

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

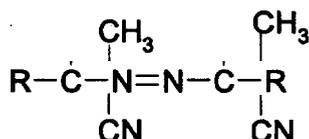
Summary

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 ATBN 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.

<u>Chemical Name</u>	<u>CAS Registry Number</u>	<u>Common Name</u>	<u>Name to be used in this Document</u>	<u>R=</u>
Butanenitrile, 2,2'-azobis(2-methyl-	13472-08-7	2,2'-azobis-(2-methylbutyronitrile)	AMBN	CH ₃ CH 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.

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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 (**CH₃**) in AIBN with ethyl groups (**CH₃CH₂**). 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:



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°C** and **64°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 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.

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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	√	√
Stability in Water	√	√
Transport (Fugacity)	√	√
Biodegradation	- ¹	√
ECOTOXICITY		
Acute Toxicity to Fish	- ¹	√
Acute Toxicity to Invertebrates	- ¹	√
Acute Toxicity to Aquatic Plants	√	√/-
MAMMALIAN TOXICITY		
Acute Toxicity	√	√
Repeated Dose Toxicity	- ¹	√
Developmental Toxicity	- ¹	√
Reproductive Toxicity	- ¹	√
Genetic Toxicity Bacterial Gene Mutations	√	√
Genetic Toxicity Chromosomal Aberrations (<i>in vitro</i>)	-	√
Genetic Toxicity <i>in vivo</i> Micronucleus	√	√
√ = Data are available and considered adequate. - = No data available. √/- = Data are available, but considered inadequate. N/A = Not Applicable. ¹ Data is available for structurally similar test substance, AIBN.		

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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 49.4°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, while the experimentally measured log Kow is 2.07. 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. **All required SIDS physical and chemical characteristic data points are complete for both azonitriles, and no further testing is recommended.**

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	392 mg/L (measured) 4.9 mg/L @ 25°C (model estimate)	350 mg/L @ 25°C (measured) 85 1.1 mg/L @ 25°C (model estimate)
Melting Point	49.4°C	100-103°C
Boiling Point	Not Applicable	Not Applicable
Vapor Pressure	0.354 Pa @ 25°C (flow rate 10 mL/min) 0.408 Pa @ 25°C (flow rate 8 mL/min)	0.81 Pa @ 25°C (measured) 0.19 Pa (model estimate)
Density	1.1	~ 1.1
Partition Coefficient (log Kow)	2.07 (measured) 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

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Law Constant for AMBN is calculated from vapor pressure and water solubility data to be $1.97 \times 10^{-6} \text{ atm} \cdot \text{m}^3/\text{mole}$, and the estimated half-life from a river is 412 hours. Measured half-lives for AIBN in water ranged from 2 to 10 to 304 days at 25°C, and were dependent on the bioconcentration factor (BCF) for AMBN was estimated as 7.8 (log BCF = 0.894). Therefore, AMBN is estimated to have a high to moderate potential for persistence and a low 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. Therefore, AIBN is considered not to be readily biodegradable. The fugacity model predicts that both AMBN and AIBN will distribute primarily to the soil, air, and water when emissions are to air only, to water primarily when emissions are to water only, and to soil primarily when emissions are to soil only. The rate constant for the reaction of AMBN vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be $2.97 \times 10^{-12} \text{ cm}^3/\text{molecular} \cdot \text{sec}$, 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. **All required SIDS environmental fate data points are complete for both azonitriles, and no further testing is recommended.**

Table 3: Environmental Fate

	AMBN	AIBN
Bioaccumulation *	Low potential for bioaccumulation BCF = 7.8	Low potential for bioaccumulation BCF = 1.403
Biodegradation	No Data	Not readily biodegradable
Fugacity *	When released 100% to air: Air 22.3% Water 16.2% Soil 61.4% Sediment 0.07% When released 100% to water: Air 0.12% Water 99.1% Soil 0.32% Sediment 0.43% When released 100% to soil: Air 0.21% Water 10.3% Soil 89.4% Sediment 0.04%	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		

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No information regarding aquatic toxicity to fish or invertebrates is 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 LC₅₀ of 580 mg/L (greater than water solubility) in fish, and a 48-hour EC₅₀ of 397 mg/L in *Daphnia* (greater than water solubility). A 72-hour EC₅₀ 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. Modeled values were compared for AMBN and AIBN. The similarity in modeled values for the two compounds, and good agreement between modeled values and actual measured data, support the use of AIBN as an appropriate analog for AMBN. Where measured values for AMBN are lacking, AIBN data provide a reliable surrogate. ECOSAR estimates of acute toxicity to fish, daphnids, and algae indicated algae were predicted to be more sensitive to the effects of AMBN or AIBN than fish or daphnids. Therefore, an algae test following OECD Guideline 201 was conducted with AMBN. The 72-hour EC₅₀ was 38.1 mg/L, based on cell count. Based on the results of the ECOSAR predictions for AMBN and AIBN, and the actual algal test results with AMBN, the acute toxicity to fish, daphnids, and algae has been adequately characterized for both compounds. **No further ecotoxicity testing is recommended.**

Table 4: Eco toxicity

	AMBN	AIBN
Toxicity to Fish (96-LC₅₀ value)	122.5 mg/L (ECOSAR; log Kow = 2.07)	853.9 mg/L (ECOSAR; log Kow = 1.1) 580 mg/L (nominal)
Toxicity to Invertebrates (48-EC₅₀ value)	13 1.9 mg/L (ECOSAR; log Kow = 2.07)	859.8 mg/L (ECOSAR; log Kow = 1.1) 397 mg/L (nominal)
Toxicity to Algae (EC₅₀ value)	82.8 mg/L (96-hour ECOSAR; log Kow = 2.07) 72-hour EC ₅₀ , Healthy cell count: 38.1 mg/L	5 10.4 mg/L (96-hour ECOSAR; log Kow = 1.1) 72-hour EC ₅₀ > 9.4 mg/L

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 LC₅₀ 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.**

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Table 5: Acute Mammalian Toxicity

	AMBN	AIBN
Oral LD₅₀ (rat)	337 mg/kg	360 mg/kg
Inhalation LC₅₀ (rat)	>8.9 mg/L (4-hour)	> 7.78 mg/L (1-hour)
Dermal LD₅₀ (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 a_{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/m³, 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

	AMB	AIB
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 AMB nor AIB induce mutations in bacteria. AIB was not clastogenic when tested in an *in vitro* study in Chinese hamster lung cells. Neither AMB nor AIB was active when tested in an *in vivo* mouse micronucleus study. **Therefore, no further genetic toxicity testing is recommended.**

Table 7: Genetic Toxicity

	AMB	AIB
Mutagenicity	Not mutagenic (Ames test)	Not mutagenic (Ames test)
Clastogenicity	Not clastogenic (<i>in vivo</i> mouse micronucleus assay)	Not clastogenic (Chromosomal aberration test in CHL/IU cells; <i>in vivo</i> mouse micronucleus assay)

In the absence of available literature, a model was used to determine potential metabolic pathways for AMB and AIB. 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.

AMB

Potential initial pathways for metabolism of AMB 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 abstractable 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

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at high exposure concentrations. In the case of AMBN there are two nonequivalent 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 nonsymmetrical 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 carboncentered 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. The major manufacturers of AMBN practice Responsible Care®. Specific manufacturing procedures and industrial hygiene programs in place at manufacturing sites limit the potential for employee exposure through use of engineering controls, environmental controls, and personal protective equipment. DuPont has set an Acceptable Exposure Limit (AEL) of 1 mg/mg³ TWA for both AMBN and AIBN. DuPont also 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.

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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. Overall the toxicology datasets for AMBN and AIBN are complete, and no further testing is recommended for purposes of the HPV Program.

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TEST PLAN FOR AMBN

	Acceptable Data for AIBN (CAS No. 7867-1)	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	Y	
Boiling Point	N/A	N/A	N/A
Vapor Pressure	Y	Y	
Partition Coefficient	Y	Y	
Water Solubility	Y	Y	
ENVIRONMENTAL FATE			
Photodegradation	Y	Y	
Stability in Water	Y	Y	
Transport (Fugacity)	Y	Y	
Biodegradation	Y	Y ³	N
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y ³	N
Acute Toxicity to Invertebrates	Y	Y ³	N
Acute Toxicity to Aquatic Plants	N	Y	
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y	Y ³	N
Developmental Toxicity	Y	Y ³	N
Reproductive Toxicity	Y	Y ³	N
Genetic Toxicity Bacterial Gene Mutations	Y	Y	N
Genetic Toxicity Chromosomal Aberrations	Y	Y	N
N/A = Not Applicable. ¹ Testing was performed to fulfill required endpoint for HPV program. ² Model was re-run using measured values obtained in tests to fulfill required endpoints for HPV program. ³ Data is available for the analog AIBN.			

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APPENDIX A

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

CAS NO. 13472-08-7

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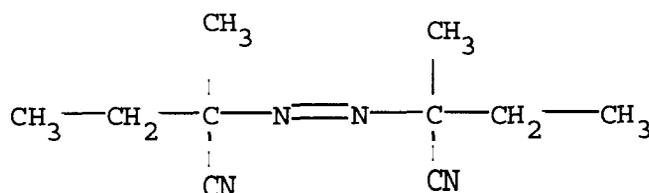
The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as related references in this document.

1.0 Substance Information

CAS Number: 13472-08-7

Chemical Name:

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/m³, 8-hour TWA and 0.7 mg/m³, 12-hour TWA:
DuPont Acceptable Exposure Limit (AEL)

2.0 Physical - Chemical Properties

2.1 Melting Point

Value: 49.4°C
Decomposition: The MSDS for this test substance states that the compound should not be heated above 50°C due to violent decomposition with self ignition.
Pressure: No Data
Method: The procedures used in this test were based on the recommendations of the following guideline: U.S. EPA Product Properties Test Guidelines OPPTS 830.7200.

A preliminary test was performed to determine the approximate melting point of the test substance. A Mettler FP900 Thermosystem was used, and the preliminary test was performed in triplicate. Due to the potentially dangerous reaction of the test substance when subjected to heat, friction, or impact, and the fact that the consistency of the test substance was already that of a powder, the test substance was not ground using a mortar and pestle. A portion of the dried test substance was loaded into the bottom of 3 melting point tubes to a depth of 4-6 mm. The 3 melting point tubes were heated from 40°C (start temperature) to 50°C (end temperature) at a rate of +0.2°C per minute. (The MSDS for this test substance states that the compound should not be heated above 50°C due to violent decomposition with self ignition.)

The definitive test was then performed. Triplicate melting point tubes containing 4-6 mm of test substance were heated from 47.5°C (start temperature) at a rate of +0.2°C per minute until the end temperature of 50°C was reached.

GLP: Yes
Reference: ABC Laboratories, Inc. (2004). Unpublished Data, ABC Study No. 48128 (DuPont-15140), "Determination of Melting Point/Melting Range for 2,2'-Azobis-(Methylbutyronitrile) CAS# 13472-08-7" (July 17).
Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for Melting Point:

DuPont Co. (2000). Material Safety Data Sheet No. DUO00905 (March 28).

2.2 Boiling Point: Not Applicable.

2.3 Density

Value: Specific gravity = 1.1; bulk density = 25 lbs/ft³
Temperature: No Data
Method: Not Available
GLP: unknown
Results: No additional data.
Reference: DuPont Co. (2000). Material Safety Data Sheet No. DUO00905 (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: 0.354 Pa at a flow rate of 10 **mL/min**
0.408 Pa at a flow rate of 8 **mL/min**
Temperature: 25°C
Decomposition: No
Method: The procedures used in the test were based on the recommendations of the following guideline: U.S. EPA Product Properties Test Guidelines OPPTS 830.7950.

A dose level of 0.1% (w/w) was chosen to ensure that the sand prepared for use in the preliminary and definitive tests would be coated with an excess of test substance. The sand and test substance solution were thoroughly mixed together by stirring. The coated sand was placed in a fume hood to allow the solvent to evaporate. The dry, treated sand was placed into a 2-L **carboy** and tumbled for a total of approximately 4 hours.

Prior to using the sand in the preliminary test, three 1 g aliquots of the sand were transferred to 20 **mL** scintillation vial. Each sand portion was extracted with 2x10 **mL** and 1x5 **mL** portions of acetonitrile. The extract volumes were pooled into graduated cylinders. The final volumes of the extracts were adjusted with acetonitrile. Each extract was diluted for analysis and was analyzed by HPLC. A single 1 g aliquot of sand not coated with the test substance was also extracted, diluted, and analyzed to serve as a control.

Preliminary Test

A preliminary test was performed, in which the dosed sand was distributed evenly into 3 vapor saturator columns labeled Test 1, 2, and 3. A control vapor saturator **column** was previously filled with a similar amount of sand that was not coated with test substance. The saturator columns containing the dosed sand and the saturator column containing the control sand were placed inside glass water jackets in an environmental chamber and were attached to a flow-controlled gas manifold. The temperature of the

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environmental chamber was maintained at 25 ± 1 °C. Nitrogen gas was passed through each saturator column overnight.

On the following day, a primary (“A”) and a secondary (“B”) vapor trap were attached end-to-end to the systems on the effluent port of each of the saturator columns with the primary vapor trap before the secondary vapor trap. No test substance was added to these traps. Three spiked traps were prepared by applying test substance solution to each trap. One spiked trap was connected to the end of each dosed saturator column after the secondary trap. A single vapor trap, containing no test substance, was connected to the effluent port of the control saturator column. All connections used for the test system were of Teflon or parafilm.

The flow rate of all systems was adjusted to 10 mL/min and measured with a digital flow meter. The temperature of the environmental chamber was measured at the same time the flow rates were measured.

The preliminary test systems were terminated after approximately 168 hours (7 days) (the saturator columns remained in the environmental chamber under nitrogen flow). The primary, secondary, and spiked cartridges were extracted and analyzed.

The HPLC analysis of the primary trap extracts indicated that the concentration of the test substance was above the standard curve. Therefore, the eluates were diluted and then analyzed by HPLC. All samples were refrigerated when not in use.

Definitive Test

The definitive test duration was chosen based on the amount of test material collected in the preliminary phase.

A primary (“A”) and a secondary (“B”) vapor trap were attached end-to-end to the systems on the effluent port of each of the saturator columns with the primary vapor trap before the secondary vapor trap. No test substance was added to these traps. Three spiked traps were prepared by applying test substance solution to each trap. One spiked trap was connected to the end of each dosed saturator

column after the secondary trap. A single vapor trap, containing no test substance, was connected to the effluent port of the control saturator column. All connections used for the test system were of Teflon or parafilm.

The flow rate of all systems was adjusted to 10 mL/min and measured with a digital flow meter. Flow rates were confirmed and adjusted several times throughout the study.

The vapor traps from the 10 mL/min definitive test were terminated after approximately 24 hours (1 day). The "A" (primary) and "B" (secondary), spiked, and control vapor traps were extracted. Each extract was diluted and then analyzed by HPLC.

The 8 mL/min definitive test was conducted exactly as described for the 10 mL/min definitive test, with the exception that the flow of nitrogen through the saturator columns was 8 mL/min.

The backpressure at the outlet to the saturator column caused by the vapor traps at nitrogen flow rates of 10 and 8 mL/min was measured at the test temperature. Three vapor traps were prepared and connected as in the preliminary and definitive tests, with the exception that no test substance was spiked onto any of the traps. The saturator column containing the control sand used in the definitive test and maintained in the 25°C environmental chamber was used. The vapor traps were placed on the saturator column, the nitrogen flow was set to 8 mL/min, and the system was equilibrated overnight.

The backpressure was measured by placing a U-tube manometer tilted with mercury between the saturator column and the vapor traps and measuring the pressure difference shown on the manometer. Flow rates were measured immediately before placing the manometer between the saturator column and the vapor traps. After letting the system equilibrate for about 10 minutes, a pressure reading was taken. This procedure was repeated twice for a total of 3 readings. The atmospheric pressure was measured using a NOVA mercury barometer. The backpressure at the outlet to the saturator column caused by the vapor traps at a nitrogen flow rate of 10 mL/min was determined as described for the 8 mL/min test. All solutions were refrigerated when not in use.

Following the termination of the definitive study, a 1 g aliquot of sand from each saturator column was extracted and analyzed for stability confirmation.

The vapor pressure was determined using the equations below:

Vapor Density:
$$d = \frac{m}{G \times t}$$

where:

d = vapor density (g/mL)

m = mass of trapped test material (g)

G = nitrogen flow rate (mL/min)

t = test duration (min)

Vapor Pressure:
$$P = d \frac{V_m (t = 273.15) P_B P_B}{M \quad 273.15 \quad P_C}$$

where:

P = vapor pressure (Pa)

d = vapor density (g/mL)

M = molecular weight of the test substance (g/mol)

V_m = molar volume of ideal gas (22.4E03 mL/mol)

t = temperature at saturator outlet ("C)

P_B = pressure of nitrogen at saturator outlet (Pa)
(atmospheric pressure + backpressure, Pa)

P_C = pressure of nitrogen at outlet of vapor traps
(atmospheric pressure)

GLP: Yes

Reference: ABC Laboratories, Inc. (2004). Unpublished Data, ABC Study No. 41829 (DuPont- 15 141), "Vapor Pressure Determination (Gas Saturation Method) for 2,2'-Azobis-(Methylbutyronitrile) CAS# 13472-08-7" (May 20).

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

Additional Reference for Vapor Pressure:

DuPont Co. (2000). Material Safety Data Sheet No. DUO00905 (March 28).

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2.5 **Partition Coefficient (log K_{ow}):**

Value: 2.07

Temperature: 20°C

Method: The procedures used in this test were based on the recommendations of the following guideline: U.S. EPA Product Properties Test Guidelines OPPTS 830.7550.

Preliminary Test

A volume of test substance solution in octanol saturated with water was added to each of 4 plastic **centrifuge** tubes. Reagent water saturated with octanol was added to each centrifuge tube. The tubes were capped, and the caps secured with electrical tape. The samples were placed on a shaker in a 20°C environmental chamber. Shaking was performed in the dark. After 2 and 24 hours, 2 samples were removed from the shaker, and were centrifuged at 20°C for 30 minutes. The octanol and aqueous phases were then separated.

The octanol phases were diluted using acetonitrile. The diluted samples were further diluted and analyzed by HPLC. The aqueous phases were also diluted for analysis.

Octanol quality control samples were prepared in triplicate, diluted, and analyzed by HPLC. Aqueous quality control samples were prepared in duplicate and diluted for analysis.

Definitive Test

The definitive test was performed at 3 volume ratios of octanol to water. The ratios were 1: 1, 2: 1, and 1:2 (v:v), or twice, the same, and ½ the volume ratio used during the preliminary test. Each volume ratio was performed in duplicate.

Volumes of the test substance solution in octanol saturated with reagent water were added to duplicate plastic centrifuge tubes. Reagent water saturated with octanol was added to each centrifuge tube. The tubes were capped, and the caps secured with electrical tape. The samples were placed on a shaker in a 20°C environmental chamber. Shaking was performed in the dark. After 24 hours, the samples were removed from the shaker, and centrifuged at 20°C for 30 minutes. The octanol and aqueous phases were then

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separated. The octanol and aqueous phases were diluted and analyzed by HPLC.

Octanol quality control samples were prepared in duplicate, diluted, and final dilutions were analyzed by HPLC. Aqueous quality control samples were prepared in duplicate and diluted for analysis.

The pH of each definitive test aqueous phase sample was measured.

The octanol/water partition was calculated from the following equation:

$$K_{ow} = \frac{C_o}{C_w}$$

where:

C_o = concentration of test substance at equilibrium in octanol phase

C_w = concentration of test substance at equilibrium in aqueous phase

GLP:	Yes
Reference:	ABC Laboratories, Inc. (2004). Unpublished Data, ABC Study No. 48 125 (DuPont- 15 138), "Determination of n-Octanol/Water Partition Coefficient (Shake Flask Method) for 2,2'-Azobis-(Methylbutyronitrile) CAS# 13472-08-r' (May 20).
Reliability:	High because a scientifically defensible or guideline method was used.
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). <u>J. Pharm. Sci.</u> , 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient: None Found.

2.6 Water Solubility

Value: 392±5 µg/mL (392±5 mg/L)
Temperature: 20°C
pH/pKa: 6.41
Method: The procedures used in this test were based on the recommendations of the following guideline: U.S. EPA Product Properties Test Guidelines OPPTS 830.7840.

Preliminary Test

Approximately 35 mg of the test substance was weighed into two plastic centrifuge tubes. Twenty mL of reagent water was added to each tube. The 2 samples were placed on a shaker in a 20°C environmental chamber. After approximately 2 hours, the samples were removed from the shaker and centrifuged for 30 minutes at 20°C to settle any undissolved test substance that remained in the tubes. The supernatant of each sample was diluted for analysis. A dilution of a separate test substance stock solution was analyzed concurrently with the samples.

Quality control samples were prepared in duplicate. The samples were placed on a shaker in the 20°C environmental chamber for 2 hours, and were diluted for analysis. All samples were refrigerated when not in use.

Definitive Test

Three test samples (replicates 1, 2, and 3) were prepared by adding test substance to plastic centrifuge tubes. Reagent water was added to each tube. The samples were capped, and the caps secured with electrical tape. They were placed on a platform shaker in a 20°C environmental chamber.

After approximately 24, 48, and 72 hours, the replicate samples 1, 2, and 3, respectively, were removed from the shaker in the 20°C environmental chamber. The samples were centrifuged for 30 minutes at 20°C. The supernatant was diluted for analysis in duplicate. Quality control samples were prepared in triplicate. The samples were placed on a shaker in the 20°C environmental chamber. One sample was removed at the 24-, 48-, and 72-hour sample points. The samples were diluted for analysis. The pH of the sample supernatants was measured after

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centrifugation. All solutions were refrigerated when not in use.

GLP: Yes
Reference: ABC Laboratories, Inc. (2004). Unpublished Data, ABC Study No. 48 126 (DuPont- 15 139), "Determination of Water Solubility by the Shake Flask Method for 2,2'-Azobis-(Methylbutyronitrile) CAS# 13472-08-7" (May 20).
Reliability: High because a scientifically defensible or guideline method was used.

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 132 10.

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.

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Additional Reference 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. DU000905 (March 28).

2.7 Flash Point: Not Applicable.

2.8 Flammability

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

Method: Autoignition Temperature = 185°C
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 = 1.5×10^6 OH/cm³).

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 $1.97 \times 10^{-6} \text{ atm} \cdot \text{m}^3/\text{mole}$ (Henry v3.10 Program, Bond SAR Method in SRC Epiwin v3.05) from its estimated vapor pressure ($6.7 \times 10^{-4} \text{ mm-Hg}$; 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 4 12 hours. 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 4616 hours (EPIWIN v. 3.11).

GLP: Not Applicable
Reference: Syracuse Research Corporation EPIWIN Version 3.11.
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	22.3%	0.12%	0.214%
	Water	16.2%	99.1%	10.3%
	Soil	61.4%	0.32%	89.4%
	Sediment	0.07%	0.43%	0.04%

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: 1.97×10^{-6} atm·m³/mole (calculated from experimentally determined water solubility and vapor pressure)

Vapor Pressure: 0.00306 mm Hg (converted from experimentally determined value of 0.408 Pa)

Log Kow : 2.07(experimentally determined)

Soil Koc : 48.2 (calc. by Level III model)

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: **7.83** (Log BCF = 0.894)
Method: Calculated by BCFWIN Computer Program, Vers. 2.15, 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. Boethhng, 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 LC₅₀**
Species: Fish
Value: 122.5 mg/L; log Kow = 2.07
Method: Modeled
GLP: Not Applicable
Test Substance:
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User’s Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 132 10.
Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type: **48-hour EC₅₀**
Species: Water flea
Value: 13 1.9 mg/L; log Kow = 2.07
Method: Modeled
GLP: Not Applicable
Test Substance: **Butanenitrile, 2,2'-azobis(2-methyl-**
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 132 10.
Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type: **72-hour EC₅₀**
Species: *Selenastrum capricornutum*
Value: Healthy Cell Count EC₅₀ = 38.1 mg/L (95% confidence limits, 32.6-44.6 mg/L); NOEC = 12.5 mg/L

Area Under the Growth Curve EC₅₀ = 31.3 mg/L (95% confidence limits, 23.6-39.1 mg/L); NOEC = 12.5 mg/L

Growth Rate EC₅₀ = 67.0 mg/L (95% confidence limits, 60.5-74.1 mg/L); NOEC = 12.5 mg/L
Method: OECD Guideline 201 (1984) and EEC Directive 92/69/EEC Annex 5, Part C.3.

The EC₅₀ value for growth rate was calculated based on 5 measured concentrations (6.20, 12.3, 24.5, 49.3, and 99.5 mg/L). DMF (100 mg/L) was used as a co-solvent.
GLP: Yes
Test Substance: Butanenitrile, 2,2'-azobis(2-methyl-, purity 97.4%
Results: The reductions in healthy cell count, area under the growth curve, and growth for *Selenastrum capricornutum* at 72 hours (3 days) indicated a dose-dependent response for increasing concentrations of the test substance. The most sensitive parameter was area under the growth curve with an EC₅₀ of 3 1.3 mg/L and a NOEC of 12.5 mg/L, based on

mean measured test concentrations. The ability to recover was assessed at measured concentrations of 49.3 and 99.5 mg/L. The test substance was determined to be algistatic at measured concentrations less than or equal to 99.5 mg/L.

Reference: DuPont Co. (2004). Unpublished Data, Haskell Laboratory Report DuPont - 11644, "Influence on Growth and Growth Rate of the Green Alga *Selenastrum capricornutum* with 2,2'-Azobis(2-methylbutyronitrile) (AMBN)" (February 12).

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

Type: 96-hour EC₅₀

Species: Green algae

Value: 82.8 mg/L; log Kow = 2.07

Method: Modeled

GLP: Not Applicable

Test Substance: Butanenitrile, 2,2'-azobis(2-methyl-

Results: No additional data.

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

Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

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 and 402 mg/kg.

GLP: Yes

Test Substance: (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

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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 (199 1). Unpublished Data, "Perkadox AMBN: Acute Oral Toxicity Study In The

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: 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. 5 13-80.

DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 5 1 1- 80.

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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.
Re ference: 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-** purity 100%
Results: The test substance produced no or conjunctival effects at any time when tested in rabbit eyes.

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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 (199 1). 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 typhimurium* 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 (199 1). 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

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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.