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October 24, 2002

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 2216

Attn: Chemical Right-to-Know Program

Dear Administrator Whitman,

E. I. du Pont de Nemours & Company, Inc. and Akzo Nobel Polymer Chemicals LLC received EPA's comments on the test plan and robust data summary for Butanenitrile, 2,2'-azobis (2-methyl-, (CAS# 13472-08-7) and are pleased to respond. We have considered the recommended revisions to the ecotoxicity studies as well as EPA's specific comments on the robust data summaries. Included with this submittal is a summary of our response to EPA's comments and a revised robust data summary. A draft protocol for the proposed algae study is also attached for forwarding to the Agency's aquatic toxicologists.

Please feel free to contact me with any questions or concerns you may have with regards to this submission at Edwin.L.Mongan-1@usa.dupont.com, or my Akzo Nobel counterpart, Jack Orr, at jack.orr@akzo-nobel.com.

Sincerely,

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

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

Summary of Response to EPA's Comments

Ecotoxicity

EPA Comment: For algae, a more definitive test should be conducted either on the analog AIBN or the sponsored chemical AMBN. In general practice, the highest exposure level should be 100 mg/L or at the chemical's aqueous water solubility limit.

Response: An algae test following OECD Guideline 201 will be conducted with AMBN at 100 mg/L or at the limit of solubility in algae medium, whichever is lower. A draft protocol for this study is attached for forwarding to the Agency's aquatic toxicologists.

Health Effects

EPA Comment: The submitter needs to supply a robust summary for the developmental toxicity endpoint. HPV Challenge Program guidance indicates that when a study addresses multiple endpoints, a robust summary is needed for each endpoint.

Response: Requested data were added to the robust summary.

24 October 2002

TEST PLAN/ROBUST SUMMARIES FOR BUTANENITRILE, 2,2'-AZOBIS(2-METHYL- WITH ITS ANALOG, PROPANENITRILE, 2,2'-AZOBIS(2-METHYL-

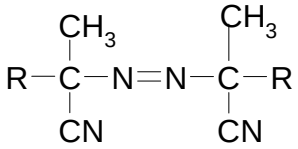
Summary

Two closely related azonitriles meet the production volume criteria for inclusion in the HPV Challenge Program:

- Butanenitrile, 2,2'-azobis(2-methyl-
  CAS Number: 13472-08-7
  Common name: 2,2'azobis-(2-methylbutyronitrile) (AMBN)
- Propanenitrile, 2,2'-azobis(2-methyl-
  CAS Number: 78-67-1
  Common name: 2,2'azobis-(2-isobutyronitrile) (AIBN)

AIBN is exempt from the HPV program because it has already been evaluated through the Organization of Economic Cooperation and Development (OECD) high production volume (HPV) program. A SIDS Initial Assessment Report (SIAR) was prepared for evaluation by the Ninth SIAM, which convened in France June 29 through July 1, 1999. While AIBN does not require any additional information for the HPV program, the data for AIBN is useful for predicting the expected properties for its homologue, AMBN. By examining these chemicals simultaneously, relevant data from both can be considered in evaluation of their environmental effects and potential toxicity, thereby minimizing redundant and unnecessary animal testing.

For purposes of this HPV document, the two azonitrile chemicals can be represented by the general structural formula:



Information regarding these chemicals is presented in the table below.

Chemical Name	**CAS Registry Number**	**Common Name**	**Name to be used in this Document**	**R=**
Butanenitrile, 2,2'-azobis(2-methyl-	13472-08-7	2,2'azobis-(2-methylbutyronitrile)	AMBN	CH_3CH_2-
Propanenitrile, 2,2'-azobis(2-methyl-	78-67-1	2,2'azobis-(2-isobutyronitrile)	AIBN	CH_3-

As shown above, AMBN and AIBN are very similar in chemical structure. The only functional groups present in these molecules are the nitrile (-CN) moiety and the azo (N=N) moiety. The nitrile and azo moieties are bonded to the same carbon atom, which also bears two alkyl groups.

The molecules are symmetric about the azo bridge, the most labile functional group. The azo bridge is easily thermally cleaved, liberating nitrogen gas and a stabilized free radical, as described below. AMBN differs from AIBN only by the replacement of methyl groups (CH_3) in AIBN with ethyl groups (CH_3CH_2). The functional groups and the alkyl groups on these two azonitriles will be expected to interact in similar fashion with other molecules, including enzymes.

Azonitriles, such as AMBN and AIBN, are designed to cleave the azo bridge to liberate nitrogen gas and form stabilized free radicals, as shown in the following equation:

$$R(CH_3)(CN)CN{=}NC(CN)(CH_3)R \rightarrow 2R(CH_3)(CN)C\bullet + N_2$$

This reactivity is the basis of the commercial utility of azonitriles as a source of free radical initiators for various chemical reactions. Azonitriles are often used as initiators for polymerization reactions, and, to a lesser degree, as a source of nitrogen gas in foam blowing applications. The reaction pathways for AMBN and AIBN are essentially the same. The synthesis routes for production of AMBN and AIBN are also the same, differing only in the ketone starting material that becomes the carbon backbone of the molecule. AMBN is produced from the four-carbon ketone, 2-butanone, and AIBN is produced from the three-carbon ketone, acetone.

The disproportionation of azonitriles to form free radicals is well understood and follows first-order kinetics. Decomposition of azonitriles in non-polymerizing solutions is a simple means of characterizing their reactivity. The temperature at which azonitriles exhibit a half-life of 10 hours has been commonly used for their classification . While this parameter does not necessarily predict the behavior of a given azonitrile in a different environment, it does provide a readily available comparative measure of various azonitriles. For AMBN and AIBN these temperatures are 67^{o}C and 64^{o}C, respectively. The similarity of AMBN and AIBN in thermal stability, reaction pathways, and reaction products all support simultaneous evaluation of these chemicals.

Scientific literature was searched and summarized. Data were identified for AIBN and AMBN (Table 1). All of the SIDS endpoints have been satisfied for AIBN. Each study was evaluated for adequacy. Robust summaries were developed for each study addressing specific SIDS endpoints. Summaries were also developed for studies either considered not adequate but provided information of relevance for hazard identification and evaluation, or covered non-SIDS endpoints. Information for AMBN and AIBN are reported in Appendix A and Appendix B, respectively.

Table 1: Matrix of Available and Adequate Data for AMBN and AIBN

	AMBN	AIBN
PHYSICAL/CHEMICAL CHARACTERISTICS		
Melting Point	√/–	√
Boiling Point	N/A	N/A
Vapor Pressure	√/–	√
Partition Coefficient	√/–	√
Water Solubility	√/–	√
ENVIRONMENTAL FATE		
Photodegradation	√[1]	√
Stability in Water	√[1]	√
Transport (Fugacity)	√[1]	√
Biodegradation	–[2]	√
ECOTOXICITY		
Acute Toxicity to Fish	–[2]	√
Acute Toxicity to Invertebrates	–[2]	√
Acute Toxicity to Aquatic Plants	–	√/–
MAMMALIAN TOXICITY		
Acute Toxicity	√	√
Repeated Dose Toxicity	–[2]	√
Developmental Toxicity	–[2]	√
Reproductive Toxicity	–[2]	√
Genetic Toxicity Bacterial Gene Mutations	√	√
Genetic Toxicity Chromosomal Aberrations (*in vitro*)	–[2]	√
Genetic Toxicity *in vivo* Micronucleus	√	√

√ = Data are available and considered adequate.
– = No data available.
√/– = Data are available, but considered inadequate.
N/A = Not Applicable.
[1] Modeled data, which will be re-evaluated when new physical/chemical data are available.
[2] Data is available for structurally similar test substance, AIBN.

Evaluation of Data Matrix

The available adequate data were broken out by discipline (physical/chemical, environmental fate, ecotoxicology, and mammalian toxicology). These comparisons were conducted to determine if a pattern existed between the two chemicals and to determine if additional testing is needed for AMBN.

AMBN and AIBN are white, odorless solids. Both AMBN (with a melting point of 45°C) and AIBN (with a melting point of 100-103°C) decompose rapidly when exposed to temperatures above the self-accelerating decomposition temperature of 50°C, with the potential for violent decomposition. The specific gravity of both chemicals is approximately 1.1, the vapor pressures are negligible at room temperature, and the chemicals have low solubility in water. The lower flammability limits in air (% by volume) for AMBN and AIBN are 0.034 and 0.02 g/L, respectively, and the upper flammability limits have not been determined. Boiling point measurement is not applicable, due to the low vapor pressure and thermal instability of the chemicals. A log Kow (log of the n-octanol-water partition coefficient) model predicts that AMBN has a log Kow of 3.86. The same log Kow model predicts that AIBN has a log Kow of 2.87, while the experimentally measured log Kow of AIBN is 1.10. **Since no methods or specific data were provided for the measurement of physical and chemical characteristics of AMBN, reanalysis of these endpoints following current GLP and/or using guideline methodology is proposed.**

Table 2: Physical and Chemical Characteristics

	AMBN	**AIBN**
Physical Appearance	White, odorless solid	White, odorless crystalline solid
Molecular Weight	192.26	164.21
Water Solubility	< 10 g/L (measured) 4.9 mg/L (model estimate)	350 mg/L (measured) 851.1 mg/L (model estimate)
Melting Point	45ºC	100-103ºC
Boiling Point	Not Applicable	Not Applicable
Vapor Pressure	Negligible at room temperature 8.9×10^{-2} Pa (model estimate)	8.1×10^{-1} Pa @ 25ºC (measured) 1.9×10^{-1} Pa (model estimate)
Density	1.1	~ 1.1

Table 2: Physical and Chemical Characteristics (cont'd)

	AMBN	AIBN
Partition Coefficient (log Kow)	3.86 (model estimate)	1.10 (measured) 2.87 (model estimate)

Empirical data regarding the environmental fate are limited for AMBN. Estimated physical and chemical properties of AMBN were used to model environmental fate endpoints. The Henry's Law Constant for AMBN is estimated to be 2.19×10^{-10} atm-m^3/mole, and the estimated half-life from a river is 3.7×10^6 hours (> 400 years). Measured half-lives for AIBN ranged from 210-304 days at 25ºC, and were dependent upon pH. It is expected that the modeled value for AMBN will more closely parallel the measured results for AIBN when more reliable model input data are available for AMBN. The bioconcentration factor (BCF) for AMBN was estimated as 185.7 (log BCF = 2.269). Therefore, AMBN is estimated to have a high to moderate potential for persistence and a moderate potential for bioaccumulation. The BCF for AIBN was estimated as 1.403 (log BCF = 0.147). Therefore it is estimated to have a high potential for persistence and a low potential for bioaccumulation. No biodegradation information was available for AMBN; however, the experimentally determined biodegradation of AIBN, the structurally similar analog, was 7% in 28 days and 15% in 110 days. The fugacity model predicts that both AMBN and AIBN will distribute primarily to the soil when emissions to soil are combined with air, sediment, and water, and to water when emissions are to water only. The rate constant for the reaction of AMBN vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be 2.97×10^{-12} cm^3/molecular-sec, which corresponds to a reaction half-life of 3.6 days. AIBN has a reaction half-life of 15.99 days when tested with photochemically generated hydroxyl radicals in the atmosphere. The comparability of modeled environmental fate parameters for AMBN and AIBN is limited by the differences in input parameters of the models. Many input parameters for AMBN were themselves values derived from modeling, whereas measured values were available for AIBN in most cases. **Since the models for the environmental fate of AMBN were run using physical and chemical characteristics of unknown reliability, models for the environmental fate endpoints will be re-run using the newly acquired AMBN measured data.**

Table 3: Environmental Fate

	AMBN	AIBN
Bioaccumulation*	Moderate potential for bioaccumulation BCF = 185.7	Low potential for bioaccumulation BCF = 1.403
Biodegradation	No Data	Not readily biodegradable
Fugacity*	When released 100% to air: Air 0.00302% Water 6% Soil 93.7% Sediment 0.0758% When released 100% to water: Air 1.98×10^{-8}% Water 98% Soil 0.000614% Sediment 1.2% When released 100% to soil: Air 3.76×10^{-7}% Water 3% Soil 96.3% Sediment 0.0449%	When released 100% to air: Air 31.0% Water 40.9% Soil 27.9% Sediment 0.2% When released 100% to water: Air 0.5% Water 98.6% Soil 0.5% Sediment 0.4% When released 100% to soil: Air 0.7% Water 28.6% Soil 70.6% Sediment 0.1%
* Modeled data.		

No information regarding aquatic toxicity to fish, invertebrates, or plants are available for AMBN. However, data are available for the structurally similar compound, AIBN, which is of low aquatic concern. Based on nominal concentration data, statistically derived results indicate a 96-hour LC_{50} of 580 mg/L in fish, and a 48-hour EC_{50} of 397 mg/L in *Daphnia* (greater than water solubility). A 72-hour EC_{50} of > 9.4 mg/L in algae was reported for AIBN (dispersed with DMF). The reported water solubility of the analog chemical, AIBN, is greater than 9.4 mg/L, which was the upper limit tested in the algae study. Therefore, an algae test following OECD Guideline 201 will be conducted with AMBN at 100 mg/L or at the limit of solubility in algae medium, whichever is lower.

Table 4: Ecotoxicity

	AMBN	AIBN
Toxicity to Fish (LC_{50} value)	No Data	580 mg/L (96-hour; nominal)
Toxicity to Invertebrates (EC_{50} value)	No Data	397 mg/L (48-hour; nominal)
Toxicity to Algae (EC_{50} value)	No Data	> 9.4 mg/L (72-hour)

AMBN and its analog, AIBN, are similar in regard to their acute mammalian toxicity. Both compounds were moderately toxic orally with acute oral toxicity values of 337 and 360 mg/kg for AMBN and AIBN, respectively. AMBN had a 4-hour inhalation acute lethal concentration (ALC) of > 8.9 mg/L, while AIBN had a 1-hour inhalation LC_{50} of > 7.78 mg/L. Neither test substance was a skin irritant nor a skin sensitizer. AIBN was not an eye irritant, while AMBN produced some irritation that cleared within 24 hours. **All required SIDS acute toxicity data points are complete for both azonitriles, and no further acute mammalian testing is recommended.**

Table 5: Acute Mammalian Toxicity

	AMBN	AIBN
Oral LD_{50} (rat)	337 mg/kg	360 mg/kg
Inhalation LC_{50} (rat)	>8.9 mg/L (4-hour)	> 7.78 mg/L (1-hour)
Dermal LD_{50} (rabbit)	No Data	5010-7940 mg/kg
Dermal Irritation	Not irritating	Not irritating
Eye Irritation	Irritation effects observed only at 1 hour after dosing	Not irritating
Dermal Sensitization	Not a sensitizer	Not a sensitizer

No information regarding repeated dose, developmental, or reproductive toxicity was available for AMBN. An OECD combined repeated dose and developmental/reproductive toxicity study in rats was performed with AIBN at doses of 0, 2, 10, and 50 mg/kg/day (Table 6). Kidney effects which included increases in eosinophilic bodies and basophilic changes of the renal

tubular epithelial cells in the kidneys were observed only in treated male rats. Accumulation of α_{2u}-macroglobulin was suspected as a cause of the male specific renal toxicity. Liver effects, including increased liver weight and centrilobular hypertrophy of hepatocytes was observed in both males and females at 10 and 50 mg/kg/day. The NOAEL was considered to be 2 mg/kg/day for the repeated dose study. The only reproductive effect was a reduction in viability and body weight of offspring after birth at 50 mg/kg/day, which was reported as most likely due to maternal toxicity. Therefore, the reproductive NOAEL was considered to be 50 mg/kg/day. No morphological abnormalities were observed in pups at any level. Liver effects were also observed in a 90-day oral toxicity study in dogs at doses of 150 and 300 ppm. Similar effects were observed in a 2-week inhalation study in rats at 80.0 mg/m^3, however, the liver effects were not detected in these rats following a 14-day recovery period. With the similarities in physical/chemical properties and acute toxicity, AMBN is expected to produce toxicological findings similar to that of AIBN. Since the database for repeated dose, developmental, and reproductive toxicity satisfies the HPV requirements for AIBN, further toxicity testing with AMBN is unlikely to provide new information on the azonitriles sufficient to warrant such testing. **Therefore, no further repeated dose, developmental, or reproductive toxicity testing is recommended.**

Table 6: Repeated Dose, Developmental, and Reproductive Toxicity

	AMBN	**AIBN**
Repeated Dose Toxicity (NOAEL)	No Data	2 mg/kg/day in a repeated dose rat study 50 ppm in a 90-day dog study 10 mg/m^3 in a 2-week inhalation study
Developmental Toxicity (NOAEL)	No Data	50 mg/kg/day
Reproductive Toxicity (NOAEL)	No Data	10 mg/kg/day (parental generation) 50 mg/kg/day (F_1 offspring)

Genetic toxicity data are similar between the two substances (Table 7). Neither AMBN nor AIBN induce mutations in bacteria. AIBN was not clastogenic when tested in an *in vitro* study in Chinese hamster lung cells. Neither AMBN nor AIBN was active when tested in an *in vivo* mouse micronucleus study. **Therefore, no further genetic toxicity testing is recommended.**

Table 7: Genetic Toxicity

	AMBN	AIBN
Mutagenicity	Not mutagenic (Ames test)	Not mutagenic (Ames test)
Clastogenicity	Not clastogenic (*in vivo* mouse micronucleus assay)	Not clastogenic (Chromosomal aberration test in CHL/IU cells; *in vivo* mouse micronucleus assay)

In the absence of available literature, a model was used to determine potential metabolic pathways for AMBN and AIBN. The predicted metabolic pathways are based on the metabolic behavior of the isolated component substructures. Since the effects of substructure connectivity on metabolic behavior of these azonitriles are unknown, the likelihood and/or prevalence of any given reaction cannot be predicted with certainty.

AMBN

Potential initial pathways for metabolism of AMBN include hydroxylation of the methyl groups to primary alcohols and *N*-oxidation of the azo moiety to an *N*-oxide. *N*-dealkylation of the azo moiety is unlikely, due to the absence of an abtractable hydrogen on the α carbon. Examples of hydroxylation of methyl groups situated β to a nitrogen function are abundant in the literature. The primary alcohol may be further oxidized to a carboxylic acid, which occurs *via* an intermediate aldehyde. The carboxylic acid may be eliminated unchanged, or may be conjugated to glucuronic acid prior to excretion. Glucuronidation is likely to be a more significant pathway at high exposure concentrations. In the case of AMBN there are two non-equivalent methyl groups, and from steric considerations hydroxylation of the terminal methyl group would likely predominate over hydroxylation of the α methyl group. In addition to these pathways, AMBN contains a methylene carbon, which may undergo hydroxylation and subsequent oxidation of the resultant secondary alcohol. Formation of *N*-oxides from 1,2-dialkylazo compounds occurs during metabolism of symmetrical and non-symmetrical dialkylhydrazines. Examples include dimethylhydrazine and procarbazine. Further metabolism of the azoxy metabolite of AMBN seems unlikely, due to the lack of an α proton.

AIBN

Similar to AMBN, biotransformation of AIBN may involve methyl hydroxylation and proceed through carboxylic acid formation and glucuronic acid conjugation. Likewise, N oxidation of the azo function is also possible with AIBN. As with AMBN, further metabolism of the azoxy metabolite of AIBN seems unlikely, due to the lack of an α proton.

In summary, biotransformation pathways for AMBN and AIBN are predicted to be very similar, differing primarily in the possibility of methylene oxidation in the case of AMBN.

Human Exposure Assessment

AMBN and its analogous compound, AIBN are solid free-radical initiators used industrially in polymerization reactions. Although the products have slightly different properties, they may, in most cases, be used interchangeably. There are no direct consumer uses of these products. Both compounds decompose when exposed to heat, releasing nitrogen gas and carbon-centered radicals. End-use applications include acrylics, resins, industrial polymers, and foams. The materials react rapidly and completely; thus, neither is recognizable in end-use products, and consumer exposure is unlikely. Transport of dry product in temperature-controlled containers is required for shipment of any amount greater than 100 grams. Exposure to either material would not occur during shipping, unless container integrity is compromised.

During manufacturing uses, the most likely exposure is to skin, with some potential of airborne exposure during material transfer operations. Specific manufacturing procedures and industrial hygiene programs in place at manufacturing sites limit the potential for employee exposure. DuPont has set an Acceptable Exposure Limit (AEL) of 1 mg/mg^3 TWA for both AMBN and AIBN. All sites that produce and use these compounds have safety, health, and environmental practices and procedures in place, and utilize engineering controls, environmental controls, and personal protective equipment to manage the risk of exposure above recommended limits. The major manufacturers practice Responsible Care®, and DuPont has a program to assess the ability of potential customers to safely handle the materials prior to commencing a commercial relationship. This assessment includes reviews and audits of PPE (personal protective equipment), safety equipment and procedures, structural integrity, and safety practices.

Conclusion

The use of AIBN data to supplement the existing data for AMBN is supported by the similarities in molecular structure, reactivity, production, physical/chemical characteristics, structure-activity predictions of metabolism, toxicity, and potential human exposure for these two azonitriles. AMBN and AIBN are nearest analogs, have the same functional groups, and are essentially chemically equivalent. The use of AIBN as an analog to AMBN is consistent with the Agency's directive to HPV participants to maximize the use of scientifically appropriate data for related chemicals. Although differences between AMBN and AIBN due to different rates of reaction and chemical structure may be expected, we believe these differences to be minimal and insufficient to warrant additional animal testing. Generation of the additional physical/chemical property data, as summarized in the following test plan, will support refined modeling of the fate of AMBN in the environment.

TEST PLAN FOR AMBN

	Acceptable Data for AIBN (CAS No. 78-67-1)		Acceptable Data for AMBN (CAS No. 13472-08-7)	Testing Recommended for AMBN
	Y/N		**Y/N**	**Y/N**
PHYSICAL/CHEMICAL CHARACTERISTICS				
Melting Point	Y		N	Y
Boiling Point	N/A		N/A	N/A
Vapor Pressure	Y		N	Y
Partition Coefficient	Y		N	Y
Water Solubility	Y		N	Y
ENVIRONMENTAL FATE				
Photodegradation	Y		N	Y[1]
Stability in Water	Y		N	Y[1]
Transport (Fugacity)	Y		N	Y[1]
Biodegradation	Y		Y[2]	N
ECOTOXICITY				
Acute Toxicity to Fish	Y		Y[2]	N
Acute Toxicity to Invertebrates	Y		Y[2]	N
Acute Toxicity to Aquatic Plants	N		N	Y
MAMMALIAN TOXICITY				
Acute Toxicity	Y		Y	N
Repeated Dose Toxicity	Y		Y[2]	N
Developmental Toxicity	Y		Y[2]	N
Reproductive Toxicity	Y		Y[2]	N
Genetic Toxicity Bacterial Gene Mutations	Y		Y	N
Genetic Toxicity Chromosomal Aberrations	Y		Y	N

N/A = Not Applicable.
[1] Modeled data will be re-run after completion of re-analysis of physical/chemical characteristics.
[2] Data is available for the analog AIBN.

24 October 2002

APPENDIX A

ROBUST SUMMARY FOR BUTANENITRILE, 2,2'-AZOBIS(2-METHYL- (AMBN)

CAS NO. 13472-08-7

24 October 2002

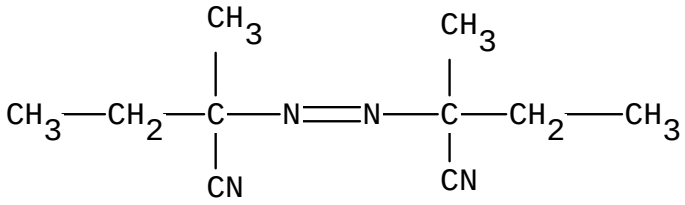
The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as related references in this document.

1.0 Substance Information

CAS Number: 13472-08-7

Chemical Name: Butanenitrile, 2,2'-azobis(2-methyl-

Structural Formula:



Other Names:
2,2'-Azobismethylethylacetonitrile
2,2'-Azobis-2-methylbutyronitrile
2,2'-Asodi(2-methylbutyronitrile)
2,2'-Azobis(2-cyanopentane)
2,2-Azobisisovaleronitrile
2,2'-Azobis(α-methylbutyronitrile)
2,2'-Dimethyl-2,2'-azodibutyronitrile
Azocatalyst M
Azostarter V 59
V 59
Perkadox AMBN
Vazo® 67
Vazo 64-A
Wako V 59

Exposure Limits: 1 mg/m^3, 8-hour TWA and 0.7 mg/m^3, 12-hour TWA: DuPont Acceptable Exposure Limit (AEL)

2.0 Physical – Chemical Properties

2.1 Melting Point

Value:	45°C
Decomposition:	Decomposition can be violent. Rapid decomposition releases nitrogen in potentially sufficient quantities to result in hazardous pressures or oxygen-deficient atmospheres in tightly confined spaces. Decomposition at temperatures above the Self-Accelerating Decomposition Temperature (SADT), 50°C, can be very rapid.
Pressure:	No Data

Method:	Not Available
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000905 (March 28).
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point: Not Applicable.

2.3 Density

Value:	Specific gravity = 1.1; bulk density = 25 lbs/ft^3
Temperature:	No Data
Method:	Not Available
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000905 (March 28).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Density:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

2.4 Vapor Pressure

Value:	Negligible at room temperature.
Temperature:	No Data
Decomposition:	No Data
Method:	Not Available
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000905 (March 28).
Reliability:	Not assignable because limited study information was available.

Value:	8.9×10^{-2} Pa
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated using the modified Grain method.
GLP:	Not Applicable
Reference:	SRC MPBPWIN v1.40 in EpiWin v3.05.

	Syracuse Research Corporation (MPBPWIN) program estimates the vapor pressure using the modified Grain method. A description of the methodology is detailed in: Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

Additional Reference for Vapor Pressure: None Found.

2.5 Partition Coefficient (log Kow):

Value:	3.86
Temperature:	No Data
Method:	Modeled. The KOWWIN computer program, version 1.66 from Syracuse Research Corporation, calculates the Log octanol/water partition coefficient (log Kow) of organic chemicals using an atom/fragment contribution method.
GLP:	Not Applicable
Reference:	The methodology is described in the following journal article: Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient: None Found.

2.6 Water Solubility

Value:	< 1 wt% (< 10 g/L)
Temperature:	No Data
pH/pKa:	No Data
Method:	Not Available
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000905 (March 28).
Reliability:	Not assignable because limited study information was available.

Value:	4.9 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Modeled

GLP: Not Applicable
Reference: WsKow v1.4 in EpiWin v3.05 (SRC Database).

WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following journal articles describe the estimation methodology:

Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.

Meylan, W. M. and P. H. Howard (1994). Upgrade of PCGEMS Water Solubility Estimation Method (May 1994 Draft); prepared for Robert S. Boethling, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, Syracuse, NY 13210.

Meylan, W. M. and P. H. Howard (1994). Validation of Water Solubility Estimation Methods Using Log Kow for Application in PCGEMS & EPI (Sept 1994, Final Report); prepared for Robert S. Boethling, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, Syracuse, NY 13210.
Reliability: Estimated value based on accepted model.

Additional Reference for Water Solubility:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

2.7 Flash Point: Not Applicable.

2.8 Flammability

Results: Flammable limits in air, % by volume: LEL = 0.034 g/L, UEL = Not determined

Autoignition Temperature = 185°C
Method: Not Available
GLP: Unknown
Reference: DuPont Co. (2000). Material Safety Data Sheet No. DU000905 (March 28).

Reliability: Not assignable because limited study information was available.

Additional Reference for Flammability:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	Not Applicable
Indirect Photolysis:	OH Half-life = 3.605 days (12-hour day; concentration of OH radicals =$1.5x10^6$ OH/cm^3).
Breakdown Products:	No Data
Method:	Calculated by AOP Computer Program, Vers. 1.90, Syracuse Research Corporation. The AOP Program, Version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and coworkers (Atkinson et al., 1987; 1995; 1996; 1984). The AOP Program is described in Meylan and Howard, 1993.
GLP:	Not Applicable
Reference:	Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19:799-828. Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695. Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334. Atkinson, R. et al. (1984). Chem. Rev., 84:437-470. Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-2299.
Reliability:	Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration:	Not Applicable
Half-life:	Estimated half-life for a model river is 422.9 years.
% Hydrolyzed:	Not Applicable
Method:	The Henry's Law constant for butanenitrile, 2,2'-azobis(2-methyl- (Vazo® 67) is estimated to be 2.19×10^{-10} atm-m^3/mole (Henry v3.10 Program, Bond SAR Method in SRC Epiwin v3.05) from its estimated vapor pressure (6.7×10^{-4} mm Hg; MPBPWIN v1.40) and estimated water solubility (4.905 mg/L; WSKOW v1.40). Based on this Henry's Law constant, the estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately 422.9 years. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 4613 years (Epiwin v. 3.05).
GLP:	Not Applicable
Reference:	Syracuse Research Corporation Epiwin Version 3.05.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media: Air, Water, Soil, Sediment

Distributions:

Compartment	Released 100% to air	Release 100% to water	Release 100% to soil
Air	0.00302%	1.98×10^{-8}%	3.76×10^{-7}%
Water	6%	98%	3%
Soil	93.7%	0.000614%	96.3%
Sediment	0.0758%	1.2%	0.0449%

Adsorption Coefficient:	Not Applicable
Desorption:	Not Applicable
Volatility:	Not Applicable
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA Model defaults.

Data Used:
Molecular Weight: 192.27
Henry's Law Constant: 2.19×10^{-10} atm-m^3/mole (model

calculation)
Vapor Pressure: $6.7x10^{-4}$ mm Hg (MPBPWIN v1.40)
Log Kow : 3.86 (KOWWIN v1.66)
Soil Koc : 200.9 (PCKOCWIN v1.66)

GLP: Not Applicable

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

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

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

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

Reliability: Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation: No Data.

3.5 Bioconcentration

Value: 185.7 (Log BCF = 2.269)

Method: Calculated by BCFWIN Computer Program, Vers. 2.14, Syracuse Research Corporation (based on reference below).

GLP: Not Applicable

Reference: The estimation methodology used by BCFWIN is described in the following document prepared for the U. S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2^{nd} Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.

Reliability: Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish: No Data.

4.2 Acute Toxicity to Invertebrates: No Data.

4.3 Acute Toxicity to Aquatic Plants: No Data.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Acute Oral Toxicity**
Species/Strain:	Rat/Sprague-Dawley CD
Value:	337 mg/kg
Method:	OECD 401; doses administered were 202, 254, 320, and 402 mg/kg.
GLP:	Yes
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Perkadox AMBN), purity 98.5%
Results:	The incidence of mortality was 0, 0, 50, and 80% at 202, 254, 320, and 402 mg/kg. All mortality occurred by day 2. Clinical signs of toxicity, which were seen in surviving and dead animals at all dose levels, included lethargy, staggered gait, muscle tremor, piloerection, salivation, and hunched posture. The surviving animals had no clinical signs of toxicity by day 6. The gross necropsy of dead animals showed abnormal gastrointestinal contents and a single observation of dark areas on the glandular mucosa of the stomach. There were no significant changes observed in the gross necropsy of surviving animals.
Reference:	Akzo Chemicals International BV (1991). Unpublished Data, "Perkadox AMBN: Acute Oral Toxicity Study In The Rat" (8/5/91).
Reliability:	High because a scientifically defensible and guideline method were used.

Additional Reference for Acute Oral Toxicity:

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

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 577-78.

Type:	**Inhalation ALC**
Species/Strain:	Male rats/Crl:CD®
Exposure Time:	4 hours
Value:	>8.9 mg/L
Method:	Groups of 6 rats (7-8 weeks old) were exposed nose-only for single, 4-hour periods to dust atmospheres of the test substance in air at concentrations of 1.8, 3.7, and 8.9 mg/L (the highest concentration that could be generated). Rats were weighed and observed daily for 14 days post exposure, weekends included when deemed necessary. Dust atmospheres were generated and calibrated volumes of test atmosphere were drawn through pre-weighed glass fiber filters. Atmospheric concentration was determined from filter weight gain. Percent and mass median diameter of respirable particulate were determined during each exposure. Chamber temperature was monitored.
GLP:	No
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Vazo® 67), purity >98%
Results:	No mortality was observed at any exposure level tested. The % respirable particulates <10 μm was 11, 25 or 31, and 24 at 1.8, 3.7, and 8.9 mg/L, respectively. The % respirable particulates <5 μm was 2.0, 8.2 or 10, and 8.2 at 1.8, 3.7, and 8.9 mg/L, respectively. The mass median diameter of respirable particulate (μm), calculated for particles less than 10 μm, was 6.8 or 7.5, and 5.1 at 3.7 and 8.9 mg/L, respectively. The mass median diameter of respirable particulate for the 1.8 mg/L group could not be calculated. All rats exhibited slight to severe weight loss 1 day post-exposure. At 8.9 mg/L, 1 rat continued to lose weight for 1 more day. Weight loss was followed by normal weight gain. Rats exposed to 1.8 and 3.7 mg/L exhibited red to brown ocular and/or nasal discharge for 1 day post-exposure. No other adverse clinical signs were observed.
Reference:	DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 368-83.
Reliability:	Medium because a suboptimal study design was used. Only a small percentage of particles in the exposure atmospheres were of respirable size.

Additional References for Acute Inhalation Toxicity: None Found.

Type: **Dermal Toxicity:** No Data.

Type:	**Dermal Irritation**
Species/Strain:	Rabbits/New Zealand White
Method:	OECD 404. A 0.5 g sample was applied directly to the skin, and covered by a gauze patch, for a 4-hour exposure period. The control site was covered by a similar semi-occlusive dressing.
GLP:	Yes
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Perkadox AMBN), purity 98.5%
Results:	There was no irritation seen in any of the three animals used in the study during the 72-hour observation period.
Reference:	Akzo Chemicals International BV (1991). Unpublished Data, "Perkadox AMBN: Acute Dermal Irritation/Corrosion Test In The Rabbit" (7/26/91).
Reliability:	High because a scientifically defensible and guideline method was used.

Additional References for Dermal Irritation:

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

DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 513-80.

DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 511-80.

Type:	**Dermal Sensitization**
Species/Strain:	Guinea pigs/Duncan Hartley
Method:	The primary irritation test was conducted on 10 guinea pigs by applying 0.05 mL of an 80% and an 8% suspension of the test substance in dimethyl phthalate (DMP) on shaved, intact shoulder skin. The induction phase for sensitization was a series of 4 sacral intradermal injections of 0.1 mL of a 1.0% suspension in DMP, 1 each week beginning 2 days after the test for primary irritation. After a 13-day rest period, the test guinea pigs were challenged for sensitization by applying and lightly rubbing in 0.05 mL of an 80% and an 8% suspension of the test substance in DMP on shaved intact shoulder skin. At the same time 10 unexposed guinea pigs (controls) of the same age received identical topical application. Reactions were observed at 24 and 48 hours.
GLP:	No

Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Vazo® 67), purity 100%
Results:	The test substance caused no irritation on shaved intact skin of guinea pigs at 24 or 48 hours. None of the test guinea pigs showed a sensitization response.
Reference:	DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 511-80.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Male rabbits/Albino
Method:	The solid test substance (28.4 mg) was placed into the right conjunctival sac of each of 2 male albino rabbits. After 20 seconds, 1 treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris, and conjunctiva were made with a hand-slit lamp at 1 and 4 hours, and at 1, 2, and 3 days. Fluor-i-strip® stain and a biomicroscope were used at examinations after the day of treatment.
GLP:	No
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Vazo® 67), purity 100%
Results:	The test substance produced no corneal, iritic, or conjunctival effects at any time when tested in rabbit eyes.
Reference:	DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 514-80.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**Eye Irritation**
Species/Strain:	Rabbits/New Zealand White
Method:	OECD 404. A 0.1 g sample was instilled into the right eye of the animals. The left eye was untreated.
GLP:	Yes
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Perkadox AMBN), purity 98.5%
Results:	There was no irritation seen in any of the three animals used in the study at the 24-hour observation period until the end of the study (72-hour observation period). There was irritation of the conjunctiva and slight chemosis seen in all animals, and iritis seen in two animals at the 1-hour observation period.
Reference:	Akzo Chemicals International BV (1991). Unpublished

	Data, "Perkadox AMBN: Acute Eye Irritation Test In The Rabbit" (8/5/91).
Reliability:	High because a scientifically defensible and guideline method was used.

Additional References for Eye Irritation: None Found.

5.2 Repeated Dose Toxicity: No Data.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Assay**
Tester Strains:	*Salmonella typhmurium* strains TA98, TA100, TA1535, TA1537
Exogenous Metabolic Activation:	Rat liver S-9
Exposure Concentrations:	50-5000 μg/plate
Method:	OECD 471. Positive controls used were benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, 9-aminoacridine, and sodium azide. The solvent was DMSO.
GLP:	Yes
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl- (Perkadox AMBN), purity 98.5%
Results:	Negative
Remarks:	No evidence of mutagenic activity was detected, with or without metabolic activation.
Reference:	Akzo Chemicals International BV (1991). Unpublished Data, "Perkadox AMBN: Assessment Of Mutagenic Potential In Histidine Auxotrophs Of *Salmonella Typhimurium* (The Ames Test)" (7/25/91).
Reliability:	High because a scientifically defensible and guideline method was used.

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

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

Takenaka, S. I. et al. (1993). J. Toxicol. Sci., 18(4):418.

Type:	***In vitro* Clastogenicity Studies:** No Data.
Type:	***In vivo* Mouse Micronucleus Assay**
Species/Strain:	Mice/ddY
Sex/Number:	Male
Route of Administration:	Oral
Concentrations:	Not Available
Method:	The micronucleus test using acridine orange staining method was performed in male mice (8-weeks old) following double oral administration.
GLP:	Unknown
Test Substance:	Butanenitrile, 2,2'-azobis(2-methyl-, purity not specified
Results:	Negative
Remarks:	At 24 and 48 hours after treatment, the test substance did not produce a significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of the treated mice.
Reference:	Takenaka, S. I. et al. (1993). J. Toxicol. Sci., 18(4):418.
Reliability:	Not assignable because limited study information was available.

Additional References for *In vivo* Studies: None Found.

24 October 2002

APPENDIX B

ROBUST SUMMARY FOR PROPANENITRILE, 2,2'-AZOBIS(2-METHYL- (AIBN)

CAS NO. 78-67-1

24 October 2002

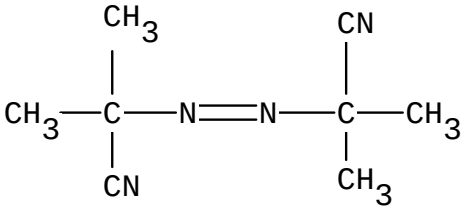
AIBN is exempt from the HPV program because it has already been evaluated through the Organization of Economic Cooperation and Development (OECD) high production volume (HPV) program. A SIDS Initial Assessment Report (SIAR) was prepared for evaluation by the Ninth SIAM convened in France June 29 through July 1, 1999. The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as related references in this document.

1.0 Substance Information

CAS Number: 78-67-1

Chemical Name: Propanenitrile, 2,2'-azobis(2-methyl-

Structural Formula:



Other Names:
Vazo® 64
Alpha, alpha'-azobis(isobutyronitrile)
Alpha,alpha'-azodiisobutyronitrile
Alpha,alpha'-azodiisobutyric acid dinitrile
Azobis(isobutyronitrile)
Azodiisobutyronitrile
Azodiisobutyrodinitrile
2,2'-Azobis(2-methylpropionitrile)
2,2'-Azo-bis(isobutyronitrile)
2,2'-Dicyano-2,2'-azopropane
2,2'-Dimethyl-2,2'-azopropionitrile
Aceto AZIB
Aceto AZDH
Aceto AZDN
AIBN
Genitron®
Genitron® AZDN
Pianofor AN
Porofor N
Porofor-57
Purifier N

Exposure Limits: 1 mg/m^3, 8-hour TWA and 0.7 mg/m^3, 12-hour TWA: DuPont Acceptable Exposure Limit (AEL)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	100-103ºC
Decomposition:	No
Sublimation:	No
Pressure:	No Data
Method:	No Data
GLP:	No
Reference:	MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Melting Point:

DuPont Co. (2000). Material Safety Data Sheet No. B0000109 (March 28).

2.2 Boiling Point: Not Applicable.

2.3 Density

Value:	Specific gravity = ~ 1.1; bulk density = ~25 lbs/ft^3
Temperature:	No Data
Method:	Not Available
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. B0000109 (March 28).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Density:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

2.4 Vapor Pressure

Value:	8.1×10^{-1} Pa
Temperature:	25ºC
Decomposition:	No Data
Method:	OECD Guideline 104

	The purity of the test substance was 99.6%.
GLP:	Yes
Reference:	MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Value:	$1.9x10^{-1}$ Pa
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated using the modified Grain method.
GLP:	Not Applicable
Reference:	SRC MPBPWIN v1.40 in EpiWin v3.05. Syracuse Research Corporation (MPBPWIN) program estimates the vapor pressure using the modified Grain method. A description of the methodology is detailed in: Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

Additional References for Vapor Pressure:

DuPont Co. (2000). Material Safety Data Sheet No. B0000109 (March 28).

2.5 Partition Coefficient (log Kow)

Value:	1.10
Temperature:	25ºC
Method:	OECD Guideline 107; purity of the test substance was 98%.
GLP:	Yes
Reference:	MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

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

2.6 Water Solubility

Value:	350 mg/L (slightly soluble)
Temperature:	25ºC
pH/pKa:	No Data
Method:	OECD Guideline 105; purity of the test substance was 99.6%.
GLP:	Yes
Reference:	MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Value:	851.1 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Modeled
GLP:	Not Applicable
Reference:	WsKow v1.4 in EpiWin v3.05 (SRC Database).
	WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following journal article describes the estimation methodology:
	Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Water Solubility:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

DuPont Co. (2000). Material Safety Data Sheet No. B0000109 (March 28).

2.7 Flash Point: Not Applicable

2.8 Flammability

Results:	Flammable limits in air, % by volume: LEL = 0.02 g/L, UEL = Not determined
	Autoignition Temperature = 295°C

Method:	Not Available
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. B0000109 (March 28).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Flammability:

DuPont Co. (n.d.). Vazo® Polymerization Initiators: Properties, Uses, Storage, and Handling (also cited in TSCA Fiche OTS0000937).

3.0 Environmental Fate

3.1 Photodegradation:

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	Not Applicable
Indirect Photolysis:	OH Half-life = 15.99 days (12-hour day; concentration of OH radicals =$1.5x10^6$ OH/cm^3).
Breakdown Products:	No Data
Method:	Calculated by AOP Computer Program, Vers. 1.90, Syracuse Research Corporation. The AOP Program, Version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and coworkers (Atkinson et al., 1987; 1995; 1996; 1984). The AOP Program is described in Meylan and Howard, 1993.
GLP:	Not Applicable
Reference:	Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19:799-828. Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695. Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334. Atkinson, R. et al. (1984). Chem. Rev., 84:437-470. Meylan, W. M. and P. H. Howard (1993). Chemosphere,

26:2293-2299.
Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration: No Data
Half-life: 263 days @ pH 4 and 25ºC
304 days @ pH 7 and 25ºC
210 days @ pH 9 and 25ºC
% Hydrolyzed: No Data
Method: OECD Guideline 111; purity of the test substance was 99.6%.
GLP: Yes
Reference: MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media: Air, Water, Soil, Sediment
Distributions:

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	31.0%	0.5%	0.7%
Water	40.9%	98.6%	28.6%
Soil	27.9%	0.5%	70.6%
Sediment	0.2%	0.4%	0.1%

Adsorption Coefficient: No Data
Desorption: No Data
Volatility: No Data
Method: Fugacity Level III
GLP: Not Applicable
Reference: MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability: Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value:	Propanenitrile, 2,2'-azobis(2-methyl- (Perkadox AIBN) biodegraded 7% at day 28 (with silica gel). There was no biodegradation at day 20. The biodegradation only slightly increased to about 15% in the prolonged study of approximately 110 days.
Breakdown Products:	Not Applicable
Method:	OECD Guideline 301. Secondary activated sludge was used as the inoculum. The concentration of the test substance used was 0.7 mg/L. The vehicle was dichloromethane.
GLP:	Yes
Reference:	Akzo Nobel Chemicals (n.d.). Unpublished Data, "Biodegradability Of Perkadox AIBN In The Closed Bottle Test."
Reliability:	High because a scientifically defensible and guideline method was used.

Additional Reference for Biodegradation:

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

MITI, JAPAN (n.d.). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

3.5 Bioconcentration

Value:	1.403 (Log BCF = 0.147)
Method:	Calculated by BCFWIN Computer Program, Vers. 2.14, Syracuse Research Corporation (based on reference below).
GLP:	Not Applicable
Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U. S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2nd Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil

	Gouchie; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-Hour LC_{50}**
Species:	*Brachydanio rerio* (Zebra fish)
Value:	580 mg/L (based on nominal test concentrations)
Method:	OECD Guideline 203. Fish (7/dose group) were exposed to 62.5, 125, 250, 500, or 1000 mg/L under semi-static conditions. The temperature was 22.5-23.5°C. The oxygen concentrations were 8.6-8.9 mg/L. The pH ranged from 7.9-8.2. The water hardness was 12°dH. The fish had an average size of 3.1 cm and an average weight of 0.31 g.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl- (Perkadox AIBN), purity 99.2%
Results:	There were no mortality or signs of toxicity observed at concentrations of 62.5, 125, and 250 mg/L. There was 29% mortality at 500 mg/L and 100% mortality at 1000 mg/L. The NOEC was 250 mg/L.
Reference:	Akzo Nobel Chemicals (1996). Unpublished Data, "Acute Toxicity Of Perkadox AIBN To The Freshwater Fish *Brachydanio Rerio*" (3/21/96).
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Additional References for Acute Toxicity to Fish:

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

DuPont Co. (1997). Unpublished Data, Haskell Laboratory Report No. 1997-01184.

Environment Agency of Japan (1996). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

4.2 Acute Toxicity to Invertebrates

Type:	**48-hour EC_{50}**
Species:	*Daphnia magna*
Value:	397 mg/L (95% confidence interval, 195-811 mg/L)
Method:	*Daphnia magna* were exposed to the test substance in a static, acute 48-hour screening test. Nominal concentrations tested were 0, 0.5, 1.0, 50, 500, and 5000 mg/L, with replicate test chambers used at each dose level. Dissolved oxygen and pH were reported at test initiation (0 hours) and test completion (48 hours).
GLP:	No
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl- (Vazo® 64) purity not specified
Results:	The test substance exhibited slight toxicity in a 48-hour, unaerated, static acute test using *Daphnia magna*. Based on visual observations, the water control solution was clear and had no color, and the 0.5, 1.0, 50, 500, and 5000 mg/L test solutions all had undissolved test material present throughout the test. Immobilities were 0, 0, 0, 0, 60, and 100% at 0, 0.5, 1.0, 50, 500, and 5000 mg/L, respectively. All water quality parameters were within acceptable limits. Dissolved oxygen at test initiation and completion was 8.4 mg/L. The pH ranged from 7.7-7.8 and 7.9-8.2 at test initiation and completion, respectively.
Reference:	DuPont Co. (1997). Unpublished Data, Haskell Laboratory Report No. 1997-01185.
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Additional References for Acute Toxicity to Invertebrates:

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

Environment Agency of Japan (1995). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

Service Analyse Environment (France). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

4.3 Acute Toxicity to Aquatic Plants

Type:	**72-hour EC_{50} Biomass**
Species:	*Selenastrum capricornutum* ATCC 22662
Value:	> 9.4 mg/L
Method:	OECD Guideline 201 (1984) was performed. The EC_{50} value for growth rate (% inhibition) was calculated based on 5 measured concentrations (0.46, 0.71, 2.1, 4.2, and 9.4 mg/L). DMF of 100 mg/L was used as a solubilizer.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.3%
Results:	The NOEC was 4.2 mg/L.
Reference:	Environment Agency of Japan (1996). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Toxicity to Aquatic Plants:

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

Service Analyse Environment (France). (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral LD_{50}**
Species/Strain:	Rats/Sprague Dawley
Value:	360 mg/kg (95% confidence limits, 340-380 mg/kg)
Method:	Male and female Sprague Dawley rats (5/dose level) were given single oral doses of a 10.0% solution-suspension in corn oil at doses of 251, 316, 398, and 501 mg/kg. Clinical signs of toxicity were recorded. Survivors were killed 14 days later and gross autopsy was performed.
GLP:	No
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity not specified
Results:	Mortality was 0/5, 1/5, 4/5, and 5/5 at 251, 316, 398, and 501 mg/kg. Mortality occurred in 1 to 5 days, with most deaths within 2 days. Clinical signs of toxicity included reduced appetite and activity (2-3 days in survivors),

	increasing weakness, tremors, collapse, and death. Gross autopsy of animals that died revealed hemorrhagic areas of the lungs and liver, and acute gastrointestinal inflammation. The viscera appeared normal in survivors.
Reference:	Monsanto (1974). Younger Laboratories, Inc. Report No. Y-74-61 (TSCA Fiche OTS0545441).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Acute Oral Toxicity:

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

DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 27-62 (also cited in TSCA Fiche OTS0546516 and OTS0000937).

DuPont Co. (1947). Unpublished Data, Haskell Laboratory Report No. 25-47.

Budavari, S. et al. (eds.) (1989). The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals, p. 146, Merck & Co., Inc., Rahway, NJ.

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Eastman Kodak Co. (1960). TSCA Fiche OTS0555369.

Rusin, V. Y. (1958). Tr. Naunchn. Sess. Leningr. Nauchno. – Issled. Inst. Gig. Tr. Profzabol., pp. 247-251.

Type:	**Inhalation LC_{50}**
Species/Strain:	Male and female rats/ Crl:CD®
Exposure Time:	1 hour
Value:	> 7.78 mg/L
Method:	The method was in accordance with the International Maritime Dangerous Code (IMDG code, pg. 6003-1,2). Male and female Crl:CD® rats (10/exposure level) were exposed nose only to the test substance at concentrations of 1.57, 3.40, and 7.78 mg/L. All rats were weighed and observed daily for 2 weeks post-exposure, except for the Saturday and Sunday of the 2nd week post-exposure. At approximately 10-minute intervals, calibrated volumes of test atmospheres were drawn through pre-weighed glass fiber filters, and atmospheric concentrations were determined. Percent respirability (≤10 μm) was determined during each exposure. Percent respirability was 7.96, 10.0,

	and 6.65 at 1.57, 3.40, and 7.78 mg/L, respectively.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl- (Vazo® 64), purity >98%
Results:	One male rat died 1 day after exposure to 1.57 mg/L. No other deaths occurred throughout the study. Most rats exhibited moderate to severe weight losses 1 or 2 days after exposure, followed by a return to a normal weight gain rate. Approximately ½ of the rats exhibited wet or stained perineal areas for 1 to 2 days after exposure. Most females exhibited sporadic weight loss during the 2-week observation period. Seven of 10 female rats exposed to 7.78 mg/L had hair loss, mainly around the head, face, and forelegs. No male rats had hairloss at this concentration. Two males and 1 female had back or foreleg hair loss after exposure to 3.40 mg/L; no rats had hair loss after exposure to 1.57 mg/L. During exposures, rats' faces were covered with dust, which was removed from the fur after the exposure. A dried red discharge around the facial area was observed in some rats a day after exposure, but was not considered test substance-related.
Reference:	DuPont Co. (1984). Unpublished Data, Haskell Laboratory Report No. 196-84.
Reliability:	Medium because a suboptimal study design was used. Only a small percentage of particles in the exposure atmospheres were of respirable size.

Additional References for Acute Inhalation Toxicity:

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

DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 88-62 (also cited in TSCA Fiche OTS0000937).

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 40-81 (also cited in TSCA Fiche OTS0000937).

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Type:	**Dermal ALD**
Species/Strain:	Rabbits/New Zealand White
Exposure Time:	24 hours
Value:	5010-7940 mg/kg

Method:	The test substance was applied as a 40.0% solution-suspension in corn oil to the skin of rabbits (1 male or 1 female) for a 24-hour exposure. Survivors were killed 14 days later.
GLP:	No
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity not specified
Results:	The animal dosed with 5010 mg/kg survived, while the rabbit dosed with 7940 mg/kg died within 9 days. Clinical signs observed included reduced appetite and activity (4 days in the survivor), increasing weakness, collapse, and death. Gross autopsy of the rabbit that died revealed hemorrhagic areas of the lungs, liver hyperemia, enlarged gall bladder, discolored kidneys, and gastrointestinal inflammation. The viscera of survivors appeared normal.
Reference:	Monsanto (1974). Younger Laboratories, Inc. Report No. Y-74-61 (TSCA Fiche OTS0545441).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Acute Dermal Toxicity:

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

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Eastman Kodak Co. (1960). TSCA Fiche OTS0555369.

Rusin, V. Y. (1958). Tr. Naunchn. Sess. Leningr. Nauchno. – Issled. Inst. Gig. Tr. Profzabol., pp. 247-251.

Type:	**Dermal Irritation**
Species/Strain:	Rabbits/New Zealand White
Method:	OECD Guideline No. 404 and EC Guideline 92/69/E.E.C., B_4.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.2%
Results:	The test material was not irritating to rabbit skin.
Reference:	ELF Atochem (1996). Laboratory study number 14350 TSG (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Irritation:

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

Eastman Kodak Co. (1960). TSCA Fiche OTS0555369.

Monsanto (1974). Younger Laboratories, Inc. Report No. Y-74-61 (TSCA Fiche OTS0545441).

Data from these additional sources were not summarized because it was not the species of choice.

DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 88-62 (also cited in TSCA Fiche OTS0000937).

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Rusin, V. Y. (1958). Tr. Naunchn. Sess. Leningr. Nauchno. – Issled. Inst. Gig. Tr. Profzabol., pp. 247-251.

Type:	**Dermal Sensitization (MaximizationTest)**
Species/Strain:	Guinea pigs/Duncan Hartley
Method:	OECD Guideline No. 406 and EC Guideline 92/69/E.E.C., B_6.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.2%
Results:	The test substance was not sensitizing to guinea pigs.
Reference:	ELF Atochem (1996). Laboratory study number 14352 TSG (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**Human Patch Test**
Species/Strain:	Human
Method:	Patch testing was performed on 173 humans as described in Kanerva et al., 1988; Estlander, 1990; and Jolanki, 1991, with 2 days occlusion and 3 readings (usually on Days 2, 3, and 4-6). Allergic reactions were scored according to ICDRG recommendations, +, ++, and +++ reactions being

	considered allergic. Irritant reactions were also recorded. Reactions scored as doubtful (?+) or irritant (IR) were classified as irritant.
GLP:	Unknown
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity not specified
Results:	At a dose of 1.0% (w/w), the test substance produced no allergic reactions. It produced an irritant reaction in 1 of 173 humans (6%).
Reference:	Kanerva, L. et al. (1997). Contact Dermatitis, 37:301-302.
	Kanerva, L. et al. (1988). Int. Arch. Occup. Environ. Health, 60:89-94.
	Estlander, T. (1990). Acta Dermato-venereologica, Suppl. 155:1-84.
	Jolanki, R. (1991). Acta Dermato-venereologica, Suppl. 155:1-80.
Reliability:	Not assignable because limited study information was available.

Additional References for Dermal Sensitization:

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

DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 88-62 (also cited in TSCA Fiche OTS0000937).

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Kanerva, L. et al. (1999). Acta Dermato-Venereologica, 79(4):296-300 (BIOSIS/99/24592).

Type:	**Eye Irritation**
Species/Strain:	Rabbits/New Zealand White
Method:	OECD Guideline No. 405 and EC Guideline 92/69/E.E.C., B_5.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.2%
Results:	The test material was not irritating to the rabbit eye.
Reference:	ELF Atochem (1996). Laboratory study number 14351 TSG (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile),

	http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation:

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

DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 88-62 (also cited in TSCA Fiche OTS0000937).

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

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Monsanto (1974). Younger Laboratories, Inc. Report No. Y-74-61 (TSCA Fiche OTS0545441).

5.2 Repeated Dose Toxicity

Type:	**Combined Repeat Dose and Reproductive Toxicity Screening Test**
Species/Strain:	Rats/Crj:CD(SD)
Sex/Number:	Male and female/Number not specified
Exposure Period:	Males: 42 days Females: 14 days before mating to day 3 lactation
Frequency of Treatment:	Daily by gavage
Exposure Levels:	0, 2, 10, and 50 mg/kg/day
Method:	OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Tests Guideline 422.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity, 99.9%
Results:	*Males:* Temporary salivation was induced at ≥10 mg/kg. Decrease in body weight gain and food consumption was observed at 50 mg/kg/day. In the kidneys, absolute and relative weight was increased in all treatment groups, and in ≥ 10 mg/kg/day groups, respectively. In addition, increases in eosinophilic bodies and basophilic changes of the renal tubular epithelial cells were observed in all treatment groups and granular casts in the lower nephrons were observed at

≥ 10 mg/kg/day. As these pathological changes were observed only in males, accumulation of α_{2u}-macroglobulin was suspected as a cause of male specific renal toxicity. Liver weights were significantly increased by 14 and 66% for absolute weight (14 and 74% for relative weight) in the 10 and 50 mg/kg/day group, respectively. Centrilobular hypertrophy of hepatocytes was observed in the 10 and 50 mg/kg/day groups (±: 4 in 13, +:9 in 13 for 10 mg/kg, ++: 13 in 13 for 50 mg/kg, compared to no changes in the 0 and 2 mg/kg groups). In blood analysis, there were several changes at 50 mg/kg, such as an elevation of platelet and white blood cell counts, increases in total protein, albumin, total cholesterol, Ca, and inorganic phosphorus, and decreases in the A/G ratio and Cl concentration.

Females: One animal died on postpartum day 3 at 50 mg/kg/day. Decrease in body weight gain and food consumption was observed at ≥ 10 mg/kg/day. In the kidneys, absolute and relative weights were increased at 50 mg/kg/day. Liver weights were significantly increased by 43% for absolute weight (51% for relative weight) only at 50 mg/kg/day. However, centrilobular hypertrophy of hepatocytes was observed in the 10 and 50 mg/kg/day groups (±: 6 in 13, +: 1 in 13 at 10 mg/kg; ±: 1 in 13, +: 11 in 13, ++: 1 in 13 at 50 mg/kg/day, compared to no changes at 0 and 2 mg/kg/day).

The NOAEL for males and females was 2 mg/kg/day, and the LOAEL for males and females was 10 mg/kg/day.

Reference: MHW, Japan (1997). Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 5:65 (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

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

Type: **90-Day Subacute Oral Toxicity**
Species/Strain: Dogs/Purebred beagle
Sex/Number: Male and female/4 per dose
Exposure Period: 90 days
Frequency of Treatment: 7 days/week
Exposure Levels: 0, 50, 150, 300, 1000 ppm
Method: The test substance was incorporated into a stock diet and fed

to dogs 7 days/week. Initially, the body weight of each dog was determined and recorded. Thereafter, weighings were conducted weekly for the duration of the test. Food consumption was recorded. Dogs were examined daily for clinical sings or symptoms indicative of systemic toxicity. Five hematologic, 7 blood chemistry, and 7 urinalysis parameters were measured just prior to the inception of the study, after 42 days, and/or after 85 days for the 0, 50, 150, and 300 ppm groups. The parameters were measured in dogs at 1000 ppm just prior to the inception of the study, and on all surviving dogs after 28 days. At the conclusion of the study, animals were sacrificed and given a complete gross necropsy. Nine organ weights were collected, and representative specimens of approximately 35 organs/tissues were saved for histopathologic examination.

GLP: No

Test Substance: Propanenitrile, 2,2'-azobis(2-methyl-, purity not specified

Results: After the death of 1 animal, the surviving 1000 ppm group animals were sacrificed *in extremis* on Day 28 of the investigation. These animals exhibited body weight losses or no body weight gain, and a reduction in the amount of food consumed during the 4 weeks. The animals were asthenic after 3 weeks on test.

The results of the blood chemistry studies conducted on samples collected from the 1000 ppm animals just prior to sacrifice revealed significant increases in serum alkaline phosphatase, serum glutamic-pyruvic transaminase, and serum glutamic-oxalacetic transaminase activities. The results of the hematologic studies and urinalyses conducted on samples obtained from the 1000 ppm animals revealed no unusual findings. The organ weight and ratio data revealed significant increases in liver and kidney to body weight ratios. Histopathologic examination of a series of tissues from the animals fed 1000 ppm revealed morphologic changes in the liver sections.

No deaths occurred in the 0, 50, 150, or 300 ppm groups. No test substance related findings in body weight/gain, food consumption, or clinical signs were observed at 0, 50, 150, or 300 ppm.

The 300 ppm group animals exhibited an increase in serum alkaline phosphatase activity. The females fed 300 ppm exhibited a slight increase in blood thiocyanate. No test substance-related findings were observed in hematologic or

urinalysis parameters at 300 ppm, nor were there any test substance-related findings in hematologic, blood chemistry, or urinalysis parameters at 50 or 150 ppm.

Marginal increases in liver to body weight ratios were observed at 300 ppm, and in 1 male at 150 ppm. No other organ weight effects were observed. Histopathologic examination revealed test substance-related morphologic changes in the liver of some animals at 150 and 300 ppm. The number of animals with this finding was greater at 300 ppm, but the finding was regarded to be an adaptive response of the liver. No histopathologic findings were observed at 50 ppm.

Reference: Monsanto Co. (1974). Industrial Bio-Test Laboratories, Inc. Report, BTL No. 73-54, IBT No. 651-04494 (TSCA Fiche OTS0545629).

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

Type: **Subacute Inhalation Toxicity**

Species/Strain: Rats/ Crl:CD®

Sex/Number: Males/10 per concentration level

Exposure Period: 2 weeks

Frequency of Treatment: 6 hours/day, 5 days/week

Exposure Levels: 0, 10.0, 80.0 mg/m^3

Method: Groups of rats were exposed head-only. Five rats/group were randomly selected for sacrifice after the 10th exposure, while the remaining 5 rats/group were sacrificed after a 14-day recovery-observation period. Rats were weighed and observed daily (except weekends) throughout the exposure and recovery period.

Dust atmospheres of the test substance were generated and atmospheric concentration of test substance was determined from weight gain of the filters.

An overnight (16 hour) urine specimen was collected from 10 rats in groups exposed to 0 and 10.0 mg/m^3 and 9 rats exposed to 80.0 mg/m^3 after the 9th exposure. Blood was taken from these rats after the 10th exposure, then 5 rats from the groups exposed to 0 and 10.0 mg/m^3 and 4 rats exposed to 80.0 mg/m^3 were sacrificed for pathological examination. Fourteen days later (recovery), blood and urine samples were collected from the rats remaining in each group. Approximately 12 hematologic parameters were measured or

calculated.

After the 10th exposure, 5 rats from each group were sacrificed for gross and histopathological examination. Remaining rats were sacrificed on the 14th day of recovery for identical follow-up examination. Seven organs were weighed and 22 tissues/organs were saved for histologic evaluation.

GLP: No

Test Substance: Propanenitrile, 2,2'-azobis(2-methyl- (Vazo® 64), purity 99%

Results: The mean TWA concentration was 9.80 and 79.5 mg/m^3 for the 10.0 and 80.0 mg/m^3 design concentrations. Mass median diameter ranged from 8.0-11.5 µ at 80.0 mg/m^3.

One rat was sacrificed *in extremis*, following the 4th exposure to 80 mg/m^3. This rat exhibited lung noise, poor righting reflex, stained fur, labored breathing, and sluggishness prior to sacrifice. Pathological examination could not explain the cause of death, however, it was not attributed solely to test substance administration.

When compared with controls, rats exposed to 10 mg/m^3 showed a normal rate of weight gain during both the exposure and recovery periods. Mean body weight gain of rats exposed to 80.0 mg/m^3 was significantly reduced on days 2-4 of the exposure period. For the remainder of the test period, these rats exhibited a normal rate of weight gain. No test substance-related clinical signs were noted.

All exposed rats tended to have higher serum total proteins than the unexposed controls after 10 exposures. Urine osmolality was lower in rats exposed to 80.0 mg/m^3. Following the 14-day recovery period, no effect was observed in rats at 10.0 mg/m^3, but rats at 80.0 mg/m^3 continued to have higher serum total proteins.

No test substance-related pathological lesions occurred in rats exposed to 10.0 mg/m^3. The 80.0 mg/m^3 rats sacrificed after the 10th exposure exhibited a compound-related liver effect, increased cytoplasmic basophilia of hepatocytes. However, this liver effect was not detected in these rats following a 14-day recovery period. The mean relative liver-to-body weight ratios of exposed rats was significantly higher than the control group after exposure 10. This effect was no longer evident after a 14-day recovery period.

Reference:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 40-81 (also cited in TSCA Fiche OTS0000937).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

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

DuPont Co. (1947). Unpublished Data, Haskell Laboratory Report No. 25-47.

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Motoc, F. et al. (1971). Arch. Mal. Prof. Med. Trav. Secur. Soc., 32(10-11):653-658 (CA76:122561y).

Preussmann, R. et al. (1969). Ann. N.Y. Acad. Sci., 163(2):697-716 (CA73:12854b).

Boyland, E. and S. Sargent (1951). Br. J. Cancer, 5:433-439.

5.3 Developmental Toxicity

Species/Strain:	Rats/Cjr:CD(SD)
Sex/Number:	Male and female/Number not specified
Route of Administration:	Gavage
Exposure Period:	Males: From 14 days before mating to 14 days after mating Females: From 14 days before mating to day 3 of lactation
Frequency of Treatment:	Daily
Exposure Levels:	0, 2, 10, 50 mg/kg
Method:	OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test Guideline 422
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.9%
Results:	There were no adverse effects of the test substance on copulation and fertility, duration of pregnancy, gestation index, or parturition of all treated groups. Three of 12 dams at 50 mg/kg showed difficulty of nursling, and 2 of them let all their offspring die within the first 4 days after birth. The test substance had no adverse effects on viability, sex ratio,

	or body weight gain of pups. However, viability of newborns at birth and body weight of nurslings on postnatal day 4 was lower than the control level at 50 mg/kg/day. These changes were considered to be caused by maternal toxicity. There were no morphological abnormalities in pups of any treatment group. The NOAEL for the parental generation was 10 mg/kg/day. The NOAEL for the F_1 offspring was 50 mg/kg/day.
Reference:	MHW, Japan (1997). Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 5:65 (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Developmental Toxicity: None Found.

5.4 Reproductive Toxicity

Species/Strain:	Rats/Cjr:CD(SD)
Sex/Number:	Male and female/Number not specified
Route of Administration:	Gavage
Exposure Period:	Males: From 14 days before mating to 14 days after mating Females: From 14 days before mating to day 3 of lactation
Frequency of Treatment:	Daily
Exposure Levels:	0, 2, 10, 50 mg/kg
Method:	OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test Guideline 422
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.9%
Results:	There were no adverse effects of the test substance on copulation and fertility, duration of pregnancy, gestation index, or parturition of all treated groups. Three of 12 dams at 50 mg/kg showed difficulty of nursling, and 2 of them let all their offspring die within the first 4 days after birth. The test substance had no adverse effects on viability, sex ratio, or body weight gain of pups. However, viability of newborns at birth and body weight of nurslings on postnatal day 4 was lower than the control level at 50 mg/kg/day. These changes were considered to be caused by maternal

toxicity. There were no morphological abnormalities in pups of any treatment group.

The NOAEL for the parental generation was 10 mg/kg/day. The NOAEL for the F_1 offspring was 50 mg/kg/day.

Reference: MHW, Japan (1997). Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 5:65 (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).

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

Additional References for Reproductive Toxicity: None Found.

5.5 Genetic Toxicity

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

Tester Strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA97 (without S9 mix)
Escherichia coli WP2 *uvr*A

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

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

Without metabolic activation: 0, 313, 625, 1250, 2500, 5000 µg/plate

Method: Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 471 and 472. The positive control for tests with metabolic activation was 2-aminoanthracene (5 strains). Positive controls for tests without metabolic activation included sodium azide (TA1535), 9-aminoacridine (TA1537 and TA97), and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98, and WP2).

GLP: Yes

Test Substance: Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.9%

Results: Negative

Remarks: Toxicity was not observed when tested with or without exogenous metabolic activation. Precipitation was observed at concentrations of 1250 and 2500 µg/plate when tested with and without metabolic activation, respectively. The test substance was negative for induction of mutations when

	tested with and without metabolic activation.
Reference:	MHW, Japan (1997). Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 5:65 (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

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

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

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 89-76 (also cited in TSCA Fiche OTS0000937).

Eastman-Kodak Co. (n.d.). Toxicity and Health Hazard Summary (TSCA Fiche OTS0001156).

Takenaka, S. et al. (1993). J. Toxicol. Sci., 18(4):418 (Abstract P-223).

Eder, E. et al. (1989). Naunyn-Schmiedeberg's Arch. Pharmacol., 339(Suppl.):R26 (Abstract 102) and Eder, E. et al. (1989). Toxicol. Lett., 48:225-234).

Type:	***In vitro* Chromosomal Aberration Test**
Cell Type:	Chinese hamster lung (CHL/IU) cells
Exogenous Metabolic Activation:	With and without phenobarbital and 5,6-benzoflavone rat liver induced S9
Exposure Concentrations:	0, 0.40, 0.80, 1.6 mg/mL
Method:	Guide for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guideline No. 473. The short-term treatment was 6 hours, and the continuous treatment was 24 and 48 hours. The positive controls were cyclophosphamide and mitomycin for the tests with and without activation, respectively.
GLP:	Yes
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity 99.9%
Results:	Negative
Remarks:	Cytotoxicity was not observed. The test substance was negative for clastogenicity and polyploidy when tested both

	in the presence and absence of metabolic S9 activation.
Reference:	MHW, Japan (1997). Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 5:65 (cited in OECD SIDS Dossier for 2,2'-Azobis(2-methylpropionitrile), http://www1.oecd.org/ehs/sidstable/index.htm, accessed January 28, 2002).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Clastogenicity: None Found.

Type:	***In vivo* Mouse Micronucleus Assay**
Species/Strain:	Mice/ddY
Sex/Number:	Male/Number not specified
Route of Administration:	Oral
Concentrations:	No Data
Method:	A micronucleus test was performed using groups of male mice orally administered 2 doses of the test substance.
GLP:	Unknown
Test Substance:	Propanenitrile, 2,2'-azobis(2-methyl-, purity not specified
Results:	Negative
Remarks:	At both 24 and 48 hours after treatment, the test substance did not produce a significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of the treated mice.
Reference:	Takenaka, S. et al. (1993). J. Toxicol. Sci., 18(4):418 (Abstract P-223).
Reliability:	Not assignable because limited study information was available.

Additional References for *In vivo* Genetic Toxicity: None Found.

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STUDY PROTOCOL

AMBN: Influence on Growth and Growth Rate of the Green Alga *Selenastrum capricornutum*

WORK REQUEST NUMBER 14011
DUPONT NUMBER «HLHLO»

Testing Facility

E. I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
P. O. Box 50, Elkton Road
Newark, Delaware 19714-0050

Study Sponsor

E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

and

Akzo-Nobel Chemicals, Inc.
5 Livingstone Ave.
Dobbs Ferry, NY 10522-3407
U.S.A.

Study Director: Terry Lee Sloman, B.S.

GENERAL INFORMATION

A. Test Guidelines Followed in This Protocol

This study will investigate the influence of AMBN on the growth and growth rate of the green alga, *Selenastrum capricornutum*. This protocol is designed to meet the US EPA or OECD Test Guideline 201,[1-7] except as noted in the study records and final report. This study will be conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).[8]

B. Test Sponsor

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

And

Akzo Nobel Chemicals, Inc.
Dobbs Ferry, NY 10522-3407

Date of Sponsor's Approval: <>

C. Testing Facility and Administrative Officials

Testing Facility: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
P.O. Box 50, Elkton Road
Newark, Delaware 19714-0050

Administrative Officials: Robert W. Rickard, Ph.D., D.A.B.T.
Manager
Haskell Laboratory for Health and Environmental Sciences

Robert A. Hoke, Ph.D.
Manager, Aquatic Toxicology
Haskell Laboratory for Health and Environmental Sciences

D. Organizations to Which Study may be Submitted

EPA, EU, OECD

E. Proposed Study Schedule

Proposed Experimental Start of Test: <>
Proposed End of Test: <>
Proposed Issue of Report: <>

OBJECTIVES

This study will investigate the influence of AMBN on the growth and growth rate of the green alga, *Selenastrum capricornutum*.

TEST SUBSTANCE INFORMATION

A. Identity

Name of the chemical: butanenitrile, 2,2.-azobis(2-methyl-
CAS Number: 13472-08-7
Common name: 2,2.azobis-(2-methylbutyronitrile) (AMBN)

The test substance will be characterized according to the US EPA Good Laboratory Practice Standards (40 CFR Part 792). The lot number, chemical purity, and source will be documented in the study records.

The test substance and an analytical standard of known purity will be supplied by the sponsor and will be assigned a Haskell Laboratory Number.

B. Solubility

Prior to the start of the study, the solubility of AMBN in synthetic algal-assay-procedure (AAP) nutrient medium will be determined.

TEST SYSTEM

Selenastrum capricornutum is a test species that meets several important criteria including representing an important taxonomic group (green algae), and biological factors that facilitate testing. It is a freshwater, unicellular, non-motile, green alga. It is often utilized for testing because of its ready availability, ease of culture, and fast growth rate (rendering it possible to conduct a multi-generation test in a short period of time).

The source of the original culture and the stock culture maintenance conditions, including the composition of the nutrient medium; the type of growth vessels; the type, duration, and intensity of the illumination; shaking speed; the temperature; and the transfer frequency, will be documented in the study records.

MATERIALS PREPARATION

A. AAP Nutrient Medium for *Selenastrum capricornutum*

Synthetic algal-assay-procedure (AAP) nutrient medium[9] will be used as the culture medium and the test diluent. The exact ingredients and preparation of the AAP nutrient medium will be documented in the study records. The AAP nutrient medium is not routinely analyzed for contaminants because it is

prepared using reagent grade chemicals and Milli-Q® (deionized) water. Therefore, no contaminants are expected in the medium that would interfere with the purpose or conduct of the study.

B. Test Vessels

Sterilized Erlenmeyer flasks fitted with foam stoppers typically will be used as the test vessels. The purpose of the foam stopper is to permit the necessary gas exchange (i.e., O_2 and CO_2) while maintaining aseptic conditions. Any size flask may be used as long as the 5:1 or 5:2 ratio of the flask volume:medium volume is maintained.

Each test vessel will be uniquely identified and documented in the study records. Each test vessel containing *Selenastrum capricornutum* will be randomly assigned a number to eliminate bias while determining growth.

C. Test Substance

The desired concentration(s) of the test substance will be prepared in filter-sterilized AAP nutrient medium. The actual preparation of test solution(s) to obtain the desired concentration(s) will be documented in the study records.

A blank (normal culture medium) control, which contains only filter-sterilized AAP nutrient medium and *Selenastrum capricornutum*, will be included in each test.

An abiotic (stability) control, which contains test substance in filter-sterilized AAP nutrient medium but no *Selenastrum capricornutum*, will be included in the definitive test. The purpose of the abiotic control is to determine the stability of the test substance under testing conditions without the presence of the test species.

RANGE-FINDING TEST(S)

Unless previous work provides enough information to determine whether a Tier 1 or Tier 2 definitive test will be performed, range-finding test(s) will be conducted prior to the start of the study. The maximum test concentration tested will be the highest concentration recommended by the US EPA (100 mg/L) or at the maximum solubility limit. In addition to this maximum concentration, 2 or more lower concentrations (e.g., 2 to 100 times lower) may be tested. Actual test concentrations will be documented in the study records.

DEFINITIVE TEST

If the growth inhibition relative to the control at the maximum test concentration in the range-finder is less than 50%, a Tier 1 definitive (limit) test will be conducted. If a 50% or greater growth inhibition is exhibited, a Tier 2 definitive (dose-response) test will be conducted.

If a Tier 1 definitive test is to be conducted, the following treatments are normally tested: the highest concentration recommended by the specific guidelines or the maximum solubility limit of the test substance; a blank control; and an abiotic control. The pH of each solution will be measured at test

initiation prior to the addition of the test species and at test termination. Each of the test substance concentration and controls will be replicated 3 to 6 times.

If a Tier 2 definitive test is to be conducted, the following treatments are normally tested: at least 5 concentrations of test substance; a blank control; and an abiotic control. Test concentrations may be selected based on information from the range-finding test(s) and documented in the study records. The pH of each solution will be measured at test initiation and termination, prior to the addition of the test species. Each of the test substance concentrations and controls will be replicated 3 to 6 times.

RECOVERY TEST

Recovery will be assessed for each test concentration from the Tier 2 definitive test exhibiting a 50% or greater growth inhibition relative to the control. However, at the scientific discretion of the study director or when there is a business need, the recovery criteria may be lowered (e.g. 45%). Any such changes will be documented in the study records. Assessment of recovery is conducted to distinguish algicidal effects from algistatic effects.[10]

An aliquot from each of the replicate flasks for each of the selected test concentration(s) will be removed and combined into a single sterile flask containing enough untreated filter-sterilized AAP nutrient medium to dilute the test substance to a concentration (less than the NOEC) that theoretically should not inhibit algal growth and growth rate. A blank will be prepared for comparison by diluting an aliquot from a randomly selected, single blank control replicate with filter-sterilized AAP nutrient medium. The final volume of each recovery test solution will be 50 or 100 mL. The actual aliquot volumes of each selected test solution and filter-sterilized AAP nutrient medium used for testing will be documented in the study records.

Each recovery test solution will be placed in a single sterile 250 mL Erlenmeyer flask, and will be incubated under the same conditions as used in the Tier 2 definitive test. At recovery test initiation (day 0 or 0-hour), every third or fourth day, and the termination of the recovery test, a sample will be removed from the flasks for the determination of the number of cells per milliliter (cell count). If growth is evident from an increase in cell count relative to cell count at the initiation of the recovery test, the test will be terminated and effects will be deemed algistatic. If no increase in cell count, relative to cell count at the initiation of the recovery test, is observed by the 9^{th} or 10^{th} day of the recovery test, the test will be terminated and the effects will be labeled algicidal.

INOCULATION AND INCUBATION

The range-finding and definitive tests will be initiated by aseptically adding an aliquot of algal inoculum from a logarithmically growing stock culture to test vessels each containing the appropriate volume of test or control solution. The recovery test will be initiated by aseptically adding a volume of algal inoculum from each of the replicate(s) of the selected test concentration(s) and blank control to test vessels containing the appropriate volume of untreated, filter-sterilized AAP nutrient medium.

The inoculation and incubation conditions for each type of test are summarized in the following table. The actual cell count, volume of algal inoculum, and the time interval for the inoculation at test initiation (day 0 or 0-hour) will be documented in the study records. The actual incubation conditions followed also will be documented in the study records.

Guideline	Study Duration	Initial Population for Each Test Type	Illumination (lumens/m^2 =lux)	Photo-period (hrs)	Shaking Speed (rpms)	Temperature (°C)
EU/OECD	3 to 13 Days	Definitive: 10,000 cells/mL Recovery: variable; based on the definitive test termination day counts from the selected test concentrations	6,000 to 10,000	24	100	24 ± 2

Each working day the test vessels will be non-systematically or randomly, if required, repositioned to minimize any variations due to position within the incubator or chamber.

Selenastrum capricornutum will be illuminated with cool-white fluorescent lights using a 24-hour constant light photoperiod.

TEST SOLUTION ANALYSIS

To determine the initial and final concentrations of the test substance in the definitive test concentration and the control solutions, aliquots (samples) of each solution will be given to the analytical chemist at test initiation (day 0 or 0-hour) and on test termination day. The concentration of the working stock solution prepared on day 0 also will be determined. Aliquots of each test concentration and blank control will be taken prior to the addition of *Selenastrum capricornutum* to provide the day 0 samples for chemical analysis and initial pH measurement. An aliquot of the abiotic control will be taken to provide the day 0 sample for chemical analysis and initial pH measurement. After the completion of the definitive test, the replicates from the abiotic control will be pooled to provide the termination-day sample for chemical analysis and the final pH measurement. After the completion of the definitive test and/or the initiation of the recovery test, the replicates from each test concentration(s) and blank control will be pooled to provide the termination-day sample for chemical analysis and the final pH measurement. If the samples are not analyzed immediately, storage conditions will be documented in the study records. Storage stability data will be provided, if necessary.

Test solution analysis will be performed at Haskell Laboratory for Health and Environmental Sciences and/or the DuPont Experimental Station (exact location will be documented in the study records). The actual methods used to analyze the test substance concentrations will be documented in the study records.

MEASUREMENTS AND GROWTH EFFECT EVALUATION

A. Test Substance Concentrations

The growth effect analysis will be based upon the nominal concentrations of the test substance at the initiation of the study, unless otherwise indicated in the study records and the final report.

B. Cell Counts

The growth of *Selenastrum capricornutum* will be quantified visually approximately every 72 to 120 hours during the course of the range-finding test(s), approximately every 24 hours during the course of the definitive test (4 to 5 days), and approximately every 72 hours during the course of the recovery test (maximum 10 days).

Samples (approximately 0.2 to 0.4 mL) from the test flasks will be collected aseptically using sterile pipettes or pipette tips. Samples will not be returned to the test flasks. Cell counts will be recorded using an Improved Neubauer Hemacytometer (0.1 mm deep) or a Coulter Counter. Actual methods used will be recorded in the study records. Any algal abnormalities observed also will be recorded. Most counts will be made at approximately (within 4 hours) the same time each counting day. After the initial (day 0 or 0-hour) count, subsequent readings will include test incubator or chamber temperature, healthy cell count (cells/mL), and the time duration of the actual counting.

C. Area under the Growth Curve (E_bC)

The mean healthy cell counts for each test concentration and for the blank control are plotted against time to produce growth curves. The area under the growth curves for each interval will be calculated according to the following formula:

$$A = [0.5 \times t_1 \times (N_1 - N_0)] + [0.5 \times (t_2 - t_1) \times (N_2 + N_1 - 2N_0)]$$
$$+ \ldots + [0.5 \times (t_n - t_{n-1}) \times (N_n + N_{n-1} - 2N_0)]$$

where t_1, t_2, t_{n-1}, and t_n are times of observation measured from the initiation of the test and N_0, N_1, N_{n-1}, and N_n are the initial and subsequent healthy cell counts corresponding to the observation times.

D. Growth Rate (E_rC)

The mean growth rate (μ) for each test concentration and for the blank control will be calculated according to the following formula:

$$\mu = \frac{\ln (N_n / N_0)}{t_n}$$

where t_n is the time of observation measured from the initiation of the test and N_0 and N_n are the initial and subsequent healthy cell counts corresponding to the observation time.

E. Percent Inhibition (%I)

The mean healthy cell count, area under the growth curve, and growth rate at each sampling interval for each test concentration will be expressed relative to the blank control. The percent growth inhibition, % I, will be calculated according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where C is equal to the mean healthy cell count, area under the growth curve, or growth rate for the blank control at a selected sampling interval and T is the mean healthy cell count, area under the growth curve, or growth rate for each test concentration at a selected sampling interval. Negative values of inhibition indicate stimulation of growth.

F. Statistical Analysis

The means and standard errors of the healthy cell counts, areas under the growth curve, and growth rate for each test concentration and blank control will be calculated using standard procedures.[11]

For the Tier 1 definitive test, the mean and standard error for the single test concentration will be calculated using standard procedures. The percent inhibition and the corresponding 95% confidence interval will be calculated using Welch's t' statistic for ratios. The null hypothesis of 50% or greater inhibition will be tested at the 95% confidence level using Welch's t-test for ratios.

For the Tier 2 definitive test, the healthy cell counts, areas under the growth curve, and growth rates will be used to calculate the appropriate EC values (e.g., EC_{25}, EC_{50}) if applicable. This "effective concentration" is defined as the concentration producing a 25% and/or 50% inhibition of growth relative to the blank control. The appropriate EC value(s) and associated 95% confidence interval will be determined by weighted least-squares non-linear regression of the log of the test concentration against the measured parameter or another suitable method. If possible, the NOEC, defined as the highest concentration of test substance that had no significant effect on the measured parameter relative to the blank control, will be determined from a trend test (Jonckheere-Terpstra or Williams) applied in step-down manner. Such a procedure assumes a monotone dose-response. In the event of significant non-monotonicity, the NOEC will be determined by appropriate parametric or non-parametric multiple comparison methods. All tests of significance will be at $\alpha = 0.05$.

G. OECD 201 Validity Criteria of the Study

1. Cell concentration in the control cultures should have increased by a factor of at least 16 within three days.
2. pH in control cultures shall not increase more than 1.5 units.

H. Disposal of Waste Material

Solutions containing test substance will be placed in a 55 gallon barrel or plastic waste jugs and disposed of in accordance with the guidelines outlined in the Stine-Haskell Safety Manual.

I. Safety Procedures

Contact with test materials and solutions will be minimized by wearing the appropriate personal protective equipment (PPE), e.g., safety glasses with side shields, gloves, lab coat, etc.

J. Good Laboratory Practices

This study will be conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850). Signatures of the study director, collaborating scientists, and supervisory personnel will attest to the authenticity of the study. A statement of compliance will be included in the final report.

K. Records Retention

Records to be maintained will include at least the originals of all raw data, the original signed protocol, and any amendments thereto, all letters, memos, or notes pertaining to the study, and the original signed final report. All records will be retained at Haskell Laboratory, Newark, Delaware or at Iron Mountain Records Management, Wilmington, Delaware.

REPORTING

A report will be issued which shall include at a minimum the following information:

Title Page
Good Laboratory Practice Compliance Statement
General Information Describing Test Substance
Records and Sample Storage
Table of Contents
Summary
Quality Assurance Documentation
Study Personnel
Introduction
Materials and Methods
- Test Substance
- Nutrient Medium
- Test Organism Culture
- Test Methods
- Sample Preparation and Chemical Analysis
- Statistical Analysis

Results and Discussion
Conclusion
References

Tables, Figures, Appendices

REFERENCES

1. Holst, R. W.; Ellwanger T. C. *Pesticide Assessment Guidelines, Subdivision J Hazard Evaluation: Non-target Plants*; U.S. Environmental Protection Agency. U.S. Government Printing Office: Washington, DC, 1982; EPA-540/9-82-020.
2. U.S. EPA. 1993. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules §.797.1050. Algal Acute Toxicity Test.
3. U.S. Environmental Protection Agency. Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792).
4. Commission of the European Communities. Directorate - General for Agriculture. Commission Directive 96/12/EC amending Council Directive 91/414/EEC, March 8, 1996.
5. Commission of the European Communities. Annex to Directive 92/69/EEC Part C: Methods for the Determination of Ecotoxicity, Method C3: Algal Inhibition Test. OJEC L383A, 179-186. December 29, 1992.
6. Organization for Economic Cooperation and Development. 1984. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 201, Alga Growth Inhibition Test. Adopted 4 April 1984.
7. OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17.
8. MAFF Japan Good Laboratory Practice Standards (59 NOHSan No. 3850). 1985.
9. Miller, W. E.; Greene, J. C.; Shiroyama, T. *The* Selenastrum capricornutum *Printz Algal Assay Bottle Test*; U.S. Environmental Protection Agency; U.S. Government Printing Office: Washington, DC, 1978; EPA-600/9-78-018.
10. American Society for Testing and Materials (ASTM). (1990). "Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae" in *ASTM Annual Book of Standards*, E1218-90. Vol. 11.04, Philadelphia, PA.
11. Miller, R.G., Jr. *Beyond* ANOVA, *Basics of Applied Statistics*; Wiley: New York, 1986.

SIGNATURES

Approved by: _____ _____

Terry Lee Sloman, B.S. Date
Study Director