile are identical to those of n-butyronitrile and propionitrile, it is likely that this material also produces acute toxicity through liberation of cyanide.

In conclusion, adequate acute toxicity studies have been performed on all three nitriles in the category. Results of acute toxicity studies with propionitrile, n-butyronitrile and isobutyronitrile are remarkably similar (with the possible exception of priopionitrile, which is more potent by the dermal route).

3.4.3 Repeated Dose

The repeated dose toxicity of butyronitrile and isobutyronitrile have not been tested. The effect of inhalation of 60, 120 or 209 ppm propionitrile, 6 hr/day, 5 days/week over 14 weeks was tested in Sprague Dawley rats (Velasquez and Thake, 1984). A NOAEL was not established in this study, as all concentrations tested were toxic. Symptoms of neurotoxicity (ataxia, tremors and convulsions) were noted at the highest concentration tested. Labored breathing, nasal and ocular discharge, salivation, hypoactivity and/or alopecia were observed in all exposed groups. In addition, all groups had

significant decreases in red blood cells and hemoglobin values and increases in urine thiocyanate concentrations (a marker for cyanide toxicity) and spleen weights. No histopathological changes were noted in any of the organs (with the exception of hemosiderin deposits in the spleens of 11/15 females exposed to 120 ppm).

In general, symptoms of toxicity in this study are consistent with the rapid elimination of propionitrile after inhalation exposure (Biodynamics Inc, 1981d, 1987) and results of acute inhalation toxicity studies with propionitrile, n-butyronitrile and isobutyronitrile. The additional finding of toxicity to red blood cells and the spleen at a concentration that does not produce other symptoms of toxicity also is in agreement with the hypothesis that the toxicity of the nitriles is mediated through cyanide (Willhite and Smith, 1981). Heme in the hemoglobin of red blood cells avidly binds cyanide; therefore, red blood cells are targets of cyanide toxicity. Since the acute toxicity profiles of propionitrile, n-butyronitrile and isobutyronitrile are similar, the repeated dose toxicity profiles at similar concentrations of these materials should also be similar. Therefore, repeated dose toxicity testing of n-butyronitrile and isobutyronitrile is not necessary.

3.4.4 Genetic Toxicity

The genetic toxicity of the alkyl nitriles is summarized in Table 7.

Table 7. Genetic Toxicity of Alkyl Nitriles

Category Member	Ames Test (w/wout activation)	Mammalian Cell Mutagenesis[a]	In vitro Cytogenicity[b]	In vivo Cytogenicity[c]
Propionitrile CAS No. 107-12-0	Negative[d]	Negative[e] Positive[f]	No data	Negative
Butyronitrile CAS No. 109-74-0	Negative	No data	Negative	No data
Isobutyronitrile CAS No. 78-82-0	Negative	No data	Negative	No data

Study details are located in the dossiers (Sections 5.5 and 5.6). [a] mouse lymphoma cell assay; [b] Chinese Hamster Ovary Cell ; [c] mouse bone marrow cells; [d] Study was given a reliability rating of 4; [e] Test material contained 97.8% propionitrile, 0.3% adiponitrile, 0.1% paranitrosophenylamine, 0.1% water and <0.1% acrylonitrile or solids; [f] Test material contained 97.0% propionitrile, 1.2 % adiponitrile, 0.12% paranitrosophenylamine, 0.04% water and 0.02% acrylonitrile.

3.4.4.1 Mutagenicity

Propionitrile tested negative at concentration of up to 10,000 micrograms/ml in an Ames test conducted with *S. typhimurium* strains TA98, TA100, TA1535, and TA1538 (Flowers, 1977). This test was given a reliability rating of 4 (and therefore is not adequate), due to limited documentation and other inadequacies.

The results of two well-conducted mammalian cell mutagenicity studies with cultured mouse lymphoma L5178Y cells are mixed (Microbiological Associates, 1982a,b). The only difference between the two studies is that the negative one was conducted with a commercial material containing 97.8% propionitrile, 0.3% adiponitrile, 0.1% paranitrosophenylamine, 0.1% water and <0.1% acrylonitrile or solids, and the positive one was conducted with a material containing 97.0% propionitrile, 1.2 % adiponitrile, 0.12% paranitrosophenylamine, 0.04% water and 0.02% acrylonitrile. In addition to producing a positive response, the latter material was much more toxic to the cells than the former.

Concentrations of up to 5000 micrograms/plate n-butyronitrile and isobutyronitrile were tested for mutagenicity in EEC Guideline bacterial mutagenicity studies conducted with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and E. coli strain WP2uvra- in the presence and absence of metabolic activation (Covance Laboratories Inc, 1999a,c). The results of these tests were negative.

3.4.4.2 Chromosomal aberration

In vitro

n-Butyronitrile and isobutyronitrile have been tested for the ability to cause chromosomal aberrations in an OECD Test Guideline 473 study performed with cultured Chinese Hamster Ovary Cells (CHO WBL line) (Covance Laboratories Inc., 1999c,d). In both studies, there was no significant effect of the test material on the number of cells with aberrations (with and without metabolic activation). In the tests with n-butyronitrile and isobutyronitrile, the highest concentrations of test material used (701 and 700 micrograms/ml, respectively) did not cause a significant reduction in the mitotic index. Higher concentrations were not tested because 700 micrograms/ml was the highest recommended concentration for the assay.

In vivo

The ability of propionitrile (100 or 200 mg/kg orally) to cause chromosomal aberrations in bone marrow cells was tested in Sprague-Dawley rats (Hazleton Biotechnologies Corporation, 1985). There was no effect of treatment on the frequency of chromosomal aberrations, mean chromosome number or mitotic index. The study was conducted according to GLP, but was given a reliability rating of 2 (valid with restrictions) since only 2 animals were available for analysis at 200 mg/kg.

In conclusion, adequate studies have been performed which show that the nitriles are not genotoxic. The results of studies with propionitrile indicate that small amounts of contaminants present in commercial preparations of this material may be mutagenic.

3.4.5 Reproductive and Developmental Toxicity

3.4.5.1 Reproductive Toxicity

Reproductive toxicity tests with n-butyronitrile or isobutyronitrile have not been performed. The effect of propionitrile on female and male fertility was tested in two separate studies that were given reliability ratings of 1 (valid without restriction)(Kier, 1984a,b). In the female fertility study, up to 210

ppm propionitrile was administered by inhalation, 6 hrs/day, 7 days/week, to females only, 21 days prior to mating and during mating until copulation was confirmed. For the study in males, up to 200 ppm propionitrile was given to males only by inhalation, 6 hours/day, 5 days a week, for 57 days (46 days prior to mating and 11 days during mating). In either study, there was no effect of propionitrile on fertility. In both studies, signs of acute cyanide toxicity (arched back, hypoactivity, salivation, lacrimation and/or labored breathing) were observed in high and mid dose animals. Signs of toxicity generally abated by the morning after exposure.

The signs of toxicity observed in this study are consistent with those observed in the repeated dose study with propionitrile and acute toxicity studies with all three nitriles. Since the category members have similar structures, metabolism and acute toxicity profiles, it is expected that they would have a similar reproductive toxicity profile. Therefore, results of the fertility study with propionitrile should be predictive of those for n-butyronitrile and isobutyronitrile. No additional testing is necessary.

3.4.5.2 Developmental Toxicity

Results of developmental toxicity tests with the aliphatic nitriles are summarized in Table 8.

Table 8. Developmental Toxicity of Alkyl Nitriles

Category Member	Animal	Treatment	Effects
Propionitrile CAS No. 107-12-0	Sprague-Dawley rat	Gavage, 20, 40, 80 mg/kg/day, GD 6-19	NOAEL (maternal) = 40 mg/kg/day NOAEL (teratogenicity) = 80 mg/kg/day NOAEL (fetotoxicity) = 40 mg/kg/day. Decreased fetal body weight and increased skeletal variants at 80 mg/kg/day
Butyronitrile CAS No. 109-74-0	Sprague-Dawley rat	Inhalation, 50, 100, 150, 200 ppm 6 hr/day, GD 6-20	NOAEL (maternal) = 200 ppm NOAEL (teratogenicity) = 200 ppm NOAEL (fetotoxicity) = 150 ppm. Decreased fetal body weight (females) at 200 ppm.
Isobutyronitrile CAS No. 78-82-0	Sprague-Dawley rat	Inhalation, 50, 100, 200, 300 ppm 6 hr/day, GD 6-20	NOAEL (maternal) = 200 ppm NOAEL (teratogenicity) = 200 ppm NOAEL (fetotoxicity) = 100 ppm. Decreased fetal body weight at 200 and 300 ppm.

Study details are located in the dossiers (Section 5.8.2). GD = gestation day; NOAEL = No observable adverse effect level

Propionitrile has been tested for the ability to cause developmental toxicity at oral concentrations up to 80 mg/kg/day (IRDC, 1981). This study was given a reliability rating of 2 (valid with restrictions), since maternal body weights or weight gains were not analyzed statistically. The authors concluded that the NOAEL for teratogencity was 80 mg/kg, since there was no effect of treatment with up to 80 mg/kg on the incidence of malformations. The summary preparer established a NOAEL for embryotoxicity of 40 mg/kg, since there was a significant increase in the number of early resorptions at 80 mg/kg. The NOAEL for fetotoxicity also was 40 mg/kg, since offspring of dams treated with 80 mg/kg had lower body weights and increased incidences of some skeletal variations compared to controls. This dose appeared to be the NOAEL for maternal toxicity, since the authors stated that dams

treated with 80 mg/kg had a slight to moderate reductions in weight gain. However, statistical evidence to support this conclusion was not supplied.

The developmental toxicity of n-butyronitrile has been evaluated in a study similar to OECD test guideline 414, which was given a reliability rating of 1 (valid without restriction) (Stump, 2001b). Groups of pregnant Sprague-Dawley rats were exposed to 50, 100, 150 or 200 ppm n-butyronitrile. vapor 6 hr/day, from Days 6 – 20 of gestation. On Day 21, all animals were euthanized, and the uterus and contents were weighed. The numbers of viable and non-viable fetuses, resorptions, and implantation sites were also recorded. Each fetus was weighed, sexed and examined for external abnormalities. One half of the fetuses were examined for visceral abnormalities, and the other half was examined for skeletal anomalies. There was no evidence of maternal toxicity (as assessed by body weight changes only) or embryotoxicity, and no effect of treatment on the number of viable fetuses. No visceral or skeletal anomalies were attributed to treatment. The only effect observed in fetuses was reduced body weight of female fetuses from dams treated with 200 ppm. The NOAELs for maternal toxicity and teratogenicity were 200 ppm, and the NOAEL for fetotoxicity was 150 ppm.

The toxicity of isobutyronitrile was examined in a developmental study identical to that described above, with the exception that 50, 100, 200 and 300 ppm were tested (Saillenfait et al., 1993). No visceral or skeletal anomalies were attributed to treatment. The NOAEL for maternal toxicity was 100 ppm, since administration of 200 and 300 ppm caused lethality in a few animals. The NOAEL for fetotoxicity also was 100 ppm, since a reduction in fetal body weight was observed in female offspring from dams treated with 200 ppm, and both sexes at 300 ppm. The NOAEL for teratogenicity was 200 ppm, since 300 ppm caused an increase in embryolethality.

In conclusion, none of the nitriles tested were teratogenic (i.e. caused malformations). Embrolethality occurred after exposure to isobutyronitrile or propionitrile at concentrations that also induced maternal toxicity (300 ppm or 80 mg/kg, respectively). Fetotoxicity (as evidenced by reduced fetal body weights and/or increased incidences of skeletal variants) also was observed in offspring of rats treated with maternally toxic concentrations of all three nitriles. The fetotoxic effects that were observed were nonspecific in nature, and are commonly observed in offspring from dams with lower than normal body weight gains.

3.4.6 Test Plan for Mammalian Toxicity

Adequate acute, genetic and developmental toxicity tests have been performed on all of the category members. Repeated dose and reproductive toxicity studies have been performed with propionitrile. Results of these studies show that the profile of toxicity noted with repeated exposure to propionitrile is consistent with repeated exposure to acutely toxic concentrations that are rapidly eliminated, and that exposure of males and females to concentrations of propionitrile that produce acute symptoms of cyanide toxicity has no effect on fertility. Additional repeated dose or reproductive toxicity testing of the nitriles that have not been tested (n-butyronitrile and isobutyronitrile) is not planned, due to the physical, metabolic and acute toxicological similarities of these materials with propionitrile, and the lack of reproductive toxicity and cumulative repeated dose toxicity with this material.

4. Summary

Physical properties

Measured data are available for melting point, boiling point, vapor pressure and water solubility for all category members. Estimated data for octanol/water partition coefficients are available. Existing data are sufficient to characterize physical property endpoints for these chemicals. As discussed in Section 3.1, the data indicate that the category members behave similarly with respect to physical properties, and serve to support the category approach for these members.

Environmental fate properties

Estimated values are available for all category members with respect to the hydroxyl radical induced photolysis rate constant and atmospheric half-life, Henry's Law Constant, and Fugacity Level III environmental transport parameters. No hydrolysis studies were located for propionitrile, butyronitrile or isobutyronitrile. The theoretical hydrolysis of propionitrile and several other chemicals has been examined by Dr. Lee Wolfe at the USEPA Environmental Research Laboratory in Athens, Georgia. The results of these analyses were published in a report that indicated that propionitrile can hydrolyze slowly. According to a study cited in the Hazardous Substances Data Bank, the chemical hydrolysis of acetonitrile at pH 7 is more than 150,000 yrs. Taken together, these data suggest that hydrolysis of propionitrile, butyronitrile or isobutyronitrile at environmentally relevant pHs will occur too slowly to be a significant means of degradation. Adequate biodegradation tests with all three materials indicate that environmentally relevant concentrations of these materials can degrade over time. Overall, the existing data for environmental fate properties are adequate and demonstrate the similar behaviors of category members.

Aquatic toxicity

Adequate aquatic toxicity data exist for all 3 category members (with the exception of algal toxicity for propionitrile). Available data indicate that the other category members have a similar degree of toxicity toward algae, and that ECOSAR-estimated values for algae are similar. Therefore, it is likely that data for the other nitriles (esp. isobutyronitrile, which has the lowest EC_{50} value) are sufficient to fill the endpoint for propionitrile. The LC/EC_{50} values obtained in the studied species are consistent with the materials being of low level for concern.

Mammalian toxicity

Adequate tests have been performed for all three category members (with the exception of repeated dose and reproductive toxicity). All three nitriles are industrial intermediates with low potential for exposure to the general public. Results of repeated dose and reproductive studies performed with propionitrile will be predictive of those for n-butyronitrile and isobutyronitrile since all three materials have a similar mechanism of action of mammalian toxicity and are virtually identical in structure. No additional testing is necessary.

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Data Set

Existing Chemical	:	ID: 107-12-0
CAS No.	:	107-12-0
EINECS Name	:	propiononitrile
EC No.	:	203-464-4
TSCA Name	:	Propanenitrile
Molecular Formula	:	C3H5N

Producer related part

Company	:	Solutia Inc.
Creation date	:	06.06.2003

Substance related part

Company	:	Solutia Inc.
Creation date	:	06.06.2003
Status	:	
Memo	:	
Printing date	:	06.10.2003
Revision date	:	13.11.2003
Date of last update	:	30.10.2003
Number of pages	:	58
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	C(#N)CC
Molecular formula	:	C3H5N
Molecular weight	:	55.079
Petrol class	:	

11.06.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	liquid
Purity	:	= 99.6 % v/v
Colour	:	clear
Odour	:	pungent

11.06.2003 (32)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

cyanoethane

10.07.2003

ethyl cyanide

10.07.2003

propanenitrile

10.07.2003

propionic nitrile

10.07.2003

propionitrile

10.07.2003

propylnitrile

10.07.2003

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category :

Reliability : (2) valid with restrictions
11.06.2003

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information :

11.06.2003

Type : TSCA
Additional information :

11.06.2003

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= -92.8 °C
Sublimation	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed, published data.
Flag	:	Critical study for SIDS endpoint

11.06.2003 (29)

2.2 BOILING POINT

Value	:	= 97 °C at 1013 hPa
Decomposition	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed, published data.
Flag	:	Critical study for SIDS endpoint

10.07.2003 (29)

2.3 DENSITY

Type	:	relative density
Value	:	= .7818 g/cm³ at 20 °C
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed, published data.

10.07.2003 (37)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 52 hPa at 20 °C
Decomposition	:	
Method	:	other (measured)
Year	:	
GLP	:	no

Test substance : as prescribed by 1.1 - 1.4

Remark : Purity of the test material is 99.6%.
Reliability : (2) valid with restrictions
Source of data is a MSDS.
Flag : Critical study for SIDS endpoint
29.07.2003 (32)

Value : = 53.3 at 22 °C
Decomposition :
Method : other (measured): not specified
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Purity of the material is unknown.
Result : Value is 40 mm Hg, which converts to 53.3 hPa.
Source : Hazardous Substances Data Bank for Priopionitrile, dated 5/13/99.
Reliability : (2) valid with restrictions
Primary source is peer reviewed and published.
10.07.2003 (10)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = .16 at °C
pH value :
Method : other (measured)
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Test condition : The value was obtained using the Shake-Flask method. The aqueous phase was octanol-saturated water. The concentration of material in the aqueous phase was measured using gas-liquid chromatography.
Test substance : Purity of the test material was not mentioned.
Reliability : (2) valid with restrictions
Data were from a peer reviewed, published source.
Flag : Critical study for SIDS endpoint
13.08.2003 (31)

Partition coefficient : octanol-water
Log pow : = .35 at 20 °C
pH value :
Method : other (calculated)
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program are CAS No., melting point (-92.8 degrees C), boiling point (97 degrees C), vapor pressure (39 mm Hg), and water solubility (93380 mg/l).
Reliability : (2) valid with restrictions
Data were obtained by modeling.
13.08.2003 (17)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : water
Value : = 93380 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not specified
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Test substance : Purity of the test material was not mentioned.
Reliability : (2) valid with restrictions
Data were from a peer reviewed, published source.
Flag : Critical study for SIDS endpoint
13.08.2003 (38)

Solubility in : water
Value : = 119 g/l at 40 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not specified
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Purity of material is unknown.
Reliability : (2) valid with restrictions
Source is peer-reviewed, published data.
15.08.2003 (37)

Solubility in : water
Value : = 55650 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: calculated
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program are CAS No., melting point (-92.8 degrees C), boiling point (97 degrees C), vapor pressure (39 mm Hg), and water solubility (93380 mg/l).
Reliability : (2) valid with restrictions
Data were obtained by modeling.
15.08.2003 (18)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	= 16 °C
Type	:	open cup
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of the test material is unknown.
Source	:	Hazardous Substances Data Bank for propionitrile, dated 5/13/99.
Reliability	:	(2) valid with restrictions Primary reference is peer-reviewed and published.

10.07.2003 (23)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .0000000000001938 cm³/(molecule*sec)
Degradation	:	= 50 % after 55.2 day(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program are CAS No., melting point (-92.8 degrees C), boiling point (97 degrees C), vapor pressure (39 mm Hg), and water solubility (93,380 mg/l).
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

15.08.2003 (13)

3.1.2 STABILITY IN WATER

Type	:	abiotic
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN Hydrowin cannot calculate hydrolysis rate constants for nitriles. The theoretical hydrolysis of propionitrile and several other chemicals has been examined by Dr. Lee Wolfe at the USEPA Environmental Research Laboratory in Athens, Georgia. The results of these analyses were published in a report by Dr. Wolfe that could not be located. In a personal communication, Dr. Wolfe stated that propionitrile can hydrolyze (albeit slowly). According to a study cited in the Hazardous Substances Data Bank, the chemical hydrolysis of the related material acetonitrile in water is base-catalyzed (the rate constant for base catalyzed hydrolysis is 5.8X10-3/M-hr), but the half-life at pH 7 is more than 150,000 yrs (Ellington et al., 1988). Acetonitrile ($CH_3C{\equiv}N$, CAS No. 75-05-8) is the 2-carbon analog of the category members, possessing the same functionality, but having one less carbon than propionitrile. Taken together, these data suggest that hydrolysis of propionitrile at environmentally relevant pHs will occur too slowly to be a significant means of degradation.
Reliability	:	(2) valid with restrictions Experimental results for the test material could not be located. Results are for a related material.

07.08.2003 (16)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air, water, soil and sediment
Air	:	14.4 % (Fugacity Model Level I)
Water	:	48.7 % (Fugacity Model Level I)
Biota	:	.0821 % (Fugacity Model Level II/III)
Soil	:	36.9 % (Fugacity Model Level II/III)
Method	:	other
Year	:	2003
Remark	:	Measured inputs to the program are CAS No., melting point (-92.8 degrees C), boiling point (97 degrees C), vapor pressure (39 mm Hg), and water solubility (93,380 mg/l).
Result	:	Henry's Law Constant (Bond Est) is estimated to be 4.06E-005 atm-m3/mole. The soil/sediment constant Koc is 8.3 as estimated by the EPIWIN PCKOC Program (v1.66).
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

15.08.2003 (15)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other: activated sludge
Concentration	:	1000 mg/l related to COD (Chemical Oxygen Demand)
Contact time	:	6 hour(s)
Result	:	other: biodegradable
Deg. product	:	
Method	:	other
Year	:	1960
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The test conditions state that the test was performed for 6 hours, but the results for 10 hours are listed. The study results show that biodegradation occurred, but the results are not related to TOD. Based on the TOD of the previous study (1670 mg/l), the percentage of material that biodegraded in 10 hours was approximately 24%.
Result	:	The oxygen uptake was as follows: Time (hrs) Approximate O2 uptake (mg/l)

2	100
4	210
6	300
8	350
10	400

The study results show that 400 mg/l O2 out of a possible 1000 mg/l (COD) was utilized (40%).

The kinetics of the metabolism of propionamide and propionic acid suggested that propionitrile was sequentially metabolized to these materials.

Test condition : Bacteria: Activated sludges were grown in 1.5 liter pilot plants under 23 hour aeration. The material was allowed to settle for 1 hour. After settling, 1.0 liter of supernatant liquor was withdrawn, 1.0 liter of tap water, organics and buffer were added, and aeration was resumed. This cycle was repeated 7 days a week for the duration of the study.

Test material: The quantity of feed was 1000 mg/l (based on COD). Occasionally, at the end of the aeration period, 25 ml of mixed liquor was passed through a membrane filter, which was then dried at 103 degrees C and reweighed to determine the amount of suspended solids. The filter was then analyzed for COD and all forms of inorganic nitrogen.

Washed sludge, grown on propionitrile, was placed in a Warburg Respirometer with low concentrations of several possible breakdown products. Oxygen uptake was then measured for 6 hours. After this time, the contents of each flask were passed through a membrane filter so that the filtrate could be analyzed for COD and inorganic nitrogen forms.

Reliability : (2) valid with restrictions
Purity of the test material was not given.

Flag : Critical study for SIDS endpoint

10.08.2003 (34)

Type : aerobic
Inoculum : other: activated sludge
Concentration : 500 mg/l
Contact time : 72 hour(s)
Degradation : = 0 (±) % after 72 hour(s)
Result : other: material was toxic
Deg. product :
Method : other
Year : 1970
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : TOD for propionitrile was 1670 mg/l. Test material was toxic to all three sludges. No biodegradation occurred.

Test condition : Bacteria: The activated sludges were obtained from the municipal plant at Franklin, TN, the municipal plant at Nashville TN and the plant at Bordeaux, a suburb of Nashville. Mixed liquor from the aeration tanks was collected in the morning, the day of the Warburg run. Each sample was packed in ice and transported to the laboratory within 1 hour of collection. Before the run began, the sludge sample was blended for 10 sec and the homogenous blend was analyzed for concentration of SS (not defined, but assumed suspended solids), using a membrane-filter technique. The original sample was adjusted to a SS concentration of 2,500 mg/l.

Test conduct: Test material was added to a Warburg flask (125 ml) in order to obtain a final concentration of 500 mg/l in the reaction compartment (final volume of 20 ml). KOH (1.0 ml, 20%) was added to the

center well. A 20 ml volume of activated sludge was then introduced. The test was performed in duplicate. Flasks were incubated for 72 hours (constant motion) at 20 degrees C. All three sludges were tested. Oxygen uptake curves were plotted. Respiration of the sludge alone was plotted as the control curve.

Theoretical O2 demand (the mg/l O2 required to completely oxidize the test material) was calculated on the basis of the test material to CO2 and water, plus nitrate according to the following equation: [TOD = moles of O2 required to balance the equation x molecular weight of O2 x concentration of test material/ (moles of test material required to balance the oxidation equation x molecular weight of the test material)]. The percentage of TOD was to be calculated as follows: % TOD = 100 x D (the difference in mg/l of O2 uptake between substrate and control)/ TOD. The material was considered toxic if D was less than 0.

Reliability : (2) valid with restrictions
Purity of the test material was not given.

08.08.2003 (26)

Type : aerobic
Inoculum : other: mixed microbial culture
Concentration : 1000 mg/l
Contact time : 48 hour(s)
Result : other: biodegradable
Deg. product :
Method : other
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The final protein and ammonia concentrations and pH were 10.20 mg/l, 76.9 micromoles/ml and 8.66, respectively, indicating that the mixed culture could use this material as a growth substrate.

Test condition : A mixed microbial culture (protein concentration of 0.085 mg/l) was isolated from an environment contaminated with organic cyanides and polychlorinated biphenyls. This was grown for 48 hours on phosphate buffer (ph 7.0, 30 degrees C) containing propionitrile (1 g/l) as the sole source of carbon and nitrogen. The final concentration of protein, ammonia and pH were determined.

Test substance : Test material was obtained from Aldrich Chemical Co. It is presumed that the material has high purity.

Reliability : (4) not assignable
The study shows that the test material was used as a substrate (and therefore was metabolized); however, the extent to which the test material biodegraded is difficult to determine from the study.

07.08.2003 (9)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 1520
EC50	:	= 1520
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other: following the U.S. EPA Committee on Methods for Toxicity Tests with Aquatic Organisms
Year	:	1990
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Result : None of the controls or fish exposed to measured concentrations < = 887 mg/l died or had abnormal behavior. One fish exposed to 1100 mg/l died by 24 hours. All fish exposed to 2188 mg/l died by24 hours. Affected fish lost schooling behavior, were darkly colored and lost equilibrium prior to death. The 96 hour LC50 and EC50 values (with confidence intervals) were the same (1520 and 1450-1580 mg/l, respectively).

The average (+/- SD) temperature, dissolved oxygen, hardness, alkalinity and pH of the water in the test chambers were 24.4 +/- 0.63 degrees C, 7.3 +/- 0.22 mg/l, 47.0 +/- 0.44 mg/l CaCO3, 40.1 +/- 1.04 mg/l CaCO3, and 7.6 +/- 0.21. It is not known whether these variables were affected by test material concentration.

Average and ranges of analytical concentrations of the chambers treated with 0, 455, 700, 1080, 1660 and 2550 mg/l material were <5, 375, 610, 885, 1098 and 2184 mg/l, respectively. When corrected for recovery (99.8 %), test material concentrations were < 5.01, 375, 611, 887, 1100 and 2188 mg/l.

The mean length and weight (+/- SD) of the fish at study termination were 20.8 +/- 1.673 mm and 0.092 +/- 0.0244 g.

Test condition : Newly hatched minnows from adults reared in flow-through tanks were held at 25 degrees C in flowing water with a 16-hr photoperiod and were fed brine shrimp nauplii three times daily (twice on weekends). They were cultured in filtered Lake Superior water or dechlorinated water from the city of Superior, WI (exact source not given) The two waters were similar in all measured chemical parameters. This water was used for test material dilution and all tests.

Healthy fish (32 days old) were fasted for 24 hours before treatment. They were pooled together in one tank and randomly distributed among the exposure chambers. Tests were initiated by adding 20 fish per treatment (455, 700, 1080, 1660 and 2550 mg/l) and control to test chambers containing 1.0 liter of water. Fish loading was 0.1278 g/l. The rate of exchange was 14.4 volumes of test water per day.

Observations of fish behavior and toxic signs were made at 2-8, 24, 48, 72 and 96 hr and recorded on checklists specifically formatted to convert observational data for approximately 100 endpoints into a numerically coded form. Death (cessation of opercular movements and inability to respond when prodded) was recorded at 24, 48, 72 and 96 hours. Dead fish were removed. At study termination, individual control fish were weighed (wet) and measured.

All test exposure chambers were sampled for test material concentration at the beginning of the test and daily thereafter. Concentrations of test material were analyzed using gas-liquid chromatography. All analyses included one spike and one duplicate sample for every 6 to 12 water samples.

Five water quality parameters were routinely measured for each test: temperature, dissolved oxygen, total hardness, total alkalinity, and pH. The desired test temperature was 25 +/- 1 degrees C. Daily measurements of oxygen concentration were taken in each treatment and the control exposure chambers if fish were present. The control and one or more treatment chambers were sampled once for total hardness and alkalinity. The pH was measured once in the control and in one to five of the treatment tanks (specific times were not stated).

The estimated LC50 and EC50 values, with corresponding 95% confidence intervals were calculated using the corrected average of the analyzed tank concentrations and the Trimmed Spearman-Karber Method. The EC50 values were based on loss of equilibrium manifested by an inability of the fish to remain in an upright position when swimming. The mean concentrations used in the calculations were corrected for analytical recoveries of spiked water samples.

Test substance : Purity of test material was 99 %.
Reliability : (1) valid without restriction.
The study was comparable to a guideline study.
Flag : Critical study for SIDS endpoint
10.08.2003 (20)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : < 10 measured/nominal
LC50 : = 41 measured/nominal
Limit test : no
Analytical monitoring : no
Method : other: APHA, Standard Methods for Examination of Water and Wastewater, 14th Ed., 1975
Year : 1981
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Although the dissolved oxygen concentration in all 3 tested solutions dropped below 40% saturation at 96 hours, the authors concluded that this did not have an effect on mortality.
Result : None of the negative control fish died. Ten percent of the fish exposed to 10 or 18 mg/l died by 96 hours. The mortality rate for fish exposed to 32 mg/l was 20% at 24 hours, 20% at 48 hours, 40% at 72 hours, and 50% at 96 hours. The mortality rate for fish exposed to 56 mg/l was 0% at 24 hours, 40% at 48 and 72 hours, and 50% at 96 hours. The mortality rate for fish exposed to 100 mg/l was 50% at 24 hours, and 90% at 48, 72 and 96 hours. The 24, 48 and 96 hour LC50 values for propionitrile (with confidence limits if applicable) were > 100 mg/l, 56 (43 - 78) mg/l and 41 (28 - 66) mg/l, respectively.

Negative control fish appeared normal at all observations. One fish in each of the 10 and 18 mg/l groups was surfacing at 96 hours. All fish that did not die after exposure to 32 mg/l appeared normal (with the exception of 1 fish that had an illegible observation at 48 hours). All survivors exposed to 56 and 100 mg/l were observed to be surfacing at 96 hours. The weight and

length of the fish at the end of the test were 0.12 +/- 0.02 g and 19 +/- 1.5 mm, respectively.

The temperature was 22 degrees C for all water samples. The dissolved oxygen concentration was 9.0 in each tested solution at 0 hours. Dissolved oxygen concentration ranged from 6.0 - 8.5 at 48 hours, and 2.0 - 4.3 at 96 hours. The water containing 100 mg/l propionitrile had the lowest dissolved oxygen concentration at 48 and 96 hours. The pH ranged from 7.1 - 7.7. Total ammonia concentrations were < 0.1 mg/l at each measurement. All temperatures, pH values and ammonia concentrations were within acceptable limits. Dissolved oxygen concentrations at 96 hours were not within acceptable limits (as defined by the protocol as 40-100% saturation).

The 24, 48 and 96 hour LC50 values for the positive control (with confidence limits if applicable) were > 0.00014 mg/l, 0.00012 mg/l and 0.00010 (0.000075 - 0.00014) mg/l, respectively. The LC50 values for the positive control were within the 95 % confidence limits reported in the literature.

Test condition : Test organisms: The bluegill sunfish used in the study were obtained from Osage Catfisheries, Inc., Osage Beach, MO. All fish were on a 16 hour daylight photoperiod and observed for at least 14 days prior to testing. Fish received a standard commercial fish food daily until 48 hours prior to testing. The mean weight and length of the fish at the end of the test were 0.12 +/- 0.02 g and 19 +/- 1.5 mm, respectively. Maximum loading was 0.8 g fish/liter of solution.

Test material: Test concentrations were prepared based on the total compound. They were obtained by transferring appropriate aliquots from a working standard (150 mg/ml test material in absolute ethanol) directly to the test chambers. A preliminary, 48-hour range finding test was conducted with 1, 10 and 100 mg/l. The definitive, 96 hour test was conducted with 5 concentrations of test material ranging from 10 - 100 mg/l. The negative control chamber received an ethanol aliquot equivalent to the highest amount used in the test solutions. A positive control (Antimycin A), also was tested at concentrations ranging from 0.000014 to 0.00014 mg/l.

Test water: The well water from which the reconstituted water was prepared contained < 0.01 ppm aluminum, copper and zinc, <0.001 ppm arsenic, cadmium, and cobalt, 0.001 ppm chromium, 0.012 ppm iron, 0.009 ppm lead, <0.0001 ppm mercury, 0.0157 ppm nickel, and <0.3 ppb of commonly analyzed pesticides. The water was reconstituted to contain 48 mg/l $NaHCO_3$, 30 mg/l $CaSO_4$, 30 mg/l $MgSO_4$, and 2 mg/l KCl. The hardness, alkalinity and initial pH of the water were 45 mg/l (as $CaCO_3$), 35 mg/l (as $CaCO_3$) and 7.3, respectively. The dissolved oxygen concentration at the start of the test was 9.0 mg/l. The temperature of the water was kept at 22 +/- 1 degrees C.

Test conduct: Tests were conducted in 5 gallon glass vessels containing 15 liters of reconstituted water. The test fish (10 per test concentration) were acclimated to the dilution water for 48 hours prior to testing. They were not fed during this acclimation period or during the test. The test concentrations (10, 18, 32, 56 and 100 mg/l) were chosen based on the results of a preliminary study. Two additional groups of 10 fish were exposed to the negative or positive control. Fish were added randomly within 30 minutes of preparation of the test solutions. All fish were observed at 24, 48, 72 and 96 hours for mortality and abnormal behavior. The pH, dissolved oxygen concentration, and temperature of water in the negative control, and 10 mg/l and 100 mg/l test vessels were determined at the beginning of the test and after 48 and 96 hours. Total ammonia concentration of water in these 3 vessels was determined at the beginning and end of the test.

Statistical analysis: Concentration vs. lethality data were analyzed by a computer program which utilized the binomial, moving average and probit tests to determine the LC50 values (and 95% confidence limits) at 24, 48 and 96 hours. The method of calculation selected for presentation (the probit method for propionitrile and the binomial method for Actinomycin A) was the one that gave the narrowest confidence limit.

Test substance : The purity of the test material (lot # 34) was 96.1%. Contaminants were not mentioned.

Reliability : (2) valid with restrictions
Test concentrations were not analytically confirmed.

07.08.2003 (2)

Type : static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 180 measured/nominal
LC50 : = 340 measured/nominal
Limit test : no
Analytical monitoring : no
Method : other: Committee on Methods for Toxicity Tests With Aquatic Organisms, EPA-660/3-75-009, 1975
Year : 1981
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : A brown film was present on the surface of water containing the highest 2 concentrations of propionitrile.

Result : None of the negative control fish or fish exposed to 100 or 180 mg/l died. The mortality rate for fish exposed to 320 mg/l was 10% at 24 hours, 20% at 48 hours, 30% at 72 hours, and 40% at 96 hours. The mortality rate for fish exposed to 560 or 1000 mg/l was 100% by 24 hours. The 24, 48 and 96 hour LC50 values for propionitrile (with confidence limits if applicable) were 400 (320 - 560) mg/l, 380 (180 - 560) mg/l and 340 (180 - 560) mg/l, respectively.

Negative control fish and fish exposed to 100 and 180 mg/l appeared normal at all observations. Surfacing was observed in some of the fish exposed to 320 mg/l at 48 hours (N = 8), 72 hours (N = 2) and 96 hours (N = 1). An illegible abnormality was observed in one fish exposed to 320 mg/l for 24 hours. The weight and length of the fish at the end of the test were 0.12 +/- 0.02 g and 19 +/- 1.5 mm, respectively.

The temperature was 12 degrees C for all water samples. The dissolved oxygen concentration was 9.4 and 8.7 in each tested solution at 0 and 48 hours, respectively. Dissolved oxygen concentration ranged from 8.0 - 9.1 at 96 hours. The pH ranged from 6.8 - 7.7 in all water samples tested (except for the water containing 1000 mg/l, which had a pH of 9.1 at 0 hours). Total ammonia concentrations were < 0.1 mg/l in the control and 100 mg/l solution at 0 hours, 0.21 mg/l in the control solution at 96 hours, 0.27 mg/l in the 1000 mg/l solution at 0 hours and the 100 mg/l solution at 96 hours, and 0.52 mg/l in the 320 mg/l solution at 96 hours. All temperatures, pH values, and dissolved oxygen and ammonia concentrations were within acceptable limits.

The 24, 48 and 96 hour LC50 values for the positive control (with confidence limits if applicable) were 0.00014 (0.00010 - 0.00047) mg/l, 0.000041 (0.000032 - 0.000052) mg/l and 0.000030 (0.000024 - 0.000042) mg/l, respectively. The LC50 values for the positive control were within the 95 % confidence limits reported in the literature.

Test condition : Test organisms: The rainbow trout used in the study were obtained from

Spring Creek Trout Hatchery in Lewistown, Montana. All fish were on a 16 hour daylight photoperiod and observed for at least 14 days prior to testing. Fish received a standard commercial fish food daily until 48 hours prior to testing. The mean weight and length of the negative control fish at the end of the test were 1.16 +/- 0.37 g and 44 +/- 3.7 mm, respectively. Maximum loading was 0.8 g fish/liter of solution.

Test material: Test concentrations were prepared based on the total compound. They were obtained by transferring appropriate aliquots from a working standard (150 mg/ml test material in absolute ethanol) directly to the test chambers. A preliminary, 48-hour range finding test was conducted with 10 and 100 mg/l. The definitive, 96 hour test was conducted with 5 concentrations of test material in a logarithmic series ranging from 100 - 1000 mg/l. The negative control chamber received an ethanol aliquot equivalent to the highest amount used in the test solutions. A positive control (Antimycin A), also was tested at concentrations ranging from 0.000014 to 0.00014 mg/l.

Test water: The well water from which the reconstituted water was prepared contained < 0.01 ppm aluminum, copper and zinc, <0.001 ppm arsenic, cadmium, and cobalt, 0.001 ppm chromium, 0.012 ppm iron, 0.009 ppm lead, <0.0001 ppm mercury, 0.0157 ppm nickel, and <0.3 ppb of commonly analyzed pesticides. The water was reconstituted to contain 48 mg/l $NaHCO_3$, 30 mg/l $CaSO_4$, 30 mg/l $MgSO_4$, and 2 mg/l KCl. The hardness, alkalinity and initial pH of the water were 45 mg/l (as $CaCO_3$), 35 mg/l (as $CaCO_3$) and 7.3, respectively. The dissolved oxygen concentration at the start of the test was 9.4 mg/l. The temperature of the water was kept at 12 +/- 1 degrees C.

Test conduct: Tests were conducted in 5 gallon glass vessels containing 15 liters of reconstituted water. The test fish (10 per test concentration) were acclimated to the dilution water for 48 hours prior to testing. They were not fed during this acclimation period or during the test. The test concentrations (100, 180, 320, 560 and 1000 mg/l) were chosen based on the results of a preliminary study. Two additional groups of 10 fish were exposed to the negative or positive control. Fish were added randomly within 30 minutes of preparation of the test solutions. All fish were observed at 24, 48, 72 and 96 hours for mortality and abnormal behavior. The pH, dissolved oxygen concentration, temperature of water and total ammonia concentration in the negative control, and 100 mg/l and 1000 mg/l test vessels were determined at the beginning of the test. At 48 and 96 hours, these variables were tested in control water and water containing 100 and 320 mg/l test material (with the exception that total ammonia was not measured at 48 hours).

Statistical analysis: Concentration vs. lethality data were analyzed by a computer program which utilized the binomial, moving average and probit tests to determine the LC50 values (and 95% confidence limits) at 24, 48 and 96 hours. The method of calculation selected for presentation (the binomial method) was the one that gave the narrowest confidence limit.

Test substance : The purity of the test material (lot # 34) was 96.1%. Contaminants were not mentioned.

Reliability : (2) valid with restrictions
Test concentrations were not analytically confirmed.

07.08.2003 (1)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)

Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 100 measured/nominal
EC50	:	= 250 measured/nominal
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: Committee on Methods for Toxicity Tests With Aquatic Organisms, EPA-660/3-75009, 1975
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	A no observable effect level of 100 mg/l was listed by the authors although abnormal behavior was noted in 1/20 daphnids exposed to this concentration.
Result	:	None of the controls or daphnids exposed to 100 mg/l died during the study. One daphnid in one vessel containing 180 mg/l died between 24 and 48 hours. Three fish exposed to this concentration in another vessel died (one by 24 hours). Therefore, the overall death rate of daphnids exposed to 180 mg/l was 5% and 24 hours and 20% at 48 hours. Ten out of 20 daphnids exposed to 320 mg/l (4 in one vessel and 6 in the other) died within 24 hours, and 15 died by 48 hours (8 in one vessel and 7 in the other). Therefore, the overall death rate of daphnids exposed to 320 mg/l was 50% and 24 hours and 75% at 48 hours. All daphnids exposed to 560 or 1000 mg/l died within 24 hours. The 24 and 48 hour LC50 values (with confidence limits) were 310 (270 - 350) mg/l and 250 (210 - 290) mg/l, respectively. In one vessel containing daphnids exposed to 100 mg/l, 1 daphnid was observed on the surface at 48 hours. Two daphnids exposed to 320 mg/l (in one vessel) were on the surface at 24 hours. Behavior of other daphnids was normal. The initial temperature, dissolved oxygen concentration and pH of the control water were 20 degrees C, 8.8 mg/l and 8.6. The temperature, dissolved oxygen concentration and pH of all water assayed at 48 hours were 21 degrees C, 7.8 - 8.3 mg/l and 8.6 - 8.7. All temperatures, dissolved oxygen concentrations and pH values were within acceptable limits.
Test condition	:	Test organisms: The Daphnia magna used in the study were obtained from an in-house culture. The adults were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. All daphnids were held at 20 +/- 2 degrees C, under a 16 hour daylight photoperiod. First instar daphnids (< 24 hours old) were used in the test. Test daphnids were not fed during the study. Test material: Test concentrations were not corrected for sample purity. A primary standard of 20 mg/ml was prepared in water. Appropriate volumes of this standard were added to test water to obtain test concentrations. Test water: The water used in the study was from a deep well source. It contained < 0.01 ppm aluminum, copper and zinc, <0.001 ppm arsenic, cadmium, and cobalt, 0.001 ppm chromium, 0.012 ppm iron, 0.009 ppm lead, <0.0001 ppm mercury, 0.0157 ppm nickel, and <0.3 ppb of commonly analyzed pesticides. The hardness, alkalinity, conductivity, dissolved oxygen concentration and initial pH of the well water were 255 ppm (as CaCO3), 368 ppm (as CaCO3), 50 micromhos/ cm, 9.2 ppm, and 7.8, respectively. The temperature of the water was kept at 20 +/- 1 degrees C. Test conduct: Tests were conducted in 250 ml glass beakers containing 200 ml of well water. The test organisms (10 per test concentration) were added randomly to the test water within 30 minutes of addition of test material. The test concentrations (100, 180, 320, 560 and 1000 mg/l) were

based on the results of a preliminary study performed with 1, 10 and 100 mg/l. One additional group of 10 organisms was exposed to water only served as a control. Each condition was tested in duplicate. All organisms were observed initially and after 24 and 48 hours of exposure for mortality and abnormal behavior (surfacing or loss of equilibrium). The pH, dissolved oxygen concentration and temperature of the control water were determined at the beginning and end of the study. Water containing 100, 320 and 1000 mg/l was analyzed for pH, dissolved oxygen concentration and temperature at the end (but not the beginning) of the study.

Statistical analysis: Concentration vs. lethality data were analyzed by a computer program which utilized the binomial, moving average and probit tests to determine the LC50 value (and 95% confidence limit) at 24 and 48 hours. The method of calculation selected for presentation (probit) was the one that gave the narrowest confidence limit.

Test substance : The purity of the test material (lot # 34) was 96.1%. Contaminants were not mentioned.

Reliability : (2) valid with restrictions
Test concentrations were not analytically confirmed.

Flag : Critical study for SIDS endpoint

01.08.2003 (3)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Endpoint : biomass
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 789.303 calculated
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program are CAS No., melting point (-92.8 degrees C), boiling point (97 degrees C), vapor pressure (39 mm Hg), and water solubility (93,380 mg/l).

This is a supporting study for the SIDS endpoint.

Reliability : (2) valid with restrictions

15.08.2003 (14)

Species : Selenastrum capricornutum (Algae)
Endpoint : other: biomass and growth rate
Exposure period : 72 hour(s)
Unit : mg/l
NOEC : = 87.8 measured/nominal
EC50 : > 87.8 measured/nominal
Limit test : yes
Analytical monitoring : yes
Method : other: OECD: TG-201 and EEC/Annex V C.3
Year : 1999
GLP : yes
Test substance : other TS

Remark : No protocol deviations were noted. The EbC50 (0-72 hr) and the ErC50 (0-72 hr) were inestimable as greater than 50% inhibition in growth and/or biomass was not achieved. The significant loss (up to 80.7% over the course of the study) in test material was attributed to volatilization.

This is a supporting study for the SIDS endpoint for propionitrile.

Result : Algae exposed to test material exhibited normal growth with respect to control. No deformed cells were noted. At the end of the test, the mean cell density in treated cultures was 1.365 x 10E6 cells /ml (compared to 1.356 x 10E6 cells in control).

The average concentrations of material in the test flasks at the beginning of the test and after 72 hours were 200.68 and 38.65 mg/l, respectively. Approximately 80.74% of the material was lost over the course of the experiment. The mean concentration was 87.82 mg/l. This concentration was listed as the NOEC.

Results of the photostability tests were similar to those in flasks containing test material and algae. The control solutions that were exposed to light or were in the dark exhibited 78.11% and 69.35% losses of test material.

The mean temperature and illumination were 24 degrees C and 746 foot-candles (range 744 - 748 foot-candles) throughout the test. The pH ranged from 7.42 - 7.88. The shaker speed was maintained at 100 rpm.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 136-fold within 72 hours.

Test condition : A 4-day culture of Selenastrum capricornutum SF-3148 (passage 5 in liquid algal medium) was used as the test algae. Several passages were performed prior to the test to confirm exponential growth.

Test medium: Sterile growth medium was prepared using high quality distilled water. The pH of the medium was measured and adjusted to 7.5 (+/- 0.1) using 0.01N NaOH.

Test material stock solution: Test material (0.156 ml) was added to 600.0 g of algal growth medium (to produce a nominal concentration of 200 mg/l). The solution was immediately capped and stirred for 1-2 minutes. An aliquot of the solution was removed for analysis of concentration at time 0.

Test conduct: All steps were carried out aseptically in a hood to prevent contamination. Test vessels were sterile 250 ml Erlenmeyer flasks. Test material stock solution (100 ml) was added to 5 flasks and test medium that did not contain test material was added to 3 flasks. Algae (515 microliters of algal stock culture to achieve an initial cell density of 1 x 10E4 cells/ml) were added to 3/5 flasks that contained test material and the three that did not. The two flasks that contained test material but were not inoculated served as photostability controls. One of the flasks was exposed to light and one was wrapped in foil to shield it from light. All flasks were secured with foam stoppers and transferred to a shaking incubator (24 degrees C, 100 rpm). They were illuminated at an average of 746.2 footcandles throughout the study.

Temperature, light intensity, and shaker speed (rpm) were assessed at the 0, 24, 48, and 72 hours. Concentrations of test material in the flasks that contained algae also were assessed at these times. The pH and was assessed at time 0 and after 72 hours. Concentrations of test material in the photostability controls also were measured at 0 and 72 hours. Concentrations of test material were analyzed using gas chromatography with flame ionization detection (GC/FID).

The exposure concentration was calculated as the geometric mean of the test concentrations analyzed at the 4 time points. Cell counts were performed after 24, 48 and 72 hours of exposure using a calibrated Coulter Counter. The mean algal cell count for the test and control curves was calculated. Two measures of growth [biomass (area under the growth

curve) and growth rate] were used to determine the effect of the material on algae. The concentrations that produced a 50% inhibition of biomass (EbC50) and growth rate (ErC50) relative to control were to be calculated by fitting linear regression models to the data.

The test was considered valid if the mean cell concentration in the control cultures increased by a factor of at least 16 within 72 hours. exhibited 78.11% and 69.35% losses of test material.

The mean temperature and illumination were 24 degrees C and 746 foot-candles (range 744 - 748 foot-candles) throughout the test. The pH ranged from 7.42 - 7.88. The shaker speed was maintained at 100 rpm.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 136-fold within 72 hours.

Test substance : The test material was isobutyronitrile (CAS No. 78-82-0). Purity was 99.9%.

Conclusion : The results of this study indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.

Reliability : (1) valid without restriction
This was a well-documented OECD-study conducted under GLP assurances.

07.08.2003 (11)

Species : Selenastrum capricornutum (Algae)
Endpoint : other: biomass and growth rate
Exposure period : 72 hour(s)
Unit : mg/l
NOEC : = 133.4 measured/nominal
EC50 : > 133.4 measured/nominal
Limit test : yes
Analytical monitoring : yes
Method : other: OECD: TG-201 and EEC/Annex V C.3
Year : 1999
GLP : yes
Test substance : other TS

Remark : Results of a pilot study conducted prior to this test indicated that a limit test design would be appropriate for the material.

The EbC50 (0-72 hr) and the ErC50 (0-72 hr) were inestimable as greater than 50% inhibition in growth and/or biomass was not achieved. No protocol deviations were noted.

This is a supporting study for the SIDS endpoint for propionitrile.

Result : Algae exposed to test material exhibited normal growth with respect to control. At the end of the test, the mean cell density in treated cultures was 9.5 x 10E5 cells /ml (compared to 9.0 x 10E5 cells in control).

The average concentrations of material in the test flasks at the beginning of the test and after 72 hours were 206.0 and 85.7 mg/l, respectively. The mean concentration was 133.4 mg/l. This concentration was listed as the NOEC, EbC50 and ErC50.

Results of the photostability tests were similar to those in flasks containing test material and algae. The control solutions that were exposed to light or were in the dark exhibited 58% and 50% losses of test material.

The mean temperature and illumination were 24 degrees C and 747 foot-candles (+/- 5.5 foot-candles) throughout the test. The pH ranged from 7.4 - 7.6.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 90.2-fold within 72 hours.

Test condition : Test Organisms: A 4-day culture of Selenastrum capricornutum SF-3148 (passage 3 in liquid algal medium) was used as the test algae. Several passages were performed prior to the test to confirm exponential growth. The density of cells in the stock culture was 2.58 x 10E6 cells/ml prior to use.

Test medium: Sterile growth medium was prepared using high quality distilled water. The pH of the medium was measured and adjusted to 7.5 (+/- 0.1) using 0.1N NaOH prior to use.

Test material stock solution: Approximately 0.151 ml (120 mg) of the test material was added to 600 ml of algal growth medium with a gas tight Hamilton syringe (to produce a nominal concentration of 200 mg/l). The solution was stirred for approximately 1 minute. An aliquot (1.0) of the solution was removed for analysis of concentration.

Test conduct: All steps were carried out aseptically in a hood to prevent contamination. Test vessels were sterile 250 ml Erlenmeyer flasks. Test material stock solution (100 ml) was added to 5 flasks and test medium that did not contain test material was added to 3 flasks. Algae (388 microliters of algal stock culture to achieve an initial cell density of 1 x 10E4 cells/ml) were added to 3/5 flasks that contained test material and the three that did not. The two flasks that contained test material but were not inoculated served as photostability controls. One of the flasks was exposed to light and one was wrapped in foil to shield it from light. All flasks were secured with foam stoppers and transferred to a shaking incubator (24 degrees C, 100 rpm). They were illuminated at 747 (+/- 5.5) footcandles throughout the study.

Temperature, light intensity, and shaker speed (rpm) were assessed at the 0, 24, 48, and 72 hours. Concentrations of test material in the flasks that contained algae also were assessed at these times. The pH and was assessed at time 0 and after 72 hours. Concentrations of test material in the photostability controls also were measured at 0 and 72 hours. Concentrations of test material were analyzed using gas chromatography with flame ionization detection (GC/FID). The exposure concentration was calculated as the geometric mean of the test concentrations analyzed at the 4 time points. Cell counts were performed after 24, 48 and 72 hours of exposure using a calibrated Coulter Counter. The mean algal cell count for the test and control curves. Two measures of growth [biomass (area under the growth curve) and growth rate] were used to determine the effect of the material on algae. The concentrations that produced a 50% inhibition of biomass (EbC50) and growth rate (ErC50) relative to control were to be calculated by fitting linear regression models to the data.

The test was considered valid if the mean cell concentration in the control cultures increased by a factor of at least 16 within 72 hours.

Test substance : The test material was butyronitrile (CAS No. 109-74-0). Purity was 99.9% (GC/FID).

Conclusion : The results of this study indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.

Reliability : (1) valid without restriction
This was a well-documented OECD-study conducted under GLP assurances.

07.08.2003 (12)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Excretion
Species : rat
Number of animals
Males : 3
Females : 3
Doses
Males : approximately 1.65 mg 14C-labeled priopionitrile
Females : approximately 1.65 mg 14C-labeled priopionitrile
Vehicle :
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other
Year : 1987
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The overall 14C recovery was 92.05 %. The majority of the material was eliminated in exhaled air or urine by 24 hours. An average of 27.05% of test material was detected in the Aquasol-2 bubbler (represents volatile organics) 0.5 hours after administration of 14C-labeleled propionitrile by gavage. By 3 hours, recovery in the 1N sodium hydroxide bubbler (material that had been exhaled as either 14CO2 or free cyanide) ranged from 38.54 to 49.25%. At 24 hours, the total 14C recovery in the urine (which represented a parent metabolite or thiocyanate) ranged from 0.76 to 5.83%. At 72 hours, a small percentage (< 2%) was found in the liver and kidneys.
Test substance : Purity of the 14C-labeled propionitrile was 98.4%. The specific activity was 4.0 mCi/mmol.
Conclusion : The material is rapidly absorbed from the GI tract and eliminated through expired air as parent material, free CO2 or free cyanide.
Reliability : (4) not assignable
The study was given a reliability rating of 4 because it was not reviewed in detail.

10.08.2003 (7)

In Vitro/in vivo : In vivo
Type : Excretion
Species : rat
Number of animals
Males : 3
Females : 3
Doses
Males : 5.74 mg/kg, 7.9 microcuries
Females : 7.35 mg/kg, 7.9 microcuries
Vehicle :
Route of administration : gavage
Exposure time :
Product type guidance :

Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The total 14C-activity eliminated in 72 hours was 93.98 % and 74.73% of the administered dose in males and females, respectively. After 72 hours, the 14C-activity found in the urine of 3 males and 3 females averaged 6.2% and 4.4% of dose, respectively. The cumulative 14C-activity after 72 hours found in the feces averaged 0.9% and 1.1% of the dose in males and females, respectively. The percentage of the dose recovered in whole blood cells was 0.38% and 0.31% for males and females, respectively. The majority of the 14C-activity (81.31 % and 63.26% of the administered dose for males and females, respectively) was in respired air trapped in a sodium hydroxide bubbler (which represents CO2 or free cyanide). The amount of volatile material trapped in Aquasol-2 (which represented volatile organics) was 5.55% and 5.93% of dose for males and females, respectively.

It was thought that the lower recovery in females was due to loss of 78 ml of NaOH from the bubbler for one rat. Assuming that this was not lost, the amount of material in the bubbler would have been 76.25 % of the dose. The total amount of 14C recovered in this animal would be 85.59%, which is in agreement with the average total amount of material recovered from the other 2 females (84.3%).
Test substance : Purity of the 14C-labeled priopionitrile was 97.5%.
Reliability : (4) not assignable
The study is given a reliability rating of 4 because it was not reviewed in detail.

05.08.2003 (8)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 40 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 60
Vehicle :
Doses : 25.1, 31.6, 39.8, 50.1, 63.1 and 79.4 mg/kg bw
Method : other
Year : 1980
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Whether the study was done according to GLP was not stated in the study documents. However, an accompanying letter from the company that commissioned the study requested that the study should be performed according to GLP. Therefore, it is assumed that it was.
Result : The numbers of animals that died after each of the doses is as follows:

Dose (mg/kg) Number of Deaths (all within 1 day)

25.1	1/5 males, 0/5 females
31.6	2/5 males, 1/5 females
39.8	4/5 males, 1/5 females
50.1	5/5 males, 2/5 females
63.1	3/5 males, 5/5 females
79.4	5/5 males, 4/5 females

The LD50 values [with 95% confidence limits (CL)] and slopes of the curves were as follows:

Sex	LD50 (mg/kg)	95 % CL (mg/kg)	slope
M/F	40	35 - 46	4.9
M	38	28 - 51	3.5
F	52	43 - 63	5.6

Signs of toxicity included increasing weakness and collapse (both sexes), and tremors (in 2 males). Necropsies of animals that died revealed hemorrhagic lungs and liver, discoloration of liver, kidneys and spleen, and acute gastrointestinal inflammation. Average weights of survivors at days 7 and 14 were higher than initial weights. Viscera appeared normal in survivors.

Test condition : Thirty rats/sex were fasted overnight and divided into 6 groups of 5 rats/sex. These groups were given a single oral dose (undiluted) of propionitrile at 25.1, 31.6, 39.8, 50.1, 63.1 or 79.4 mg/kg. The average initial weights of males and females were 200 and 184 g, respectively. Animals were observed for 14 days, and weighed on days 7 and 14. It is presumed that they were observed daily. Animals that died were necropsied upon discovery. Survivors were euthanized and necropsied on day 14. The LD50 value, 95% confidence interval and slope of the curve were calculated by an unknown method.

Test substance : The approximate composition of the test material was 97% propionitrile, 1-3% acrylonitrile, 1% adiponitrile and 2% water.

Reliability : (2) valid with restrictions
The method of calculating the LC50 value was not listed.

13.08.2003 (39)

Type : LD50
Value :
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 60
Vehicle :
Doses : 50.1, 63.1, 79.4, 100, 126, 158 mg/kg (males); 158, 200, 251, 316 mg/kg (females)
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The numbers of animals that died after each of the doses is as follows:

Dose (mg/kg)	Number of Deaths (all in 1 - 3 days)
50.1	0/5 males
63.1	2/5 males
79.4	4/5 males
100	3/5 males

126	3/5 males
158	4/5 males, 0/5 females
200	1/5 females
251	1/5 females
316	4/5 females

The LD50 values [with 95% confidence limits (CL)] and slopes of the curves were as follows:

Sex	LD50 (mg/kg)	95 % CL (mg/kg)	slope
M	75	58 - 98	3.9
F	270	235 - 310	9.0

Signs of toxicity in both sexes included weight loss (one to three days in survivors), increasing weakness, ocular discharge, tremors, convulsions, collapse and death. Diarrhea and dyspnea were also observed in females. Animals that died had hemorrhagic lungs, liver hyperemia and/or discoloration (in some instances), and gastrointestinal inflammation (acute in some instances). Viscera appeared normal in survivors.

Test condition : Thirty males and 20 females were divided into 6 groups of 5 rats/sex. Males were given a single oral dose (undiluted) of propionitrile at 50.1, 63.1, 79.4, 100, 126, or 158 mg/kg, and females were given 158, 200, 251 or 316 mg/kg. The average initial weights of males and females were 237.5 and 242.5 g, respectively. Animals were observed for 14 days. It is presumed that they were observed daily. Animals that died were necropsied upon discovery. Survivors were euthanized and necropsied on day 14. The LD50 value, 95% confidence interval and slope of the curve for each sex were calculated by an unknown method.

Test substance : A data sheet containing information about the test material listed the purity to be 90+%. Impurities included 0.05% acrylonitrile, < 0.1% water, and 0.16% adiponitrile. Additional impurities (other than an illegible one at 510 ppm) were not listed.

Reliability : (2) valid with restrictions
Test conditions are not described in detail.

10.08.2003 (40)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : = 3.3 mg/l
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 60
Vehicle :
Doses : 1.58, 2.51, 3.98, 6.31, 10.0, 15.8 mg/l
Exposure time : 4 hour(s)
Method : other
Year : 1978
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The NOAEL in ppm can be calculated using the following equation [ppm = 3300 mg/m3 x 24.45/55.09(MW)]. For this experiment, the value is 1464.6.

Result : The numbers of animals that died after exposure to propionitrile is as follows:

Concentration (mg/l)	Number of Deaths
1.58	0/5 males, 0/5 females (0/10)
2.51	5/5 males 0/5 females (5/10)
3.98	5/5 males, 0/5 females (5/10)
6.31	5/5 males, 3/5 females (8/10)
10.0	5/5 males, 5/5 females (10/10)
15.8	5/5 males, 5/5 females (10/10)

All deaths occurred within 4 - 24 hours (with the exception of 1 male exposed to 3.98 mg/l, that died after 13 days).

The LD50 value for males and females combined [with 95% confidence limits (CL)] was 3.3 (2.6 - 4.3) mg/l. The slope of the curve was 4.1. These values were not calculated for males and females individually.

Signs of toxicity included salivation, lethargy, increasing weakness, tremors and convulsions, and collapse. Animals that died had hemorrhagic lungs, discolored liver (mottled in some) and acute gastrointestinal inflammation. Necropsies of survivors were normal.

Test condition : Sixty rats (30/sex) were divided into 6 groups (5/sex/group). Each group was exposed to 1.58, 2.51, 3.98, 6.31, 10.0 or 15.8 mg/l by inhalation. During the test, males and females receiving the same concentration were placed together in 9 inch x 16 inch x 7 inch cages that were suspended in the middle of 210-liter drum-like chambers that were equipped with circulating fans. Test material was introduced into the chamber through a port equipped with a needle and syringe. No supplementary air was introduced. The average temperature and relative humidity inside the chambers were 24-25 degrees C and 70%, respectively.

Animals were observed for signs of toxicity during exposure and for 14 subsequent days. Animals that died were necropsied upon discovery. On day 14, survivors were euthanized and necropsied.

The LC50 value, 95% confidence limits and slope of the curve were calculated according to the method of DeBeer (reference was not given).

Test substance : The purity of the material was not listed. However, in a study conducted by the same laboratory in 1979, a data sheet containing information about the test material listed the purity to be 90+%. Impurities included 0.05% acrylonitrile, < 0.1% water, and 0.16% adiponitrile. Additional impurities (other than an illegible one at 510 ppm) were not listed.

Reliability : (2) valid with restrictions
Basic data and methodologies are given.

Flag : Critical study for SIDS endpoint

10.08.2003 (41)

Type : LC100
Value : = 90.3 mg/l
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals : 6
Vehicle :
Doses : 90.3 mg/l
Exposure time : 1.25 hour(s)
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : All six rats exposed to 90.3 mg/l for 1.25 hours died. The NOAEL in ppm can be calculated using the following equation [ppm = 90300 mg/m3 x

24.45/55.09(MW)]. For this experiment, the value is 40076.87.

Test substance : A data sheet containing information about the test material listed the purity to be 90+%. Impurities included 0.05% acrylonitrile, < 0.1% water, and 0.16% adiponitrile. Additional impurities (other than an illegible one at 510 ppm) were not listed.

Reliability : (4) not assignable
The study was given a reliability rating of 4 because it was not reviewed in detail.

10.08.2003 (40)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : = 40 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 40
Vehicle :
Doses : 12.5, 25, 50 and 100 mg/kg
Method : other: 40 CFR, Part 163.81-2
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The numbers of animals that died after each of the doses is as follows:

Dose (mg/kg)	Number (and time) of Deaths
12.5	0/10
25	0/10
50	9/10 (4/5 M, 5/5 F), 3-23 hr
100	10/10, 2-7.5 hr

The LD50 values [with 95% confidence limits (CL)] were as follows:

Sex	LD50 (mg/kg)	95 % CL (mg/kg)
M/F	40	29 - 51
M	43	24 - 62
F	35	(could not be calculated)

Signs of toxicity included ataxia, convulsions, tremors, respiratory abnormalities (hyperpnea, hypopnea, dyspnea and arrythmic respiration), hypoactivity, prostration, hypothermia, nasal or ocular discharge, fecal staining, nasal discharge, soft stool, reddened nictitating membrane or reduced food consumption. Most animals in the 12.5 and 25 mg/kg dose groups were free of significant abnormalities from days 3 to 14.

Necropsies of animals that died revealed reddening of the nictitating membrane, mottling of the liver, GI abnormalities (reddened walls, black foci in the mucosa, black material adhered to the mucosa), and red interstitial walls or intestinal contents. Necropsies of survivors were normal.

Average weights of survivors at days 7 and 14 were generally higher than initial weights; however a few animals exhibited slight weight losses (0.1 or

0.2 kg). No dose-related differences in weight gain were apparent. Six and 3 of the animals given 12.5 and 25 mg/kg (respectively) had no dermal irritation. The other animals in these groups had only slight or very slight erythema without edema.

Test condition : Forty animals were acclimated for 38 days, then randomized to four groups of 5/sex. They were given food and water ad libitum. Animals considered unsuitable due to poor health or outlying weights were excluded. Pretest body weights were 2.6 - 3.3 kg for males and 2.5 - 3.6 kg for females. Approximately 18 hours prior to treatment, the hair on each rabbit was closely clipped from the trunk (dorsal and ventral surface and sides from scapular to pelvic area). Skin was not abraded. Test material (12.5, 25.0, 50.0 or 100 mg/kg) was applied directly onto the exposed skin and was spread evenly over the entire area. A layer of 8-ply gauze was then wrapped around the animal to cover the application site. This was wrapped in an impervious plastic sleeve, which was secured with masking tape. Elizabethan collars were placed on all animals.

Wrappings were removed after 24 hours, and the test site was wiped free of test material. Skin was scored for irritation according to the method of Draize 30 minutes after removal of the dressing. Animals were observed for toxicity at 1, 2, and 4 hours and daily thereafter for 14 days. Animals were weighed prior to treatment and 7 and 14 days after treatment. Animals that did not survive for 14 days were weighed and examined grossly at the time they were found dead. All animals surviving to day 14 were euthanized at this time and examined grossly.

The LD50 values (and 95% confidence intervals) for males, females and both sexes together were calculated using logarithmic-probit graph paper.

Test substance : The purity of the test material was 94.5%. It also contained 1.1% adiponitrile, < 0.1% acrylonitrile, < 0.1% water, < 0.1 % solids and 0.07% para nitrosodiphenylamine.

Reliability : (1) valid without restriction
The test was performed according to an established guideline.
Documentation is thorough.

10.08.2003 (4)

Type : LD50
Value : = 56 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 40
Vehicle :
Doses : 12.5, 25, 50 and 100 mg/kg
Method : other: 40 CFR, Part 163.81-2
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The numbers of animals that died after each of the doses is as follows:

Dose (mg/kg)	Number (and time) of Deaths
12.5	0/10
25	0/10
50	4/10 (3/5 M, 1/5 F), 3.5 - 21.5 hr
100	9/10 (5/5 M, 4/5 F), 4-21 hr

The LD50 values [with 95% confidence limits (CL)] were as follows:

Sex	LD50 (mg/kg)	95 % CL (mg/kg)
M/F	56	40 - 72
M	45	19 - 71
F	70	44 - 96

Signs of toxicity included ataxia, convulsions, respiratory abnormalities (hypopnea, dyspnea and gasping), hypoactivity, prostration, nasal or ocular discharge, fecal staining or reduced food consumption. Necropsies of animals that died revealed reddening of the nictitating membrane, red or black foci or patches in the gastric mucosa, discoloration of liver or kidneys, red material in the GI tract or thickening of the abdominal wall. Necropsies of survivors were normal. Average weights of survivors at days 7 and 14 were higher than initial weights and were not affected by dose of test material. Approximately one half of the survivors at 24 hours had no dermal irritation. The others had only slight or very slight erythema without edema.

Test condition : Forty animals were acclimated for 35 days, then randomized to four groups of 5/sex. They were given food and water ad libitum. Animals considered unsuitable due to poor health or outlying weights were excluded. Pretest body weights were 2.7 - 3.2 kg for males and 2.6 - 3.4 kg for females. Approximately 18 hours prior to treatment, the hair on each rabbit was closely clipped from the trunk (dorsal and ventral surface and sides from scapular to pelvic area). Skin was not abraded. Test material (12.5, 25.0, 50.0 or 100 mg/kg) was applied directly onto the exposed skin and was spread evenly over the entire area. A layer of 8-ply gauze was then wrapped around the animal to cover the application site. This was wrapped in an impervious plastic sleeve, which was secured with masking tape. Elizabethan collars were placed on all animals.

Wrappings were removed after 24 hours, and the test site was wiped free of test material. Skin was scored for irritation according to the method of Draize 30 minutes after removal of the dressing. Animals were observed for toxicity at 1, 2, and 4 hours and daily thereafter for 14 days. Animals were weighed prior to treatment and 7 and 14 days after treatment. Animals that did not survive for 14 days were weighed and examined grossly at the time they were found dead. All animals surviving to day 14 were euthanized at this time and examined grossly.

The LD50 values (and 95% confidence intervals) for males, females and both sexes together were calculated using logarithmic-probit graph paper.

Test substance : The purity of the test material was not listed. It was used as received from Monsanto. The density was listed as 0.7978 g/ml.

Reliability : (2) valid with restrictions
Purity of the material and documentation of GLP were not provided.

10.08.2003 (6)

Type : LD50
Value : = 90 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 40
Vehicle :
Doses : 12.5, 25, 50 and 100 mg/kg
Method : other: 40 CFR, Part 163.81-21
Year : 1981
GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Result : The numbers of animals that died after each of the doses is as follows:

Dose (mg/kg)	Number (and time) of Deaths
12.5	0/10
25	0/10
50	1/10 (0/5 M, 1/5 F), 23.5 hr
100	6/10 (3/5 M, 3/5 F), 2.5-23.5 hr

The LD50 values [with 95% confidence limits (CL)] were as follows:

Sex	LD50 (mg/kg)	95 % CL (mg/kg)
M/F	90	65 - 115
M	90	49 - 131
F	85	35 - 135

Signs of toxicity included ataxia, convulsions, tremors, respiratory abnormalities (hyperpnea and dyspnea), hypoactivity, prostration, red eyes, nasal discharge, soft stool, fecal staining or reduced food consumption. Most animals in the 12.5 and 25 mg/kg dose groups and most survivors in the 50 and 100 mg/kg dose groups were free of significant abnormalities from days 2 to 14. Necropsies of animals that died revealed reddening of the nictitating membrane, pale liver, and GI abnormalities (reddened walls, black foci in the mucosa, and/or white film on the mucosa). Necropsies of survivors were normal.

Average weights of survivors at days 7 and 14 were higher than initial weights and were not affected by dose of test material. Approximately one half of the survivors at 24 hours had no dermal irritation. The others had only slight or very slight erythema, generally without edema.

Test condition : Forty animals were acclimated for 31 days, then randomized to four groups of 5/sex. They were given food and water ad libitum. Animals considered unsuitable due to poor health or outlying weights were excluded. Pretest body weights were 2.5 - 3.1 kg for males and 2.7 - 3.4 kg for females. Approximately 25 hours prior to treatment, the hair on each rabbit was closely clipped from the trunk (dorsal and ventral surface and sides from scapular to pelvic area). Skin was not abraded. Test material (12.5, 25.0, 50.0 or 100 mg/kg) was applied directly onto the exposed skin and was spread evenly over the entire area. A layer of 8-ply gauze was then wrapped around the animal to cover the application site. This was wrapped in an impervious plastic sleeve, which was secured with masking tape. Elizabethan collars were placed on all animals.

Wrappings were removed after 24 hours, and the test site was wiped free of test material. Skin was scored for irritation according to the method of Draize 30 minutes after removal of the dressing. Animals were observed for toxicity at 1, 2, and 4 hours and thereafter for 14 days. Animals were weighed prior to treatment and 7 and 14 days after treatment. Animals that did not survive for 14 days were weighed and examined grossly at the time they were found dead. All animals surviving to day 14 were euthanized at this time and examined grossly.

The LD50 values (and 95% confidence intervals) for males, females and both sexes together were calculated using logarithmic-probit graph paper.

Test substance : The purity of the test material was not listed. It was used as received from Eastman Kodak. The density was listed as 0.7885 g/ml.

Reliability : (2) valid with restrictions

Purity of the material and documentation of GLP were not provided.

10.08.2003 (5)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	14 weeks (total of 63 exposure days)
Frequency of treatm.	:	6 hours/day, 5 days per week
Post exposure period	:	
Doses	:	60, 120, 209 ppm
Control group	:	yes
NOAEL	:	< 60 ppm
LOAEL	:	= 60 ppm
Method	:	other
Year	:	1984
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : Test material concentrations: The nominal concentrations (+/- SD) were 56.0 +/- 8.8, 119.1 +/- 16.9 and 203.0 +/- 19.6. Corresponding analytical concentrations were 60.2 +/- 1.0, 120.3 +/- 1.1 and 209.0 +/- 1.3 ppm. Since the nominal analytical ratios were close to 1, the material was a true vapor. There was no indication that the test material was unstable. Distribution was uniform (> 96%) for each of the exposure concentrations. Airflow, temperature and relative humidity ranged from 1719-1765 l/min, 21.0 - 26.1 degrees C and 17-50%, respectively.

Effects at all exposure concentrations: Signs of toxicity (labored breathing, nasal discharge, salivation, discharge from the eyes, hypoactivity and/or alopecia) were observed in all exposed groups. Incidences of these signs increased in a dose-dependent manner. Males and/or females in all exposed groups had significant decreases in red blood cells and hemoglobin values. Urine thiocyanate concentrations increased in all exposed groups, with concentrations from animals exposed to 120 ppm similar to or higher than those exposed to 210 ppm. However, since a dose-dependent diuresis occurred, the total amount of urine thiocyanate present (concentration x urine volume) increased with increasing concentrations.

Effects at 209 ppm: Three males died or were killed in extremis (2 between exposures 2 and 3 and one between exposures 18 and 19). Arched back, ataxia, tremors or convulsions, biting, pawing or rubbing chin

against cage, irritation of the conjunctiva and breathing difficulties were noted in a few animals during exposure. Males and females exhibited significant decreases in body weight throughout the study (P <= 0.01). Average final body weights of males and females were lower than controls (377.5 g in exposed males vs. 435.4 g in controls and 255.0 g in exposed females vs. 276.6 g in controls). Absolute and/or relative heart, liver, spleen and kidney weights were increased in males and/or females. Absolute testes weights were decreased in males. Mean corpuscular hemoglobin concentrations (males only) were lower than control (all P <= 0.05). Serum alkaline phosphatase (males and females), SGOT (males only) and SGPT (males only) were increased, and BUN concentrations were decreased. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 10/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 120 ppm: Ataxia was observed during exposure in 2 females. Males had significant body weight decreases (P < = 0.05) at two time points. Absolute and/or relative liver weights were increased in males and females and absolute and/or relative spleen weights were increased in males. Mean corpuscular hemoglobin concentrations (males only, P < = 0.05) were lower than control. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 11/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 60 ppm: Absolute and relative spleen weights were increased in males. Serum thiocyanate concentrations were marginally increased in males and females.

Test condition : Animals: Rats were acclimated for at least 10 days prior to use. Two days prior to the start of the study, males weighed 174-200 g and females weighed 132-145 g. On the first day of the study, the animals were 43 days old. Animals were randomly allocated by body weight into 4 groups of 15 animals/sex/group. Animals were individually housed in suspended mesh cages and given food and water ad libitum (except during exposure). Animal rooms were maintained at 70-74 degrees C and 35-60% relative humidity, with a 12 hour light/dark cycle.

Exposure conditions: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Rats were placed individually in wire mesh cages that were suspended in the chambers by 3-tiered racks. Males were placed on one side and females on the other. The concentrations of material in the chambers (20, 120 or 210 ppm) were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. The bubbler was connected to a side port in the vertical particle-size separator, which was in turn connected to the air inlet at the top of the inhalation chamber. One bubbler was used in each of the generation systems. Airflow was maintained at a constant flow of 1727 liters/min. Nominal concentration measurements were determined daily for each chamber following exposure, by dividing the amount of test material delivered to the chamber (the difference between the pre- and post-exposure weights) over the 6-hr exposure period by the total air volume during the same period. Concentrations of test material in the chambers were measured 4 times daily using a Miran 1A General Purpose Gas Analyzer. Additional samples of atmosphere from 9 specified locations in each chamber were also taken at 3 different times to determine if the vapor was distributed uniformly.

Test conduct: Animals were observed for clinical signs between the second and fifth hour of each exposure. Estimations of the percentages of animals exhibiting hypoactivity, eye irritation and breathing difficulties were made. All animals were individually examined for gross signs of toxicity preceding

and following each exposure and checked for mortality. Each animal was weighed and given a thorough examination for gross signs of toxicity on a weekly basis.

Animals were euthanized after 14 total weeks on the study. Terminal body weights were obtained (following an overnight fast). Blood and urine were collected. Whole blood was treated with an anticoagulant and was analyzed for total and differential erythrocyte count, total leukocyte count, platelet count, hematocrit, hemoglobin, and red blood cell indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration). Serum was analyzed for albumin, globulin, total protein, blood urea nitrogen, total bilirubin, glucose, glutamic pyruvic transaminase (SGPT), alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), T3, T4, thiocyanate and lactate dehydrogenase. Urine was analyzed for the presence of thiocyanates.

Detailed necropsies were conducted on all rats that died during the course of the study, those that were killed moribund, and those that survived to study termination. The adrenal glands (both together), testes (with epididymides, heart, kidneys, liver, pituitary and spleen were weighed. The aforementioned organs and the following tissues were fixed in 10% neutral formalin: abdominal aorta, bone and bone marrow (femur), brain, esophagus, ovaries, colon, ileum, lung, lymph nodes (mesenteric), mammary gland, nasal turbinates, pancreas, thyroid/parathyroid, prostate, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, stomach, thymus, trachea, urinary bladder, uterus (with cervix) and gross lesions. Eyes (with optic nerve) were fixed in a solution of 2% glutaraldehyde and 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, cut at five microns, stained with hematoxylin and examined microscopically.

Statistical analyses: In life and terminal body weights and organ weight data were analyzed using Dunnett's test. Organ to body weight ratios were analyzed using the Mann-Whitney test, with the Bonferroni inequality. Data for frequencies of microscopic lesions were evaluated with the Fisher's exact test with the Bonferroni inequality. Hematological and serum and urine chemistry variables were examined using Dunnett's test.

Test substance : The purity of the test material was 96%. Impurities were not listed.
Reliability : (2) valid with restrictions
The study is comparable to a guideline study; however, a NOAEL was not established.
Flag : Critical study for SIDS endpoint
10.08.2003 (35)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 18 days
Frequency of treatm. : 6 hr/day, 5 days per week
Post exposure period : none
Doses : 44, 115, 329 ppm
Control group : yes
NOAEL : = 115 ppm
LOAEL : = 329 ppm
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Thirteen males and one female died. High dose animals exhibited

decreases in mean body weight, ruffled fur, irritability, tremors, and discharges around the nose and eyes. Hematological measurements were also depressed in high dose animals. No signs of toxicity or changes in hematological measurements were observed in animals exposed to 44 or 115 ppm. There was no effect of treatment on clinical chemistries or urinalyses. No treatment-related gross or microscopic lesions were observed.

Test condition : Groups of 18 animals/sex were exposed to 0 (control) 44, 115 or 329 ppm, 6 hr/day for 18 days.

Test substance : Purity of the test material was 94.87%.

Reliability : (4) not assignable
The study was given a reliability rating of 4 because it was not reviewed in detail.

10.08.2003 (30)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mammalian cell gene mutation assay
System of testing : L5178Y Mouse Lymphoma Cells
Test concentration : 2143 to 5000 micrograms/ml
Cytotoxic concentr. : > 5000 micrograms/ml
Metabolic activation : without
Result : negative
Method : other
Year : 1982
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : An initial toxicity test showed that complete toxicity was not reached at 5000 micrograms/ml.

Result : First study: After the 2 day expression period, ten cultures (2 per concentration) were cloned at 2714, 3286, 3857, 4429, and 5000 micrograms/ml. All cultures exhibited mutant frequencies similar to controls (ranged from 0.2 - 0.4 per 10E4 surviving cells) and the total growth ranged from 61 to 106%.

Second study: After the 2 day expression period, twelve cultures (2 per concentration) were cloned at 2143, 2714, 3286, 3857, 4429, and 5000 micrograms/ml. All cultures exhibited mutant frequencies similar to controls (ranged from 0.2 - 0.3 per 10E4 surviving cells) and the total growth ranged from 69 to 94%.

Based on the combined results of both assays, none of the test concentrations induced a positive response (all p values were > 0.01) . The linear component of the dose-response curve also was not statistically significant.

The test was considered valid, as the control frequencies were within the required range (0.2 - 0.3 per 10E4 surviving cells in the two studies) and EMS induced 9.2 and 7.6 mutants per 10E4 surviving cells at 0.5 microliters/ml and 28.4 and 24.5 mutants per 10E4 surviving cells at 0.5 microliters/ml and 1.0 microliters/ml, in the 2 studies, respectively.

Test condition : Cell preparation: Prior to use in the test, L5178Y cells that were actively growing in culture were cleansed as described by Clive et al. (Mutat Res 31:17-29, 1975). Three ml of thymidine, hypoxanthine, methotrexate and glycine (THMG) stock solution was added to 100 ml cell suspension containing 0.1 x 10E6 cells/ml. The culture was gassed with 5% CO2 in air and placed in a shaking incubator (125 rpm, 37 degrees C). After 24 hours, the THMG was removed by pelletizing the cells and decanting off the supernatant. The cells were rinsed in 20 ml of F10 P (Fisher's Media for

leukemic cells with 10% heat inactivated horse serum) and reinstated in culture at 3 x 10E 4 cells/ml in 100 ml of F10 P plus 1 ml of THMG stock solution.

Test conduct: The test material was solubilized and diluted to produce dose levels from 2714 to 5000 micrograms/ml. Each concentration was tested in duplicate, and the test was performed twice. An additional concentration of 2143 micrograms/ml was tested in one of the experiments. All stock solutions were freshly prepared. Four ml of FoP was added to each tube. This yielded a final cell suspension of 0.6 x 10E6 cells/ml. Two control tubes received solvent only (DMSO). Positive controls were treated with ethyl methane sulfate (1.0 and 0.5 microliters/ml). All tubes were gassed with 5% CO2 in air and placed on a roller drum apparatus for 4 hours at 37 degrees C. All steps were carried out under amber lighting and the cells were incubated in the dark for 4 hours. After 4 hours, the cells were washed twice with 10 ml of F10P, then resuspended in 20 ml of F10P gassed with 5% CO2 in air, and replaced on a roller drum apparatus at 37 degrees C.

After the initial exposure, the cells were incubated for 2 days with a cell population adjustment (to 0.3 x 10E6 cells/ml) at 24 and 48 hours. At the end of this expression period, cells (a 2 x 10E-2 dilution of 1.5 x 10E6 cells/ml) were placed in 100 ml cloning medium containing 0.37% noble agar, and incubated in a shaking incubator at 37 degrees C. Trifluorothymidine (TFT; final concentration of 3 micrograms/ml) was added to one of the duplicate flasks per concentration. After 15 minutes, the flasks were removed, and 33 ml of the cell suspension from each flask was pipetted into 3 100 mm Petri plates. To accelerate the gelling process, the plates were stored at 4 degrees C for 20 min. The plates were then removed and incubated at 37 degrees C in a humidified 5% CO2 atmosphere for 10-12 days.

After the incubation period, the plates were scored for total number of colonies per plate. The plates that did not contain TFT served as viability controls. Each plate was counted 3 times by an automatic colony counter, and the median count was recorded. The mutation frequency was determined by dividing the average number of colonies in the 3 TFT plates by the average number of colonies x 10E4 in the 3 viability control plates and multiplying the quotient by 2.

Validity criteria: The test was considered acceptable if the positive control induced at least a 2-fold increase in the frequency of mutants with respect to the solvent control and resulted in a viability rate of 10-80%. The solvent control frequency also had to be 0.2 - 1 per 10E 4 cells, the plating efficiency of the control at least 50%, and the material tested to either 500 micrograms/ml, or at the limit of 10% viability or solubility. A test was considered positive if at least 2 concentrations that caused no more than 90% toxicity caused 2-fold increases in the frequency of mutants with respect to solvent controls and the response was concentration-dependent ($p < 0.01$) for at least 2 concentrations that did not cause > 90% cytotoxicity. A test was considered negative if none of the concentrations caused a significant increase in the frequency of mutations ($p > 0.01$) and the linear component of the dose-response curve was not significant ($p > 0.1$) for test concentrations resulting in at least 10% relative total growth.

Test substance : The test material was propionitrile, used as received from Kodak. The purity was listed as 97.8%. Impurities included adiponitrile (0.3%), paranitrosophenylamine (0.1%), water (0.1%), acrylonitrile (<0.1%), and solids (<0.1%).

Reliability : (1) valid without restriction
The study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (27)

Type : Mammalian cell gene mutation assay
System of testing : L5178Y Mouse Lymphoma Cells
Test concentration : 100 to 1457 micrograms/ml
Cytotoxic concentr. : 1457 micrograms/ml
Metabolic activation : without
Result : positive
Method : other
Year : 1982
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : An initial toxicity test showed that complete toxicity was caused by 5000 micrograms/ml. Based on this test, the maximum concentration tested in the mutagenesis study was 1186 micrograms/ml. In the second mutagenicity test, one plate at 100 micrograms/ml and one solvent control were lost due to human error. This did not have a bearing on the outcome of the test.

Result : First study: After the 2 day expression period, ten cultures (2 per concentration) were cloned at 100, 371, 643, 914, 1186 and 1457 micrograms/ml. All cells incubated with 1457 micrograms/ml died. The cultures cloned at 643, 914 and 1186 micrograms/ml exhibited mutant frequencies greater than 2 times the average number in the vehicle controls (1.0, 1.7 - 1.9, and 2.8 - 4.0 per 10E4 surviving cells, respectively). The % total growth of these cultures ranged from 3 to 37% (toxicity increased with increasing concentration). The four remaining cultures exhibited mutant frequencies similar to solvent controls, with a total growth range of 71% to 87%.

Second study: After the 2 day expression period, ten cultures (2 per concentration) were again cloned at 100, 371, 643, 914, 1186 and 1457 micrograms/ml. All cells incubated with 1457 micrograms/ml died. The cultures cloned at 643, 914 and 1186 micrograms/ml exhibited mutant frequencies greater than 2 times the average number in the vehicle controls (1.0, 1.6 - 1.9, and 2.2 - 2.4 per 10E4 surviving cells, respectively). The % total growth of these cultures ranged from 4 to 38% (toxicity increased with increasing concentration). The four remaining cultures exhibited mutant frequencies similar to solvent controls, with a total growth range of 60% to 83%.

The combined statistical analysis of the 2 studies indicated a positive response. The mean mutation frequencies of cultures cloned at 643 and 914 micrograms/ml (each with greater than 10% relative growth), are significantly greater than that of the solvent control ($p < 0.01$). The linear component of the dose-response relationship also was statistically significant ($p < 0.01$) and exhibited a positive slope for test concentrations with at least 10% relative total growth.

The test was considered valid, as the control frequencies were within the required range (0.3 - 0.4 per 10E4 surviving cells in the two studies) and EMS induced 9.2 and 7.3 mutants per 10E4 surviving cells at 0.5 microliters/ml and 28.4 and 38.4 mutants per 10E4 surviving cells at 0.5 microliters/ml and 1.0 microliters/ml, in the two studies, respectively.

Test condition : Cell preparation: Prior to use in the test, L5178Y cells that were actively growing in culture were cleansed as described by Clive et al. (Mutat Res 31:17-29, 1975). Three ml of thymidine, hypoxanthine, methotrexate and glycine (THMG) stock solution was added to 100 ml cell suspension containing 0.1 x 10E6 cells/ml. The culture was gassed with 5% CO2 in air and placed in a shaking incubator (125 rpm, 37 degrees C). After 24 hours, the THMG was removed by pelletizing the cells and decanting off the supernatant. The cells were rinsed in 20 ml of F10 P (Fisher's Media for leukemic cells with 10% heat inactivated horse serum) and reinstated in

culture at 3 x 10E 4 cells/ml in 100 ml of F10 P plus 1 ml of THMG stock solution.

Test conduct: The test material was solubilized and diluted to produce dose levels from 100 to 1457 micrograms/ml. Each concentration was tested in duplicate, and the test was performed twice. All stock solutions were freshly prepared. Four ml of FoP was added to each tube. This yielded a final cell suspension of 0.6 x 10E6 cells/ml. Two control tubes received solvent only (DMSO). Positive controls were treated with ethyl methane sulfate (1.0 and 0.5 microliters/ml). All tubes were gassed with 5% CO2 in air and placed on a roller drum apparatus for 4 hours at 37 degrees C. All steps were carried out under amber lighting and the cells were incubated in the dark for 4 hours. After 4 hours, the cells were washed twice with 10 ml of F10P, then resuspended in 20 ml of F10P gassed with 5% CO2 in air, and replaced on a roller drum apparatus at 37 degrees C.

After the initial exposure, the cells were incubated for 2 days with a cell population adjustment (to 0.3 x 10E6 cells/ml) at 24 and 48 hours. At the end of this expression period, cells (a 2 x 10E-2 dilution of 1.5 x 10E6 cells/ml) were placed in 100 ml cloning medium containing 0.37% noble agar, and incubated in a shaking incubator at 37 degrees C. Trifluorothymidine (TFT; final concentration of 3 micrograms/ml) was added to one of the duplicate flasks per concentration. After 15 minutes, the flasks were removed, and 33 ml of the cell suspension from each flask was pipetted into 3 100 mm Petri plates. To accelerate the gelling process, the plates were stored at 4 degrees C for 20 min. The plates were then removed and incubated at 37 degrees C in a humidified 5% CO2 atmosphere for 10-12 days.

After the incubation period, the plates were scored for total number of colonies per plate. The plates that did not contain TFT served as viability controls. Each plate was counted 3 times by an automatic colony counter, and the median count was recorded. The mutation frequency was determined by dividing the average number of colonies in the 3 TFT plates by the average number of colonies x 10E4 in the 3 viability control plates and multiplying the quotient by 2.

Validity and positive assay criteria: The test was considered acceptable if the positive control induced at least a 2-fold increase in the frequency of mutants with respect to the solvent control and resulted in a viability rate of 10-80%. The solvent control frequency also had to be 0.2 - 1 per 10E 4 cells, the plating efficiency of the control at least 50%, and the material tested to either 500 micrograms/ml, or at the limit of 10% viability or solubility. A test was considered positive if at least 2 concentrations that caused no more than 90% toxicity caused 2-fold increases in the frequency of mutants with respect to solvent controls and the response was concentration-dependent (p< 0.01) for at least 2 concentrations that did not cause > 90% cytotoxicity.

Test substance : The test material was "propionitrile tails", used as received from Monsanto. This contained propionitrile (97.0%), adiponitrile (1.2%), paranitrosophenylamine (0.12%), water (0.04%), and acrylonitrile (0.02%).

Reliability : (1) valid without restriction
The test is comparable to a guideline study.

10.08.2003 (28)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535 and TA1538
Test concentration : up to 10,000 micrograms per plate
Cytotoxic concentr. : > 10,000 micrograms per plate
Metabolic activation : with and without
Result : negative

Method : other
Year : 1977
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The authors stated that the test was negative, even though there was a positive response with metabolic activation at one concentration in one strain.

Result : Experiment 1: There was no effect of treatment with test material on the number of revertants in any strain in the absence or presence of S-9. The numbers of revertants per plate in control strains TA98, TA100, TA1535 and TA1538 without microsomes were 13, 42, 11 and 11, respectively. The numbers of revertants in strains TA98, TA100, TA1535 and TA1538 treated with 5 to 1000 micrograms/plate test material without microsomes ranged from 3-12, 24-47, 6-19 and 5-9, respectively. The numbers of revertants per plate in control strains TA98, TA100, TA1535 and TA1538 with microsomes were 47, 47, 11 and 40, respectively. The numbers of revertants in strains TA98, TA100, TA1535 and TA1538 treated with 5 to 1000 micrograms/plate test material with microsomes ranged from 33-50, 24-44, 7-13 and 12-39, respectively. The positive controls induced 11, 104 and 26 revertants per plate in strains TA98, TA100 and TA1538 without microsomes and 358, 832 and 202 revertants/plate with microsomes.

Experiment 2: There was no effect of treatment with test material on the number of revertants in any strain in the absence of S-9. The numbers of revertants per plate in control strains TA98, TA100, TA1535 and TA1538 without microsomes were 7, 37, 7 and 8, respectively. The numbers of revertants in strains TA98, TA100, TA1535 and TA1538 treated with 2500 to 10000 micrograms/plate test material without microsomes ranged from 4-7, 3-36, 7-12 and 4-11, respectively. The numbers of revertants per plate in control strains TA98, TA100, TA1535 and TA1538 with microsomes were 36, 34, 5 and 26, respectively. The numbers of revertants in strains TA98, TA100, and TA1538 treated with 2500 to 10000 micrograms/plate test material with microsomes ranged from 28-41, 25-31, and 19-31, respectively. In strain 1535 in the presence of microsomes, 4, 555, 7 and 9 revertants/plate were found after incubation with 2500, 500, 7500 and 10000 micrograms/plate. The response at 5000 micrograms/plate appeared to be an aberration, since it was not dose-dependent. The positive controls induced 15, 1011 and 20 revertants per plate in strains TA98, TA100 and TA1538 without microsomes and 331, 1395 and 299 revertants/plate with microsomes.

The enzyme controls and histidine response check all gave predicted responses.

Test condition : Two plate tests were performed - one with 5, 10, 25, 50, 100, 500 and 1000 micrograms/plate and another with 2500, 5000, 7500 and 10000 micrograms/plate. The vehicle for the test material was water. Both tests were conducted in the presence and absence of liver microsome preparations from Aroclor-induced rats (sex not stated). The positive controls for strains TA98, TA100, TA1535 and TA1538 were benzo(a)pyrene, 2-aminoanthracene, diethyl sulfate, and 2-aminoanthracene, respectively (doses were not stated). Enzyme controls and histidine response checks also were performed.

Reliability : (4) not assignable
Purity of the test material was not given. Only 4 strains were tested. Numerical results for the positive control in strain TA1535 were not given (only listed as a positive sign). Documentation is limited. It appears that the assay was not performed in triplicate. The positive control for TA98 without microsomes did not induce an increase in revertants in the first experiment. Criteria for a positive test were not mentioned. The test that gave a positive result at one concentration should have been repeated.

Type	:	Unscheduled DNA synthesis
System of testing	:	Primary rat liver cell cultures
Test concentration	:	up to 2500 micrograms/ml
Cytotoxic concentr.	:	5000 micrograms/ml
Metabolic activation	:	
Result	:	negative
Method	:	other
Year	:	1985
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The hepatocytes would be expected to metabolize the material, due to the presence of cytochrome p450 and other enzymes in the cells. A positive control (2-acetylaminofluorene) which is known to induce unscheduled DNA synthesis after metabolic conversion to its active form was positive in the test.
Test substance	:	Purity of the test material was 97 +/- 1%.
Reliability	:	(4) not assignable The study is given a reliability rating of 4 because it was not reviewed in detail.

07.08.2003 (33)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	6, 24 or 48 hours
Doses	:	0, 100 and 200 mg/kg
Result	:	negative
Method	:	other
Year	:	1985
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The doses used in this study were based on a preliminary study performed with 30, 100, 300, 500 and 750 mg/kg, which showed abnormal clinical signs and loss of body weight at doses > = 100 mg/kg, and no effect on mitotic index at 100 or 300 mg/kg. Initially, 200 mg/kg was chosen as the dose for both males and females. However, since 9/15 males administered 200 mg/kg died within 24 hours, the dose in males was reduced to 100 mg/kg.
Result	:	The number of metaphases analyzed in control animals at each time point ranged from 475-500 cells (from a total of 10 animals). Two hundred and fifty metaphases from 5 animals were analyzed at each time point for animals treated with propionitrile (with the exception of 100 cells isolated at 48 hours from 2 females treated with 200 mg/kg). There was no effect of treatment with propionitrile on the frequency of chromosomal aberrations (0 - 0.4 % aberrant cells per group in treated vs. 0 - 0.2 % in controls and 0 - 0.004 aberrations per cell in treated vs. 0 - 0.002 in control), mean chromosome number (treated ranged from 41.74 - 41.84 and control ranged from 41.75 - 41.83) or mitotic index (treated ranged from 0.52 - 2.40 and control ranged from 1.20 - 2.48) at any time point. A significant increase in the percentage of aberrant cells (25.52 in treated vs. 0 in control) and average number of aberrations per cell (1.401 in treated vs. 0

in control), and a decrease in mitotic index (0.28 vs. 1.20 in control) was observed in cells from animals treated with cyclophosphamide (the positive control).

Three females treated with 200 mg/kg died. All males and females treated with propionitrile exhibited signs of toxicity. Males exhibited depression, red stains on nose/eyes, soft feces, dilated pupils and urine stains, and females exhibited depression, cold to touch, red stains on nose/eyes, wheezing, urine stains and tremors. Reduced body weights were observed in treated males at 24 hours and females at 24 and 48 hours.

Test condition : Eighty one Sprague-Dawley rats/sex (approximately 46-51 days old) were acclimated for 19 days prior to treatment. Food and water was supplied ad libitum. Eighty animals/sex were randomized and 35 per sex were assigned to the study. A single dose of test material (10 ml/kg, corrected for 100% purity) was administered by oral gavage to 2 groups of 15 male rats each at 0 (corn oil vehicle control) or 100 mg/kg and 2 groups of 15 females at 0 (vehicle control) or 200 mg/kg. Five animals per sex were euthanized at approximately 6, 24 and 48 hours after administration of test material. An additional 5 animals per sex were given the positive control cyclophosphamide (40 mg/kg) and euthanized 24 hours after treatment.

Animals were observed twice daily for general appearance, behavior, and clinical signs. Body weights of all animals were recorded just prior to administration of test material and just prior to colchicine administration (for animals that were to be killed 24 and 48 hours after treatment).

Approximately 4, 22 and 46 hours after test material was given, the appropriate groups of animals received a single intraperitoneal injection of colchicine (2.0 mg/kg body weight, dosing factor 5 ml/kg) to inhibit mitosis and arrest cells in metaphase. The colchicine was dissolved in Hank's Balanced Salt Solution (HBSS). Animals were euthanized approximately 2 hours after colchicine injection.

Bone marrow cells were collected from both femurs of each animal by aspiration into 5 ml of HBSS heated to 37 degrees C. The aspirate was spun in a centrifuge for 5 minutes at approximately 1100 rpm. The supernatant was decanted and 5.0 ml of preheated 0.075 M KCl was added to each tube. After 25 minutes, five drops of freshly prepared fixative (methanol:acetic acid, 3:1) were added to each tube. The tubes were capped, inverted and spun in a centrifuge for 5 minutes at 1100 rpm. The cells were resuspended in 5 ml of fixative, and again spun in a centrifuge for 5 min at 1100 rpm. This procedure was repeated two more times, and the cells were suspended in fresh fixative and refrigerated. After chilling, the cells were spun in a centrifuge at 1100 rpm for 5 minutes, the supernatant was decanted, and the cells were resuspended in 0.5 - 2 ml fresh fixative. Several drops of the final cell suspension were dispersed onto microscope slides and air-dried. Two to four slides were made per animal and were marked with the animal's identification number.

The cells on the slides were stained in fresh Giemsa for 10 minutes, rinsed twice with distilled water, air-dried, and mounted with glass coverslips. Code numbers were then blindly assigned to the slides. The slides were not decoded until all had been analyzed.

At least 50 cells in metaphase were analyzed per animal (if possible). Otherwise, as many spreads as possible were analyzed. The slides were scanned with a low power objective (10 or 25 X) and the chromosomes were analyzed with a high power oil immersion lens (100X). Only those cells in metaphase were analyzed for cytogenetic abnormalities. The following items were recorded for each animal: numbers and types of

chromosome aberrations (chromatid and chromosome breaks, chromatid and chromosome gaps, exchanges, cells with >= 10 aberrations, and pulverized cells), mitotic index, chromosome number for each metaphase and the vernier location of each metaphase containing damage.

The mean mitotic indices, chromosome numbers, percent aberrant cells and the mean number of aberrations per cell for each group were statistically compared using the Kruskal-Wallis nonparametric analysis of variance and nonparametric pairwise group comparisons. Body weight data were analyzed by the analysis of covariance. All tests were evaluated at the one-tailed 95% confidence interval ($p < 0.05$).

Test substance : Purity of the test material was 95.7 %. Impurities included 3.0% acrylonitrile, 0.9% adiponitrile, and 0.1% solids (not identified).

Reliability : (2) valid with restrictions
Only two animals were available for analysis at the highest dose tested (200 mg/kg).

07.08.2003 (21)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Fertility
Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 21 to 33 days (depending on day of mating)
Frequency of treatm. : 6 hr/day, 7 days/week
Premating exposure period
Male : 0 days
Female : 21 days
Duration of test : to gestation days 13-15
No. of generation studies :
Doses : 60, 120 and 210 ppm
Control group : yes
NOAEL parental : = 60 ppm
other: NOAEL : = 210 ppm
Reproductive Toxicity
Method : other
Year : 1984
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : Exposure concentrations: The average mean daily analytical exposure concentrations (60.1, 120.2 and 209.2 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25 degrees C and 26-29%, respectively.

Signs of toxicity: None of the animals died. There was no effect of test material on body weight. Animals exposed to 210 ppm exhibited arched back (N = 4 on days 1-10 and N=2 on days 11-20), lacrimation (N = 2 on days 1-10 and N = 1 on days 21-30), salivation (N= 15 on days 1-10, N = 22 on days 11-20 and N = 21 on days 21-30) hypoactivity (N = 13 on days 1-10, N = 5 on days 11-20 and N = 3 on days 21-30), staining of facial fur (N = 2 on days 1-10, N = 4 on days 11-20 and N = 4 on days 21-30) and red nasal encrustation (N = 1 on days 1-10, N = 5 on days 11-20 and N = 5

on days 21-20) after exposure. Animals exposed to 120 ppm also exhibited salivation (N = 6 on days 11-20 and N = 4 on days 21-30, staining of facial fur (N = 7 on days 1-10, N = 5 on days 11-10 and N = 2 on days 21-30) and red nasal encrustation (N = 2 on days 1 1-0, N = 8 on days 11-20 and N = 6 on days 21-30). A few animals in the 60 ppm group also exhibited red nasal encrustation (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-30) and staining of facial fur (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-20). One control animal had stained facial fur on days 21-30 and another had red nasal encrustation on days 1-10. Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 2 controls, N = 3 low dose, N = 5 mid dose, N = 9) at one or more of their weekly physical examinations.

The only remarkable findings at gross necropsy were bilateral uterine hydrometra in one animal exposed to 210 ppm and hydrometra in the left uterine horn of one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on fertility. Efficiency of mating (32.0%, 32.0%, 30.7% and 25.0% in the control, low, mid and high dose groups) and pregnancy rate (100%, 95.8%, 100% and 91.3% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 13.4 - 13.9), resorptions (ranged from 0.6 - 0.8), nidations (ranged from 14.1 - 14.5), corpora lutea (ranged from 13.0 - 15.2), preimplantation loss (4-8%) and postimplantation loss (4-6%). Evaluation of the vaginal smears of 2 females that did not copulate showed one that did not cycle (but was pregnant at necropsy), and another that only went through the cycling stage of proestrus.

Test condition : Animals: Virgin female Sprague Dawley rats (43 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of ten females and ten males that were taken upon receipt were 128-144 g and 178-233 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to females during exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 7 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Due to inclement weather and building equipment failures, 2 exposures (days 2 and 16) were only for 4 hours and one exposure (day 1) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 16) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Twenty four females per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when females were 63 days old. Animals were observed during exposure for signs of toxicity. After 21 days of exposure (which was sufficient to cover 3-4 estrus cycles), females were randomly mated (1:1) to an untreated male that had been assigned to the corresponding treatment group (30 males were assigned per group). At night, after exposure, females were caged with their assigned male until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or

5 nights without confirmed copulation. Females that failed to mate with the assigned male were mated with another male that had copulated with another female in the same group. Nightly co-housing with the second male occurred until copulation was confirmed (or for a maximum of 7 nights). The day on which copulation was confirmed was considered gestation day 0. Exposure of females continued until copulation was confirmed or a maximum of 12 nights of cohabitation with males without signs of copulation. Vaginal smears were taken on 5 consecutive days for females that did not exhibit copulation.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Females were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities.

Females were killed on gestation day 13 (or the nearest working day after gestation day 13, up to gestation day 15). Females without confirmed copulation ware euthanized in the second week after the last day of co-housing. Each female was given an external examination and weighed. The tissues and organs of the thoracic and abdominal cavities were examined for gross lesions. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted. The ovaries and uteri of females were preserved in 10% neutral buffered formalin. Males were killed after mating and were not examined.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (< 0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : The authors concluded that the incidences of red nasal encrustation in the low dose animals, alopecia in the mid and high dose animals and staining of facial fur in all treated groups were too low to be definitely related to administration of test material. There was no effect of treatment on fertility of females.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (24)

Type : Fertility
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 46 to 57 days (depending on day of mating)
Frequency of treatm. : 6 hours/day, 5 days/week
Premating exposure period
Male : 46 days
Female : 0 days
Duration of test : to gestation day 13-15
No. of generation studies :
Doses : 60, 120 and 210 ppm
Control group : yes
NOAEL parental : = 60 ppm

other: NOAEL : = 210 ppm

Reproductive Toxicity

Method : other

Year : 1985

GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Result : Exposure concentrations: The average mean daily analytical exposure concentrations (60.2, 120.4 and 208.9 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25.5 degrees C and 24-27%, respectively.

Signs of toxicity: One of animals exposed to 210 ppm died after 2 days of exposure. On the previous day, this animal exhibited labored breathing, hypoactivity, poor control of the hind limbs, difficulty in standing, body tremors and involuntary movements. No unusual findings were observed at necropsy.

Body weights of males exposed to 210 ppm were approximately 6-9% lower than those of the control group during most of the exposure period, and remained lower than control (but were not significantly different) until the end of the study.

Animals exposed to 210 ppm exhibited signs of toxicity such as arched back (N = 8 on days 1-10, N = 3 on days 11-20 and 51-57, and N = 5 on days 41-50), hypoactivity (N = 12-15 at each 10-day interval up to day 50, and N = 4 from days 51-57), labored breathing (N = 10 on days 1-10, N = 3 on days 11-20 and 31-40, N = 5 on days 21-30 and N = 1 on days 51-57), and salivation (N = 3 on days 1-10, and N = 10 - 12 at all other intervals). A few high dose animals (individual numbers were not stated) also exhibited abnormal behavior such as grinding of teeth, head bobbing, body tremors, involuntary movements, and pawing at the cage. A few of the animals exposed to 120 ppm exhibited salivation (N = 3-8 at all intervals) and hypoactivity (N = 3 at days 11-20). Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 1 control, N = 2 low dose, N = 1 mid dose, N = 5 high dose) at one or more of their weekly physical examinations. No unusual treatment-related signs were observed in rats exposed to 60 ppm. The only remarkable finding at gross necropsy was a small right testis in one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on male fertility. Efficiency of mating (34.4%, 30.6%, 29.8% and 27.1% in the control, low, mid and high dose groups) and pregnancy rate (90.5%, 97.6%, 90.0% and 97.4% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 12.7 - 13.9), resorptions (ranged from 0.7 - 1.1), nidations (ranged from 13.8 - 14.9), corpora lutea (ranged from 13.1 - 15.2), preimplantation loss (4-8%) and postimplantation loss (5-10%).

Test condition : Animals: Virgin female Sprague Dawley rats (28 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of fifteen females and ten males that were taken upon receipt were 155-181 g and 80-103 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to males during exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. A

scheduled exposure day was cancelled due in clement weather. A new exposure day (exposure day 41) was used in its place. Due to inclement weather and building equipment failures, 2 exposures (days 33 and 43) were only for 4 hours and one exposure (day 32) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 43) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Fifteen males per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when males were 43 days old. Mating was initiated when males and females were 16 and 12 weeks old, respectively. At this time, males had been 69 days on the study (which was sufficient to cover the spermatogenesis cycle of the rat), and had 46 days of exposure. Males were randomly mated (1: 1) with three untreated females (consecutively) that had been assigned to the corresponding treatment group (45 females were assigned per group). Exposure of males continued until the day after the last mating opportunity (57 exposure days). At night, after exposure, males were caged with their assigned female until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or 5 nights without confirmed copulation. The day on which copulation was confirmed was considered gestation day 0.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Males were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities (except for one day prior to mating when inclement weather permitted observations).

One half of the males of each group were euthanized on each of the 2 consecutive days at the end of the study. They had not been exposed to propionitrile for about 2 weeks prior to termination. Each male was given an external examination and weighed. The tissues and organs of the thoracic, scrotal and abdominal cavities were examined for gross lesions and the testes, epididymides, prostate glands and seminal vesicles were preserved in 10% neutral buffered formalin. Females that were not mated with males were euthanized and were not examined.

Mated females were euthanized on gestation day 13 (or the nearest workday up to gestation day 15). Females that were co-housed with males without confirmed copulation were euthanized during the second week after the last day of co-housing. Gross necropsies were performed on females that had copulated and those that had not. The tissues and organs of the thoracic and abdominal cavities were examined. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (<

0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : There was no effect of treatment on fertility of males.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (25)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Days 6 through 19 of gestation
Frequency of treatm. : daily
Duration of test : to Day 20 of gestation
Doses : 20, 40, 80 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 40 mg/kg bw
NOAEL teratogen. : = 80 mg/kg bw
NOAEL Fetotoxicity : = 40 mg/kg bw
NOAEL Embryotoxicity : = 40 mg/kg bw
Method : other
Year : 1981
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Doses used in this study were chosen based on the results of a pilot study (International Research and Developmental Corporation study IR-79-163, dated December 22, 1980) conducted with 5, 10, 20, 37.5 and 75 mg/kg/day. In this study, 75 mg/kg caused a moderate to severe decrease in maternal weight gain. Two out of 5 dams treated with 75 mg/kg/day also had an increased number of resorptions (16 early in one animal and 7 late in the other).

In the main study, the number of fetuses and litters with sternebrae 5 and/or 6 unossified in the mid dose group (55 and 35, respectively) was "nearly comparable" to historical controls (3.8 - 23.7 in 13.0 - 73.9 litters). Since this was the only change observed in the mid dose fetuses, it is not considered to be indicative of fetotoxicity. Therefore, this dose was chosen as the NOAEL for fetotoxicity.
The NOAEL for teratology was established by study personnel. The summary preparer established the NOAELs for maternal toxicity, fetotoxicity and embryotoxicity.

Result : Maternal: One rat in the high dose group died of an undetermined cause on gestation day 9. Survival was 100% in the other groups. There was a slight to moderate reduction in mean maternal body weight gain over the entire treatment period for the high dose group (118 g in treated vs. 134 g in control). This decrease was predominantly due to decreased body weight gain from days 6 to 9 (2 g in treated vs. 9 g in control). The mean maternal adjusted body weight gain (body weight minus the uterus and contents) in the high dose group also was slightly reduced during the gestation period (52 g in treated vs. 60 g in control). Weights of animals in other treated groups were similar to controls.

There was no effect of treatment on the number of pregnancies, mean number of viable fetuses (all were viable), late resorptions, total implantations, or corpora lutea. There was a significant increase in the number of early resorptions (2.0 +/- 2.63 in treated vs. 0.7 +/- 0.81 in

control) and a corresponding increase in the number of postimplantation losses in the high dose group. None of the animals aborted.

Fetal: There was no effect of treatment on fetal sex ratio. Average mean fetal body weight of fetuses from high dose animals were significantly less than controls (3.0 +/- 0.40 g in treated vs. 3.5 +/- 0.28 g in control). The number of control, 20 mg/kg/day, 40 mg/kg/day and 80 mg/kg/day fetuses (and litters) with malformations were 5 (3), 1 (1), 1 (1) and 0 (0), respectively. One fetus in the low dose group and one in the mid dose group had a diaphragmatic hernia. An increase in the number of fetuses and litters with sternebrae 5 and/or 6 unossified was noted in the mid dose group (55 and 35, respectively) and high dose group (92 and 66.7, respectively) when compared to the study control (21 and 14, respectively). An increase in the number of fetuses and litters with sternebrae 1 and/or 2, 3, and 4 unossified was also found in the high dose group (8 in 2 treated litters vs. 1 in control).

Test condition : Rats: One hundred virgin female COBS CD rats (approximately 14 weeks old) were used in the study. They were acclimated for at least 10 days prior to mating. One female was mated with one male. Copulation was verified by the presence of a copulatory plug or sperm in a vaginal smear. The day that evidence of mating was detected was designated day 0 of gestation. Mated females were assigned in a block design to a vehicle control group and 3 treatment groups consisting of 25 rats each.

Test material: Dosing solutions of propionitrile were prepared daily at concentrations that permitted administration of 20, 40 and 80 mg/kg/day at a constant volume of 10 ml/kg. The material was dissolved in distilled water and shaken by hand to ensure dissolution. The test material was administered orally by gavage as a single daily dose on days 6 through 19 of gestation. The control group received distilled water at 10 ml/kg. Individual dosages were determined using body weights that were taken on gestation day 6.

Maternal observations: Prior to treatment, the dams were observed daily for mortality and overt changes in appearance and behavior. They were also observed daily from days 6 through 20 of gestation. Individual body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20. On gestation day 20, all survivors were euthanized. The uterus was excised and weighed, and the number and location of viable and nonviable fetuses, early and late resorption and total number of implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined grossly. Any dam that did not survive to scheduled termination also was necropsied. Maternal tissues were preserved when deemed necessary according to gross findings. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy.

Fetal observations: All fetuses (total of 326, 302, 319 and 272 in the control, low, mid and high dose groups) were individually weighed and examined for external malformations and variations. Each fetus was sexed. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning. The other fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination.

Statistical analyses: The sex distribution and number of litters with malformations in treated animals were compared to controls using a Chi-square test with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test. The numbers of early and late resorptions and postimplantation losses were compared by the Mann-Whitney U-test. The mean number of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by Bartlett's test for homogeneity

of variance, analysis of variance (one-way), and the appropriate t-test (for equal or unequal variances). Dunnett's multiple comparison tables were used to judge significance of differences. The level of significance was $p < 0.05$.

Test substance : The purity of the test material was > 90%.

Reliability : (1) valid without restriction
Significant differences between weights and weight gains of treated dams and controls were not designated.

Flag : Critical study for SIDS endpoint

10.08.2003 (22)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Mechanistic Studies
Study descr. in chapter :
Reference :
Type :
Species : mouse
Sex : male
Strain : CD-1
Route of admin. : i.p.
No. of animals :
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : n-Butyronitrile also was tested in this study. The results with this material were similar to those of propionitrile.

This study is considered to be valid without restriction. The study was conducted and documented in a thorough manner.

Result : In the first study, the mortality rate for animals treated only with 45 mg/kg propionitrile was 9/10. Co-treatment with sodium nitrite reduced the rate to 5/9. None of the 10 animals that were co-treated with sodium thiosulfate died.

In the carbon tetrachloride study, the mortality rate for animals treated only with 45 mg/kg propionitrile was 8/10. None of the animals co-treated with carbon tetrachloride died.

Cyanide concentrations in liver and brain of mice co-treated with sodium thiosulfate or carbon tetrachloride were significantly less than those of mice treated with propionitrile alone. In mice receiving propionitrile only, 26.7 +/- 8.0 (mean +/- SD) and 12.8 +/- 5.4 nmol/g cyanide were found in liver and brain, respectively. In mice receiving propionitrile plus sodium thiosulfate, 2.0 +/- 0.7 and 0.8 +/- 0.8 nmol/g cyanide were found in liver and brain, respectively. In mice receiving propionitrile plus carbon tetrachloride, 0.9 +/- 1.1 and 1.5 +/- 1.4 nmol/g cyanide were found in liver and brain, respectively.

Test condition : Male CD mice (30 g) were divided into 3 groups of ten animals each. One group received 45 mg/kg i.p. propionitrile only, another received i.p. injections of 75 mg/kg sodium nitrite (a cyanide antagonist) 20 minutes before and 100 minutes after i.p. injection of 45 mg/kg propionitrile, and another received i.p. injections of 1 g/kg sodium thiosulfate (a cyanide antagonist) 20 minutes before and 80 and 180 minutes after i.p. injection of

45 mg/kg propionitrile.

Two other groups of 10 mice received either 0.2 ml of vegetable oil or 0.2 ml of 20% carbon tetrachloride (a hepatotoxic dose) in vegetable oil subcutaneously, 24 hours before i.p. treatment with 45 mg/kg propionitrile.

In both experiments, animals were observed for 7 days. Mortality data were analyzed statistically by the chi-square test. The criterion for significance was $p < 0.05$.

In an additional study, the concentrations of cyanide in liver and brain were determined in a) 5 mice treated only with 28 mg/kg propionitrile (i.p.), b) 5 mice given 1 g/kg sodium thiosulfate 20 minutes before and 80 minutes after 28 mg/kg propionitrile (i.p.), and c) 5 mice given 0.2 ml of 20% carbon tetrachloride subcutaneously 24 hours before i.p. treatment with 28 mg/kg propionitrile. All mice were killed 2.5 hours after propionitrile injection (if still alive at this time). The livers and brains were excised as soon as possible after death, quick-frozen and weighed. Cyanide concentrations were determined by the method of Bruce et al. (Anal Chem 27: 1346-1347, 1955). Results were analyzed using an unpaired t-test.

Test substance : The purity of the test material was 99%. No free cyanide was found in solutions made in distilled, deionized water.

Conclusion : Propionitrile is activated by the liver to release cyanide, which is responsible for acute toxicity.

05.08.2003 (36)

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

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Data Set

Existing Chemical	:	ID: 109-74-0
CAS No.	:	109-74-0
EINECS Name	:	Butyronitrile
TSCA Name	:	Butanenitrile
Molecular Formula	:	C4H7N

Producer related part

Company	:	Eastman Chemical Company
Creation date	:	24.06.2003

Substance related part

Company	:	Eastman Chemical Company
Creation date	:	24.06.2003
Status	:	
Memo	:	
Printing date	:	06.10.2003
Revision date	:	13.11.2003
Date of last update	:	06.10.2003
Number of pages	:	43
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	C(N#)CCC
Molecular formula	:	C4H7N
Molecular weight	:	69.11
Petrol class	:	

15.08.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	liquid
Purity	:	>= % w/w
Colour	:	clear
Odour	:	

Reliability : (2) valid with restrictions
28.07.2003

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1-Cyanopropane

Butanenitrile

Butyric acid nitrile

n Butyl nitrile

n-Butyronitrile

n-Propyl cyanide

Propyl cyanide

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use	:	industrial
Category	:	Chemical industry: used in synthesis
Remark	:	Chemical intermediate

02.07.2003 (5)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= -112 °C
Sublimation	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

28.07.2003 (29)

2.2 BOILING POINT

Value	:	= 117.5 °C at 1016 hPa
Decomposition	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Value is boiling point at 760 mm Hg. Purity of material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

28.07.2003 (29)

2.3 DENSITY

Type	:	relative density
Value	:	= .7954 g/cm³ at 15 °C
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of the material is unknown.
Reliability	:	(2) valid with restrictions Source is peer-reviewed published data.

28.07.2003 (29)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 26 hPa at 25 °C
Decomposition	:	
Method	:	other (measured): not specified
Year	:	
GLP	:	no data

Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of the material is unknown. Data were obtained from Hazardous Substances Data Bank Number: 5013. Last revision date: 9/21/1999.
Result	:	Value is 19.5 mmHg
Reliability	:	(2) valid with restrictions Primary source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

28.07.2003 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	
Log pow	:	= .53 at °C
pH value	:	
Method	:	other (measured)
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The value obtained from the experiment was 0.53. A value of 0.60 with a 0.10 confidence limit also was listed.
Test condition	:	The test was performed at ambient temperature (20-25 degrees C). The value was obtained using the Shake-Flask method. The aqueous phase was octanol-saturated water. The concentration of material in the aqueous phase was measured using gas-liquid chromatography.
Test substance	:	Purity of the test material was not mentioned.
Reliability	:	(2) valid with restrictions Data were from a peer reviewed, published source.
Flag	:	Critical study for SIDS endpoint

13.08.2003 (25)

Partition coefficient	:	octanol-water
Log pow	:	= .837 at 25 °C
pH value	:	= 7
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program are CAS No., melting point (-112 degrees C), boiling point (117.5 degrees C), vapor pressure (19.5 mm Hg), and water solubility (33,000 mg/l).
Reliability	:	(2) valid with restrictions Approved model to calculate log Kow.

13.08.2003 (17)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	water
Value	:	= 33000 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	

Deg. product	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of material is unknown. Data obtained from Hazardous Substances Data Bank Number: 5013. Last revision date: 9/21/1999.
Result	:	Moderate (10-100 g/L)
Reliability	:	(2) valid with restrictions Primary source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

15.08.2003 (23)

Solubility in	:	water
Value	:	= 27840 at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other:calculated
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program are CAS No., melting point (-112 degrees C), boiling point (117.5 degrees C), vapor pressure (19.5 mm Hg), and water solubility (33,000 mg/l).
Reliability	:	(2) valid with restrictions Data were obtained by modeling.

15.08.2003 (19)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .000000000000498 cm³/(molecule*sec)
Degradation	:	= 50 % after 21.5 day(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program are CAS No., melting point (-112 degrees C), boiling point (117.5 degrees C), vapor pressure (19.5 mm Hg), and water solubility (33,000 mg/l).
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

07.08.2003 (14)

3.1.2 STABILITY IN WATER

Type	:	abiotic
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN Hydrowin cannot calculate hydrolysis rate constants for nitriles. The theoretical hydrolysis of the related material propionitrile and several other chemicals has been examined by Dr. Lee Wolfe at the USEPA Environmental Research Laboratory in Athens, Georgia. The results of these analyses were published in a report by Dr. Wolfe that could not be located. In a personal communication, Dr. Wolfe stated that propionitrile can hydrolyze (albeit slowly). According to a study cited in the Hazardous Substances Data Bank, the chemical hydrolysis of the related material acetonitrile in water is base-catalyzed (the rate constant for base catalyzed hydrolysis is 5.8X10-3/M-hr), but the half-life at pH 7 is more than 150,000 yrs (Ellington et al., 1988). Acetonitrile ($CH_3C{\equiv}N$, CAS No. 75-05-8) is the 2-carbon analog of the category members, possessing the same functionality, but having one less carbon than propionitrile. Taken together, these data suggest that hydrolysis of butyronitrile at environmentally relevant pHs will occur too slowly to be a significant means of degradation.
Reliability	:	(2) valid with restrictions Experimental results for the test material could not be located. Results are for a related material.

28.07.2003 (16)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air, water, soil and sediment
Air	:	15 % (Fugacity Model Level I)
Water	:	47.9 % (Fugacity Model Level I)
Biota	:	.0821 % (Fugacity Model Level II/III)
Soil	:	37 % (Fugacity Model Level II/III)
Method	:	other: calculated
Year	:	2003
Remark	:	Measured inputs to the program are CAS No., melting point (-112 degrees C), boiling point (117.5 degrees C), vapor pressure (19.5 mm Hg), and water solubility (33,000 mg/l).
Result	:	EPIWIN Henry Program (v3.10) estimates a Henry's Law Constant of 5.38E-005 atm-m3/mol. EPIWIN Pckocwin Program (v1.66) estimates a Koc of 15.3.
Reliability	:	(2) valid with restrictions Approved model for estimated environmental transport values.
Flag	:	Critical study for SIDS endpoint

15.08.2003 (18)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	
Deg. product	:	
Method	:	other: Directive 92/69/EEC, C.5 (BOD5) and Directive 92/69/EEC, C.6 (COD)
Year	:	1998
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Refer to Section 3.6 below.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

02.07.2003

Type	:	aerobic
Inoculum	:	other: activated sludge
Concentration	:	500 mg/l
Contact time	:	72 hour(s)
Deg. product	:	
Method	:	other
Year	:	1970

GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : TOD for n-butyronitrile was 1679 mg/l. The test material was degraded by Franklin sludge (10.5% TOD at 72 hours) and Nashville sludge (0.8 and 1.0% TOD at 24 and 72 hours, respectively). The test material was toxic to Bordeaux sludge.

Test condition : Bacteria: The activated sludges were obtained from the municipal plant at Franklin, TN, the municipal plant at Nashville TN and the plant at Bordeaux, a suburb of Nashville. Mixed liquor from the aeration tanks was collected the morning of the day the Warburg run began. Each sample was packed in ice and transported to the laboratory within 1 hour of collection. Before the run began, the sludge sample was blended for 10 sec and the homogenous blend was analyzed for concentration of SS (not defined, but assumed suspended solids), using a membrane-filter technique. The original sample was adjusted to a SS concentration of 2,500 mg/l.

Test conduct: Test material was added to a Warburg flask (125 ml) in order to obtain a final concentration of 500 mg/l in the reaction compartment (final volume of 20 ml). KOH (1.0 ml, 20%) was added to the center well. A 20 ml volume of activated sludge was then introduced. The test was performed in duplicate. Flasks were incubated for 72 hours (constant motion) at 20 degrees C. All three sludges were tested. Oxygen uptake curves were plotted. Respiration of the sludge alone was plotted as the control curve.

Theoretical O2 demand (the mg/l O2 required to completely oxidize the test material) was calculated on the basis of the test material to CO2 and water, plus nitrate according to the following equation: (TOD = moles of O2 required to balance the equation x molecular weight of O2 x concentration of test material/ (moles of test material required to balance the oxidation equation x molecular weight of the test material). The percentage of TOD was to be calculated as follows: % TOD = 100 x D (the difference in mg/l of O2 uptake between substrate and control)/ TOD. The material was considered toxic if D was less than 0.

Reliability : (2) valid with restrictions
Purity of the test material was not given

07.08.2003 (22)

Type : aerobic
Inoculum : other: mixed microbial culture
Concentration : 1000 mg/l related to
related to
Contact time : 48 hour(s)
Result : other: biodegradable
Deg. product :
Method : other
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The final protein and ammonia concentrations and pH were 10.10 mg/l, 74.6 micromoles/ml and 8.69, respectively, indicating that the mixed culture could use this material as a growth substrate.

Test condition : A mixed microbial culture (protein concentration of 0.085 mg/l) was isolated from an environment contaminated with organic cyanides and polychlorinated biphenyls. This was grown for 48 hours on phosphate buffer (pH 7.0, 30 degrees C) containing propionitrile (1 g/l) as the sole source of carbon and nitrogen. The final concentration of protein, ammonia and pH were determined.

Test substance : Test material was obtained from Aldrich Chemical Co. It is presumed that

the material has high purity.

Reliability : (4) not assignable
The study shows that the test material was used as a substrate (and therefore was metabolized); however, the extent to which the test material biodegraded is difficult to determine from the study.

07.08.2003 (1)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method : Directive 92/69/EEC, C.5
Year : 1998
GLP : yes
COD
Method : Directive 92/69/EEC, C.6
Year : 1998
COD : = 2130 mg/g substance
GLP : yes
RATIO BOD5 / COD
BOD5/COD : = .47

Remark : Method is similar to OECD: TG-301C: Modified MITI Test. The study is the critical study for the biodegradation endpoint.

Result : BOD analysis: The values obtained for the 3 different concentrations were 1.00, 1.01 and 1.03 g BOD/g test material at 5 days and 1.76, 2.07 and 1.86 g BOD/g test material at 20 days. The average BOD5 and BOD20 values were 1.0 grams BOD/gram of test substance and 1.9 grams BOD/gram of test substance, respectively.

COD analysis: The values obtained for the 3 replicates were 2.139, 2.064 and 2.184 g COD/g test material. The average value was 2.13 g COD/g test material. The percent recovery of the reference sample was 93.5%.

BOD5/COD: The BOD5/COD ratio is 0.47 (1.0/2.13).

Test condition : Test conditions: COD Determination: A 0.50 N potassium dichromate solution was used to standardize the ferrous ammonium sulfate titrant. Mercuric sulfate was added to minimize chloride interference (if any). Three separate replicates were tested. A potassium phthalate standard was analyzed as a positive control. The test was considered valid if the recovery of the standard fell between the limits of 83.47 - 116.06%. The COD was calculated by subtracting the amount of titrant needed for the sample from the amount of titrant needed for a blank. This result was multiplied by the normality of the titrant and the equivalent weight of oxygen. The product was then divided by the sample weight.

BOD Determination: The test was performed according to an Eastman Kodak Company protocol [Need to have protocol for details]. Three separate concentrations (0.000195%, 0.000397% and 0.000592%) were tested. The 5-day BOD was calculated by subtracting the final dissolved oxygen reading and the 5-day seeded dilution water drop from the initial dissolved oxygen reading. The 20-day BOD was calculated by subtracting the average 20-day seeded dilution water drop from the total dissolved oxygen drop over 20 days. The results were multiplied by 100. The products were divided by the product of the percent concentration of the stock solution in the BOD bottle and the concentration of test chemical in the stock solution. The products were divided by 1,000,000. The results were in units of grams of BOD per gram of test substance for the 5-day (or 20-day) incubation. The test was considered to be valid if the average BOD of glucose-glutamic acid standards was 198 mg/l +/- 30 mg/l.

		BOD5/COD ratio: This ratio was calculated by dividing the average 5-day BOD value by the average COD value.
Test substance	:	Purity was 99.94%.
Conclusion	:	The test material is not considered to be "Readily Biodegradable" based on a BOD5/COD ratio of <0.5 (1000/2130 = 0.47)
Reliability	:	(1) valid without restriction This was a well-documented guideline study conducted under GLP assurances.

10.08.2003 (6) (7)

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 107 measured/nominal
LC0	:	measured/nominal
LC50	:	> 107 measured/nominal
Limit test	:	yes
Analytical monitoring	:	yes
Method	:	other: OECG:TG-203 and EEC/Annex V C.1.
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	No significant protocol deviations were noted that would affect study results. The fact that the temperature (21 - 22 degrees C) was slightly higher than that specified in the protocol (20 +/- 1 degrees C) was not considered to have adversely affected the study.
Result	:	No mortality occurred and all fish exhibited normal behavior and appearance. No precipitation of test material was observed. The mean concentration of test material was 107 mg/l. The analyzed percent loss of the test material ranged from 0 - 9.1%. The temperatures of all solutions ranged from 21 - 22 degrees throughout the test. The pH and dissolved oxygen values ranged from 7.9 - 8.4 and 7.2 - 8.7 mg/l, respectively. The temperature, pH and dissolved oxygen values were considered to be acceptable for the organisms used in the test. The test was considered to be valid.
Test condition	:	Organisms: Juvenile fathead minnows were acclimated to test water for at least two weeks prior to testing. They were randomized to 6 sets of 7 fish each. Two sets of minnows (7/set) were killed before the start of the test to determine average wet weight (0.35 and 0.37 g/set) and mean standard length (2.97 cm/set). Test water: The water was pumped from Lake Ontario into a large underground storage vessel. Water from this vessel was subsequently pumped into the laboratory where it passed through polystyrene filter tubes, activated carbon filter tubes, and another set of polystyrene filter tubes. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then heated to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Representative values for hardness and total alkalinity (both as CaCO3) were 120.0 and 87.4 mg/l, respectively. Test material: A stock solution of test material was prepared in a 200 ml volumetric flask containing test water. The exposure solutions were prepared at a nominal concentration of 120 mg/l by adding the appropriate amount of stock solution to glass vessels (30.5 cm Pyrex seamless, cuboidal chromatography jars) containing 20 liters of test water. The solutions in each test vessel were stirred with a stir rod prior to adding fish. Duplicate test and dilution water control vessels were prepared. Test conduct: Immediately after stirring, fish were placed into each of the replicate test and control vessels (7 per vessel). Glass lids were placed on top of each test vessel and sealed with Parafilm. Biological loading within test vessels was kept below 1.0 g wet weight/l test solution. The vessels were placed in a certified hood under 8.5 hours of fluorescent lighting/day. Animals were observed for mortality and signs of stress at 0,

24, 48, 72 and 96 hours. Temperature, dissolved oxygen concentration and pH of each solution also were measured at these times. Concentrations of test material in the test vessels at 0 and 96 hours were analyzed by GC/MS. The geometric mean of the concentrations was calculated. Since no mortality was observed, statistical analyses were not performed.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was 20 +/- 1 degrees C and there were no abnormal occurrences that could influence the outcome.

Test substance : Purity was 99.94%

Conclusion : The LC50 value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

Flag : Critical study for SIDS endpoint

07.08.2003 (10)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 110 measured/nominal
EC0 : measured/nominal
EC50 : > 110 measured/nominal
Limit Test : yes
Analytical monitoring : yes
Method : other: OECD: TG-202 and EEC/Annex V C.2
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Nine organisms (instead of 10) were used in one test vessel. No other protocol deviations were noted.

Result : One out of 10 daphnids in one study was immobile after 48 hours of exposure to test material. This was not considered to be significant (< = 10%). All other daphnids exposed to test article exhibited behavior comparable to controls. No precipitation of test material was observed. The geometric mean of the test concentration at 0 and 48 hours was 110 mg/l. The analyzed percent loss of the test material was 0%. The temperatures of all solutions were maintained at 22 degrees throughout the test. The pH and dissolved oxygen values ranged from 8.2 - 8.4 and 8.6 - 8.7 mg/l, respectively. The temperature, pH and dissolved oxygen values were considered to be acceptable for the organisms used in the test. The test was considered to be valid.

Test condition : Organisms: Adult Daphnia magna were reared within the testing facility in 100-l culturing flasks. Gravid daphnids used to produce test animals were obtained from rearing tanks that had been established for at least two weeks. Prior to the study, approximately 100 gravid daphnids were transferred by net into two glass bowls containing test water and food. After 18 hours in the bowls, all adult daphnids were removed. Neonates were collected by pipette and transferred directly into exposure vessels. A total of 10 daphnids were placed into each of the replicate test and control vessels.

Test water: The water was pumped from Lake Ontario into a large

underground storage vessel. Water from this vessel was subsequently pumped into the laboratory where it passed through polystyrene filter tubes, activated carbon filter tubes, and another set of polystyrene filter tubes. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then heated to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Representative values for hardness and total alkalinity (both as CaCO3) were 120.0 and 87.4 mg/l, respectively.

Test material: A stock solution of test material was prepared in a 200 ml volumetric flask containing test water. The exposure solutions were prepared at a nominal concentration of 120 mg/l by adding the appropriate amount of stock solution to glass vessels (300 ml Pyrex glass, lipless beakers) containing 20 L of test water. The solutions in each test vessel were stirred with a stir rod prior to adding daphnids. Duplicate test and dilution water control vessels were prepared.

Test conduct: Immediately after stirring, daphnids were placed into each of the replicate test and control vessels 10 per vessel). Watch glasses were placed on top of each test vessel and sealed with Parafilm. The vessels were placed in a certified hood under 8.5 hours of fluorescent lighting/day. Animals were observed for mortality and signs of stress at 0, 24 and 48 hours. Temperature, dissolved oxygen concentration, and pH of each solution were measured at 0 (prior to adding organisms) and 48 hours. Concentrations of test material in the test vessels at 0 and 48 hours were analyzed by GC/MS. The geometric mean of the concentrations was calculated. Since no mortality was observed, statistical analyses were not performed.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was 20 +/- 2 degrees C, test daphnids in the control groups were not trapped at the surface of the water and there were no abnormal occurrences that could influence the outcome.

Test substance : Purity was 99.94%

Conclusion : The 48-hour EC50 value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

Flag : Critical study for SIDS endpoint

07.08.2003 (9)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : other: biomass and growth rate
Exposure period : 72 hour(s)
Unit : mg/l
NOEC : = 133.4 measured/nominal
EC50 : > 133.4 measured/nominal
Limit test : yes
Analytical monitoring : yes
Method : other: OECD: TG-201 and EEC/Annex V C.3
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Results of a pilot study conducted prior to this test indicated that a limit test design would be appropriate for the material.

The EbC50 (0-72 hr) and the ErC50 (0-72 hr) were inestimable as greater than 50% inhibition in growth and/or biomass was not achieved. No protocol deviations were noted.

Result : Algae exposed to test material exhibited normal growth with respect to control. At the end of the test, the mean cell density in treated cultures was 9.5 x 10E5 cells /ml (compared to 9.0 x 10E5 cells in control).

The average concentrations of material in the test flasks at the beginning of the test and after 72 hours were 206.0 and 85.7 mg/l, respectively. The mean concentration was 133.4 mg/l. This concentration was listed as the NOEC, EbC50 and ErC50.

Results of the photostability tests were similar to those in flasks containing test material and algae. The control solutions that were exposed to light or were in the dark exhibited 58% and 50% losses of test material.

The mean temperature and illumination were 24 degrees C and 747 foot-candles (+/- 5.5 foot-candles) throughout the test. The pH ranged from 7.4 - 7.6.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 90.2-fold within 72 hours.

Test condition : Test Organisms: A 4-day culture of Selenastrum capricornutum SF-3148 (passage 3 in liquid algal medium) was used as the test algae. Several passages were performed prior to the test to confirm exponential growth. The density of cells in the stock culture was 2.58 x 10E6 cells/ml prior to use.

Test medium: Sterile growth medium was prepared using high quality distilled water. The pH of the medium was measured and adjusted to 7.5 (+/- 0.1) using 0.1N NaOH.

Test material stock solution: Approximately 0.151 ml (120 mg) of the test material was added to 600 ml of algal growth medium with a gas tight Hamilton syringe (to produce a nominal concentration of 200 mg/l). The solution was stirred for approximately 1 minute. An aliquot (1.0) of the solution was removed for analysis of concentration.

Test conduct: All steps were carried out aseptically in a hood to prevent contamination. Test vessels were sterile 250 ml Erlenmeyer flasks. Test material stock solution (100 ml) was added to 5 flasks and test medium that did not contain test material was added to 3 flasks. Algae (388 microliters of algal stock culture to achieve an initial cell density of 1 x 10E4 cells/ml) were added to 3/5 flasks that contained test material and the three that did not. The two flasks that contained test material but were not inoculated served as photostability controls. One of the flasks was exposed to light and one was wrapped in foil to shield it from light. All flasks were secured with foam stoppers and transferred to a shaking incubator (24 degrees C, 100 rpm). They were illuminated at 747 (+/- 5.5) footcandles throughout the study.

Temperature, light intensity, and shaker speed (rpm) were assessed at the 0, 24, 48, and 72 hours. Concentrations of test material in the flasks that contained algae also were assessed at these times. The pH and was assessed at time 0 and after 72 hours. Concentrations of test material in the photostability controls also were measured at 0 and 72 hours. Concentrations of test material were analyzed using gas chromatography with flame ionization detection (GC/FID). The exposure concentration was calculated as the geometric mean of the test concentrations analyzed at

the 4 time points. Cell counts were performed after 24, 48 and 72 hours of exposure using a calibrated Coulter Counter. The mean algal cell count for the test and control curves. Two measures of growth [biomass (area under the growth curve) and growth rate] were used to determine the effect of the material on algae. The concentrations that produced a 50% inhibition of biomass (EbC50) and growth rate (ErC50) relative to control were to be calculated by fitting linear
egression models to the data.

The test was considered valid if the mean cell concentration in the control cultures increased by a factor of at least 16 within 72 hours.

Test substance : Purity was 99.9% (GC/FID).
Conclusion : The results of this study indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Reliability : (1) valid without restriction
This was a well-documented OECD-study conducted under GLP assurances.
Flag : Critical study for SIDS endpoint
07.08.2003 (8)

Species : other algae: green algae
Endpoint :
Exposure period : 96 hour(s)
Unit : mg/l
EC0 : = 364.857 calculated
Limit test :
Analytical monitoring : no
Method : other: calculated
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program are CAS No., melting point (-112 degrees C), boiling point (117.5 degrees C), vapor pressure (19.5 mm Hg), and water solubility (33,000 mg/l). The class of compound for estimation in the model was neutral organic.
Reliability : (2) valid with restrictions
Data were obtained by modeling.
07.08.2003 (15)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 50 - 100 mg/kg bw
Species : rat
Strain : other: unknown
Sex : no data
Number of animals : 21
Vehicle : other: corn oil
Doses : 25-3200 mg/kg bw
Method : other
Year : 1960
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : Whether any deaths occurred at 50 mg/kg was not listed. Mortality was noted in all animals at dose of 100 mg/kg and above. Death was noted to have occurred between 2 ½ hours and 1 day. Clinical signs included: moderate to very weak, severe tremors, vasodilatation, rough coat, sides caved in, labored respiration, and convulsive movements progressing to convulsions. Surviving animals were noted to have gained weight. There was no gross evidence of adverse pathology in rats treated with 50 mg/kg.
Test condition : A total of 21 rats were orally administered doses of n-butyronitrile ranging from 25-3200 mg/kg. Material was administered undiluted and at 10% in corn oil vehicle. Animals were monitored for clinical observations and weight change for 14 days after exposure. Two rats that were given 50 mg/kg were killed 4 days after administration and the liver and kidneys were examined for micropathology.
Test substance : The boiling point of the test material was 116.4 -117.3 degrees C and the index of refraction at 25 degrees was 1.3820.
Conclusion : Material is considered highly toxic.
Reliability : (2) valid with restrictions
Basic data are given. Purity of the test material is unknown.

07.08.2003 (12)

Type : LD50
Value : = 111 mg/kg bw
Species : rat
Strain : other: Carworth-Wistar
Sex : male
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1962
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : The LD50 value was 0.14 (0.10 - 0.19) ml/kg. Based on a density of 0.7954 (at 15 degrees C), the LD50 value in mg/kg is 111.
Test condition : Test material was given by gavage to groups of 5 nonfasted rats (4-5 weeks old, 90-120 g). Dosages (not listed) were arranged in a logarithmic series differing by a factor of 2. The material was administered in a suitable vehicle (water, corn oil, 1% Tergitol Penetrant 7, or semi-solid agar). The number of deaths was monitored over 14 days. The LD50

value and its confidence interval was estimated by the method of Thompson (Bacteriol Rev 11:115, 1947) using the tables of Weil (Biometrics 8:249, 1952).

Reliability : (2) valid with restrictions
Purity of the material, test concentrations and the number of deaths at each concentration were not mentioned.

06.08.2003 (26)

5.1.2 ACUTE INHALATION TOXICITY

Type : other: LC10
Value : = 1848 ppm
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 40
Vehicle :
Doses : 1972, 4421, 6296 and 8261 ppm
Exposure time : 1 hour(s)
Method : OECD Guide-line 403 "Acute Inhalation Toxicity"
Year : 1987
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : LC10 value: LC10 (+/- 95% confidence interval) = 1848 (695-2656) ppm (both sexes combined)

Exposure concentrations: Actual concentrations were 1972 +\- 57, 4421 +\- 161, 6296 +\- 110, and 8261 +\- 204 ppm. No aerosol was present. Overall mean chamber temperature was maintained at 21 degrees C and relative humidity varied from 52 - 56%.

Deaths at each dose:
1972 ppm: 1/10; 1 male on Day 1
4421 ppm: 7/10; 5 males and 2 females on Day 1
6296 ppm: 9/10; 5 males and 4 females on Day 1
8261 ppm: 9/10; 5 males and 4 females on Day 1

Remarks: Clinical observations noted at 2000 ppm included gait disturbance, poor condition, and sialorrhea (only seen in the one male that died). One 2000 ppm-exposed female was lethargic and had sialorrhea. Lethargy was noted prior to the death of all males at higher exposure levels. At 4000 ppm and above, females exhibited lethargy, poor body condition, sialorrhea, and porphyrin-like discharge around the nose and face. These clinical signs were seen during or just after exposure cessation. Their severity was dose-related, and they resolved after 24-hours. The mean body weight increased in all animals during the 14-day observation period. No compound-related gross pathology was noted in animals that died spontaneously or in animals terminated on Day 14.

Test condition : Rats [CRL:CD(SD)BR] were exposed to target vapor concentrations of 2000, 4000, 6000 and 8000 ppm (5/sex/concentration). Males weighed 182-217 g and females weighed 171-194 g at the start of the study. Food and water were available ad libitum (except during exposure).

Exposures were conducted in 420 l stainless steel and glass inhalation chambers. Chambers were maintained under negative pressure (-0.5" water) and at 12 air changes per hour. Vapors were generated by metering the test material dropwise into a heated glass bead-packed column supplied with metered dried oil-free compressed air. Chamber vapor concentrations were determined 4-5 times per hour with an infrared

analyzer equipped for automated sampling and analyses. Temperature and humidity were measured twice per hour. Once during exposure, the concentration of background nongaseous material was measured in the 6000 ppm chamber relative to a chamber containing air in order to insure that exposures were to vapor and not aerosol. Distribution of test material was determined by measuring the concentrations in 10 different positions throughout the chamber and comparing them to a fixed reference position. Subsequently, the chamber vapor concentration was measured from the fixed reference position.

Exposure was for 1-hour in duration followed by a 14-day observation period. Rats visible through the chamber windows were observed for signs of toxicity during exposure. Each rat was removed from its cage before and after exposure and twice daily thereafter (on each workday) and examined. Animals that died during the study were necropsied as soon as possible after discovery of death. Survivors were fasted for 16 hours prior to euthanization and necropsy on Day 15. The following tissues were examined: trachea, lungs, heart, liver, esophagus, stomach, duodenum, jejunum, ileum , cecum, colon, pancreas, liver, salivary glands, kidneys, urinary bladder, pituitary gland, adrenal, thyroid, parathyroid, thymus, spleen, mesenteric lymph nodes, bone marrow (femoral), brain, testes, epididiymides, male accessory sex glands, ovaries, vagina, uterus, and Fallopian tubes. Mortality data were evaluated by Probit analysis.

Test substance : Purity was 99.5%

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

Flag : Critical study for SIDS endpoint

07.08.2003 (13)

Type : LC50
Value : > 1147 ppm
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Doses : 1147 ppm (males), 1220 ppm (females)
Exposure time : 1 hour(s)
Method : other: DOT Final Rule 49 CFR (parts 172 and 173)
Year : 1986
GLP :
Test substance : as prescribed by 1.1 - 1.4

Remark : The study was conducted according to GLP with the exceptions that stability was not determined, reserve samples were not collected and the degree of absorption was not determined (because it was not applicable).

Result : All animals survived to the end of the study. Mean vapor concentrations of the test material were 1147 +/- 24 and 1220 +/- 28 ppm for male and female rats, respectively. The saturated vapor concentration at 20 degrees C was 28,553 ppm. The temperature and relative humidity of the room ranged from 68-70 degrees F and 55-60%, respectively. An aerosol was not present.

Test condition : Groups of 5 male (231-242 g) and 5 female (212-224 g) rats [CRL:CD(SD)BR] were exposed to a target vapor concentration of 1200 ppm for 1 hour. Feed and water were available ad libitum (except during exposure). Rats were observed for mortality during exposure and twice daily on subsequent workdays for 14 days.

Chamber atmospheres were produced by passing dried, oil-free compressed air through a glass bead-packed distilling column into which the test material was pumped dropwise. The exposure began when the

vapor concentration reached 1000 ppm. Males and females were exposed in separate 20-l bell jars. Four samples of the atmosphere were analyzed for n-butyronitrile concentration with an infrared analyzer. Two samples of atmosphere were analyzed with a particle analyzer to insure that an aerosol was not present. The temperature was monitored 4 times during exposure.

Test substance : Purity of the test material was 99.5% (GC-MS). 4-heptanone (0.5%) was the only trace component identified.

Conclusion : The material is not subject to regulation under 49 CFR Parts 172 and 173, since the LC50 value was greater than 1000 ppm.

Reliability : (1) valid without restriction
The study is comparable to an OECD guideline, GLP study.

07.08.2003 (11)

5.1.3 ACUTE DERMAL TOXICITY

Type :
Value : = 398 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals :
Vehicle :
Doses :
Method : other: one-day cuff method of Draize
Year : 1962
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : The LD50 value (with confidence interval) was 0.50 (0.37 - 0.68) ml/kg. Based on a density of 0.7954 (at 15 degrees C), the LD50 value in mg/kg is 398.

Test condition : Fur was clipped from the entire trunk of rabbits (2.5 to 3.5 kg) and the material was applied beneath an impervious plastic film. Groups of 4 animals were exposed to various concentrations (not listed). The animals were immobilized during the 24-hour contact period. The film was then removed and the animals were caged for the subsequent 14 day observation period. The LD50 value and its confidence interval was estimated by the method of Thompson (Bacteriol Rev 11:115, 1947) using the tables of Weil (Biometrics 8:249, 1952).

Reliability : (2) valid with restrictions
Purity of the material, test concentrations and the number of deaths at each concentration were not mentioned.

06.08.2003 (26)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	14 weeks (total of 63 exposure days)
Frequency of treatm.	:	6 hours/day, 5 days per week
Post exposure period	:	none
Doses	:	60, 120, 209 ppm
Control group	:	yes
NOAEL	:	< 60 ppm
LOAEL	:	= 60 ppm
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	other TS

Result : Test material concentrations: The nominal concentrations (+/- SD) were 56.0 +/- 8.8, 119.1 +/- 16.9 and 203.0 +/- 19.6. Corresponding analytical concentrations were 60.2 +/- 1.0, 120.3 +/- 1.1 and 209.0 +/- 1.3 ppm. Since the nominal analytical ratios were close to 1, the material was a true vapor. There was no indication that the test material was unstable. Distribution was uniform (> 96%) for each of the exposure concentrations. Airflow, temperature and relative humidity ranged from 1719-1765 l/min, 21.0 - 26.1 degrees C and 17-50%, respectively.

Effects at all exposure concentrations: Signs of toxicity (labored breathing, nasal discharge, salivation, discharge from the eyes, hypoactivity and/or alopecia) were observed in all exposed groups. Incidences of these signs increased in a dose-dependent manner. Males and/or females in all exposed groups had significant decreases in red blood cells and hemoglobin values. Urine thiocyanate concentrations increased in all exposed groups, with concentrations from animals exposed to 120 ppm similar to or higher than those exposed to 210 ppm. However, since a dose-dependent diuresis occurred, the total amount of urine thiocyanate present (concentration x urine volume) increased with increasing concentrations.

Effects at 209 ppm: Three males died or were killed in extremis (2 between exposures 2 and 3 and one between exposures 18 and 19). Arched back, ataxia, tremors or convulsions, biting, pawing or rubbing chin against cage, irritation of the conjunctiva and breathing difficulties were noted in a few animals during exposure. Males and females exhibited significant decreases in body weight throughout the study (P <= 0.01). Average final body weights of males and females were lower than controls (377.5 g in exposed males vs. 435.4 g in controls and 255.0 g in exposed females vs. 276.6 g in controls). Absolute and/or relative heart, liver, spleen and kidney weights were increased in males and/or females. Absolute testes weights were decreased in males. Mean corpuscular hemoglobin concentrations (males only) were lower than control (all P <= 0.05). Serum alkaline phosphatase (males and females), SGOT (males only) and SGPT (males only) were increased, and BUN concentrations were decreased. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 10/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 120 ppm: Ataxia was observed during exposure in 2 females. Males had significant body weight decreases (P < = 0.05) at two time points. Absolute and/or relative liver weights were increased in males and

females and absolute and/or relative spleen weights were increased in males. Mean corpuscular hemoglobin concentrations (males only, P < = 0.05) were lower than control. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 11/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 60 ppm: Absolute and relative spleen weights were increased in males. Serum thiocyanate concentrations were marginally increased in males and females.

Test condition : Animals: Rats were acclimated for at least 10 days prior to use. Two days prior to the start of the study, males weighed 174-200 g and females weighed 132-145 g. On the first day of the study, the animals were 43 days old. Animals were randomly allocated by body weight into 4 groups of 15 animals/sex/group. Animals were individually housed in suspended mesh cages and given food and water ad libitum (except during exposure). Animal rooms were maintained at 70-74 degrees C and 35-60% relative humidity, with a 12 hour light/dark cycle.

Exposure conditions: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Rats were placed individually in wire mesh cages that were suspended in the chambers by 3-tiered racks. Males were placed on one side and females on the other. The concentrations of material in the chambers (20, 120 or 210 ppm) were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. The bubbler was connected to a side port in the vertical particle-size separator, which was in turn connected to the air inlet at the top of the inhalation chamber. One bubbler was used in each of the generation systems. Airflow was maintained at a constant flow of 1727 liters/min. Nominal concentration measurements were determined daily for each chamber following exposure, by dividing the amount of test material delivered to the chamber (the difference between the pre- and post-exposure weights) over the 6-hr exposure period by the total air volume during the same period. Concentrations of test material in the chambers were measured 4 times daily using a Miran 1A General Purpose Gas Analyzer. Additional samples of atmosphere from 9 specified locations in each chamber were also taken at 3 different times to determine if the vapor was distributed uniformly.

Test conduct: Animals were observed for clinical signs between the second and fifth hour of each exposure. Estimations of the percentages of animals exhibiting hypoactivity, eye irritation and breathing difficulties were made. All animals were individually examined for gross signs of toxicity preceding and following each exposure and checked for mortality. Each animal was weighed and given a thorough examination for gross signs of toxicity on a weekly basis.

Animals were euthanized after 14 total weeks on the study. Terminal body weights were obtained (following an overnight fast). Blood and urine were collected. Whole blood was treated with an anticoagulant and was analyzed for total and differential erythrocyte count, total leukocyte count, platelet count, hematocrit, hemoglobin, and red blood cell indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration). Serum was analyzed for albumin, globulin, total protein, blood urea nitrogen, total bilirubin, glucose, glutamic pyruvic transaminase (SGPT), alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), T3, T4, thiocyanate and lactate dehydrogenase. Urine was analyzed for the presence of thiocyanates.

Detailed necropsies were conducted on all rats that died during the course of the study, those that were killed moribund, and those that survived to

study termination. The adrenal glands (both together), testes (with epididymides, heart, kidneys, liver, pituitary and spleen were weighed. The aforementioned organs and the following tissues were fixed in 10% neutral formalin: abdominal aorta, bone and bone marrow (femur), brain, esophagus, ovaries, colon, ileum, lung, lymph nodes (mesenteric), mammary gland, nasal turbinates, pancreas, thyroid/parathyroid, prostate, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, stomach, thymus, trachea, urinary bladder, uterus (with cervix) and gross lesions. Eyes (with optic nerve) were fixed in a solution of 2% glutaraldehyde and 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, cut at five microns, stained with hematoxylin and examined microscopically.

Statistical analyses: In life and terminal body weights and organ weight data were analyzed using Dunnett's test. Organ to body weight ratios were analyzed using the Mann-Whitney test, with the Bonferroni inequality. Data for frequencies of microscopic lesions were evaluated with the Fisher's exact test with the Bonferroni inequality. Hematological and serum and urine chemistry variables were examined using Dunnett's test.

Test substance : Test material was propionitrile (CAS No. 107-12-0). The purity of the test material was 96%. Impurities were not listed.

Reliability : (1) valid without restriction
The study is comparable to a guideline study; however, a NOAEL was not established.

Flag : Critical study for SIDS endpoint

10.08.2003 (27)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay

System of testing : Salmonella typhimurium/TA98, 100, 1535, 1537, and Escherichia coli/WP2uvrA(pKM101)

Test concentration : 100, 333, 1000, 3330 and 5000 micrograms/plate

Cytotoxic concentr. : > 5000 micrograms/plate

Metabolic activation : with and without

Result : negative

Method : other: EEC Annex V Guideline number B.14, "Other Effects-Mutagenicity Salmonella typhimurium-Reverse Mutation Assay", and Guideline number B.13, Other Effects-Mutagenicity, Escherichia coli-Reverse Mutation Assay"

Year : 1999

GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Remark : This is the critical study for the mutagenesis endpoint.

Result : No positive responses were induced in any of the tester strains. None of the concentrations tested caused toxicity. No precipitate was observed at the maximum concentration tested. All criteria for a valid test were met.

Test condition : Test strains: The S. typhimurium and E. coli strains were obtained from Dr. Bruce Ames, University of California Berkeley and the National Collection of Industrial Bacteria, Torry Research Station, Scotland, respectively. Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 ml/ml culture) and freezing small aliquots at < = -70 degrees C. Master plates were prepared by streaking each test strain from a frozen permanent stock onto minimal agar supplemented with histidine, biotin, ampicillin and/or tryptophan (depending on the strain). Tester strain master plates were stored at 5 +/- 3 degrees C. Overnight cultures were inoculated by transferring colonies from the master plates to a flask containing culture medium. Inoculated flasks were placed in

a shaker/incubator (125 +/- 25 rpm, 37 +/- 2 degrees C). Cultures were harvested once a predetermined turbidity was reached (at least 0.5 x 10E9 cells/ml). Test stains were checked for rfa wall mutation (all Salmonella strains), pKM101 plasmid R-factor (Salmonella TA98 and TA100 and E. coli only), and characteristic number of spontaneous revertants (all strains) on the day the mutagenesis test was conducted.

Test medium: The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution supplemented with 2.5% Oxoid Nutrient Broth No. 2. Bottom agar was Vogel-Bonner minimal medium E supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Overlay agar contained 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with either 10 ml of 0.5 mM histidine/biotin solution or 0.5 mM tryptophan solution.

S-9 mix: S9 homogenate was purchased from Molecular Toxicology Inc. This was prepared from male Sprague-Dawley rats that had been injected i.p. with 500 mg/kg Aroclor 1254. S-9 mix was prepared immediately prior to use.

Concentrations of test material: The concentrations tested (100, 333, 1000, 3330 and 5000 micrograms/plate) were selected based on the results of a dose range-finding study using test strains TA100 and WP2uvrA(pKM101) and 10 doses of test material ranging from 6.67 to 5000 micrograms/plate (both in the presence and absence of S-9 mix).

Positive, negative and sterility controls: Positive controls [2 aminoanthracene (2.5 and 5.0 micrograms/plate), 2-nitrofluorene (1.0 micrograms/plate), sodium azide (2.0 micrograms/plate), ICR-191 (2.0 micrograms/plate), and 4-nitroquinoline-N-oxide (2 micrograms/plate)] were run concurrently. DMSO (50 microliters) was used as a vehicle and vehicle control. The most concentrated test material dilution and S-9 mix were tested for sterility by plating a 50 microliter aliquot on selective agar.

Test conduct: A plate incorporation methodology was used. Test material or positive control (50 microliters), test strains (100 microliters) and S-9 mix or vehicle (500 microliters) were combined in 2.0 ml of molten, selective top agar. This was overlaid onto 25 ml of minimal agar that had been plated into 15 x 100 mm Petri dishes. All concentrations of test material, vehicle controls and positive controls were plated in triplicate. Revertant colonies were counted after 48 +/- 8 hours of inverted incubation at 37 +/- 2 degrees C. The condition of the background lawn was evaluated for evidence of cytotoxicity and precipitate.

Evaluation: The mean number of revertants and standard deviation were calculated. Various criteria were established to constitute a valid assay (test strain integrity, characteristic number of spontaneous revertants, cell density > = 0.5 x 10E9, at least a 3-fold increase in revertants in positive controls, and a minimum of 3 non-toxic doses). A positive response was indicated by a 2-3 fold increase in mean revertant number depending on the bacterial tester strain.

Test substance : Purity was 99.9%
Conclusion : Material was not genotoxic under conditions of this assay.
Reliability : (1) valid without restriction
This was a well-documented EEC Annex guideline study conducted under GLP assurances.

10.08.2003 (3)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary (CHO) Cells
Test concentration : up to 701 micrograms/ml
Cytotoxic concentr. : >701 microgams/ml
Metabolic activation : with and without

Result : negative
Method : OECD Guide-line 473
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : This is the critical study for the chromosomal aberration endpoint.
Result : No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures. No precipitate was observed at the maximum concentration tested. None of the concentrations caused a significant reduction in the mitotic index. All criteria for validity were met.
Test condition : Cells: The Chinese hamster ovary cells used in the assay (CHO-WBL) were from a permanent cell line originally obtained from Dr. S. Wolff, University of California, San Francisco. Stock cultures were maintained for up to 8 weeks after thawing. Mycoplasma testing was performed twice during this period. Cells were grown at 37 +/- 2 degrees C (in 5% +/- 1.5% Co2 in air) in McCoy's 5a culture medium which was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin G and 100 micrograms/ml streptomycin.

S-9 mix: S-9 was isolated from the liver of rats (sex not stated) 5 days after i.p. treatment with 500 mg/kg Aroclor 1254. S-9 was stored frozen at < = -70 degrees C until use. S-9 mix was prepared by adding an energy-producing system (NADP plus isocitric acid) to S-9.

Test material and negative and positive controls: The test material was dissolved in DMSO. The top concentration tested (approximately 700 micrograms/ml or 10 mM) was the recommended high dose for the assay. The negative control was 10 microliters/ml DMSO. The positive controls were mitomycin C (without activation) and cyclophosphamide (with activation).

Initial test: Cultures were initiated by seeding approximately 1.2 x 10E6 cells per 75 cm2 flask into 10 ml of complete McCoy's 5a medium. For the test without metabolic activation, cultures were incubated with test material for 3.0 hrs at 37 degrees C. For the test with metabolic activation, cells were incubated for approximately 3.0 hours with test material and S-9 mix in McCoy's 5a medium that did not contain fetal bovine serum. Replicate cultures for each concentration of test material (4.77, 6.81, 9.73, 13.9, 19.9, 28.4, 40.5, 57.8, 82.6, 118, 169, 241, 344, 491 and 701 micrograms/ml), positive control (0.75 and 1.5 micrograms/ml mitomycin C and 5.0 and 10.0 micrograms/ml cyclophosphamide), vehicle and untreated controls were prepared. Cultures with or without S-9 were then washed with buffered saline, and incubated with complete McCoy's 5a medium for 17 hours. Colcemid (0.1 micrograms/ml) was present during the last 2 hours of incubation. Cells were visually inspected for cytotoxicity prior to harvest. Cells were then trypsinized and spun in a centrifuge. The supernatant was discarded and the cells swollen with 75 mM KCl hypotonic solution. The cells were then fixed with an absolute methanol: glacial acetic acid (3:1, v:v) fixative. They were then placed on glass slides and air-dried. Cells were stained with 5% Giemsa and analyzed for mitotic index and chromosomal aberrations.

Confirmatory assay: As in the previous test, cells were harvested after 20 total hours of incubation. In this test, the test with metabolic activation was conducted the same as in the initial test, but with different concentrations of test material (217, 289, 385, 513 and 684 micrograms/ml). In the test without metabolic activation, the test material (27.3, 54.5, 109, 217, 289, 385, 513 and 684 micrograms/ml), positive control (0.20 or 0.40 micrograms/ml mitomycin C or 5.0 or 10.0 micrograms/ml cyclophosphamide) and negative controls were incubated with the cells for

17.8 hours (instead of 3), and colcemid was present for the last 2.2 hours of harvest (instead of 2). The slides were prepared as described for the previous test.

Evaluation: Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 21 +/-2 were analyzed. One hundred cells (if possible) were analyzed from each replicate of the vehicle control, 4 concentrations of the test material (the highest 4 concentrations tested), and one concentration of positive control for the different types of chromosomal aberrations. At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations. The number of mitotic cells in 1000 cells was determined and the ratio expressed as percentage of mitotic cells. Percent polyploidy and endoreduplication were analyzed by evaluating 100 metaphases (if possible). Chromatid and isochromatid gaps were noted but were not used in calculating the total number of aberrations.

Acceptance criteria: The assay was considered valid if the negative (untreated) and vehicle controls contained < 5% cells with aberrations, the positive control result was significantly higher than that of the vehicle control, a high dose of 10 mM or the highest soluble concentration was used if the material did not cause a reduction of the mitotic index at the tested concentrations, and at least 3 concentrations were analyzed.

Data analysis: The statistical analysis employed a Cochran-Armitage test for linear trends and Fisher's Exact Test to compare the percentage of cells with aberrations. Data for polyploidy and/or endoreduplication were also analyzed separately. A test was considered positive if a significant increase in the number of cells with aberrations (p < = 0.01) was observed at one or more concentrations.

Test substance : Purity was 99.9%
Conclusion : Material was not genotoxic under conditions of this assay.
Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

06.08.2003 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Fertility
Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 21 to 33 days (depending on day of mating)
Frequency of treatm. : 6 hr/day, 7 days/week
Premating exposure period
Male : 0 days
Female : 21 days
Duration of test : to gestation days 13-15
No. of generation studies :
Doses : 60, 120 and 210 ppm

Control group : yes
NOAEL parental : = 60 ppm
other: NOAEL : = 210 ppm
Reproductive Toxicity
Method : other
Year : 1984
GLP : yes
Test substance : other TS

Result : Exposure concentrations: The average mean daily analytical exposure concentrations (60.1, 120.2 and 209.2 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25 degrees C and 26-29%, respectively.

Signs of toxicity: None of the animals died. There was no effect of test material on body weight. Animals exposed to 210 ppm exhibited arched back (N = 4 on days 1-10 and N=2 on days 11-20), lacrimation (N = 2 on days 1-10 and N = 1 on days 21-30), salivation (N= 15 on days 1-10, N = 22 on days 11-20 and N = 21 on days 21-30) hypoactivity (N = 13 on days 1-10, N = 5 on days 11-20 and N = 3 on days 21-30), staining of facial fur (N = 2 on days 1-10, N = 4 on days 11-20 and N = 4 on days 21-30) and red nasal encrustation (N = 1 on days 1-10, N = 5 on days 11-20 and N = 5 on days 21-20) after exposure. Animals exposed to 120 ppm also exhibited salivation (N = 6 on days 11-20 and N = 4 on days 21-30, staining of facial fur (N = 7 on days 1-10, N = 5 on days 11-10 and N = 2 on days 21-30) and red nasal encrustation (N = 2 on days 1 1-0, N = 8 on days 11-20 and N = 6 on days 21-30). A few animals in the 60 ppm group also exhibited red nasal encrustation (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-30) and staining of facial fur (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-20). One control animal had stained facial fur on days 21-30 and another had red nasal encrustation on days 1-10. Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 2 controls, N = 3 low dose, N = 5 mid dose, N = 9) at one or more of their weekly physical examinations.

The only remarkable findings at gross necropsy were bilateral uterine hydrometra in one animal exposed to 210 ppm and hydrometra in the left uterine horn of one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on fertility. Efficiency of mating (32.0%, 32.0%, 30.7% and 25.0% in the control, low, mid and high dose groups) and pregnancy rate (100%, 95.8%, 100% and 91.3% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 13.4 - 13.9), resorptions (ranged from 0.6 - 0.8), nidations (ranged from 14.1 - 14.5), corpora lutea (ranged from 13.0 - 15.2), preimplantation loss (4-8%) and postimplantation loss (4-6%). Evaluation of the vaginal smears of 2 females that did not copulate showed one that did not cycle (but was pregnant at necropsy), and another that only went through the cycling stage of proestrus.

Test condition : Animals: Virgin female Sprague Dawley rats (43 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of ten females and ten males that were taken upon receipt were 128-144 g and 178-233 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to females during exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 7 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Due to inclement weather and building equipment failures, 2 exposures (days 2 and 16) were only for 4 hours and one exposure (day 1) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 16) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Twenty four females per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when females were 63 days old. Animals were observed during exposure for signs of toxicity. After 21 days of exposure (which was sufficient to cover 3-4 estrus cycles), females were randomly mated (1:1) to an untreated male that had been assigned to the corresponding treatment group (30 males were assigned per group). At night, after exposure, females were caged with their assigned male until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or 5 nights without confirmed copulation. Females that failed to mate with the assigned male were mated with another male that had copulated with another female in the same group. Nightly co-housing with the second male occurred until copulation was confirmed (or for a maximum of 7 nights). The day on which copulation was confirmed was considered gestation day 0. Exposure of females continued until copulation was confirmed or a maximum of 12 nights of cohabitation with males without signs of copulation. Vaginal smears were taken on 5 consecutive days for females that did not exhibit copulation.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Females were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities.

Females were killed on gestation day 13 (or the nearest working day after gestation day 13, up to gestation day 15). Females without confirmed copulation ware euthanized in the second week after the last day of co-housing. Each female was given an external examination and weighed. The tissues and organs of the thoracic and abdominal cavities were examined for gross lesions. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted. The ovaries and uteri of females were preserved in 10% neutral buffered formalin. Males were killed after mating and were not examined.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : The test material was propionitrile (CAS No. 107-12-0). Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (< 0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : The authors concluded that the incidences of red nasal encrustation in the low dose animals, alopecia in the mid and high dose animals and staining of facial fur in all treated groups were too low to be definitely related to

administration of test material. There was no effect of treatment on fertility of females.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (20)

Type : Fertility
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 57 days
Frequency of treatm. : 6 hours/day, 5 days/week
Premating exposure period
Male : 46 days
Female : 0 days
Duration of test : to gestation day 13-15
No. of generation studies :
Doses : 60, 120 and 210 ppm
Control group : yes
NOAEL parental : = 60 ppm
other: NOAEL : = 210 ppm
Reproductive Toxicity
Method : other
Year : 1985
GLP : yes
Test substance : other TS

Result : Exposure concentrations: The average mean daily analytical exposure concentrations (60.2, 120.4 and 208.9 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25.5 degrees C and 24-27%, respectively.

Signs of toxicity: One of animals exposed to 210 ppm died after 2 days of exposure. On the previous day, this animal exhibited labored breathing, hypoactivity, poor control of the hind limbs, difficulty in standing, body tremors and involuntary movements. No unusual findings were observed at necropsy.

Body weights of males exposed to 210 ppm were approximately 6-9% lower than those of the control group during most of the exposure period, and remained lower than control (but were not significantly different) until the end of the study.

Animals exposed to 210 ppm exhibited signs of toxicity such as arched back (N = 8 on days 1-10, N = 3 on days 11-20 and 51-57, and N = 5 on days 41-50), hypoactivity (N = 12-15 at each 10-day interval up to day 50, and N = 4 from days 51-57), labored breathing (N = 10 on days 1-10, N = 3 on days 11-20 and 31-40, N = 5 on days 21-30 and N = 1 on days 51-57), and salivation (N = 3 on days 1-10, and N = 10 - 12 at all other intervals). A few high dose animals (individual numbers were not stated) also exhibited abnormal behavior such as grinding of teeth, head bobbing, body tremors, involuntary movements, and pawing at the cage. A few of the animals exposed to 120 ppm exhibited salivation (N = 3-8 at all intervals) and hypoactivity (N = 3 at days 11-20). Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 1 control, N = 2 low dose, N = 1 mid dose, N = 5 high dose) at one or more of their weekly physical examinations. No unusual treatment-related signs were observed in rats exposed to 60 ppm. The only remarkable finding at gross necropsy was a small right testis in one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on male fertility. Efficiency of mating (34.4%, 30.6%, 29.8% and 27.1% in the control, low, mid and high dose groups) and pregnancy rate (90.5%, 97.6%, 90.0% and 97.4% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 12.7 - 13.9), resorptions (ranged from 0.7 - 1.1), nidations (ranged from 13.8 - 14.9), corpora lutea (ranged from 13.1 - 15.2), preimplantation loss (4-8%) and postimplantation loss (5-10%).

Test condition : Animals: Virgin female Sprague Dawley rats (28 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of fifteen females and ten males that were taken upon receipt were 155-181 g and 80-103 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to males during exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. A scheduled exposure day was cancelled due in clement weather. A new exposure day (exposure day 41) was used in its place. Due to inclement weather and building equipment failures, 2 exposures (days 33 and 43) were only for 4 hours and one exposure (day 32) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 43) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Fifteen males per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when males were 43 days old. Mating was initiated when males and females were 16 and 12 weeks old, respectively. At this time, males had been 69 days on the study (which was sufficient to cover the spermatogenesis cycle of the rat), and had 46 days of exposure. Males were randomly mated (1: 1) with three untreated females (consecutively) that had been assigned to the corresponding treatment group (45 females were assigned per group). Exposure of males continued until the day after the last mating opportunity (57 exposure days). At night, after exposure, males were caged with their assigned female until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or 5 nights without confirmed copulation. The day on which copulation was confirmed was considered gestation day 0.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Males were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities (except for one day prior to mating when inclement weather permitted observations).

One half of the males of each group were euthanized on each of the 2 consecutive days at the end of the study. They had not been exposed to

propionitrile for about 2 weeks prior to termination. Each male was given an external examination and weighed. The tissues and organs of the thoracic, scrotal and abdominal cavities were examined for gross lesions and the testes, epididymides, prostate glands and seminal vesicles were preserved in 10% neutral buffered formalin. Females that were not mated with males were euthanized and were not examined.

Mated females were euthanized on gestation day 13 (or the nearest workday up to gestation day 15). Females that were co-housed with males without confirmed copulation were euthanized during the second week after the last day of co-housing. Gross necropsies were performed on females that had copulated and those that had not. The tissues and organs of the thoracic and abdominal cavities were examined. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : Test material was propionitrile (CAS No. 107-12-0). Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (< 0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : There was no effect of treatment on fertility of males.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (21)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : Days 6-20 of gestation
Frequency of treatm. : 6 hr/day
Duration of test : to day 21 of gestation
Doses : 50, 100, 150, and 200 ppm
Control group : yes, concurrent no treatment
NOAEL maternal tox. : = 200 ppm
NOAEL teratogen. : = 200 ppm
NOAEL Fetotoxicity : = 150 ppm
NOAEL Embryotoxicity : = 200
Result : butyronitrile was not teratogenic
Method : other: similar to OECD 414
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Four different concentrations of acetonitrile, priopionitrile, isobutylnitrile, acrylonitrile, allylnitrile, methacrylonitrile and 2-chloroacrylonitrile also were tested in this study.

The methodology described in the manuscript is essentially identical to that under OECD TG-414. However, information is lacking in the report in

regard to food intake. Therefore, it is unknown if reduced fetal body weights were a consequence of reduced material feed intake. In addition, it is unknown as to whether the study was conducted under GLP assurances. However, based on the date in which this study was completed it is likely to have been a GLP study. All other parameters noted in the guideline appear in the manuscript.

In a preliminary study, 300 ppm induced mortality in 6 of 6 pregnant rats. Since a repeated dose toxicity study has not been conducted, and whether inhalation of 200 ppm caused toxicity to dams was not assessed in the developmental study (other than effect on body weight), the actual maternal NOAEL may be less than 200 ppm.

Result : Maternal: All animals survived and there was no effect on weight gain. Indices of pregnancy were comparable among groups. There was no significant effect of treatment on the mean numbers of implantations, or incidences of nonsurviving implants and resorptions.

Fetal: There was no effect of treatment on the mean numbers of live fetuses or sex ratio. There was a concentration-related trend towards a decrease in fetal body weights, with weights of females from the 200 ppm group significantly less than control (5.08 g/litter in treated vs. 5.41/litter in control). A single case of skeletal malformation (fused ribs) was observed at 100 ppm. The incidences of visceral and skeletal variations in treated fetuses were similar to controls.

Test condition : Animals: Male (350 g) and primiparous female (200-220 g) were acclimated for 1-2 weeks prior to breeding. Females were then placed with males (one male: 3 females) overnight and examined by vaginal smear for the presence of sperm the following morning. Sperm-positive females were considered to be at Day 0 of gestation. These animals were randomly assigned to groups of 20-23 rats each.

Exposure conditions: Exposures were conducted in 200 -liter stainless-steel inhalation chambers at an air flow of 10-20 m3/hr. Chambers were maintained at a negative pressure of < = 3 mm water. Chamber temperatures and humidities were 23 +/- 2 degrees and 50 +/- 5%, respectively. Vapor was generated by bubbling an additional air flow through a flask containing test material. The vapor was mixed with filtered room air to achieve the desired concentration. Analytical concentrations were determined by analyzing the atmosphere once/hour (by gas-liquid chromatography) during each 6 hour exposure. The nominal concentrations of n-butyronitrile were 50, 100, 150 and 200 ppm. Corresponding analytical concentrations were 53 +\- 2.3, 104 +/- 4.5, 154 +/- 11.5, and 208 +/- 98.7 ppm.

Test conduct: Animals were exposed 6 hours/day on Days 6 through 20 of gestation. Control animals were exposed concurrently to filtered room air in an adjacent chamber with flow characteristics identical to those of the treated groups. Food and water were available ad libitum (except during exposure). All rats were observed daily and maternal body weights were recorded on Days 0, 6 and 21 of gestation. Females were euthanized on Day 21 of gestation and the uterus was removed and weighed. The uterus horns were then opened and the numbers of implantation and absorption sites and live and dead fetuses were recorded. Live fetuses were removed and weighed, examined for external anomalies (including those of the oral cavity) and sexed. The numbers of fetuses (and litters) examined for external anomalies in the 0, 50, 100, 150 and 200 ppm groups were 227 (17), 239 (17), 219 (18), 280 (21) and 187 (15), respectively. Half of the fetuses from each litter were fixed in Bouin's solution and examined microscopically for visceral abnormalities. The remaining half were fixed in 70% ethanol, eviscerated, macerated in 1% KOH , stained in alizarin red S, and examined microscopically for skeletal anomalies.

Statistical analysis: Depending on the parameter evaluated: one-way analysis of variance followed by Dunnett's test, Wilcoxon test after arc-sine-square root transformation, Fisher's test, or least squares analysis. The litter was used as the basis for analysis of fetal variables.

Test substance : Purity was > 99%.

Reliability : (1) valid without restriction
The study was comparable to a guideline study.

Flag : Critical study for SIDS endpoint

10.08.2003 (24)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Mechanistic Studies
Study descr. in chapter :
Reference :
Type :
Species : mouse
Sex : male
Strain : CD-1
Route of admin. : i.p.
No. of animals :
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Propionitrile also was tested in this study. The results with this material were similar to those of n-Butyronitrile.

This study is considered to be valid without restriction. The study was conducted and documented in a thorough manner.

Result : In the first study, the mortality rate for animals treated only with 55 mg/kg n-butyronitrile was 8/10. Co-treatment with sodium nitrite or sodium thiosulfate reduced the rate to 2/10 and 1/10, respectively.

In the carbon tetrachloride study, the mortality rate for animals treated only with 55 mg/kg n-butyronitrile was 10/10. None of the animals co-treated with carbon tetrachloride died.

Cyanide concentrations in liver and brain of mice co-treated with sodium thiosulfate or carbon tetrachloride were significantly less than those of mice treated with n-butyronitrile alone. In mice receiving n-butyronitrile only, 21.4 +/- 16.1 (mean +/- SD) and 16.8 +/- 12.6 nmol/g cyanide were found in liver and brain, respectively. In mice receiving n-butyronitrile plus sodium thiosulfate, 2.4 +/- 0.8 and 2.4 +/- 2.6 nmol/g cyanide were found in liver and brain, respectively. In mice receiving propionitrile plus carbon tetrachloride, 1.3 +/- 1.5 and 1.6 +/- 2.0 nmol/g cyanide were found in liver and brain, respectively.

Test condition : Male CD mice (30 g) were divided into 3 groups of ten animals each. One group received 55 mg/kg i.p. n-butyronitrile only, another received i.p. injections of 75 mg/kg sodium nitrite (a cyanide antagonist) 20 minutes before and 100 minutes after i.p. injection of 55 mg/kg n-butyronitrile, and another received i.p. injections of 1 g/kg sodium thiosulfate (a cyanide antagonist) 20 minutes before and 80 and 180 minutes after i.p. injection of 55 mg/kg n-butyronitrile.

Two other groups of 10 mice received either 0.2 ml of vegetable oil or 0.2 ml of 20% carbon tetrachloride (a hepatotoxic dose) in vegetable oil subcutaneously, 24 hours before i.p. treatment with 55 mg/kg n-butyronitrile .

In both experiments, animals were observed for 7 days. Mortality data were analyzed statistically by the chi-square test. The criterion for significance was $p < 0.05$.

In an additional study, the concentrations of cyanide in liver and brain were determined in a) 5 mice treated only with 38 mg/kg n-butyronitrile (i.p.), b) 5 mice given 1 g/kg sodium thiosulfate 20 minutes before and 80 minutes after 38 mg/kg n-butyronitrile (i.p.), and c) 5 mice given 0.2 ml of 20% carbon tetrachloride subcutaneously 24 hours before i.p. treatment with 38 mg/kg n-butyronitrile. All mice were killed 2.5 hours after n-butyronitrile injection (if still alive at this time). The livers and brains were excised as soon as possible after death, quick-frozen and weighed. Cyanide concentrations were determined by the method of Bruce et al. (Anal Chem 27: 1346-1347, 1955). Results were analyzed using an unpaired t-test.

Test substance : The purity of the test material was 98%. No free cyanide was found in solutions made in distilled, deionized water.

Conclusion : n-Butyronitrile is activated by the liver to release cyanide, which is responsible for acute toxicity.

06.08.2003 (28)

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-14860B1

IUCLID

Data Set

RECEIVED
OPPT CBIC
03 NOV 25 PM 1:45

Existing Chemical	:	ID: 78-82-0
CAS No.	:	78-82-0
EINECS Name	:	Isobutyronitrile
TSCA Name	:	Propanenitrile, 2-methyl-
Molecular Formula	:	C4H7N

Producer related part

Company	:	Eastman Chemical Company
Creation date	:	02.07.2003

Substance related part

Company	:	Eastman Chemical Company
Creation date	:	02.07.2003
Status	:	
Memo	:	
Printing date	:	06.10.2003
Revision date	:	13.11.2003
Date of last update	:	06.10.2003
Number of pages	:	43
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	C(#N)C(C)C
Molecular formula	:	C4H7N
Molecular weight	:	69.11
Petrol class	:	

15.08.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

-Methylpropanenitrile

-Methylpropionitrile

1-Cyano-1-methylethane

2-Cyanopropane

2-Methylpropanenitrile

2-Methylpropionitrile

Dimethylacetonitrile

Isobutanenitrile

Isopropyl cyanide

Isopropyl nitrile

Propanenitrile, 2-methyl

Propanoic acid, 2-methyl-, nitrile

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use	:	industrial
Category	:	Chemical industry: used in synthesis
Remark	:	Chemical intermediate
Reliability	:	(2) valid with restrictions

(5)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= -71.5 °C
Sublimation	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of the material is unknown. Data obtained from Hazardous Substances Data Bank Number: 5221. Last revision date: 9/21/1999.
Reliability	:	(2) valid with restrictions Primary source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

(31)

2.2 BOILING POINT

Value	:	= 103.8 °C at 1016 hPa
Decomposition	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Purity of the material is unknown. Data obtained from Hazardous Substances Data Bank Number: 5221. Last revision date: 9/21/1999
Reliability	:	(2) valid with restrictions Primary source is peer-reviewed published data.
Flag	:	Critical study for SIDS endpoint

06.08.2003 (31)

2.3 DENSITY

Type	:	relative density
Value	:	= .77 at 20 °C
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Source of data is a Material Safety Data Sheet.

(5)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 55.2 hPa at 20 °C
Decomposition	:	
Method	:	other (measured)
Year	:	1986

GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The study results were reported in Appendix 2 of a GLP acute inhalation toxicity study discussed below under acute toxicity effects.
Result : Vapor pressures at 40, 23, and 20 degrees C were 92.1, 47.0, 41.4 and mmHg, respectively.
Test condition : The vapor pressures at 40 and 23 degrees C were determined by head space gas chromatography with flame ionization detection. The vapor pressure at 20 degrees C was calculated by linear extrapolation.
Test substance : Purity was 99.7%
Reliability : (2) valid with restrictions
Basic data are given.
Flag : Critical study for SIDS endpoint
06.08.2003 (6)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = .46 at °C
pH value :
Method : other (measured)
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The value obtained from the experiment was 0.46. The confidence limit was 0.25.
Test condition : The test was performed at ambient temperature (20-25 degrees C). The value was obtained using the Shake-Flask method. The aqueous phase was octanol-saturated water. The concentration of material in the aqueous phase was measured using gas-liquid chromatography.
Test substance : Purity of the test material was not mentioned.
Reliability : (2) valid with restrictions
Data were from a peer reviewed, published source.
Flag : Critical study for SIDS endpoint
13.08.2003 (27)

Partition coefficient : octanol-water
Log pow : = .76 at 25 °C
pH value : = 7
Method : other (calculated)
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program were the CAS No., melting point = - 71.5 degrees C, boiling point = 103.8 degrees C, water solubility = 39,000 mg/l, and vapor pressure = 41.4 mm Hg.
Reliability : (2) valid with restrictions
Approved program for estimating Log Kow.
13.08.2003 (20)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : water
Value : = 39000 mg/l at 25 °C
pH value :

concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Seventeen ml of isobutyronitrile (IBN) was added to a beaker containing 500 ml of distilled water that was being stirred with a stir bar at moderate speed. A thermometer was placed in the beaker to monitor temperature (25 degrees C). IBN was added in 2 ml increments under constant stirring, and the water was inspected for saturation after each addition. After 25 ml IBN had been added, IBN was added in 1 ml increments because of noticeable signs of reaching the saturation point. A total of 27 ml IBN was added. The solubility was determined by analyzing 20 ml of the final solution with a nitrogen analyzer. This instrument measured the amount of nitrogen dissolved in the water. The concentration of IBN = 1.049 g N/100 g solution x 69 g IBN/14g N x 0.755 (instrument response factor derived from nitrogen standard) = 3.90 wt % IBN.
Test substance	:	Purity of the test material was not listed. According to a MSDS from the supplier (dated 1/9/2002), the purity of the material is 100%.
Reliability	:	(2) valid with restrictions The study is comparable to a guideline study.
Flag	:	Critical study for SIDS endpoint

23.09.2003 (4)

Solubility in	:	water
Value	:	= 32,500 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: calculated
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program were the CAS No., melting point = - 71.5 degrees C, boiling point = 103.8 degrees C, water solubility = 39,000 mg/l, and vapor pressure = 41.4 mm Hg.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.

11.09.2003 (22)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .0000000000007032 cm³/(molecule*sec)
Degradation	:	= 50 % after 15.2 day(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured inputs to the program were the CAS No., melting point = - 71.5 degrees C, boiling point 103.8 degrees C, water solubility = 39,000 mg/l, and vapor pressure = 41.4 mm Hg.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

19.08.2003 (17)

3.1.2 STABILITY IN WATER

Type	:	abiotic
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN Hydrowin cannot calculate hydrolysis rate constants for nitriles. The theoretical hydrolysis of the related material propionitrile and several other chemicals has been examined by Dr. Lee Wolfe at the USEPA Environmental Research Laboratory in Athens, Georgia. The results of these analyses were published in a report by Dr. Wolfe that could not be located. In a personal communication, Dr. Wolfe stated that propionitrile can hydrolyze (albeit slowly). According to a study cited in the Hazardous Substances Data Bank, the chemical hydrolysis of the related material acetonitrile in water is base-catalyzed (the rate constant for base catalyzed hydrolysis is 5.8X10-3/M-hr), but the half-life at pH 7 is more than 150,000 yrs (Ellington et al., 1988). Acetonitrile ($CH_3C{\equiv}N$, CAS No. 75-05-8) is the 2-carbon analog of the category members, possessing the same functionality, but having one less carbon than propionitrile. Taken together, these data suggest that hydrolysis of butyronitrile at environmentally relevant pHs will occur too slowly to be a significant means of degradation.
Reliability	:	(2) valid with restrictions Experimental results for the test material could not be located. Results are for a related material.

19.08.2003 (19)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air, water, soil and sediment
Air	:	17.8 % (Fugacity Model Level I)
Water	:	47.3 % (Fugacity Model Level I)
Biota	:	.0809 % (Fugacity Model Level II/III)
Soil	:	34.7 % (Fugacity Model Level II/III)
Method	:	other: calculated
Year	:	2003
Remark	:	Measured inputs to the program were the CAS No., melting point = - 71.5 degrees C, boiling point = 103.8 degrees C, water solubility = 39,000 mg/l, and vapor pressure = 41.4 mm Hg.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

19.08.2003 (21)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	
Deg. product	:	
Method	:	other
Year	:	1998
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Refer to Section 3.6 below.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

06.08.2003

Type	:	aerobic
Inoculum	:	
Contact time	:	14 day(s)
Result	:	readily biodegradable
Deg. product	:	
Method	:	other: MITI protocol
Year	:	1978

GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Isobutylnitrile was shown to biodegrade readily using the Japanese MITI protocol (2 week incubation, 100 ppm concentration), with BODs of 53.9 - 66.3%.
Source : Hazardous Substances Data Bank for 2-methylpropanenitrile.
Reliability : (4) not assignable
The primary references were not consulted.

10.08.2003 (25) (28)

Type : aerobic
Inoculum : other: mixed microbial culture
Concentration : 1000 mg/l
Contact time : 48 hour(s)
Result : other: biodegradable
Deg. product :
Method : other
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The final protein and ammonia concentrations and pH were 6.76 mg/l, 51.6 micromoles/ml and 8.21, respectively, indicating that the mixed culture could use this material as a growth substrate.
Test condition : A mixed microbial culture (protein concentration of 0.085 mg/l) was isolated from an environment contaminated with organic cyanides and polychlorinated biphenyls. This was grown for 48 hours on phosphate buffer (pH 7.0, 30 degrees C) containing propionitrile (1 g/l) as the sole source of carbon and nitrogen. The final concentration of protein, ammonia and pH were determined.
Test substance : Test material was obtained from Aldrich Chemical Co. It is presumed that the material has high purity.
Reliability : (4) not assignable
The study shows that the test material was used as a substrate (and therefore was metabolized); however, the extent to which the test material biodegraded is difficult to determine from the study.

07.08.2003 (1)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method : other: Method C.5., "Degradation, Biochemical Oxygen Demand", Official Journal of the European Communities, No. L251/212, 19.9.84
Year : 1998
BOD5 : mg/l
GLP : yes
COD
Method : other:Method C.6., "Degradation, Chemical Oxygen Demand", Official Journal of the European Communities, No. L383A/227, 29 December 1992.
Year : 1998
COD : = 1910 mg/g substance
GLP : yes
RATIO BOD5 / COD
BOD5/COD : = .28

Remark : Method is similar to OECD: TG-301C: Modified MITI Test. The study is the critical study for the biodegradation endpoint. One of the two 5-day dilution water bottles had a dissolved oxygen drop greater than 0.2 ppm. This was a protocol deviation. It was not considered significant enough to negate

the test.

Result : BOD analysis: The values obtained for the 2 different concentrations were 0.567 and 0.494 g BOD/g test material at 5 days and 2.78 and 2.51 g BOD/g test material at 20 days. The average BOD5 and BOD20 values were 0.53 grams BOD/gram of test substance and 2.6 grams BOD/gram of test substance, respectively.

COD analysis: The values obtained for the 3 replicates were 2.126, 1.625 and 1.964 g COD/g test material. The average value was 1.91 g COD/g test material. The percent recovery of the reference sample was 93.5%.

BOD5/COD: The BOD5/COD ratio is 0.28 (0.53/1.91)

Test condition : Test conditions: COD Determination: A 0.50 N potassium dichromate solution was used to standardize the ferrous ammonium sulfate titrant. Mercuric sulfate was added to minimize chloride interference (if any). Three separate replicates were tested. A potassium phthalate standard was analyzed as a positive control. The test was considered valid if the recovery of the standard fell between the limits of 83.47 - 116.06%. The COD was calculated by subtracting the amount of titrant needed for the sample from the amount of titrant needed for a blank. This result was multiplied by the normality of the titrant and the equivalent weight of oxygen. The product was then divided by the sample weight.

BOD Determination: The test was performed according to an Eastman Kodak Company protocol [Need to have protocol for details]. Two separate concentrations (0.00033%, 0.00050%) were tested. The 5-day BOD was calculated by subtracting the final dissolved oxygen reading and the 5-day seeded dilution water drop from the initial dissolved oxygen reading. The 20-day BOD was calculated by subtracting the average 20-day seeded dilution water drop from the total dissolved oxygen drop over 20 days. The results were multiplied by 100. The products were divided by the product of the percent concentration of the stock solution in the BOD bottle and the concentration of test chemical in the stock solution. The products were divided by 1,000,000. The results were in units of grams of BOD per gram of test substance for the 5-day (or 20-day) incubation.

BOD5/COD ratio: This ratio was calculated by dividing the average 5-day BOD value by the average COD value.

Test substance : Purity was 99.8%.

Conclusion : The test material is not considered to be "Readily Biodegradable" based on a BOD5/COD ratio of <0.5 (0.53/1.91 = 0.28)

Reliability : (1) valid without restriction
This was a well-documented guideline study conducted under GLP assurances.

10.08.2003 (7) (8)

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 102.1 measured/nominal
LC50	:	> 102.1 measured/nominal
Limit test	:	yes
Analytical monitoring	:	yes
Method	:	other: OECG:TG-203 and EEC/Annex V C.1.
Year	:	1998
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The dissolved oxygen concentration was slightly less than 60% of the initial values at the end of the test. This did not adversely affect the outcome. Through 48 hours, solutions containing test material were clear and colorless. At 72 hours through the end of the test, exposure solutions were slightly cloudy; however no slicks or precipitates were observed. The LC50 value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Result	:	No mortality occurred and all fish exhibited normal behavior and appearance. The mean concentration of test material was 102.1 mg/l. The analyzed percent loss of the test material ranged from 12.3 - 21.5%. The temperatures of all solutions ranged from 20 - 21 degrees throughout the test. The pH and dissolved oxygen values ranged from 7.8 - 8.4 and 4.7 - 8.5 mg/l, respectively. The temperature, pH and dissolved oxygen values were considered to be acceptable for the organisms used in the test. The test was considered to be valid.
Test condition	:	Organisms: Juvenile fathead minnows were acclimated to test water for at least two weeks prior to testing. They were randomized to 6 sets of 7 fish each. Two sets of minnows (7/set) were killed before the start of the test to determine average wet weight (0.17 and 0.16 g/set) and mean standard length (2.91 and 2.90 cm/set). Test water: The water was pumped from Lake Ontario into a large underground storage vessel. Water from this vessel was subsequently pumped into the laboratory where it passed through polypropylene filter tubes, activated carbon filter tubes, and another set of polypropylene filter tubes. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then heated to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Representative values for hardness and total alkalinity (both as CaCO3) were 120.0 and 89.6 mg/l, respectively. Test material: A stock solution of test material (12 mg/l) was prepared in a 500 ml volumetric flask containing test water. The exposure solutions were prepared at a nominal concentration of 120 mg/l by adding the appropriate amount of stock solution to glass vessels (30.5 cm Pyrex seamless, cuboidal chromatography jars) containing 20 liters of test water. The solutions in each test vessel were stirred with a stir rod prior to adding fish. Duplicate test and dilution water control vessels were prepared. Test conduct: Immediately after stirring, fish were placed into each of the replicate test and control vessels (7 per vessel). Glass lids were placed on

top of each test vessel and sealed with Parafilm. Biological loading within test vessels was kept below 1.0 g wet weight/l test solution. The vessels were placed in a certified hood under 8 hours of fluorescent lighting/day. Animals were observed for mortality and signs of stress at 0, 4, 24, 48, 72 and 96 hours. Temperature, dissolved oxygen concentration and pH of each solution also were measured at 24 hour intervals. Concentrations of test material in the test vessels at 0 and 96 hours were analyzed by GC/MS. The geometric mean of the concentrations was calculated. Since no mortality was observed, statistical analyses were not performed.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was 20 +/- 1 degrees C and there were no abnormal occurrences that could influence the outcome.

Test substance : Purity was 99.8%.
Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.
Flag : Critical study for SIDS endpoint
07.08.2003 (11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 94.3 measured/nominal
EC50 : > 94.3 measured/nominal
Limit Test : yes
Analytical monitoring : yes
Method : other: OECD: TG-202 and EEC/Annex V C.2
Year : 1998
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : No protocol deviations were noted.
Result : All daphnids exposed to test-article exhibited behavior comparable to controls. No immobility was observed. No precipitation of test material was observed. The concentrations of test material at time 0 were 108.7 and 115.5 mg/l and the concentrations at 48 hours were 76.9 and 81.9 mg/l. The geometric mean of the test concentrations at 0 and 48 hours was 94.3 mg/l. The analyzed percent loss of the test material over 48 hours was 29.2%. The temperatures of all solutions were maintained at 21 degrees C throughout the test. The pH and dissolved oxygen values ranged from 8.2 - 8.5 and 8.3 - 8.5 mg/l, respectively. The temperature, pH and dissolved oxygen values were considered to be acceptable for the organisms used in the test. The test was considered to be valid.
Test condition : Organisms: Adult Daphnia magna were reared within the testing facility in 100-l culturing flasks. Gravid daphnids used to produce test animals were obtained from rearing tanks that had been established for at least two weeks. Prior to the study, approximately 100 gravid daphnids were transferred by net into two glass bowls containing test water and food. After 18 hours in the bowls, all adult daphnids were removed. Neonates were collected by pipette and transferred directly into exposure vessels. A total of 10 daphnids were placed into each of the replicate test and control vessels.

Test water: The water was pumped from Lake Ontario into a large underground storage vessel. Water from this vessel was subsequently

pumped into the laboratory where it passed through polypropylene filter tubes, activated carbon filter tubes, and another set of polypropylene filter tubes. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then heated to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Representative values for hardness and total alkalinity (both as CaCO3) were 120.0 and 89.6 mg/l, respectively.

Test material: A stock solution of test material (12 mg/l) was prepared in a 500 ml volumetric flask containing test water. The exposure solutions were prepared at a nominal concentration of 120 mg/l by adding the appropriate amount of stock solution to glass vessels (300 ml Kimax glass, lipless beakers) containing 20 liters of test water. The solutions in each test vessel were stirred with a stir rod prior to adding daphnids. Duplicate test and control vessels were prepared.

Test conduct: Immediately after stirring, daphnids were placed into each of the replicate test and control vessels (10 per vessel). Watch glasses were placed on top of each test vessel and sealed with Parafilm. The vessels were placed in a certified hood under 8 hours of fluorescent lighting/day. Animals were observed for mortality and signs of stress at 0, 24 and 48 hours. Temperature, dissolved oxygen concentration, and pH of each solution were measured at 0 (prior to adding organisms) and 48 hours. Concentrations of test material in the test vessels at 0 and 48 hours were analyzed by GC/MS. The geometric mean of the concentrations was calculated. Since no mortality was observed, statistical analyses were not performed.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was 20 +/- 2 degrees C, test daphnids in the control groups were not trapped at the surface of the water and there were no abnormal occurrences that could influence the outcome.

Test substance : Purity was 99.8%.

Conclusion : The 48-hour EC50 value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

Flag : Critical study for SIDS endpoint

31.07.2003 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : other: biomass and growth rate
Exposure period : 72 hour(s)
Unit : mg/l
NOEC : = 87.8 measured/nominal
EC50 : > 87.8 measured/nominal
Limit test : yes
Analytical monitoring : yes
Method : other: OECD: TG-201 and EEC/Annex V C.3
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : No protocol deviations were noted. The EbC50 (0-72 hr) and the ErC50 (0-

72 hr) were inestimable as greater than 50% inhibition in growth and/or biomass was not achieved. The significant loss (up to 80.7% over the course of the study) in test material was attributed to volatilization.

Result : Algae exposed to test material exhibited normal growth with respect to control. No deformed cells were noted. At the end of the test, the mean cell density in treated cultures was 1.365 x 10E6 cells /ml (compared to 1.356 x 10E6 cells in control).

The average concentrations of material in the test flasks at the beginning of the test and after 72 hours were 200.68 and 38.65 mg/l, respectively. Approximately 80.74% of the material was lost over the course of the experiment. The mean concentration was 87.82 mg/l. This concentration was listed as the NOEC.

Results of the photostability tests were similar to those in flasks containing test material and algae. The control solutions that were exposed to light or were in the dark exhibited 78.11% and 69.35% losses of test material.

The mean temperature and illumination were 24 degrees C and 746 foot-candles (range 744 - 748 foot-candles) throughout the test. The pH ranged from 7.42 - 7.88. The shaker speed was maintained at 100 rpm.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 136-fold within 72 hours.

Test condition : Test Organisms: A 4-day culture of Selenastrum capricornutum SF-3148 (passage 5 in liquid algal medium) was used as the test algae. Several passages were performed prior to the test to confirm exponential growth.

Test medium: Sterile growth medium was prepared using high quality distilled water. The pH of the medium was measured and adjusted to 7.5 (+/- 0.1) using 0.01N NaOH.

Test material stock solution: Test material (0.156 ml) was added to 600.0 g of algal growth medium (to produce a nominal concentration of 200 mg/l). The solution was immediately capped and stirred for 1-2 minutes. An aliquot of the solution was removed for analysis of concentration at time 0.

Test conduct: All steps were carried out aseptically in a hood to prevent contamination. Test vessels were sterile 250 ml Erlenmeyer flasks. Test material stock solution (100 ml) was added to 5 flasks and test medium that did not contain test material was added to 3 flasks. Algae (515 microliters of algal stock culture to achieve an initial cell density of 1 x 10E4 cells/ml) were added to 3/5 flasks that contained test material and the three that did not. The two flasks that contained test material but were not inoculated served as photostability controls. One of the flasks was exposed to light and one was wrapped in foil to shield it from light. All flasks were secured with foam stoppers and transferred to a shaking incubator (24 degrees C, 100 rpm). They were illuminated at an average of 746.2 footcandles throughout the study.

Temperature, light intensity, and shaker speed (rpm) were assessed at the 0, 24, 48, and 72 hours. Concentrations of test material in the flasks that contained algae also were assessed at these times. The pH and was assessed at time 0 and after 72 hours. Concentrations of test material in the photostability controls also were measured at 0 and 72 hours. Concentrations of test material were analyzed using gas chromatography with flame ionization detection (GC/FID).

The exposure concentration was calculated as the geometric mean of the test concentrations analyzed at the 4 time points. Cell counts were performed after 24, 48 and 72 hours of exposure using a calibrated Coulter Counter. The mean algal cell count for the test and control curves was

calculated. Two measures of growth [biomass (area under the growth curve) and growth rate] were used to determine the effect of the material on algae. The concentrations that produced a 50% inhibition of biomass (EbC50) and growth rate (ErC50) relative to control were to be calculated by fitting linear regression models to the data.

The test was considered valid if the mean cell concentration in the control cultures increased by a factor of at least 16 within 72 hours.

Test substance : Purity was 99.9%.
Conclusion : The results of this study indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Reliability : (1) valid without restriction
This was a well-documented OECD-study conducted under GLP assurances.
Flag : Critical study for SIDS endpoint
07.08.2003 (9)

Species : other algae: green algae
Endpoint : biomass
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 429.46 calculated
Method : other: model calculation
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Measured inputs to the program were melting point = - 71.5 degrees C, boiling point = 103.8 degrees C, water solubility = 39,000 mg/l and vapor pressure = 41.4 mm Hg.

Model compound class is neutral organic.
Reliability : (2) valid with restrictions
Data were obtained by modeling.
19.08.2003 (18)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= .1 ml/kg bw
Species	:	rat
Strain	:	other: Carworth-Wistar
Sex	:	male
Number of animals	:	5
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1962
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The LD50 value (with confidence interval) was 0.10 ml/kg (77 - 130 mg/kg). Based on a relative density of 0.77 (at 20 degrees C), the LD50 value in mg/kg is 77 mg/kg.
Test condition	:	Test material was given by gavage to groups of 5 nonfasted rats (4-5 weeks old, 90-120 g). Dosages (not listed) were arranged in a logarithmic series differing by a factor of 2. The material was administered in a suitable vehicle (water, corn oil, 1% Tergitol Penetrant 7, or semi-solid agar). The number of deaths was monitored over 14 days. The LD50 value and its confidence interval was estimated by the method of Thompson (Bacteriol Rev 11:115, 1947) using the tables of Weil (Biometrics 8:249, 1952).
Reliability	:	(2) valid with restrictions The number of deaths at each concentration and the purity of the test material were not mentioned.

08.07.2003 (29)

Type	:	LD50
Value	:	= 50 mg/kg bw
Species	:	rat
Strain	:	other: unknown
Sex	:	no data
Number of animals	:	20
Vehicle	:	water
Doses	:	10 - 3200 mg/kg bw
Method	:	other
Year	:	1961
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The LD50 value was 50 mg/kg. The numbers of deaths at each dose were not reported. However, death was noted to have occurred between 15 minutes and one day. Clinical signs included moderate to very weak, rapid and labored respiration, prostration, and very severe vasodilation (especially at lower does). Survivors gained weight over the observation period.
Test condition	:	A total of 20 rats were administered oral doses of isobutyronitrile ranging from 10-3200 mg/kg (either undiluted or 10% in water). Animals were monitored for clinical observations and weight change for 14 days after exposure.
Conclusion	:	Material is considered highly toxic
Reliability	:	(2) valid with restrictions

Basic data are given. Purity of the material is unknown.

10.08.2003 (13)

Type	:	LD50
Value	:	= 50 - 100 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	5
Vehicle	:	other: 1/2% sodium cellulose sulfate in water
Doses	:	25 - 400 mg/kg bw
Method	:	other
Year	:	1957
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The LD50 value was 50-100 mg/kg. The numbers of deaths at each dose were not reported. However, death was noted to have occurred between 1 to 4 hours. Clinical signs included weakness, ataxia, pink feet and ears, and occasional kicking. Survivors gained weight over the 2 week observation period.
Test condition	:	A total of 5 rats were administered oral doses of isobutyronitrile ranging from 25-400 mg/kg (as a 10% emulsion in 1/2% sodium cellulose sulfate in water). Animals were monitored for clinical observations and weight change for 14 days after exposure.
Conclusion	:	The material is moderately toxic in rats.
Reliability	:	(2) valid with restrictions Basic data are given. Purity of the material is unknown.

10.08.2003 (14)

Type	:	LD50
Value	:	> 50 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	2
Vehicle	:	other: 1/2% sodium cellulose sulfate in water
Doses	:	50 and 100 mg/kg bw
Method	:	other
Year	:	1957
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Neither of the animals died. Both animals gained weight during the observation period. Urine from rats given 50 mg/kg bw orally contained 0.1 mg thiocyanate per cc, indicating the material was converted to cyanide in the body. The amount of thiocyanate in urine from rats given 25 mg/kg bw was not listed.
Test condition	:	A total of 2 rats were administered 25 and 50 mg/kg bw isobutyronitrile (as a 10% emulsion in 1/2% sodium cellulose sulfate in water). Animals were monitored for clinical observations and weight change for 14 days after exposure. Urine was collected for 24 hours after exposure.
Reliability	:	(4) not assignable Not enough animals were tested for an accurate LD50 determination.

31.07.2003 (14)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	other: LC10
Value	:	= 1173 ppm

Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 30
Vehicle :
Doses : target vapor concentrations were 1200, 1800, and 2700 ppm
Exposure time : 1 hour(s)
Method : OECD Guide-line 403 "Acute Inhalation Toxicity"
Year : 1986
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : A pulmonary function test on 12 male rats (4/treatment group) was conducted in conjunction with this test. All four animals exposed to 2709 ppm and two exposed to 1778 ppm died on day 4. All animals exposed to 1248 ppm lived. The most consistent finding in the survivors (5/6) was a decrease in dynamic compliance (up to 76% compared to pre-exposure values). The forced expiratory flow at 10% vital capacity also was decreased (up to 67%) in the 2 survivors exposed to 1778 ppm. The changes observed were associated with pulmonary edema or congestion.

Result : LC10 value: LC10 (+/- 95% confidence interval) = 1143 ppm (males), 1630 ppm (females), 1173 ppm (combined).

Exposure concentrations: Actual concentrations were 1248 +\- 62, 1778 +\- 16, and 2709 +\- 34 ppm. No aerosol was present. Overall mean chamber temperature and relative humidity varied from 22 - 23 degrees C and 51 - 59%, respectively.

Deaths at each dose:

1248 ppm: 1/10; 1 male on Day 1
1778 ppm: 5/10; 4 males and 1 female on Day 1
2709 ppm: 8/10; 5 males on Day 1 and 3 females (2 on Day 1 and one on Day 2)

Remarks: Lethargy was noted at 1248 ppm (4/4 males and 1/5 females). The lethargic female also exhibited sialorrhea, which resolved after Day 2. At 1778 ppm, all animals exhibited lethargy. At 2709 ppm, all animals exhibited lethargy, gait disturbances and narcosis. These clinical signs were seen during or just after exposure cessation, the severity was dose-related, and they resolved after 24-hours. The mean body weight increased in all animals during the 14-day observation period. No compound-related gross pathology was noted in animals that died spontaneously or in animals terminated on Day 14.

Test condition : Rats [CRL:CD(SD)BR] were exposed to target vapor concentrations of 1200, 1800, and 2700 ppm (5/sex/concentration). Males weighed 232-263 g and females weighed 210-237 g at the start of the study. Food and water were available ad libitum (except during exposure).

Exposures were conducted in 420 l stainless steel and glass inhalation chambers. Chambers were maintained under negative pressure (-0.5" water) and at 13 air changes per hour. Vapors were generated by metering the test material dropwise into a heated glass bead-packed column supplied with metered, dried, oil-free compressed air. Chamber vapor samples were continuously collected and analyzed with an infrared analyzer equipped for automated sampling and analyses. Temperature and humidity were measured twice per hour. Twice during exposure, a particle counter sampled the chamber atmosphere for nongaseous airborne material. Particles greater than 0.3 microns were counted. The results were compared to those of the air control chamber. Distribution of test material was determined by measuring the concentrations in 27 different positions throughout the chamber and comparing them to a fixed

reference position. Subsequently, the chamber vapor concentration was measured from the fixed reference position.

Exposure was for 1-hour in duration followed by a 14-day observation period. Rats visible through the chamber windows were observed for signs of toxicity during exposure. Each rat was removed from its cage before and after exposure and twice daily thereafter and examined. Animals that died during the study were necropsied as soon as possible after discovery of death. Survivors were fasted for 16 hours prior to euthanization and necropsy on Day 15. The following tissues were examined: trachea, lungs, heart, liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pancreas, liver, salivary glands, kidneys, urinary bladder, pituitary gland, adrenal, thyroid, parathyroid, thymus, spleen, mesenteric lymph nodes, bone marrow (femoral), brain, testes, epididiymides, male accessory sex glands, ovaries, vagina, uterus, and Fallopian tubes. Mortality data were evaluated by Probit analysis.

Test substance : Purity was 99.7%

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

Flag : Critical study for SIDS endpoint

10.08.2003 (15) (16)

Type : LC50
Value : > 1000 ppm
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Doses : 1200 ppm (nominal)
Exposure time : 1 hour(s)
Method : other: DOT Final Rule 49 CFR (parts 172 and 173
Year : 1986
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Isolation and housing room low temperatures were 69 and 68 degrees F, respectively, exceeding tolerances specified in the protocol (73 +/- 3 degrees F). This deviation did not affect the outcome of the study.

Result : All animals survived to the end of the study. Mean vapor concentrations of the test material were 1233 +/- 15 and 1177 +/- 53 ppm for male and female rats, respectively. The saturated vapor concentration at 20 degrees C was 54, 474 ppm. An aerosol was not present. The temperature and relative humidity of the room ranged from 68-70 degrees F and 55-60%, respectively.

Test condition : Groups of 5 male (243-260 g) and 5 female (220-233 g) rats [CRL:CD(SD)BR] were exposed to a target vapor concentration of 1200 ppm for 1 hour. Feed and water were available ad libitum (except during exposure). Rats were observed for mortality during exposure and twice daily on subsequent workdays for 14 days.

Chamber atmospheres were produced by passing dried, oil-free compressed air through a glass bead-packed distilling column into which the test material was pumped dropwise. The exposure began when the vapor concentration reached 1000 ppm. Males and females were exposed in separate 20-l bell jars. Four samples of the atmosphere were analyzed for iso-butyronitrile concentration with an infrared analyzer. Two samples of atmosphere were analyzed with a particle analyzer to insure that an aerosol was not present. The temperature was monitored 4 times during exposure.

Test substance : Purity of the test material was 99.7% (GC-MS). No attempt was made to

identify trace components.

Conclusion : The material is not subject to regulation under 49 CFR Parts 172 and 173, since the LC50 value was greater than 1000 ppm.

Reliability : (1) valid without restriction
The study is comparable to an OECD guideline, GLP study.

07.08.2003 (12)

Type : LC50
Value : = 500 - 1000 ppm
Species : rat
Strain : other: albino
Sex : no data
Number of animals : 18
Vehicle :
Doses :
Exposure time : 4 hour(s)
Method : other
Year : 1962
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : All six animals died after inhalation exposure to 1000 ppm, and 0/6 died after exposure to 500 ppm test material for 4 hours. All six rats exposed to a concentrated vapor for 10 minutes died.

Test substance : Purity of the test material was not listed.

Reliability : (4) not assignable
Study documentation is poor. Purity of the material is not known.

08.07.2003 (29)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : = .31 ml/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals :
Vehicle :
Doses :
Method : other: Draize test
Year : 1962
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : The LD50 value (with confidence interval) was 0.31 (0.21 - 0.46) ml/kg. Based on a density of 0.77 (at 20 degrees C), the LD50 value in mg/kg is 239.

Test condition : Fur was clipped from the entire trunk of rabbits (2.5 to 3.5 kg) and the material was applied beneath an impervious plastic film. Groups of 4 animals were exposed to various concentrations (not listed). The animals were immobilized during the 24-hour contact period. The film was then removed and the animals were caged for the subsequent 14 day observation period. The LD50 value and its confidence interval was estimated by the method of Thompson (Bacteriol Rev 11:115, 1947) using the tables of Weil (Biometrics 8:249, 1952).

Reliability : (2) valid with restrictions
Purity of the material, test concentrations and the number of deaths at each concentration were not mentioned.

08.07.2003 (29)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	14 weeks (total of 63 exposure days)
Frequency of treatm.	:	6 hours/day, 5 days per week
Post exposure period	:	
Doses	:	60, 120, 209 ppm
Control group	:	yes
NOAEL	:	< 60 ppm
LOAEL	:	= 60 ppm
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	other TS

Result : Test material concentrations: The nominal concentrations (+/- SD) were 56.0 +/- 8.8, 119.1 +/- 16.9 and 203.0 +/- 19.6. Corresponding analytical concentrations were 60.2 +/- 1.0, 120.3 +/- 1.1 and 209.0 +/- 1.3 ppm. Since the nominal analytical ratios were close to 1, the material was a true vapor. There was no indication that the test material was unstable. Distribution was uniform (> 96%) for each of the exposure concentrations. Airflow, temperature and relative humidity ranged from 1719-1765 l/min, 21.0 - 26.1 degrees C and 17-50%, respectively.

Effects at all exposure concentrations: Signs of toxicity (labored breathing, nasal discharge, salivation, discharge from the eyes, hypoactivity and/or alopecia) were observed in all exposed groups. Incidences of these signs increased in a dose-dependent manner. Males and/or females in all exposed groups had significant decreases in red blood cells and hemoglobin values. Urine thiocyanate concentrations increased in all exposed groups, with concentrations from animals exposed to 120 ppm similar to or higher than those exposed to 210 ppm. However, since a dose-dependent diuresis occurred, the total amount of urine thiocyanate present (concentration x urine volume) increased with increasing concentrations.

Effects at 209 ppm: Three males died or were killed in extremis (2 between exposures 2 and 3 and one between exposures 18 and 19). Arched back, ataxia, tremors or convulsions, biting, pawing or rubbing chin against cage, irritation of the conjunctiva and breathing difficulties were noted in a few animals during exposure. Males and females exhibited significant decreases in body weight throughout the study (P <= 0.01).

Average final body weights of males and females were lower than controls (377.5 g in exposed males vs. 435.4 g in controls and 255.0 g in exposed females vs. 276.6 g in controls). Absolute and/or relative heart, liver, spleen and kidney weights were increased in males and/or females. Absolute testes weights were decreased in males. Mean corpuscular hemoglobin concentrations (males only) were lower than control (all P <= 0.05). Serum alkaline phosphatase (males and females), SGOT (males only) and SGPT (males only) were increased, and BUN concentrations were decreased. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 10/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 120 ppm: Ataxia was observed during exposure in 2 females. Males had significant body weight decreases (P < = 0.05) at two time points. Absolute and/or relative liver weights were increased in males and females and absolute and/or relative spleen weights were increased in males. Mean corpuscular hemoglobin concentrations (males only, P < = 0.05) were lower than control. No gross lesions were observed at termination. There was mildly increased hemosiderin deposition in the spleens of 11/15 females. Other microscopic changes were considered to be spontaneous.

Effects at 60 ppm: Absolute and relative spleen weights were increased in males. Serum thiocyanate concentrations were marginally increased in males and females.

Test condition : Animals: Rats were acclimated for at least 10 days prior to use. Two days prior to the start of the study, males weighed 174-200 g and females weighed 132-145 g. On the first day of the study, the animals were 43 days old. Animals were randomly allocated by body weight into 4 groups of 15 animals/sex/group. Animals were individually housed in suspended mesh cages and given food and water ad libitum (except during exposure). Animal rooms were maintained at 70-74 degrees C and 35-60% relative humidity, with a 12 hour light/dark cycle.

Exposure conditions: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Rats were placed individually in wire mesh cages that were suspended in the chambers by 3-tiered racks. Males were placed on one side and females on the other. The concentrations of material in the chambers (20, 120 or 210 ppm) were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. The bubbler was connected to a side port in the vertical particle-size separator, which was in turn connected to the air inlet at the top of the inhalation chamber. One bubbler was used in each of the generation systems. Airflow was maintained at a constant flow of 1727 liters/min. Nominal concentration measurements were determined daily for each chamber following exposure, by dividing the amount of test material delivered to the chamber (the difference between the pre- and post-exposure weights) over the 6-hr exposure period by the total air volume during the same period. Concentrations of test material in the chambers were measured 4 times daily using a Miran 1A General Purpose Gas Analyzer. Additional samples of atmosphere from 9 specified locations in each chamber were also taken at 3 different times to determine if the vapor was distributed uniformly.

Test conduct: Animals were observed for clinical signs between the second and fifth hour of each exposure. Estimations of the percentages of animals exhibiting hypoactivity, eye irritation and breathing difficulties were made. All animals were individually examined for gross signs of toxicity preceding and following each exposure and checked for mortality. Each animal was weighed and given a thorough examination for gross signs of toxicity on a weekly basis.

Animals were euthanized after 14 total weeks on the study. Terminal body weights were obtained (following an overnight fast). Blood and urine were collected. Whole blood was treated with an anticoagulant and was analyzed for total and differential erythrocyte count, total leukocyte count, platelet count, hematocrit, hemoglobin, and red blood cell indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration). Serum was analyzed for albumin, globulin, total protein, blood urea nitrogen, total bilirubin, glucose, glutamic pyruvic transaminase (SGPT), alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), T3, T4, thiocyanate and lactate dehydrogenase. Urine was analyzed for the presence of thiocyanates.

Detailed necropsies were conducted on all rats that died during the course of the study, those that were killed moribund, and those that survived to study termination. The adrenal glands (both together), testes (with epididymides, heart, kidneys, liver, pituitary and spleen were weighed. The aforementioned organs and the following tissues were fixed in 10% neutral formalin: abdominal aorta, bone and bone marrow (femur), brain, esophagus, ovaries, colon, ileum, lung, lymph nodes (mesenteric), mammary gland, nasal turbinates, pancreas, thyroid/parathyroid, prostate, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, stomach, thymus, trachea, urinary bladder, uterus (with cervix) and gross lesions. Eyes (with optic nerve) were fixed in a solution of 2% glutaraldehyde and 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, cut at five microns, stained with hematoxylin and examined microscopically.

Statistical analyses: In life and terminal body weights and organ weight data were analyzed using Dunnett's test. Organ to body weight ratios were analyzed using the Mann-Whitney test, with the Bonferroni inequality. Data for frequencies of microscopic lesions were evaluated with the Fisher's exact test with the Bonferroni inequality. Hematological and serum and urine chemistry variables were examined using Dunnett's test.

Test substance : Test material was propionitrile (CAS No. 107-12-0). The purity of the test material was 96%. Impurities were not listed.

Reliability : (1) valid without restriction
The study is comparable to a guideline study; however, a NOAEL was not established

Flag : Critical study for SIDS endpoint

10.08.2003 (30)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay

System of testing : Salmonella typhimurium/TA98, 100, 1535, 1537, and Escherichia coli/WP2uvrA(pKM101)

Test concentration : 100, 333, 1000, 3330 and 5000 micrograms/plate

Cytotoxic concentr. : > 5000 micrograms/plate

Metabolic activation : with and without

Result : negative

Method : other: EEC Annex V Guideline number B.14, "Other Effects-Mutagenicity Salmonella typhimurium-Reverse Mutation Assay", and Guideline number B.13, Other Effects-Mutagenicity, Escherichia coli-Reverse Mutation Assay"

Year : 1999

GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Remark : This is the critical study for the mutagenesis endpoint.

Result : No positive responses were induced in any of the tester strains. None of the concentrations tested caused toxicity. No precipitate was observed at the maximum concentration tested. All criteria for a valid test were met.

Test condition : Test strains: The S. typhimurium and E. coli strains were obtained from Dr. Bruce Ames, University of California Berkeley and the National Collection of Industrial Bacteria,Torry Research Station, Scotland, respectively. Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 ml/ml culture) and freezing small aliquots at < = -70 degrees C. Master plates were prepared by streaking each test strain from a frozen permanent stock onto minimal agar supplemented with histidine, biotin, ampicillin and/or tryptophan (depending on the strain). Tester strain master plates were stored at 5 +/- 3 degrees C. Overnight cultures were inoculated by transferring colonies from the master plates to a flask containing culture medium. Inoculated flasks were placed in a shaker/incubator (125 +/- 25 rpm, 37 +/- 2 degrees C). Cultures were harvested once a predetermined turbidity was reached (at least 0.5 x 10E9 cells/ml). Test stains were checked for rfa wall mutation (all Salmonella strains), pKM101 plasmid R-factor (Salmonella TA98 and TA100 and E. coli only), and characteristic number of spontaneous revertants (all strains) on the day the mutagenesis test was conducted.

Test medium: The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution supplemented with 2.5% Oxoid Nutrient Broth No. 2. Bottom agar was Vogel-Bonner minimal medium E supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Overlay agar contained 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with either 10 ml of 0.5 mM histidine/biotin solution or 0.5 mM tryptophan solution.

S-9 mix: S9 homogenate was purchased from Molecular Toxicology Inc. This was prepared from male Sprague-Dawley rats that had been injected i.p. with 500 mg/kg Aroclor 1254. S-9 mix was prepared immediately prior to use.

Concentrations of test material: The concentrations tested (100, 333, 1000, 3330 and 5000 micrograms/plate) were selected based on the results of a dose range-finding study using test strains TA100 and WP2uvrA(pKM101) and 10 doses of test material ranging from 6.67 to 5000 micrograms/plate (both in the presence and absence of S-9 mix).

Positive, negative and sterility controls: Positive controls [2-aminoanthracene (2.5 and 5.0 micrograms/plate), 2-nitrofluorene (1.0 micrograms/plate), sodium azide (2.0 micrograms/plate), ICR-191 (2.0 micrograms/plate), and 4-nitroquinoline-N-oxide (2.0 micrograms/plate)] were run concurrently. DMSO (50 microliters) was used as a vehicle and vehicle control. The most concentrated test material dilution and S-9 mix were tested for sterility by plating a 50 microliter aliquot on selective agar.

Test conduct: A plate incorporation methodology was used. Test material or positive control (50 microliters), test strains (100 microliters) and S-9 mix or vehicle (500 microliters) were combined in 2.0 ml of molten, selective top agar. This was overlaid onto 25 ml of minimal agar that had been plated into 15 x 100 mm Petri dishes. All concentrations of test material, vehicle controls and positive controls were plated in triplicate. Revertant colonies were counted after 48 +/- 8 hours of inverted incubation at 37 +/- 2 degrees C. The condition of the background lawn was evaluated for evidence of cytotoxicity and precipitate.

Evaluation: The mean number of revertants and standard deviation were calculated. Various criteria were established to constitute a valid assay (test strain integrity, characteristic number of spontaneous revertants, cell

density > = 0.5 x 10E9, at least a 3-fold increase in revertants in positive controls, and a minimum of 3 non-toxic doses). A positive response was indicated by a 2-3 fold increase in mean revertant number depending on the bacterial tester strain.

Test substance : Purity of the test material was not confirmed in this study. However, the lot of test material used (D-7) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 99.8 - 99.9 %.

Conclusion : Material was not genotoxic under conditions of this assay

Reliability : (1) valid without restriction
This was a well-documented EEC Annex guideline study conducted under GLP assurances.

10.08.2003 (3)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary (CHO) Cells
Test concentration : up to 700 micrograms/ml
Cytotoxic concentr. : > 700 micrograms/ml
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 1999
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : This is the critical study for the chromosomal aberration endpoint.

Result : Without activation: In the initial study without metabolic activation, reductions of 30%, 31%, 6% and 6% were observed in the mitotic indices of the cultures treated with 239, 342, 489 and 669 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with these concentrations. In the confirmatory study without activation, reductions of 44%, 24%, 23%, 16% and 34% were observed in the mitotic indices of cultures treated with 222, 296, 394, 525 and 700 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 296, 394, 525 and 700 micrograms/ml. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures.

With activation: In the initial study with metabolic activation, reductions of 25% and 46% were observed in the mitotic indices of cultures treated with 342 and 489 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 239, 342, 489 and 699 micrograms/ml. No reductions in mitotic index were noted in any of the cultures in the confirmatory study. In this study, chromosomal aberrations were analyzed from cultures treated with 296, 394, 525 and 700 micrograms/ml. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures.

No precipitate was observed at the maximum concentration tested in any of the studies.

All criteria for validity were met in each study.

Test condition : Cells: The Chinese hamster ovary cells used in the assay (CHO-WBL) were from a permanent cell line originally obtained from Dr. S. Wolff, University of California, San Francisco. Stock cultures were maintained for up to 8 weeks after thawing. Mycoplasma testing was performed twice during this period. Cells were grown at 37 +/- 2 degrees C (in 5% +/- 1.5% CO2 in air) in McCoy's 5a culture medium which was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin G and 100 micrograms/ml streptomycin.

S-9 mix: S-9 was isolated from the liver of rats (sex not stated) 5 days after

i.p. treatment with 500 mg/kg Aroclor 1254. S-9 was stored frozen at < = -70 degrees C until use. S-9 mix was prepared by adding an energy-producing system (NADP plus isocitric acid) to S-9.

Test material and negative and positive controls: The test material was dissolved in DMSO. The top concentration tested (approximately 700 micrograms/ml or 10 mM) was the recommended high dose for the assay. The negative control was 10 microliters/ml DMSO. The positive controls were mitomycin C (without activation) and cyclophosphamide (with activation).

Initial test: Cultures were initiated by seeding approximately 1.2 x 10E6 cells per 75 cm2 flask into 10 ml of complete McCoy's 5a medium. For the test without metabolic activation, cultures were incubated with test material for 3.0 hrs at 37 degrees C. For the test with metabolic activation, cells were incubated for approximately 3.0 hours with test material and S-9 mix in McCoy's 5a medium that did not contain fetal bovine serum. Replicate cultures for each concentration of test material (4.73, 6.76, 9.66, 13.8, 19.7, 28.1, 40.1, 57.3, 81.9, 117, 167, 239, 342, 489 and 699 micrograms/ml), positive control (0.75 and 1.5 micrograms/ml mitomycin C and 5.0 and 10.0 micrograms/ml cyclophosphamide), vehicle and untreated controls were prepared. Cultures with or without S-9 were then washed with buffered saline, and incubated with complete McCoy's 5a medium for 17 hours. Colcemid (0.1 micrograms/ml) was present during the last 2 hours of incubation. Cells were visually inspected for cytotoxicity prior to harvest. Cells were then trypsinized and spun in a centrifuge. The supernatant was discarded and the cells swollen with 75 mM KCl hypotonic solution. The cells were then fixed with an absolute methanol: glacial acetic acid (3:1, v:v) fixative. They were then placed on glass slides and air-dried. Cells were stained with 5% Giemsa and analyzed for mitotic index and chromosomal aberrations.

Confirmatory assay: The test with metabolic activation was conducted the same as in the initial test, but with different concentrations of test material (222, 296, 394, 525 and 700 micrograms/ml). In the test without metabolic activation, the test material (27.8, 55.5, 111, 222, 296, 394, 525 and 700 micrograms/ml), positive control (0.20 or 0.40 micrograms/ml mitomycin C or 5.0 or 10.0 micrograms/ml cyclophosphamide) and negative controls were incubated with the cells for 18.3 hours (instead of 3). For both tests, Colcemid was present for the last 1.9 hours of incubation and cells were harvested after 20.2 total hours of incubation. The slides were prepared as described for the previous test.

Evaluation: Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 21 +/-2 were analyzed. One hundred cells (if possible) were analyzed from each replicate of the vehicle control, 4 concentrations of the test material, and one concentration of positive control for the different types of chromosomal aberrations. At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations. The number of mitotic cells in 1000 cells was determined and the ratio expressed as percentage of mitotic cells. Percent polyploidy and endoreduplication were analyzed by evaluating 100 metaphases (if possible). Chromatid and isochromatid gaps were noted but were not used in calculating the total number of aberrations.

Acceptance criteria: The assay was considered valid if the negative (untreated) and vehicle controls contained < 5% cells with aberrations, the positive control result was significantly higher ($p < = 0.01$) than that of the vehicle control, a high dose of 10 mM or the highest soluble concentration was used if the material did not cause at least a 50% reduction of the mitotic index at the tested concentrations, and at least 3 concentrations

were analyzed.

Data analysis: The statistical analysis employed a Cochran-Armitage test for linear trends and Fisher's Exact Test to compare the percentage of cells with aberrations. Data for polyploidy and/or endoreduplication were also analyzed separately. A test was considered positive if a significant increase in the number of cells with aberrations (p < = 0.01) was observed at one or more concentrations.

Test substance : Purity of the test material was not confirmed in this study. However, the lot of test material used (D-7) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 99.8 - 99.9 %.

Conclusion : Material was not genotoxic under conditions of this assay.

Reliability : (1) valid without restriction
This was a well-documented OECD guideline study conducted under GLP assurances.

10.08.2003 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Fertility
Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 21 to 33 days (depending on day of mating)
Frequency of treatm. : 6 hr/day, 7 days/week
Premating exposure period
Male : 0 days
Female : 21 days
Duration of test : to gestation days 13-15
No. of generation studies :
Doses : 60, 120 and 210 ppm
Control group : yes
NOAEL parental : = 60 ppm
other: NOAEL : = 210 ppm
Reproductive Toxicity
Method : other
Year : 1984
GLP : yes
Test substance : other TS

Result : Exposure concentrations: The average mean daily analytical exposure concentrations (60.1, 120.2 and 209.2 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25 degrees C and 26-29%, respectively.

Signs of toxicity: None of the animals died. There was no effect of test material on body weight. Animals exposed to 210 ppm exhibited arched back (N = 4 on days 1-10 and N=2 on days 11-20), lacrimation (N = 2 on days 1-10 and N = 1 on days 21-30), salivation (N= 15 on days 1-10, N = 22 on days 11-20 and N = 21 on days 21-30) hypoactivity (N = 13 on days

1-10, N = 5 on days 11-20 and N = 3 on days 21-30), staining of facial fur (N = 2 on days 1-10, N = 4 on days 11-20 and N = 4 on days 21-30) and red nasal encrustation (N = 1 on days 1-10, N = 5 on days 11-20 and N = 5 on days 21-20) after exposure. Animals exposed to 120 ppm also exhibited salivation (N = 6 on days 11-20 and N = 4 on days 21-30, staining of facial fur (N = 7 on days 1-10, N = 5 on days 11-10 and N = 2 on days 21-30) and red nasal encrustation (N = 2 on days 1 1-0, N = 8 on days 11-20 and N = 6 on days 21-30). A few animals in the 60 ppm group also exhibited red nasal encrustation (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-30) and staining of facial fur (N = 1 on days 1-10, N = 3 on days 11-20 and N = 1 on days 21-20). One control animal had stained facial fur on days 21-30 and another had red nasal encrustation on days 1-10. Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 2 controls, N = 3 low dose, N = 5 mid dose, N = 9) at one or more of their weekly physical examinations.

The only remarkable findings at gross necropsy were bilateral uterine hydrometra in one animal exposed to 210 ppm and hydrometra in the left uterine horn of one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on fertility. Efficiency of mating (32.0%, 32.0%, 30.7% and 25.0% in the control, low, mid and high dose groups) and pregnancy rate (100%, 95.8%, 100% and 91.3% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 13.4 - 13.9), resorptions (ranged from 0.6 - 0.8), nidations (ranged from 14.1 - 14.5), corpora lutea (ranged from 13.0 - 15.2), preimplantation loss (4-8%) and postimplantation loss (4-6%). Evaluation of the vaginal smears of 2 females that did not copulate showed one that did not cycle (but was pregnant at necropsy), and another that only went through the cycling stage of proestrus.

Test condition : Animals: Virgin female Sprague Dawley rats (43 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of ten females and ten males that were taken upon receipt were 128-144 g and 178-233 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to females during exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 7 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. Due to inclement weather and building equipment failures, 2 exposures (days 2 and 16) were only for 4 hours and one exposure (day 1) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 16) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Twenty four females per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when females were 63 days old. Animals were observed during exposure for signs of toxicity. After 21 days of exposure (which was sufficient to cover 3-4 estrus cycles), females were randomly mated (1:1) to an untreated male that had been assigned to the corresponding treatment

group (30 males were assigned per group). At night, after exposure, females were caged with their assigned male until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or 5 nights without confirmed copulation. Females that failed to mate with the assigned male were mated with another male that had copulated with another female in the same group. Nightly co-housing with the second male occurred until copulation was confirmed (or for a maximum of 7 nights). The day on which copulation was confirmed was considered gestation day 0. Exposure of females continued until copulation was confirmed or a maximum of 12 nights of cohabitation with males without signs of copulation. Vaginal smears were taken on 5 consecutive days for females that did not exhibit copulation.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Females were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities.

Females were killed on gestation day 13 (or the nearest working day after gestation day 13, up to gestation day 15). Females without confirmed copulation ware euthanized in the second week after the last day of co-housing. Each female was given an external examination and weighed. The tissues and organs of the thoracic and abdominal cavities were examined for gross lesions. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted. The ovaries and uteri of females were preserved in 10% neutral buffered formalin. Males were killed after mating and were not examined.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : The test material was propionitrile (CAS No. 107-12-0). Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (< 0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : The authors concluded that the incidences of red nasal encrustation in the low dose animals, alopecia in the mid and high dose animals and staining of facial fur in all treated groups were too low to be definitely related to administration of test material. There was no effect of treatment on fertility of females.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (23)

Type : Fertility
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 57 days
Frequency of treatm. : 6 hours/day, 5 days/week
Premating exposure period
Male : 46 days
Female : 0 days
Duration of test : to gestation day 13-15

No. of generation studies	:	
Doses	:	60, 120 and 210 ppm
Control group	:	yes
NOAEL parental	:	= 60 ppm
other: NOAEL	:	= 210 ppm
Reproductive Toxicity		
Method	:	other
Year	:	1985
GLP	:	yes
Test substance	:	other TS

Result :

Exposure concentrations: The average mean daily analytical exposure concentrations (60.2, 120.4 and 208.9 ppm) were within 1% of the target levels (60, 120 and 210 ppm, respectively). Mean temperatures and humidities ranged from 22-25.5 degrees C and 24-27%, respectively.

Signs of toxicity: One of animals exposed to 210 ppm died after 2 days of exposure. On the previous day, this animal exhibited labored breathing, hypoactivity, poor control of the hind limbs, difficulty in standing, body tremors and involuntary movements. No unusual findings were observed at necropsy.

Body weights of males exposed to 210 ppm were approximately 6-9% lower than those of the control group during most of the exposure period, and remained lower than control (but were not significantly different) until the end of the study.

Animals exposed to 210 ppm exhibited signs of toxicity such as arched back (N = 8 on days 1-10, N = 3 on days 11-20 and 51-57, and N = 5 on days 41-50), hypoactivity (N = 12-15 at each 10-day interval up to day 50, and N = 4 from days 51-57), labored breathing (N = 10 on days 1-10, N = 3 on days 11-20 and 31-40, N = 5 on days 21-30 and N = 1 on days 51-57), and salivation (N = 3 on days 1-10, and N = 10 - 12 at all other intervals). A few high dose animals (individual numbers were not stated) also exhibited abnormal behavior such as grinding of teeth, head bobbing, body tremors, involuntary movements, and pawing at the cage. A few of the animals exposed to 120 ppm exhibited salivation (N = 3-8 at all intervals) and hypoactivity (N = 3 at days 11-20). Signs of toxicity generally abated by the morning after exposure. Alopecia was observed in animals (N = 1 control, N = 2 low dose, N = 1 mid dose, N = 5 high dose) at one or more of their weekly physical examinations. No unusual treatment-related signs were observed in rats exposed to 60 ppm. The only remarkable finding at gross necropsy was a small right testis in one animal exposed to 120 ppm.

Fertility: There was no effect of treatment on male fertility. Efficiency of mating (34.4%, 30.6%, 29.8% and 27.1% in the control, low, mid and high dose groups) and pregnancy rate (90.5%, 97.6%, 90.0% and 97.4% in the respective groups) were comparable between groups. There was no difference in the numbers of live implants (ranged from 12.7 - 13.9), resorptions (ranged from 0.7 - 1.1), nidations (ranged from 13.8 - 14.9), corpora lutea (ranged from 13.1 - 15.2), preimplantation loss (4-8%) and postimplantation loss (5-10%).

Test condition :

Animals: Virgin female Sprague Dawley rats (28 days old upon receipt) and virgin male Sprague Dawley rats (50 days old upon receipt) were acclimated for one week and examined for general health and the presence of pinworms and ectoparasites. Body weights of fifteen females and ten males that were taken upon receipt were 155-181 g and 80-103 g, respectively. No significant health problems were noted during the acclimation period, and the animals were released for study. Males and females were individually caged (except during mating). Food and water were available ad libitum (except food was not available to males during

exposure). Animals were housed in a room maintained at 72 +/- 2 degrees F and 40-60% relative humidity, under a 12 hr light/dark cycle.

Exposures: Exposures (6 hr/day, 5 days/week) occurred in 10 m3 Rochester-style stainless steel and glass inhalation chambers. A scheduled exposure day was cancelled due in clement weather. A new exposure day (exposure day 41) was used in its place. Due to inclement weather and building equipment failures, 2 exposures (days 33 and 43) were only for 4 hours and one exposure (day 32) was for 5 hours. Atmospheres of propionitrile vapor were generated using bubbler systems. The concentrations of material in the chambers were controlled by either adjusting the nitrogen flow though the propionitrile in the bubblers or by changing the amount of test material in the bubblers. Concentrations of test material in the chambers were measured 4 times daily (except during day 43) using a Miran 1A General Purpose Gas Analyzer. The nominal concentration of material, temperature and humidity also were measured (at intervals that were not listed).

Study conduct: Fifteen males per group were assigned to be exposed to target concentrations of 0, 60, 120 or 210 ppm test material. Exposure began when males were 43 days old. Mating was initiated when males and females were 16 and 12 weeks old, respectively. At this time, males had been 69 days on the study (which was sufficient to cover the spermatogenesis cycle of the rat), and had 46 days of exposure. Males were randomly mated (1: 1) with three untreated females (consecutively) that had been assigned to the corresponding treatment group (45 females were assigned per group). Exposure of males continued until the day after the last mating opportunity (57 exposure days). At night, after exposure, males were caged with their assigned female until copulation was confirmed (by presence of a copulatory plug or sperm in vaginal smear) or 5 nights without confirmed copulation. The day on which copulation was confirmed was considered gestation day 0.

After assignment to the study, males and females were weighed once per week. Mated females were weighed on gestation days 0 and 13. Males were given a thorough physical examination once per week and were observed for clinical signs of toxicity before and after exposures. All animals were checked twice daily for mortality and gross abnormalities (except for one day prior to mating when inclement weather permitted observations).

One half of the males of each group were euthanized on each of the 2 consecutive days at the end of the study. They had not been exposed to propionitrile for about 2 weeks prior to termination. Each male was given an external examination and weighed. The tissues and organs of the thoracic, scrotal and abdominal cavities were examined for gross lesions and the testes, epididymides, prostate glands and seminal vesicles were preserved in 10% neutral buffered formalin. Females that were not mated with males were euthanized and were not examined.

Mated females were euthanized on gestation day 13 (or the nearest workday up to gestation day 15). Females that were co-housed with males without confirmed copulation were euthanized during the second week after the last day of co-housing. Gross necropsies were performed on females that had copulated and those that had not. The tissues and organs of the thoracic and abdominal cavities were examined. Pregnancy status was determined, nidation sites and were classified, and corpora lutea were counted.

Statistical analyses: Body weight data were analyzed using Dunnett's test. Mating and pregnancy rate data were analyzed by a Fisher's exact test and an uncorrected chi-square test. Other data were analyzed by the Mann-

Whitney U test. The Bonferroni inequality was assumed when comparing multiple treatments to control values for all tests except Dunnett's test. The critical level for significance was $p < 0.05$.

Test substance : Test material was propionitrile (CAS No. 107-12-0). Purity of the test material was 96.1%. Impurities included acrylonitrile (0.1%), adiponitrile (0.7%), p-nitrosodiphenylamine (0.08%) and water (< 0.1%). Analyses indicated no significant decomposition of the test material over the course of the study.

Conclusion : There was no effect of treatment on fertility of males.

Reliability : (1) valid without restriction
Study is comparable to a guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (24)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 6 hours/day
Frequency of treatm. : Days 6 to 20 of gestation
Duration of test : to Gestation Day 21
Doses : 50, 100, 200, and 300 ppm
Control group : yes
NOAEL maternal tox. : = 100 ppm
NOAEL teratogen. : = 300 ppm
NOAEL Fetotoxicity : = 100 ppm
NOAEL Embryotoxicity : = 200 ppm
Result : isobutyronitrile was not teratogenic
Method : other: similar to OECD 414
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Four different concentrations of acetonitrile, priopionitrile, n-butyronitrile, acrylonitrile, allylnitrile, methacrylonitrile and 2-chloroacrylonitrile also were tested in this study.

The methodology described in the manuscript is essentially identical to that under OECD TG-414. However, information is lacking in the report in regard to food intake. Therefore, it is unknown if reduced fetal body weights were a consequence of reduced material feed intake. In addition, it is unknown as to whether the study was conducted under GLP assurances. However, based on the date in which this study was completed it is likely to have been a GLP study. All other parameters noted in the guideline appear in the manuscript.

The NOAEL for fetoxicity is 100 ppm, since a reduction in fetal body weight was observed in females from the 200 ppm group. The NOAEL for embryotoxicity is 200 ppm, since 300 ppm caused an increase in embryolethality.

Result : Maternal: One out of 21 animals exposed to 200 ppm and 3/21 exposed to 300 ppm died before study termination. Maternal weight gains were similar in all groups. Indices of pregnancy were comparable among groups. There was no significant effect of treatment on the mean numbers of implantations. The indices of nonsurviving implants and embryonic resorptions (per litter) in rats treated with 300 ppm (11.07 +/- 9.29 per litter for both indices) were significantly higher than control (2.02 +/- 3.63 per litter for both indices).

Fetal: There was no effect of treatment on the mean numbers of live fetuses or sex ratio. There was a concentration- related decrease in fetal body weights, with weights of females in the 200 ppm (5.11 +/- 0.41 g per litter) and males and females in 300 ppm groups (4.96 +/- 0.37 and 4.68 +/- 0.34 g per litter, respectively) being significantly less than control (5.75 +/- 0.29 g per litter for males and 5.56 +/- 0.42 g per litter for females). A single case of unilateral hydronephrosis was observed in one fetus from the 300 ppm group. The incidences of visceral and skeletal variations in treated fetuses were not significantly different from controls.

Test condition : Animals: Male (350 g) and primiparous female (200-220 g) were acclimated for 1-2 weeks prior to breeding. Females were then placed with males (one male: 3 females) overnight and examined by vaginal smear for the presence of sperm the following morning. Sperm-positive females were considered to be at Day 0 of gestation. These animals were randomly assigned to groups of 20-23 rats each.

Exposure conditions: Exposures were conducted in 200 –liter stainless-steel inhalation chambers at an air flow of 10-20 m3/hr. Chambers were maintained at a negative pressure of < = 3 mm water. Chamber temperatures and humidities were 23 +/- 2 degrees and 50 +/- 5%, respectively. Vapor was generated by bubbling an additional air flow through a flask containing test material. The vapor was mixed with filtered room air to achieve the desired concentration. Analytical concentrations were determined by analyzing the atmosphere once/hour (by gas-liquid chromatography) during each 6 hour exposure. The nominal concentrations of isobutyronitrile were 50, 100, 200 and 300 ppm. Corresponding analytical concentrations were 54 +\- 3.9, 98 +/- 10.0, 208 +/- 12.4, and 308 +/- 18.6 ppm.

Test conduct: Animals were exposed 6 hours/day on Days 6 through 20 of gestation. Control animals were exposed concurrently to filtered room air in an adjacent chamber with flow characteristics identical to those of the treated groups. Food and water were available ad libitum (except during exposure). All rats were observed daily and maternal body weights were recorded on Days 0, 6 and 21 of gestation. Females were euthanized on Day 21 of gestation and the uterus was removed and weighed. The uterus horns were then opened and the numbers of implantation and absorption sites and live and dead fetuses were recorded. Live fetuses were removed and weighed, examined for external anomalies (including those of the oral cavity) and sexed. The numbers of fetuses (and litters) examined for external anomalies in the 0, 50, 100, 200 and 300 ppm groups were 191 (16), 235 (19), 222 (19), 185 (14) and 193 (15), respectively. Half of the fetuses from each litter were fixed in Bouin's solution and examined microscopically for visceral abnormalities. The remaining half were fixed in 70% ethanol, eviscerated, macerated in 1% KOH , stained in alizarin red S, and examined microscopically for skeletal anomalies.

Statistical analysis: Depending on the parameter evaluated: one-way analysis of variance followed by Dunnett's test, Wilcoxon test after arc-sine-square root transformation, Fisher's test, or least squares analysis. The litter was used as the basis for analysis of fetal variables.

Test substance : Purity was > 99%.

Conclusion : It was concluded that isobutyronitrile was not teratogenic. While evidence of embryolethality and fetotoxicity were noted, these effects occurred at levels that also induced maternal toxicity (300 ppm).

Reliability : (1) valid without restriction
This was a well-documented OECD-like guideline study.

Flag : Critical study for SIDS endpoint

07.08.2003 (26)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT