

SCREENING-LEVEL HAZARD CHARACTERIZATION

Dinitrile Category

Adiponitrile	CASRN 111-69-3
2-Methylglutaronitrile	CASRN 4553-62-2
Ethylsuccinonitrile	CASRN 17611-82-4

The High Production Volume (HPV) Challenge Program¹ 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^{1,2}) 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 guidance³ 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, EXTOWNET, 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. Additionally, on a case-by-base basis, EPA independently reviews references included in robust summaries, and denotes these specific reviews by including the reference information in the study summary in parentheses.

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

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

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

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 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.

Chemical Abstract Service Registry Number (CASRN)	111-69-3 4553-62-2 17611-82-4
Chemical Abstract Index Name	Hexanedinitrile Pentanedinitrile, 2-methyl- Butanedinitrile, ethyl-
Structural Formula	See Table 1, Section 1
<p style="text-align: center;">Summary</p> <p>The dinitriles category contains liquids with moderate vapor pressure and high water solubility. The dinitriles are expected to have high mobility in soil. Volatilization is considered low to moderate based on the estimated Henry's Law constants. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered negligible to slow. The dinitriles are expected to have low persistence (P1) and low bioaccumulation potential (B1).</p> <p>The acute oral toxicity of CASRN 111-69-3, 4553-62-2, and 17611-82-4 is moderate in rats. The acute inhalation toxicity of CASRN 111-69-3, 4553-62-2, and 17611-82-4 is high in rats. The acute dermal toxicity of CASRN 111-69-3 and 4553-62-2 in rats is low and moderate, respectively. Rats exposed to CASRN 111-69-3 via inhalation for 13-weeks were observed to have hematological effects (mildly decreased numbers of red blood cells and hemoglobin and hematocrit levels) at 0.1 mg/L; the NOAEC for systemic toxicity is 0.03 mg/L. Repeated CASRN 4553-62-2 exposure to rats via inhalation for 4-weeks resulted in dose-dependent decreases in body weight gain, which were biologically meaningful at 0.02 mg/L; the NOAEC for systemic toxicity is 0.005 mg/L. In a dominant lethal inhalation reproductive toxicity study in which exposed female rats were mated with unexposed males, CASRN 111-69-3 caused no systemic toxicity or adverse effects on female fertility at any dose; the NOAEC for female reproductive and systemic toxicity is 0.1 mg/L. In a dominant lethal inhalation one-generation reproductive toxicity study in which exposed male rats were mated with unexposed females, CASRN 111-69-3 caused no systemic toxicity or reproductive effects at any dose; the NOAEC for both systemic and reproductive toxicity is 0.1 mg/L. In an oral prenatal developmental toxicity study in rats, CASRN 111-69-3 caused maternal mortality, but not developmental toxicity, at 80 mg/kg/day, the highest dose tested; the NOAEL for maternal toxicity is 30 mg/kg/day and the NOAEL for developmental toxicity is 80 mg/kg/day. An <i>in vitro</i> bacterial gene mutation tests (Ames assay) was negative with CASRN 111-69-3 and CASRN 17611-82-4, and weakly positive with CASRN 4553-62-2. CASRN 111-69-3 did not induce <i>in vivo</i> genotoxic changes (rat chromosomal aberrations assay). CASRN 111-69-3 is not irritating to rabbit skin, but is irritating to rabbit eyes. CASRN 4553-62-2 is irritating to rabbit eyes.</p> <p>The 96-hour LC₅₀ of the dinitriles category members for fish is 670 mg/L. The 48-hour EC₅₀ for aquatic invertebrates ranges from 831 to > 1000 mg/L. The 48-hour EC₅₀ for aquatic plants is > 97.4 mg/L.</p>	

No data gaps were identified under the HPV Challenge Program.

The Sponsor, E.I. du Pont de Nemours & Co., Inc., submitted a Test Plan and Robust Summaries to EPA for the dinitrile category on July 11, 2001. EPA posted the submission on the ChemRTK HPV Challenge website on September 13, 2001 (<http://www.epa.gov/oppt/chemrtk/pubs/summaries/dinitrile/c13107tc.htm>). EPA comments on the original submission were posted to the website on February 19, 2002. Public comments were also received and posted to the website. The Sponsor submitted updated/revised documents on September 16, 2002, which were posted to the ChemRTK website on September 24, 2002.

Category Justification

The sponsor supports grouping CASRN 111-69-3, CASRN 4553-62-2 and CASRN 17611-82-4 into a dinitrile category based on similar structural and physical-chemical properties, environmental fate, ecological effects (aquatic toxicity) and health effects.

EPA concluded that the aquatic toxicity data for all endpoints (acute toxicity to fish, invertebrates and plants) adequately support a category approach for the three chemicals.


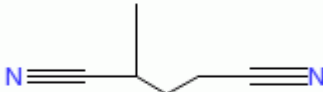
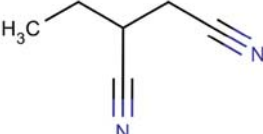
EPA concluded that the health effects data in the original test plan provided weak support for a category approach. For CASRN 111-69-3, adequate data were available for all HPV Challenge endpoints. For CASRN 4553-62-2 and CASRN 17611-82-4, data were limited to acute oral and inhalation toxicity studies for each, an acute dermal toxicity study (CASRN 4553-62-2), a gene mutation assay for each, and a chromosomal aberration assay (CASRN 4553-62-2). These were, therefore, insufficient for an adequate evaluation of their toxicities or a reliable comparison with CASRN 111-69-3. Data from a repeated-dose toxicity study of CASRN 4553-62-2 was expected to allow a more complete comparison to existing data for CASRN 111-69-3. The CASRN 4553-62-2 repeated-dose study in rats had a NOAEC of 0.005 mg/L, based on biologically meaningful decrements in body weight gain. The CASRN 111-69-3 repeated-dose study had a NOAEC of 0.03 mg/L based on quantitatively slight, but statistically significant hematological changes. Results from the 4-week, repeated-dose toxicity study (with the lower NOAEC) was used for purposes of read-across, since it provided the most conservative NOAEC.

1. Chemical Identity

1.1 Identification and Purity

The following description is taken from the final Test Plan (2001):

The dinitrile category is composed of linear straight and branched chain alkanes with a common functional group, at each end of the parent chain. This category is composed of individual isomers containing six carbon atoms that differ by the position of the terminal groups. Dinitriles included in this group are CASRN 111-69-3, CASRN 4553-62-2, and CASRN 17611-82-4. Test substance purity, when noted in the Robust Summaries, was given as $\geq 85\%$. Structures of these dinitriles are presented below.

Table 1: Cyclic Anhydrides Category Sponsored Chemical Structures		
Chemical Abstract Index Name	CASRN	Structure
Hexanedinitrile	111-69-3	
Pentanedinitrile, 2-methyl-	4553-62-2	
Butanedinitrile, ethyl-	17611-82-4	

1.2 Physical-Chemical Properties

The physical-chemical properties of the dinitriles category are summarized in Table 2, while their environmental fate properties are provided in Table 3.

Physical-Chemical Properties Characterization

The dinitriles category contains liquids with moderate vapor pressure and high water solubility.

Property	Butanedinitrile, 2-ethyl-	Pentanedinitrile, 2-methyl-	Hexanedinitrile
CASRN	17611-82-4	4553-62-2	111-69-3
Molecular Weight	108.14	108.14	108.14
Physical State	Colorless to brown liquid	Colorless liquid	Colorless liquid
Melting Point	-39 to -43°C (measured)	-44 to -48°C (measured); -45°C (measured) ²	1°C (measured)
Boiling Point	264°C (measured)	274°C (measured); 263°C (measured) ²	295°C (measured)

Vapor Pressure	1.9×10^{-2} mm Hg at 25°C (measured)	5.1×10^{-3} mm Hg at 25°C (measured)	6.8×10^{-4} mm Hg at 25°C (measured)
Water Solubility	22,000 mg/L at 23°C (measured)	40,000 mg/L at 20°C (measured)	80,000 mg/L at 20°C (measured)
Dissociation Constant (pK _a)	Not applicable	Not applicable	Not applicable
Henry's Law Constant	1.23×10^{-7} atm-m ³ /mole (estimated) ³	1.81×10^{-8} atm-m ³ /mole (estimated) ³	1.21×10^{-9} atm-m ³ /mole (estimated) ³
Log K _{ow}	0.28 (estimated)	-0.644 (estimated using CLOGP); 0.28 (estimated) ³	-0.32 (measured)

¹ E.I. DuPont de Nemours. August 20, 2002. Revised Robust Summary and Test Plan for Dinitriles. Available online from:

<http://www.epa.gov/chemrtk/pubs/summaries/dinitrle/c13107tc.htm> as of March 25, 2010.

² SRC. The Physical Properties Database (PHYSPROP). Syracuse, NY: Syracuse Research Corporation. Available online from: <http://www.srcinc.com/what-we-do/free-demos.aspx> as of March 25, 2010.

³ U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm> as of March 25, 2010.

Environmental Fate Characterization

The dinitriles are expected to have a high mobility in soil. Hexanedinitrile was shown to be readily biodegradable using a modified MITI test (OECD 301C) and was completely biodegraded within 12 days using a river die-away test. These data suggest that the other category members will not be persistent in the environment; however, their branched structures indicate that the rate of degradation may be slower as compared to hexanedinitrile. Volatilization is considered low to moderate based on the estimated Henry's Law constants. The dinitriles are considered hydrolytically stable. The rate of atmospheric photooxidation is considered negligible to slow. The dinitriles are expected to have low persistence (P1) and low bioaccumulation potential (B1).

Property	Butanedinitrile, 2-ethyl-	Pentanedinitrile, 2-methyl-	Hexanedinitrile
CASRN	17611-82-4	4553-62-2	111-69-3
Photodegradation Half-life	6.7 days (estimated) ²	10.9 days (estimated) ²	15.0 days (estimated) ²
Hydrolysis Half-life	Stable	Stable	<10% degraded after 5 days at pH 7 and 50°C (measured)
Biodegradation	No data	No data	100% after 12 days (readily biodegradable); 53–66% after 28 days (readily biodegradable) ³
Bioaccumulation Factor	BAF = 1.03 (estimated) ²	BAF = 1.03 (estimated) ²	BAF = 0.92 (estimated) ²
Log K _{oc}	1.27 (estimated) ²	1.25 (estimated) ²	1.31 (estimated) ²
Fugacity (Level III Model) ²			
Air (%)	1.7	0.4	<0.1
Water (%)	29.5	27.8	26.6
Soil (%)	68.7	71.8	73.3
Sediment (%)	0.1	0.1	0.1
Persistence ⁴	P1 (low)	P1 (low)	P1 (low)
Bioaccumulation ⁴	B1 (low)	B1 (low)	B1 (low)

¹ E.I. DuPont de Nemours. August 20, 2002. Revised Roust Summary and Test Plan for Dinitriles. Available online from:

<http://www.epa.gov/chemrtk/pubs/summaries/dinitrile/c13107tc.htm> as of March 25, 2010.

² U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: <http://www.epa.gov/opptintr/exposure/pubs/episuite4.htm> as of March 25, 2010.

³ National Institute of Technology and Evaluation. 2002. Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law. Available online from: http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html as of March 25, 2010.

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

Conclusion: The dinitriles category contains liquids with moderate vapor pressure and high water solubility. The dinitriles are expected to have high mobility in soil. Volatilization is considered low to moderate based on the estimated Henry’s Law constants. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered negligible to slow. The dinitriles are expected to have low persistence (P1) and low bioaccumulation potential (B1).

2. General Information on Exposure

2.1 Production Volume and Use Pattern

According to the 2006 IUR submissions, the dinitriles category chemicals had an aggregated production and/or import volume in the United States of greater than 1 billion pounds.

- CASRN 4553-62-2: 50 to <100 million pounds;
- CASRN 111-69-3: 1 billion pounds and greater

CASRN 17611-82-4 was not reported in the 2006 IUR.

CASRN 4553-62-2 and 111-69-3:

Non-confidential information in the IUR indicated that the industrial processing and uses of these chemicals include other basic organic chemical manufacturing as intermediates. No commercial and consumer uses were reported for this chemical.

2.2. Environmental Exposure and Fate

The environmental fate properties are provided in Table 3.

3. Human Health Hazard

A summary of health effects data submitted for SIDS endpoints is provided in Table 4. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Oral Toxicity

Adiponitrile (CASRN 111-69-3)

Sprague-Dawley rats (10 males/dose) were administered CASRN 111-69-3 as a suspension in corn oil via gavage at 100, 130, 150 or 200 mg/kg (fasted) or 250, 300, 325, 340, 370 or 400 mg/kg (non-fasted) and observed for 14-15 days. Mortality occurred in 0/10, 5/10, 7/10, and 9/10 rats in the 100, 130, 150, and 200 mg/kg fasted dose groups, respectively. Mortality occurred in 3/10, 7/10, 5/10, 3/10, 8/10, and 10/10 rats in the 250, 300, 325, 340, 370, and 400 mg/kg non-fasted dose groups, respectively.

LD₅₀ = 138 mg/kg (fasted rats)

LD₅₀ = 301 mg/kg (non-fasted rats)

2-Methylglutaronitrile (CASRN 4553-62-2)

Sprague-Dawley rats (5/sex/dose, fasted) were administered CASRN 4553-62-2 in 10% arabic gum via gavage at 0, 150, 200, 250, 300 or 400 mg/kg and observed for 14 days. Death occurred within 2-6 hours. Dose-mortality data were not provided in the robust summary.

LD₅₀ = 205 mg/kg (fasted rats)

Ethylsuccinonitrile (CASRN 17611-82-4)

Sprague-Dawley rats (5/sex/dose, fasted) were administered CASRN 17611-82-4 in deionized water via gavage at 50 mg/kg and observed for 14 days. No animals died during the exposure or observation periods.

LD₅₀ >50 mg/kg (fasted rats)

Acute Inhalation Toxicity

Adiponitrile (CASRN 111-69-3)

(1) Sprague-Dawley rats (10 males/exposure concentration) were exposed whole-body to CASRN 111-69-3 as a vapor at 0.25, 0.36, 0.71, 0.83, 1.18, 1.95, 2.47, 2.53, 2.70 or 3.03 mg/L for 4 hours, weighed and observed for 14 days. Mortality occurred in 0/10, 0/10, 5/10, 0/10, 6/10, 7/10, 9/10, 8/10, 6/10, and 7/10 rats in the 0.25, 0.36, 0.71, 0.83, 1.18, 1.95, 2.47, 2.53, 2.70, and 3.03 mg/L groups, respectively.

LC₅₀ = 1.71 mg/L

(2) Sprague-Dawley rats (10 males/exposure concentration) were exposed whole-body to CASRN 111-69-3 aerosol at 0, 2.5, 3.3, 3.4, 3.7, 4.2 or 4.6 mg/L for 4 hours and observed for up to 14 days. Mortality occurred at all doses: 2/10, 6/10, 9/10, 6/10, 6/10, and 4/10 in the 2.5, 3.3, 3.4, 3.7, 4.2 or 4.6 mg/L groups, respectively. (TSCATS - OTS0514859).

LC₅₀ = 2.9 mg/L

2-Methylglutaronitrile (CASRN 4553-62-2)

Sprague-Dawley rats (6 males/exposure concentration) were exposed whole-body to 2-methylglutaronitrile/ethyl succinonitrile (85%/14%) as a vapor at 0.33, 0.34, 0.49 or 1.11 mg/L for 4 hours and observed for 14 days. Mortality occurred at 0.33 and ≥ 0.49 mg/L.

LC₅₀ = 0.66 mg/L

Ethylsuccinonitrile (CASRN 17611-82-4)

Sprague-Dawley rats (6 males/exposure concentration) were exposed whole body to aerosolized CASRN 17611-82-4 at 0.91, 1.4 or 2.5 mg/L for 4 hours, and observed for 14-15 days.

Mortality occurred at ≥ 1.4 mg/L either during exposure or within 6 days of exposure

LC₅₀ = not determined

Acute Dermal Toxicity

Adiponitrile (CASRN 111-69-3)

(1) New Zealand White rabbits (6 males/dose) were administered 1000, 1250, 1350, 1500, 2000 or 4000 mg/kg CASRN 111-69-3 via topical application for 24 hours using occlusive wrapping. Animals were observed for 14 days. Mortality of 0/6, 0/6, 3/6, 4/6, 3/6, 4/6 was observed in the 1000, 1250, 1350, 1500, 2000, and 4000 mg/kg dose groups, respectively.

LD₅₀ = 2134 mg/kg

(2) New Zealand White rabbits (6 males/dose) were administered 2027, 2413 or 2887 mg/kg CASRN 111-69-3 via topical application for 24 hours with occlusive wrapping. Animals were

observed for up to 14 days. All six high-dose rabbits died within 1 day following exposure. (TSCATS - OTS0514992).

LD₅₀ > 2202 mg/kg

(3) Six male Albino rabbits were administered 2000 mg/kg CASRN 111-69-3 via topical application (with occlusive wrapping for 24 hours) and subsequently observed for up to 14 days. One rabbit died two days after exposure. (TSCATS - OTS0514990).

LD₅₀ > 2000 mg/kg

2-Methylglutaronitrile + Ethylsuccinonitrile (CASRN 4553-62-2 and CASRN 17611-82-4)

New Zealand White rabbits (6/dose, sex not specified) were administered CASRN 4553-62-2/CASRN 17611-82-4 (85%/14%) via topical application at 650, 725, 800, 1000 or 1500 mg/kg under occluded conditions for 24 hours and then observed for 14 days. Mortality occurred at \geq 725 mg/kg, with all rabbits dying at the highest dose (1500 mg/kg).

LD₅₀ = 776 mg/kg

Repeated-Dose Toxicity - Inhalation

Adiponitrile (CASRN 111-69-3)

Sprague-Dawley rats (15/sex/concentration) were administered CASRN 111-69-3 in inhalation chambers at 0 (control exposure), and approximately 0.01, 0.03 and 0.1 mg/L as mixed vapor and aerosol for 13 weeks (6 hours/day, 5 days/week). Urinary thiocyanate values were increased in both sexes in all dose groups. Hematologic evaluation showed statistically significant decreases in red blood cell numbers, hemoglobin, and hematocrit levels in females at 0.1 mg/L. Standard deviation or standard errors of the means were not reported. There were no exposure-related responses for clinical signs, body weight, blood chemistry, urinalysis, necropsy, organ weights or histopathology.

LOAEC = 0.1 mg/L (based on hematological effects)

NOAEC = 0.03 mg/L

2-Methylglutaronitrile (CASRN 4553-62-2)

Sprague-Dawley rats (20 males/concentration) were exposed to 4553-62-2 via inhalation by whole body exposure at 0, 5, 25 or 200 mg/m³ (~ 0.005, 0.02 or 0.2 mg/L) for 6 hours/day, 5 days/week for 4 weeks (total of 20 exposures) and observed for 4 weeks (Kelly et al. 2003). Controls were exposed to an air/nitrogen mixture. At concentrations of 0.005 and 0.02 mg/L, rats were exposed to vapor nose-only, and at the 0.2 mg/L concentration they were exposed to aerosol and vapor via whole-body. Body weights in the high dose group (0.2 mg/L) exhibited statistically significant decreases from study day 15-26. Dose-responsive decreases in body weight gain were observed during the exposure period: 6%, 12% and 20% for the low, mid, and high dose groups, respectively (calculated by EPA; statistical significance not determined). Non-dose responsive changes were observed in numbers of reticulocytes. However, an increase in the mean value of this parameter was statistically significant ($p \leq 0.05$) at the highest dose (0.2 mg/L). No toxicologically significant changes in clinical chemistry, urinalysis, or neurobehavior were observed. No quantitative data were provided on absolute and relative organ weights. However, increased heart-to-body weight ratios in all dose groups at the 28-day sacrifice were reported (neither quantitative nor statistical significance were provided). Absolute heart weight and heart-

to-brain weight changes were reported as not being significant (quantitative data were not reported). Food consumption was not measured, and no test article related gross or microscopic changes were reported.

LOAEC = 0.02 mg/L (based on decreases in body weight gain)

NOAEC = 0.005 mg/L

Reproductive Toxicity

Adiponitrile (CASRN 111-69-3)

(1) Sprague-Dawley rats (24 females/exposure group) were administered CASRN 111-69-3 via inhalation, whole body exposure, at 0 (control exposure) and approximately 0.01, 0.03 and 0.1 mg/L for 6 hours/day, 7 days/week for 22 days. Females were then mated to unexposed males (15/group) to assess female fertility. Female exposure was continued until copulation was confirmed. Females with confirmed copulation were sacrificed on gestation days 13-15. No deaths or exposure-related clinical signs or lesions were detected at gross necropsy (thoracic or abdominal cavities) among the dams. There were no significant effects on female fertility at any dose level, as evaluated by mating efficiency, pregnancy rate, numbers of corpora lutea and live implantations and pre- and post-implantation loss rates. Statistical significance was not reported. Fetuses were not examined.

NOAEC (maternal toxicity) = 0.1 mg/L (highest concentration tested)

NOAEC (reproductive toxicity) = 0.1 mg/L (highest concentration tested)

(2) Sprague-Dawley rats (12 males/exposure group) were administered CASRN 111-69-3 via whole-body inhalation chamber at 0 (control exposure) and approximately 0.01, 0.03 and 0.1 mg/L for 6 hours/day, 5 days/week for 74 days, and then mated to unexposed females to assess male fertility. One-third of the males of each treatment group were sacrificed on each of 3 consecutive days at the end of the study. There was no systemic toxicity as evaluated by mortality, clinical signs, body weight or lesions at gross necropsy (of thoracic, abdominal and scrotal cavities). There were no detectable effects on mating efficiency, pregnancy rate, numbers corpora lutea and live implantations, and pre- and post-implantation loss rates. Fetuses were not examined. Sperm parameters were not evaluated.

NOAEC (paternal toxicity) = 0.1 mg/L (highest concentration tested)

NOAEC (reproductive toxicity) = 0.1 mg/L (highest concentration tested)

Developmental Toxicity

Adiponitrile (CASRN 111-69-3)

(1) Sprague-Dawley rats (25 females/dose) were administered CASRN 111-69-3 in corn oil by gavage at 0 (control dose), 30, 50 or 80 mg/kg on days 6-19 of gestation. Surviving dams were sacrificed on gestation day 20. Mortality was observed in one dam in the 50 mg/kg/day on gestation day 17 (of undetermined causes), and two in the 80 mg/kg/day group on gestation days 9 and 20, apparently due to severe lung congestion in one dam, and peritonitis and pericarditis in the other. It is unclear if deaths during the study were due to technical error, related to misplacement of gavage cannula. No treatment-related maternal effects were observed on appearance, behavior, body weight, gross pathology, gravid uterine weight, number and location

of viable and nonviable fetuses, early and late resorptions, numbers of corpora lutea and implantations, and postimplantation loss. No treatment-related effects were observed on fetal weight, sex distribution, or fetal malformations and variations (external, visceral and skeletal).

LOAEL (maternal toxicity) = 50 mg/kg/day (based on mortality)

NOAEL (maternal toxicity) = 30 mg/kg/day

NOAEL (developmental toxicity) = 80 mg/kg/day (highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro bacterial gene mutation

Adiponitrile (CASRN 111-69-3)

(1) *Salmonella typhimurium* strains TA98, TA1535, TA1537 and TA1538 were exposed in duplicate to CASRN 111-69-3 at concentrations of 0, 100, 500, 1000, 2500, 5000, 7500 or 10,000 µg/plate in the presence and absence of metabolic activation (rat liver S-9). Positive and negative controls were tested concurrently, but responses were not provided.

CASRN 111-69-3 was not mutagenic in this assay.

(2) L5178Y TK⁺ mouse lymphoma cells were exposed in triplicate for 4 hours at 37° C to CASRN 111-69-3 at concentrations of 0, 0.3, 0.5, 0.7, 0.85, 1, 2, 3 or 5 µL/ml in the presence and absence of metabolic activation (rat liver S-9). Following a mutation expression period of 2 days, cells were seeded and then cultivated. Positive and vehicle controls were tested concurrently. The definitive test was run twice. Cytotoxicity was not observed at any dose. The test findings for CASRN 111-69-3 were reported as meeting the method's criteria for a negative (non-mutagenic) result, although no data were presented, and acceptance criteria (for assessment of test validity) were not provided.

CASRN 111-69-3 was not mutagenic in this assay.

2-Methylglutaronitrile (CASRN 4553-62-2)

Salmonella typhimurium strains TA97, TA98, TA100, TA1535 and TA1537 were exposed in triplicate to CASRN 4553-62-2 with 0, 100, 333, 1000, 3333 or 10,000 µg/plate with and without metabolic activation (S-9) using the preincubation method. Concurrent solvent and positive controls were run with each trial. No data (actual values or percent differences) were reported. The robust summary for this study stated that the assay demonstrated a weakly mutagenic response, which was apparently based on a “reproducible dose-related response of the solvent controls”.

CASRN 4553-62-2 was weakly mutagenic in this assay.

Ethylsuccinonitrile (CASRN 17611-82-4)

Using the plate incorporation method, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* were exposed to CASRN 17611-82-4 (0, 100, 333, 1000, 3333 or 5000µg/plate) with and without metabolic activation (S-9). The definitive test was run twice. In the definitive tests, all dose levels of the test substance and positive and vehicle (dimethyl sulfoxide) controls were plated in triplicate. No precipitate or toxicity was observed in any of the tests, and no positive responses were observed in the CASRN 17611-82-4-treated tester strain plates, with or without S-9.

Ethylsuccinonitrile was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vivo

Adiponitrile (CASRN 111-69-3)

Sprague-Dawley rats (15/sex/dose) were administered (by gavage) either a single dose of corn oil (0 mg/kg, vehicle control), or CASRN 111-69-3 (300 mg/kg dose) in corn oil. Positive control groups (5/sex) received a single dose of cyclophosphamide. Animals received a single intraperitoneal injection of colchicine and were sacrificed 4, 22 and 46 hours later, after which bone marrow was harvested and slides prepared for microscopic evaluation. The 300 mg/kg dose was systemically toxic to both sexes, and resulted in one male death. No statistically significant differences between bone marrow from controls and CASRN 111-69-3-treated animals with respect to mean chromosome numbers and mean mitotic indices were observed. Positive controls apparently performed as expected.

CASRN 111-69-3 did not induce chromosome aberrations in this assay.

Genetic Toxicity – Other

In vitro

Adiponitrile (CASRN 111-69-3)

Rat hepatocytes were exposed to CASRN 111-69-3 (diluted in acetone and then in culture medium) at concentrations ranging from 0.1 to 2500 µg/ml and assessed for unscheduled DNA synthesis. Positive, solvent and medium controls were included. In a rangefinder, cytotoxicity was observed at 3000 µg/ml. No cytotoxicity was observed in the definitive test. Controls yielded expected responses. Test cultures were negative for unscheduled DNA synthesis. (TSCATS - OTS0516565).

CASRN 111-69-3 did not induce unscheduled DNA synthesis in this assay.

Additional Information

Skin Irritation

Adiponitrile (CASRN 111-69-3)

(1) Six male Albino rabbits were administered undiluted CASRN 111-69-3 (0.5 ml) on clipped intact skin under semi-occluded conditions for 24 hours. No skin reactions were observed after 24 and 48 hours.

CASRN 111-69-3 was not irritating to rabbit skin in this study.

(2) Albino rabbits (two males and one female) were administered undiluted CASRN 111-69-3 under occluded conditions for 24 hours. No skin reactions were seen throughout a 7-day observation period. (TSCATS - OTS0516563).

CASRN 111-69-3 was not irritating to rabbit skin in this study.

Eye Irritation

Adiponitrile (CASRN 111-69-3)

Undiluted CASRN 111-69-3 was instilled into the conjunctival sac of eye of three albino rabbits. The eyes were rinsed with isotonic saline solution after 24 hours. Effects included slight to mild edema with mild discharge after 10 minutes and slight to mild edema with moderate discharge and slight white exudate after 1 hour, with marked improvement in 48 hours. The average maximum irritation score was 10.6/110 after 1 hour and a score of 0 by day 7. (TSCATS - OTS0516563).

CASRN 111-69-3 was slightly irritating to rabbit eyes in this study.

2-Methylglutaronitrile + Ethylsuccinonitrile (CASRN 4553-62-2 and CASRN 17611-82-4)

Undiluted CASRN 4553-62-2/CASRN 17611-82-4 (85%/14%) was instilled into the right conjunctival sac of two male albino rabbits. The treated eye of one of the rabbits was washed with water after 20 seconds. Effects in both washed and unwashed treated eyes consisted of a small area of minimal corneal opacity and mild conjunctival irritation. Effects were reversible within 2-3 days.

CASRN 4553-62-2 was mildly irritating to rabbit eyes in this study.

Conclusion: The acute oral toxicity of CASRN 111-69-3, 4553-62-2, and 17611-82-4 is moderate in rats. The acute inhalation toxicity of CASRN 111-69-3, 4553-62-2, and 17611-82-4 is high in rats. The acute dermal toxicity of CASRN 111-69-3 and 4553-62-2 in rats is low and moderate, respectively. Rats exposed to CASRN 111-69-3 via inhalation for 13-weeks were observed to have hematological effects (mildly decreased numbers of red blood cells and hemoglobin and hematocrit levels) at 0.1 mg/L; the NOAEC for systemic toxicity is 0.03 mg/L. Repeated CASRN 4553-62-2 exposure to rats via inhalation for 4-weeks resulted in dose-dependent decreases in body weight gain, which were biologically meaningful at 0.02 mg/L; the NOAEC for systemic toxicity is 0.005 mg/L. In a dominant lethal inhalation reproductive toxicity study in which exposed female rats were mated with unexposed males, CASRN 111-69-3 caused no systemic toxicity or adverse effects on female fertility at any dose; the NOAEC for female reproductive and systemic toxicity is 0.1 mg/L. In a dominant lethal inhalation one-generation reproductive toxicity study in which exposed male rats were mated with unexposed females, CASRN 111-69-3 caused no systemic toxicity or reproductive effects at any dose; the NOAEC for both systemic and reproductive toxicity is 0.1 mg/L. In an oral prenatal developmental toxicity study in rats, CASRN 111-69-3 caused maternal mortality, but not developmental toxicity, at 80 mg/kg/day, the highest dose tested; the NOAEL for maternal toxicity is 30 mg/kg/day and the NOAEL for developmental toxicity is 80 mg/kg/day. An *in vitro* bacterial gene mutation tests (Ames assay) was negative with CASRN 111-69-3 and CASRN 17611-82-4, and weakly positive with CASRN 4553-62-2. CASRN 111-69-3 did not induce *in vivo* genotoxic changes (rat chromosomal aberrations assay). CASRN 111-69-3 is not irritating to rabbit skin, but is irritating to rabbit eyes. CASRN 4553-62-2 is irritating to rabbit eyes.

Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data			
Endpoints	Adiponitrile (111-69-3)	2-Methylglutaronitrile (4553-62-2)	Ethylsuccinonitrile (17611-82-4)
Acute Oral Toxicity LD₅₀ (mg/kg)	138	205	>50 (only dose tested, no mortality observed)
Acute Inhalation Toxicity LC₅₀ (mg/L)	1.71	0.66	No LC50 determination 0.66 (RA)
Acute Dermal Toxicity LD₅₀ (mg/kg)	2000	776	No Data 776 (RA)
Repeated-Dose Toxicity NOAEC/LOAEC Inhalation (mg/L)	NOAEC = 0.03 (13-wk) LOAEC = 0.1 (13-wk)	NOAEC = 0.005 (4-wk) LOAEC = 0.02 (4-wk)	No Data NOAEC = 0.005 (4-wk) LOAEC = 0.02 (4-wk) (RA)
Reproductive Toxicity NOAEL/LOAEL Inhalation (mg/L) Systemic Toxicity Reproductive Toxicity	NOAEC = 0.1 NOAEC = 0.1	No Data NOAEC = 0.1 NOAEC = 0.1 (RA)	No Data NOAEC = 0.1 NOAEC = 0.1 (RA)
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg/day) Maternal Toxicity Developmental Toxicity	NOAEL = 30 NOAEL = 80 (highest dose tested)	No Data NOAEL = 30 NOAEL = 80 (highest dose tested) (RA)	No Data NOAEL = 30 NOAEL = 80 (highest dose tested) (RA)

Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data			
Endpoints	Adiponitrile (111-69-3)	2-Methylglutaronitrile (4553-62-2)	Ethylsuccinonitrile (17611-82-4)
Genetic Toxicity – Gene Mutation <i>In vitro</i>	Negative	Weakly Positive	Negative
Genetic Toxicity – Chromosomal Aberrations <i>In vivo</i>	Negative	No Data Negative (RA)	No Data Negative (RA)
Additional Information Skin Irritation	Not irritating	No Data	No Data
Eye Irritation	Slightly irritating	No Data	No Data

Measured data in bold text; (RA) = Read Across

4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 5. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Toxicity to Fish

Adiponitrile (CASRN 111-69-3)

(1) Fathead minnows (*Pimephales promelas*) were exposed to CASRN 111-69-3 at measured concentrations of 0, 477, 734, 1130, 1740 or 2670 mg/L under flow-through conditions for 96 hours. Affected fish (response-concentrations not reported) became excitable and stayed near the water surface with rapid fin and opercular movements.

96-h LC₅₀ = 1930 mg/L

(2) Fathead minnows (*Pimephales promelas*) were exposed to CASRN 111-69-3 at nominal concentrations of 0, 0.15, 0.2 or 0.25% (0, 1500, 2000 and 2500 mg/L, respectively) under static conditions for 96 hours. Mortality rates at concentrations of 1500, 2000 and 2500 mg/l were 1/10, 2/10 and 9/10, respectively (TSCATS submission #OTS0514973).

96-h LC₅₀ = 2140 mg/L

(3) Rainbow trout (*Salmo gairdneri*) were exposed to CASRN 111-69-3 at nominal concentrations of 0, 100, 180, 320, 560 or 1000 mg/l for up to 96 hours under static conditions (TSCATS submission #OTS0514857).

96-h LC₅₀ = 670 mg/L

(4) Bluegill sunfish (*Lepomis macrochirus*) were exposed to CASRN 111-69-3 at nominal concentrations of 0, 32, 56, 100, 180, 320, 560 or 1000 mg/l for up to 96 hours under static conditions (TSCATS submission #OTS0514858).

96-h LC₅₀ > 1000 mg/L

Acute Toxicity to Aquatic Invertebrates

Adiponitrile (CASRN 111-69-3)

Water fleas (*Daphnia magna*) were exposed to CASRN 111-69-3 at nominal concentrations of 0, 100, 180, 320, 560 or 1000 mg/L under static conditions for 48 hours. The no-effect level was 320 mg/l. Mortality was 8/20 (40%) at the highest exposure level (TSCATS submission #OTS0514860).

48-h EC₅₀ > 1000 mg/L

Ethylsuccinonitrile (CASRN 17611-82-4)

Water fleas (*Daphnia magna*) were exposed to CASRN 17611-82-4 at nominal concentrations of 1.0, 10, 100 or 1000 mg/L under static conditions for 48 hours. Exposure resulted in 0% immobility at ≤ 100 mg/L and 60% immobility at 1000 mg/L.

48-h EC₅₀ = 831 mg/L

Toxicity to Aquatic Plants

Adiponitrile (CASRN 111-69-3)

Algae (*Pseudokirchneriella subcapitata*) were exposed to CASRN 111-69-3 at nominal concentrations of 0 or 100 mg/L under unspecified conditions for 72 hours. The overall mean measured concentration was 97.4 mg/L. Exposure did not result in a reduction in either the average specific growth rate or biomass of test cultures compared to control cultures.

72-h EC₅₀ (growth) > 97.4 mg/L

72-h EC₅₀ (biomass) > 97.4 mg/L

Conclusion: The 96-hour LC₅₀ of the dinitriles category members for fish is 670 mg/L. The 48-hour EC₅₀ for aquatic invertebrates ranges from 831 to > 1000 mg/L. The 72-hour EC₅₀ for aquatic plants is > 97.4 mg/L.

Table 5. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program –Aquatic Toxicity Data

Endpoints	Adiponitrile (CASRN 111-69-3)	2-Methylglutaronitrile (CASRN 4553-62-2)	Ethylsuccinonitrile (CASRN 17611-82-4)
Fish 96-h LC₅₀ (mg/L)	670 (m)	No Data 670 (RA)	No Data 670 (RA)
Aquatic Invertebrates 48-h EC₅₀ (mg/L)	> 1000 (m)	No Data 831 (RA)	831 (m)
Aquatic Plants 72-h EC₅₀ (mg/L) (growth/biomass)	> 97.4 (m)	No Data > 97.4 (RA)	No Data > 97.4 (RA)

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

5. References

Kelly DP, Frame SR, Malley LA, Everds, NE, Kennedy Jr., GL. “Inhalation toxicity of methylglutaronitrile in rats. Drug and Chemical Toxicology 25(2): 99-115. 2003.