

## SCREENING-LEVEL HAZARD CHARACTERIZATION

### SPONSORED CHEMICAL

**2,2'-Azobis-(2-methylbutyronitrile)**  
(AMBN; CASRN 13472-08-7)

### SUPPORTING CHEMICAL

**2,2'-Azobis-(2-isobutyronitrile)**  
(AIBN; CASRN 78-67-1)

The High Production Volume (HPV) Challenge Program<sup>1</sup> was conceived as a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsored chemicals; sponsorship entailed the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data did not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set<sup>1,2</sup>) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance<sup>2,3</sup> and is based primarily on hazard data provided by sponsors; however, in preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. In order to determine whether any new hazard information was developed since the time of the HPV submission, a search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT's focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available.

OPPT does not develop HCs for those HPV chemicals which have already been assessed internationally through the HPV program of the Organization for Economic Cooperation and

---

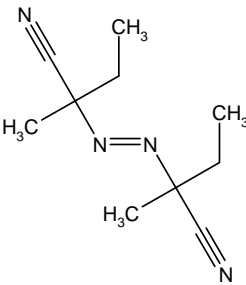
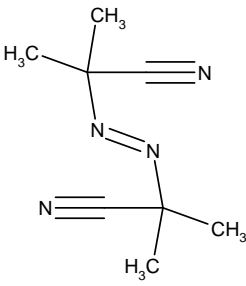
<sup>1</sup> U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

<sup>2</sup> U.S. EPA. HPV Challenge Program – Information Sources; <http://www.epa.gov/chemrtk/pubs/general/guidocs.htm>.

<sup>3</sup> U.S. EPA. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

Development (OECD) and for which Screening Initial Data Set (SIDS) Initial Assessment Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments.

These hazard characterizations are technical documents intended to inform subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public.

<p><b>Chemical Abstract Service Registry Number (CASRN)</b></p>	<p><b><u>Sponsored Chemical</u></b> <b>13472-08-7</b></p> <p><b><u>Supporting Chemical</u></b> <b>78-67-1</b></p>
<p><b>Chemical Abstract Index Name</b></p>	<p><b><u>Sponsored Chemical</u></b> <b>Butanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl-</b></p> <p><b><u>Supporting Chemical</u></b> <b>Propanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl-</b></p>
<p><b>Structural Formula</b></p>	<p><b><u>Sponsored Chemical</u></b></p>  <p><b><u>Supporting Chemical</u></b></p> 
<p style="text-align: center;"><b>Summary</b></p> <p>CASRN 13472-08-7 is a white, odorless solid at ambient temperature with moderate water solubility and moderate vapor pressure. It is expected to have moderate mobility in soil. Volatilization is considered low based on the Henry's Law constant of this substance. The rate of hydrolysis is also considered negligible based on data for the supporting chemical, CASRN 78-67-1. The rate of atmospheric photooxidation is considered low. Although azo functional groups are generally resistant to aerobic biodegradation, they are susceptible to anaerobic biodegradation. CASRN 13472-08-7 is also thermally unstable at relatively low temperatures. Therefore, CASRN 13472-08-7 is judged to have moderate persistence (P2) and low</p>	

bioaccumulation potential (B1).

The acute oral toxicity of CASRN 13472-08-7 and its supporting chemical, CASRN 78-67-1, to rats is moderate. The acute inhalation toxicity of CASRN 13472-08-7 and its supporting chemical, CASRN 78-67-1, to rats is low. The acute dermal toxicity of the supporting chemical, CASRN 78-67-1, to rabbits is low. Repeated dietary exposure of dogs to the supporting chemical CASRN 78-67-1 for 13 weeks, showed increased relative liver weights and morphologic changes in the liver at ~ 3.75 mg/kg/day; the NOAEL for systemic toxicity in dogs is ~ 1.25 mg/kg/day. Repeated inhalation exposure of rats for 2 weeks to the supporting chemical, CASRN 78-67-1, showed increased cytoplasmic basophilia of hepatocytes at 0.08 mg/L; the NOAEC for systemic toxicity in rats is 0.01 mg/L. In a combined repeated-dose/reproductive/developmental toxicity screening study, oral exposure of rats to the supporting chemical, CASRN 78-67-1, showed significantly increased liver weights in males and hepatocyte hypertrophy in females at 10 mg/kg/day; the NOAEL for systemic toxicity in rats is 2 mg/kg/day. Dams showed decreased body weight and food consumption at 10 mg/kg/day; the NOAEL for maternal toxicity is 2 mg/kg/day. No adverse effects on viability, sex ratio and body weight gain of pups were observed; the NOAEL for reproductive toxicity is 50 mg/kg/day (highest dose tested). No morphological abnormalities in pups were observed; however, a NOAEL for developmental toxicity was not determined. CASRN 13472-08-7 and the supporting chemical, CASRN 78-67-1, were not mutagenic in bacteria *in vitro* and the supporting chemical, CASRN 78-67-1, did not induce chromosomal aberrations in mammalian cells *in vitro*. CASRN 13472-08-7 and the supporting chemical, CASRN 78-67-1, did not irritate rabbit skin or eyes.

The 96-h LC<sub>50</sub> of the supporting chemical, CASRN 78-67-1, to fish is 580 mg/L. The 48-h EC<sub>50</sub> of the supporting chemical, CASRN 78-67-1, to aquatic invertebrates is 397 mg/L. The 72-h EC<sub>50</sub> values to aquatic plants from exposure to CASRN 13472-08-7 are 31.3 and 67 mg/L for biomass and growth rate, respectively.

No data gaps were identified under the HPV Challenge Program.

The sponsor, E. I. du Pont de Nemours & Company, Inc. and Akzo-Nobel Chemicals, Inc., submitted a Test Plan and Robust Summaries to EPA for butanenitrile, 2,2'-azobis(2-methyl)- (AMBN) (CASRN 13472-08-7; 9<sup>th</sup> CI name: butanenitrile, 2,2'-azobis(2-methyl)- on March 5, 2002. EPA posted the submission on the ChemRTK HPV Challenge website on April 19, 2002 (<http://www.epa.gov/chemrtk/pubs/summaries/butan22a/c13659tc.htm>). EPA comments on the original submission were posted to the website on August 16, 2002. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on May 17, 2006 and January 9, 2007, which were posted to the ChemRTK website on July 21, 2006 and February 1, 2007, respectively.

### **Justification for Supporting Chemical**

EPA agrees with the sponsor's proposal to use propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1) as a supporting chemical for CASRN 13472-08-7 for aquatic toxicity, repeated-dose, reproductive and developmental toxicity endpoints based on similar functional groups, similar molecular structure, and similar reactivity, physical/chemical properties, and available toxicity data.

An OECD SIDS Initial Assessment Report on CASRN 78-67-1 was prepared for evaluation at SIAM 9 in 1999 and is available at: <http://www.chem.unep.ch/irptc/sids/OECD/SIDS/78671.pdf>.

#### **1. Chemical Identity**

##### **1.1 Identification and Purity**

CASRN 13472-08-7 is an azonitrile compound used industrially as a solid free-radical initiator in polymerization reactions. Purity of CASRN 13472-08-7, when provided in the Robust Summaries, was greater than 98%. Purity of the supporting chemical CASRN 78-67-1, when provided, was greater than 98%.

##### **1.2 Physical-Chemical Properties**

CASRN 13472-08-7 is a white, odorless solid at ambient temperature with moderate water solubility and moderate vapor pressure.

The physical-chemical properties of CASRN 13472-08-7, as well as the supporting chemical CASRN 78-67-1 are summarized in Table 1.

<b>Property</b>	<b>Butanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl- SPONSORED CHEMICAL</b>	<b>Propanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl- SUPPORTING CHEMICAL</b>
	<b>Value</b>	<b>Value</b>
CASRN	13472-08-7	78-67-1
Molecular Weight	192.26	164.21
Physical State	White, odorless solid	White, odorless, crystalline solid
Melting Point	45°C (decomposes)	100–103°C (decomposes)
Boiling Point	Decomposes	Decomposes
Vapor Pressure	9.16×10 <sup>-4</sup> mm Hg at 25°C (estimated)	6.1×10 <sup>-3</sup> mm Hg at 25°C (measured)
Water Solubility	35 mg/L (estimated) <sup>2,3</sup>	350 mg/L (measured)
Dissociation Constant (pK <sub>a</sub> )	Not applicable	Not applicable
Henry's Law Constant	2.19×10 <sup>-10</sup> atm-m <sup>3</sup> /mole (estimated) <sup>2</sup>	1.2×10 <sup>-10</sup> atm-m <sup>3</sup> /mole (estimated) <sup>2</sup>
Log K <sub>ow</sub>	3.86 (estimated)	1.10 (measured)

<sup>1</sup> E. I. du Pont de Nemours & Co., Inc and Akzo Nobel Polymer Chemicals LLC. 2007. Revised Robust Summaries for 2,2'-Azobis-(2-Methylbutyronitrile) (AMBN). Available online at <http://www.epa.gov/chemrtk/pubs/summaries/butan22a/c13659tc.htm>.

<sup>2</sup> U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm>.

<sup>3</sup> The WATERNT program contained in EPIWIN 4.00 was employed to estimate the water solubility of AMBN using the SMILES notation of the supporting chemical (AIBN) and the measured water solubility of 350 mg/L of the supporting chemical.

## **2. General Information on Exposure**

### **2.1 Production Volume and Use**

CASRN 13472-08-7 had an aggregated production and/or import volume in the United States between 1 and 10 million pounds during calendar year 2005.

Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include paint and coating manufacturing and resin and synthetic rubber manufacturing as process regulators, used in vulcanization or polymerization processes. No commercial and consumer uses were reported.

### **2.2 Environmental Exposure and Fate**

CASRN 13472-08-7 is expected to have moderate mobility in soil. The supporting chemical, CASRN 78-67-1, is not readily biodegradable, suggesting that CASRN 13472-08-7 is also not readily biodegradable. Volatilization is considered low based on the Henry's Law constant of this substance. The rate of hydrolysis is also considered negligible based on the data for the supporting chemical. Although azo functional groups are generally resistant to aerobic

biodegradation, they are susceptible to anaerobic biodegradation. CASRN 13472-08-7 is also thermally unstable at relatively low temperatures. Therefore, CASRN 13472-08-7 is judged to have moderate persistence (P2) and low bioaccumulation potential (B1).

The environmental fate characteristics of CASRN 13472-08-7, as well as the supporting chemical CASRN 78-67-1 are summarized in Table 2.

<b>Property</b>	<b>Butanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl-SPONSORED CHEMICAL</b>	<b>Propanenitrile, 2,2'-(1,2-diazenediyl)bis[2-methyl-SUPPORTING CHEMICAL</b>
	<b>Value</b>	<b>Value</b>
CASRN	13472-08-7	78-67-1
Photodegradation Half-life	3.6 days (estimated)	16.0 days (estimated)
Hydrolysis Half-life	No data	263 days at pH 4 and 25°C (measured) 304 days at pH 7 and 25°C (measured) 210 days at pH 9 and 25°C (measured)
Biodegradation	No data	7% after 28 days (not readily biodegradable)
Bioaccumulation Factor	230 (estimated) <sup>2</sup>	2.0 (estimated) <sup>2</sup>
Log K <sub>oc</sub>	2.0 (estimated) <sup>2</sup>	1.4 (estimated) <sup>2</sup>
Fugacity (Level III Model) <sup>2</sup>		
Air (%)	<0.1	3.1
Water (%)	16.5	35.2
Soil (%)	83.4	61.6
Sediment (%)	0.1	0.1
Persistence <sup>3</sup>	P2 (moderate) <sup>4</sup>	P2 (moderate) <sup>4</sup>
Bioaccumulation <sup>3</sup>	B1 (low)	B1 (low)

<sup>1</sup>E. I. du Pont de Nemours & Co., Inc (2007) and Akzo Nobel Polymer Chemicals LLC (2006). Revised Robust Summaries for 2,2'-Azobis-(2-Methylbutyronitrile) (AMBN). Available online at <http://www.epa.gov/chemrtk/pubs/summaries/butan22a/c13659tc.htm>.

<sup>2</sup>U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at <http://www.epa.gov/opptintr/exposure/pubs/episuite.dll.htm>.

<sup>3</sup>Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

<sup>4</sup>Although azo groups, especially in azo dyes, are generally resistant to aerobic degradation the test substance is not expected to be persistent for extended periods in the environment given its thermal instability (half-life = 10 hours at 67°C in non-polymerizing solutions) and the potential for azo groups to degrade anaerobically.

**Conclusion:** CASRN 13472-08-7 is a white, odorless solid at ambient temperature with moderate water solubility and moderate vapor pressure. It is expected to have moderate mobility in soil. Volatilization is considered low based on the Henry's Law constant of this substance.

The rate of hydrolysis is also considered negligible based on data for the supporting chemical, CASRN 78-67-1. The rate of atmospheric photooxidation is considered low. Although azo functional groups are generally resistant to aerobic biodegradation, they are susceptible to anaerobic biodegradation. CASRN 13472-08-7 is also unstable at relatively low temperatures. Therefore, CASRN 13472-08-7 is judged to have moderate persistence (P2) and low bioaccumulation potential (B1).

### 3. Human Health Hazard

A summary of health effects data submitted for SIDS endpoints is provided in Table 3. The table also indicates where data for the supporting chemical are read-across (RA) to the sponsored chemical.

#### *Acute Oral Toxicity*

##### ***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

Sprague-Dawley rats (number/sex/dose not provided) were administered CASRN 13472-08-7 at 202, 254, 320 or 402 mg/kg-bw and observed for 6 days after dosing. Mortality occurred at dose levels greater than 320 mg/kg-bw within 2 days of dosing.

**LD<sub>50</sub> = 337 mg/kg-bw**

##### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

Sprague-Dawley rats (5/sex/dose) were administered CASRN 78-67-1 (10% solution in corn oil) via gavage at 251, 316, 398 or 501 mg/kg-bw and observed for 14 days after dosing. Mortality was observed at dose levels  $\geq$  316 mg/kg-bw within 5 days of dosing.

**LD<sub>50</sub> = 360 mg/kg-bw**

#### *Acute Inhalation Toxicity*

##### ***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

Male Sprague Dawley rats (6/dose) were exposed (nose-only) to airborne dust of CASRN 13472-08-7 at 1.8, 3.7 or 8.9 mg/L for 4 hours and observed for 14 days after exposure. The respirable fraction was measured and ranged from 11 to 31% for  $< 10 \mu\text{m}$  particles and 2 to 8% for  $< 5 \mu\text{m}$  particles. No mortality was observed at any dose.

**LC<sub>50</sub> > 8.9 mg/L**

##### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

Sprague Dawley rats (5/sex/dose) were exposed (nose-only) to CASRN 78-67-1 at 1.57, 3.40 or 7.78 mg/L (purity  $>98\%$ ) for 1 hour and observed for 14 days after exposure. The respirable fraction was measured and ranged from 6.6 to 10% for  $< 10 \mu\text{m}$  particles. One male rat died after 1 day of exposure at 1.57 mg/L. No other deaths occurred.

**LC<sub>50</sub> > 7.78 mg/L**

### ***Acute Dermal Toxicity***

#### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

New Zealand White rabbits (1/sex) were dermally administered CASRN 78-67-1 (40% solution in corn oil) at 5010 and 7940 mg/kg-bw and observed for 14 days after exposure. The rabbit dosed at 5010 mg/kg-bw survived, while the animal dosed at 7940 mg/kg-bw died within 9 days. **ALD = 5010 – 7940 mg/kg-bw**

### ***Repeated-Dose Toxicity***

#### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

(1) In a combined repeated-dose and reproductive/developmental toxicity screening test, Sprague-Dawley rats (12/dose) were administered CASRN 78-67-1 via gavage at 0, 2, 10 or 50 mg/kg-day (males for 42 days and females 14 days before mating to day 3 of lactation). One female rat at 50 mg/kg/day died at 3 days postpartum. Decreased body weight gain and food consumption was observed at 50 mg/kg/day in males and at  $\geq 10$  mg/kg/day in females (time period of these changes were not indicated). In males, relative kidney weight increased at  $\geq 10$  mg/kg/day and absolute kidney weight was increased in all treatment groups; granular casts, increased eosinophilic bodies and basophilic changes were observed in the renal tubular epithelial cells<sup>4</sup>. In females, both relative and absolute kidney weight increased at 50 mg/kg/day. The robust summary noted that, since changes in renal pathology were observed only in males, accumulation of  $\alpha$ -2 $\mu$ -macroglobulin may be involved and therefore kidney effects are not relevant to human health. Absolute and relative liver weight was significantly increased at 10 and 50 mg/kg/day in males and at 50 mg/kg-day in females. Centrilobular hypertrophy of hepatocytes was observed in both sexes at  $\geq 10$  mg/kg/day.

**LOAEL = 10 mg/kg/day** (based on increased liver weight in males and liver hypertrophy in males and females and decreased body weight gain in females)

**NOAEL = 2 mg/kg/day**

(2) Beagle dogs (4/sex/dose) were administered CASRN 78-67-1 via the diet at 0, 50, 150, 300, or 1000 ppm ( $\sim 0, 1.25, 3.75, 7.50$  and 25 mg/kg/day) for 90 days. After one death at 25 mg/kg/day, all animals at this dose level were sacrificed at 28 days. Decreased body weight and food consumption were observed in these animals, as well as increased relative liver and kidney weight. No other clinical signs were observed at  $\leq 7.5$  mg/kg/day. Increased relative liver weight was observed at  $\geq 3.75$  mg/kg/day and increased relative kidney weight at 25 mg/kg/day. Treatment-related morphologic changes in the liver (not described in the Robust Summary) were noted at  $\geq 3.75$  mg/kg/day. Increased blood serum enzymatic activity was noted at 25 mg/kg/day including serum alkaline phosphatase, serum glutamic-pyruvic transaminase and serum glutamic-oxalacetic transaminase. Serum alkaline phosphatase was also increased at 25 mg/kg/day.

---

<sup>4</sup> Nephropathy seen in male rats may be occurring by an alpha 2 $\mu$ -globulin-mediated mechanism (which is male rat-specific and not considered relevant to humans). EPA's Risk Assessment Forum has outlined key events and data that are necessary to demonstrate this mode of action (Alpha 2 $\mu$ -Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Rat, EPA/625/3-91/019F).

**LOAEL ~ 3.75 mg/kg/day** (based on increased relative liver weight and morphologic changes in the liver)

**NOAEL ~ 1.25 mg/kg/day**

(3) Male Sprague-Dawley rats (10/dose) were exposed to dust atmospheres of CASRN 78-67-1 at 0, 10 or 80 mg/m<sup>3</sup> (0, 0.01 or 0.08 mg/L) (head only) for 6 hours/day, 5 days/week for 2 weeks. Half of the rats were sacrificed after the last exposure and the other half were sacrificed after 14 days for pathology and histopathology exams. One rat at 80 mg/m<sup>3</sup> displayed adverse clinical signs and was sacrificed early, but the relation to exposure was unknown. Mean body weight gain was reduced at 80 mg/m<sup>3</sup> on days 2 – 4 of the treatment period, but not at other time points. Increased serum total protein and lower urine osmolality was observed at 80 mg/m<sup>3</sup>. Increased cytoplasmic basophilia of hepatocytes was observed at 80 mg/m<sup>3</sup>, but the effect resolved after recovery. Relative mean liver weights were increased at 10 mg/m<sup>3</sup>, but the effect resolved after recovery.

**LOAEL = 0.08 mg/L** (based on effects on liver and signs of increased hepatic hyperplasia)

**NOAEL = 0.01 mg/L**

### ***Reproductive and Developmental Toxicity***

#### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

In the combined repeated-dose/reproductive/developmental toxicity screening test described previously, there were no significant effects on copulation, fertility, duration of pregnancy, gestation index or parturition at any dose group. Decreased body weight gain and food consumption was observed at  $\geq 10$  mg/kg/day in females (time period of these changes were not indicated). Three of 12 dams at 50 mg/kg/day showed difficulty nursing and 2 of these litters died within 4 days of birth. There is not enough information provided to determine the significance of effects on viability of pups at birth and body weight of pups at postnatal day 4. No morphological abnormalities were noted in pups at any treatment dose.

**LOAEL (maternal toxicity) = 10 mg/kg/day** (based on decreased body weight gain)

**NOAEL (maternal toxicity) = 2 mg/kg/day**

**NOAEL (reproductive toxicity) = 50 mg/kg/day** (highest dose tested)

**LOAEL/NOAEL (developmental toxicity) = not determined**

### ***Genetic Toxicity – Gene Mutation***

#### ***In vitro***

#### ***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were exposed to CASRN 13472-08-7 in DMSO at concentrations ranging from 50 to 5000  $\mu$ g/plate in the presence and absence of metabolic activation. No evidence of mutagenic activity was noted either with or without metabolic activation. Positive controls were tested concurrently, but responses were not provided.

**CASRN 13472-08-7 was not mutagenic in this assay.**

***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA97 were exposed to CASRN 78-67-1 at concentrations of 0, 313, 625, 1250, 2500 or 5000 µg/plate in the presence and absence of metabolic activation. Cytotoxicity was not observed. CASRN 78-67-1 was negative for induction of mutation in the presence and absence of metabolic activation. Positive and negative controls were tested concurrently, but their responses were not provided.

**CASRN 78-67-1 was not mutagenic in this assay.**

***Genetic Toxicity – Chromosomal Aberrations***

***In vitro***

***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

Chinese hamster lung (CHUIU) cells were exposed to CASRN 78-67-1 at concentrations of 0, 0.40, 0.80 or 1.6 mg/mL in the presence and absence of metabolic activation. The short-term treatment was 6 hours and the continuous treatment was 24 and 48 hours. Cytotoxicity was not observed. CASRN 78-67-1 was negative for clastogenicity and polyploidy in the presence and absence of metabolic activation. Positive controls were tested concurrently, but their responses were not provided.

**CASRN 78-67-1 did not induce chromosomal aberrations in this assay.**

***Additional Information***

***Skin Irritation***

***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

CASRN 13472-08-7 (0.5 g) was applied to the skin of three New Zealand rabbits under semi-occlusive conditions for 4 hours, then assessed for up to 72 hours after exposure. No skin irritation was observed.

**CASRN 13472-08-7 was not an irritant to rabbit skin in this study.**

***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

CASRN 78-67-1 (purity 99.2%) was applied to the skin of New Zealand White rabbits. Additional details regarding the method of testing were not provided. CASRN 78-67-1 was noted as not irritating to rabbit skin.

**CASRN 78-67-1 was not an irritant to rabbit skin in this study.**

***Eye Irritation***

***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

(1) CASRN 13472-08-7 (28.4 mg) was placed in the conjunctival sac of the right eye of two male albino rabbits. The eye of one rabbit was washed 20 seconds post-treatment and the other was not washed. Observations of the cornea, iris and conjunctiva were made at 1 and 4 hours and at 1, 2 and 3 days. No irritant effects on cornea, iris or conjunctiva were observed.

**CASRN 13472-08-7 was not an irritant to rabbit eyes in this study.**

(2) CASRN 13472-08-7 (0.1 mg) was instilled in the right eye of three rabbits and washed 20 seconds post-treatment. One eye was not treated. Observations of the cornea, iris and conjunctiva were made at 1, 24 and 72 hours. At 1 hour, irritation of the conjunctiva and slight chemosis was observed in all animals and iritis was observed in two animals. No irritation was observed in the animals at the 24- and 72-hour observation period.

**CASRN 13472-08-7 was not an irritant to rabbit eyes in this study.**

***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

CASRN 78-67-1 was applied to the eyes of rabbits. Additional details regarding the method of testing were not provided.

**CASRN 78-67-1 was not an irritant to rabbit eyes in this study.**

***Sensitization***

***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

Duncan Hartley guinea pigs (10/dose) were administered CASRN 13472-08-7 in 0.05 mL of an 8 or 80% solution in dimethyl phthalate (DMP) to the surface of shaved, intact shoulder skin. Sensitization was induced with a series of four intradermal injections of 0.1 mL of a 1% suspension of DMP, one each week beginning 2 days after administration. After a 13-day rest period, animals were challenged with 0.05 mL of an 8 and 80% solution of CASRN 13472-08-7 in DMP on shaved intact shoulder skin. Reactions were observed at 24 and 48 hours post-challenge. Ten unexposed animals (controls) received the same topical application. No irritation was observed in treated animals at 24 or 48 hours and no sensitization response occurred in treated or control animals.

**CASRN 13472-08-7 was not a dermal sensitizer in guinea pigs in this study.**

***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

(1) In a maximization test, guinea pigs were exposed to CASRN 78-67-1 dermally. Additional details regarding the method of testing were not provided.

**CASRN 78-67-1 was not a dermal sensitizer in guinea pigs in this study.**

(2) Patients (173) occupationally exposed to glues and plastic were referred to a dermatology clinic in Finland for suspected skin disease. Unspecified amounts of CASRN 78-67-1 were applied to the skin in a patch test during diagnosis. Additional details regarding the method of testing were not provided. At a dose of 1%, CASRN 78-67-1 did not produce allergic skin reactions in the patients. CASRN 78-67-1 produced an irritant reaction in one test subject.

**CASRN 78-67-1 was not a dermal sensitizer (up to 1% concentrations) in humans in this study.**

**Conclusions:** The acute oral toxicity of CASRN 13472-08-7 and its supporting chemical, CASRN 78-67-1, to rats is moderate. The acute inhalation toxicity of CASRN 13472-08-7 and its supporting chemical, CASRN 78-67-1, to rats is low. The acute dermal toxicity of the supporting chemical, CASRN 78-67-1, to rabbits is low. Repeated dietary exposure of dogs to the supporting chemical CASRN 78-67-1 for 13 weeks, showed increased relative liver weights and morphologic changes in the liver at ~ 3.75 mg/kg/day; the NOAEL for systemic toxicity in dogs is ~ 1.25 mg/kg/day. Repeated inhalation exposure of rats for 2 weeks to the supporting

chemical, CASRN 78-67-1, showed increased cytoplasmic basophilia of hepatocytes at 0.08 mg/L; the NOAEC for systemic toxicity in rats is 0.01 mg/L. In a combined repeated-dose/reproductive/developmental toxicity screening study, oral exposure of rats to the supporting chemical, CASRN 78-67-1, showed significantly increased liver weights in males and hepatocyte hypertrophy in females at 10 mg/kg/day; the NOAEL for systemic toxicity in rats is 2 mg/kg/day. Dams showed decreased body weight and food consumption at 10 mg/kg/day; the NOAEL for maternal toxicity is 2 mg/kg/day. No adverse effects on viability, sex ratio and body weight gain of pups were observed; the NOAEL for reproductive toxicity is 50 mg/kg/day (highest dose tested). No morphological abnormalities in pups were observed; however, a NOAEL for developmental toxicity was not determined. CASRN 13472-08-7 and the supporting chemical, CASRN 78-67-1, were not mutagenic in bacteria *in vitro* and the supporting chemical, CASRN 78-67-1, did not induce chromosomal aberrations in mammalian cells *in vitro*. CASRN 13472-08-7 and the supporting chemical, CASRN 78-67-1, did not irritate rabbit skin or eyes.

<b>Table 3. Summary Table of the Screening Information Data Set as submitted under the U.S. HPV Challenge Program – Human Health Data</b>		
<b>Endpoints</b>	<b>SPONSORED CHEMICAL Butanenitrile, 2,2-azobis(2-methyl)- (AMBN) (13472-08-7)</b>	<b>SUPPORTING CHEMICAL Propanenitrile, 2,2'-azobis(2-methyl)- (AIBN) (78-67-1)</b>
<b>Acute Oral Toxicity LD<sub>50</sub> (mg/kg-bw)</b>	<b>337</b>	<b>360</b>
<b>Acute Inhalation Toxicity LC<sub>50</sub> (mg/L)</b>	<b>&gt; 8.9</b>	<b>&gt; 7.78</b>
<b>Acute Dermal Toxicity LD<sub>50</sub> (mg/kg-bw)</b>	No Data 5010 – 7940 (RA)	<b>5010 – 7940</b>
<b>Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg/day)</b>	No Data NOAEL = 2 LOAEL = 10 (RA)  No Data NOAEL ~ 1.25 LOAEL ~ 3.75 (RA)	<b><u>Rat</u> NOAEL = 2 LOAEL = 10</b>  <b><u>Dog</u> NOAEL ~ 1.25 LOAEL ~ 3.75</b>

<b>Table 3. Summary Table of the Screening Information Data Set                      as submitted under the U.S. HPV Challenge Program – Human Health Data</b>		
<b>Endpoints</b>	<b>SPONSORED                      CHEMICAL                      Butanenitrile, 2,2-                      azobis(2-methyl)-                      (AMBN)                      (13472-08-7)</b>	<b>SUPPORTING                      CHEMICAL                      Propanenitrile, 2,2'-                      azobis(2-methyl)- (AIBN)                      (78-67-1)</b>
<b>Repeated-Dose Toxicity                      NOAEL/LOAEL                      Inhalation (mg/L/day)</b>	No Data NOAEL = 0.08 LOAEL = 0.01 (RA)	<b>NOAEL = 0.08                      LOAEL = 0.01</b>
<b>Reproductive/Developmental                      Toxicity                      NOAEL/LOAEL                      Oral (mg/kg-bw/day)                      Maternal Toxicity                      Reproductive Toxicity                      Developmental Toxicity</b>	No Data NOAEL = 2 LOAEL = 10 (RA) No Data NOAEL = 50 (RA) -	<b>NOAEL = 2                      LOAEL = 10                      NOAEL = 50                      (highest dose tested)                      Not determined</b>
<b>Genetic Toxicity – Gene Mutation                      In vitro</b>	<b>Negative</b>	<b>Negative</b>
<b>Genetic Toxicity – Chromosomal                      Aberrations                      In vitro</b>	No Data Negative (RA)	<b>Negative</b>
<b>Additional Information                      Skin Irritation                      Eye Irritation                      Skin Sensitization</b>	<b>Not irritating                      Not irritating                      Not sensitizing</b>	<b>Not irritating                      Not irritating                      Not sensitizing</b>

Measured data in bold; (RA) = Read Across

#### 4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4. The table also indicates where data for the supporting chemical are read-across (RA) to the sponsored chemical.

##### *Acute Toxicity to Fish*

###### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

Zebra fish (*Brachydanio rerio*) were exposed to the test substance (CASRN 78-67-1) at nominal concentrations of 62.5, 125, 250, 500 or 1000 mg/L under semi-static conditions for 96 hours. Mortality rates at 96 hours were 29 and 100% for the 500 and 1000 mg/L concentrations, respectively. No mortality was noted at 62.5, 125 or 250 mg/L.

**96-h LC<sub>50</sub> = 580 mg/L**

##### *Acute Toxicity to Aquatic Invertebrates*

###### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

Water fleas (*Daphnia magna*) were exposed to the test substance (CASRN 78-67-1) at nominal concentrations of 0, 0.5, 1.0, 50, 500 or 5000 mg/L under static conditions for 48 hours. Immobilities were 60 and 100% at 500 and 5000 mg/L, respectively. Immobilities were not observed at  $\leq 50$  mg/L. Undissolved test material was observed in the 0.5, 1.0, 50, 500 and 5000 mg/L test solutions throughout the 48-hour test period.

**48-h EC<sub>50</sub> = 397 mg/L**

##### *Toxicity to Aquatic Plants*

###### ***Butanenitrile, 2,2'-azobis(2-methyl)- (CASRN 13472-08-7)***

Green algae (*Pseudokirchneriella subcapitata*) were exposed to the test substance (CASRN 13472-08-7) in dimethylformamide (DMF) at measured concentrations of 6.20, 12.3, 24.5, 49.3 or 99.5 mg/L under static conditions for 72 hours. Reductions in healthy cell count, area under the growth curve and growth for green algae indicated a dose-dependant response.

**72-h EC<sub>50</sub> (biomass) = 31.3 mg/L**

**72-h EC<sub>50</sub> (growth rate) = 67 mg/L**

###### ***Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)***

(1) Green algae (*Pseudokirchneriella subcapitata*) were exposed to the test substance (CASRN 78-67-1) under an open system for 72 hours with analytical monitoring for biomass and growth rate endpoints.

**72-h EC<sub>50</sub> (biomass) = 2.9 mg/L**

**72-h EC<sub>50</sub> (growth rate) = 6.1 mg/L**

(2) Green algae (*Pseudokirchneriella subcapitata*) were exposed to the test substance (CASRN 78-67-1) at measured concentrations of 0.46, 0.71, 2.1, 4.2, or 9.4 mg/L under an open system for 72 hours with analytical monitoring for biomass endpoint.

**72-h EC<sub>50</sub> (biomass) > 9.4 mg/L**

**Chronic Toxicity to Aquatic Invertebrates**

**Propanenitrile, 2,2'-azobis(2-methyl)- (CASRN 78-67-1, supporting chemical)**

Water fleas (*Daphnia magna*) were exposed to the test substance (CASRN 78-67-1) at nominal concentrations of 0, 0.46, 1.0, 2.2, 4.6 or 10 mg/L under semi-static conditions in closed system for 21 days. Acetone was used as a solvent. Measured concentrations were within 88-98 % of the nominal concentrations throughout the 21-d test period.

**21-d EC<sub>50</sub> = 7.5 mg/L**

**21-d LOEC = 4.6 mg/L**

**21-d NOEC = 2.2 mg/L**

**Conclusion:** The 96-h LC<sub>50</sub> of the supporting chemical, CASRN 78-67-1, to fish is 580 mg/L. The 48-h EC<sub>50</sub> of the supporting chemical, CASRN 78-67-1, to aquatic invertebrates is 397 mg/L. The 72-h EC<sub>50</sub> values to aquatic plants from exposure to CASRN 13472-08-7 are 31.3 and 67 mg/L for biomass and growth rate, respectively.

<b>Table 4. Summary Table of the Screening Information Data Set as submitted under the U.S. HPV Challenge Program – Aquatic Toxicity Data</b>		
<b>Endpoints</b>	<b>SPONSORED CHEMICAL Butanenitrile, 2,2'-azobis(2-methyl)- (AMBN) (13472-08-7)</b>	<b>SUPPORTING CHEMICAL Propanenitrile, 2,2'-azobis(2-methyl)- (AIBN) (78-67-1)</b>
<b>Fish 96-h LC<sub>50</sub> (mg/L)</b>	No data 580 (RA)	<b>580</b>
<b>Aquatic Invertebrates 48-h EC<sub>50</sub> (mg/L)</b>	No data 397 (RA)	<b>397</b>
<b>Aquatic Plants 72-h EC<sub>50</sub> (mg/L)</b>		
<b>Biomass</b>	<b>31.3</b>	<b>2.9</b>
<b>Growth rate</b>	<b>67</b>	<b>6.1</b>
<b>Chronic toxicity Aquatic Invertebrates (mg/L)</b>	No data	
<b>21-d EC<sub>50</sub></b>	7.5	<b>7.5</b>
<b>LOEC</b>	4.6	<b>4.6</b>
<b>NOEC</b>	2.2 (RA)	<b>2.2</b>

**bold** = measured data (i.e., derived from testing); (RA) = Read Across