

06 March 2002**TEST PLAN/ROBUST SUMMARIES FOR BUTANENITRILE, 2,2'-AZOBIS(2-METHYL- WITH ITS ANALOG, PROPANENITRILE, 2,2'-AZOBIS(2-METHYL-**Summary

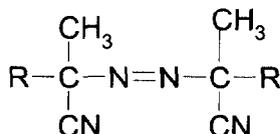
Two closely related azonitriles meet the production volume criteria for inclusion in the HPV Challenge Program:

- 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)

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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.

<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>
<b>Butanenitrile, 2,2'-azobis(2-methyl-</b>	13472-08-7	2,2'azobis-(2-methylbutyronitrile)	AMBN	CH <sub>3</sub> CH <sub>2</sub> -
<b>Propanenitrile, 2,2'-azobis(2-methyl-</b>	78-67-1	2,2'azobis-(2-isobutyronitrile)	AIBN	CH <sub>3</sub> -

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.

**06 March 2002**

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<sub>3</sub>) in AIBN with ethyl groups (CH<sub>3</sub>CH<sub>2</sub>). 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 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
<b>PHYSICAL/CHEMICAL CHARACTERISTICS</b>		
Melting Point	√/-	√
Boiling Point	N/A	N/A
Vapor Pressure	√/-	√
Partition Coefficient	√/-	√
Water Solubility	√/-	√
<b>ENVIRONMENTAL FATE</b>		
Photodegradation	√ <sup>1</sup>	√
Stability in Water	√ <sup>1</sup>	√
Transport (Fugacity)	√ <sup>1</sup>	√
Biodegradation	- <sup>2</sup>	√
<b>ECOTOXICITY</b>		
Acute Toxicity to Fish	- <sup>2</sup>	√
Acute Toxicity to Invertebrates	- <sup>2</sup>	√
Acute Toxicity to Aquatic Plants	- <sup>2</sup>	√
<b>MAMMALIAN TOXICITY</b>		
Acute Toxicity	√	√
Repeated Dose Toxicity	- <sup>2</sup>	√
Developmental Toxicity	- <sup>2</sup>	√
Reproductive Toxicity	- <sup>2</sup>	√
Genetic Toxicity Bacterial Gene Mutations	√	√
Genetic Toxicity Chromosomal Aberrations ( <i>in vitro</i> )	- <sup>2</sup>	√
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. <sup>1</sup> Modeled data, which will be re-evaluated when new physical/chemical data are available. <sup>2</sup> 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**

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

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

	AMBN	AIBN
<b>Partition Coefficient (log Kow)</b>	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  $2.19 \times 10^{-10}$  atm-m<sup>3</sup>/mole, and the estimated half-life from a river is  $3.7 \times 10^6$  hours (> 400 years). Measured half-lives for AIBN ranged from 210-304 days at 25°C, 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  $2.97 \times 10^{-12}$  cm<sup>3</sup>/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**

	<b>AMBN</b>	<b>AIBN</b>
<b>Bioaccumulation</b> *	Moderate potential for bioaccumulation BCF = 185.7	Low potential for bioaccumulation BCF = 1.403
<b>Biodegradation</b>	No Data	Not readily biodegradable
<b>Fugacity</b> *	When released 100% to air:  Air 0.00302% Water 6% Soil 93.7% Sediment 0.0758%  When released 100% to water:  Air $1.98 \times 10^{-8}$ % Water 98% Soil 0.000614% Sediment 1.2%  When released 100% to soil:  Air $3.76 \times 10^{-7}$ % 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<sub>50</sub> of 580 mg/L in fish, and a 48-hour EC<sub>50</sub> of 397 mg/L in *Daphnia* (greater than water solubility). A 72-hour EC<sub>50</sub> of > 9.4 mg/L in algae was reported for AIBN (dispersed with DMF). The expected similarity between the two azonitriles and the low aquatic toxicity of AIBN suggest that further aquatic toxicity testing with AMBN is unlikely to provide new information on the azonitriles sufficient to warrant such testing. **Therefore, no further ecotoxicity testing is recommended.**

**Table 4: Ecotoxicity**

	AMBN	AIBN
<b>Toxicity to Fish</b> (LC <sub>50</sub> value)	No Data	580 mg/L (96-hour; nominal)
<b>Toxicity to Invertebrates</b> (EC <sub>50</sub> value)	No Data	397 mg/L (48-hour; nominal)
<b>Toxicity to Algae</b> (EC <sub>50</sub> 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<sub>50</sub> 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
<b>Oral LD<sub>50</sub></b> (rat)	337 mg/kg	360 mg/kg
<b>Inhalation LC<sub>50</sub></b> (rat)	>8.9 mg/L (4-hour)	> 7.78 mg/L (1-hour)
<b>Dermal LD<sub>50</sub></b> (rabbit)	No Data	5010-7940 mg/kg
<b>Dermal Irritation</b>	Not irritating	Not irritating
<b>Eye Irritation</b>	Irritation effects observed only at 1 hour after dosing	Not irritating
<b>Dermal Sensitization</b>	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

**06 March 2002**

tubular epithelial cells in the kidneys were observed only in treated male rats. Accumulation of  $\alpha_{2u}$ -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<sup>3</sup>, 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**

	<b>AMBN</b>	<b>AIBN</b>
<b>Repeated Dose Toxicity (NOAEL)</b>	No Data	2 mg/kg/day in a repeated dose rat study  50 ppm in a 90-day dog study  10 mg/m <sup>3</sup> in a 2-week inhalation study
<b>Developmental Toxicity (NOAEL)</b>	No Data	50 mg/kg/day
<b>Reproductive Toxicity (NOAEL)</b>	No Data	10 mg/kg/day (parental generation) 50 mg/kg/day (F <sub>1</sub> 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**

	<b>AMBN</b>	<b>AIBN</b>
<b>Mutagenicity</b>	Not mutagenic (Ames test)	Not mutagenic (Ames test)
<b>Clastogenicity</b>	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 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 abstractable hydrogen on the  $\alpha$  carbon. Examples of hydroxylation of methyl groups situated  $\beta$  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  $\alpha$  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  $\alpha$  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  $\alpha$  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.

**06 March 2002**

### 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<sup>3</sup> 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<sup>®</sup>, 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
<b>PHYSICAL/CHEMICAL CHARACTERISTICS</b>			
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
<b>ENVIRONMENTAL FATE</b>			
Photodegradation	Y	N	Y <sup>1</sup>
Stability in Water	Y	N	Y <sup>1</sup>
Transport (Fugacity)	Y	N	Y <sup>1</sup>
Biodegradation	Y	Y <sup>2</sup>	N
<b>ECOTOXICITY</b>			
Acute Toxicity to Fish	Y	Y <sup>2</sup>	N
Acute Toxicity to Invertebrates	Y	Y <sup>2</sup>	N
Acute Toxicity to Aquatic Plants	Y	Y <sup>2</sup>	N
<b>MAMMALIAN TOXICITY</b>			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y	Y <sup>2</sup>	N
Developmental Toxicity	Y	Y <sup>2</sup>	N
Reproductive Toxicity	Y	Y <sup>2</sup>	N
Genetic Toxicity Bacterial Gene Mutations	Y	Y	N
Genetic Toxicity Chromosomal Aberrations	Y	Y	N
<p>N/A = Not Applicable.  <sup>1</sup> Modeled data will be re-run after completion of re-analysis of physical/chemical characteristics.  <sup>2</sup> Data is available for the analog AIBN.</p>			