

SCREENING-LEVEL HAZARD CHARACTERIZATION

N-(Methyl)-Acrylamides Category

2-Propenamide, N-(hydroxymethyl)- CASRN 924-42-5
2-Propenamide, N-(butoxymethyl)- CASRN 1852-16-0

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 guidance^{2,3} 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, EXTTOXNET, 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 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

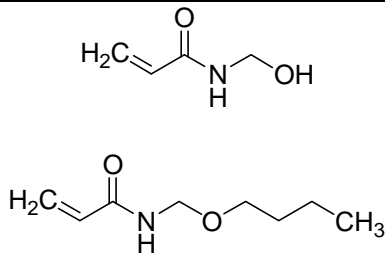
¹ U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

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>.

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 Numbers (CASRN_s)	924-42-5 1852-16-0
Chemical Abstract Index Names	2-Propenamide, <i>N</i> -(hydroxymethyl)- (NMA) 2-Propenamide, <i>N</i> -(butoxymethyl)- (NBMA)
Structural Formula	 <p>The image shows two chemical structures. The top structure is NMA (N-(hydroxymethyl)-2-propenamide), consisting of a vinyl group (H₂C=CH-) attached to a carbonyl group (C=O), which is further attached to a nitrogen atom (N-H) bonded to a hydroxymethyl group (-CH₂OH). The bottom structure is NBMA (N-(butoxymethyl)-2-propenamide), consisting of a vinyl group (H₂C=CH-) attached to a carbonyl group (C=O), which is further attached to a nitrogen atom (N-H) bonded to a butoxymethyl group (-CH₂OCH₂CH₂CH₂CH₃).</p>
<p>Summary</p> <p>The N-(methyl)-acrylamides category contains a solid CASRN 924-42-5 and a liquid CASRN 1852-16-0 ; both with moderate vapor pressure and high water solubility. These chemicals are expected to have high mobility in soil. Volatilization is considered low based on the estimated Henry’s Law constants. The rate of hydrolysis is considered negligible to slow under environmental conditions. The rate of atmospheric photooxidation is considered moderate. These category chemicals are expected to have low persistence (P1) and low bioaccumulation potential (B1).</p> <p>The acute oral toxicity of CASRN 924-42-5 is moderate in rats and mice, and for CASRN 1852-16-0, it is low in rats. The acute dermal toxicity of CASRN 924-42-5 is low in rabbits and for CASRN 1852-16-0 it is moderate. Repeated-dose, 90-day subchronic toxicity studies with CASRN 924-42-5 via gavage in rats and mice showed neurotoxic and neurobehavioral effects at 12.5 mg/kg-day (the lowest dose tested); the NOAEL for systemic toxicity is not established. A repeated-dose, 28-day toxicity study with CASRN 924-42-5 via the diet in rats showed growth retardation and hindlimb weakness at 46 mg/kg-bw/day; the NOAEL for systemic toxicity is 4 mg/kg-bw/day. In a 4-week drinking water study in rats, similar neurotoxic effects (as with those observed in the gavage studies) were seen at 33.9 mg/kg-bw/day, the lowest dose tested; the NOAEL is not established. Limited information in a 42-day dietary study in rats with CASRN 1852-16-0 indicated ataxia in both sexes at approximately 300 mg/kg-bw/day (highest dose); the NOAEL for systemic toxicity is approximately 148 mg/kg-bw/day.</p> <p>In a modified reproductive continuous breeding study in mice with CASRN 924-42-5 via drinking water, decreases in pups per litter and in number of live pups per litter were observed in F2 pups at 17 mg/kg-bw/day, the lowest dose tested; the NOAEL for both reproductive (in females) and developmental toxicity is not established. In males, decreases in testes weight and epididymal sperm concentrations were observed at 68 mg/kg-bw/day; the NOAEL for reproductive toxicity is 37 mg/kg-bw/day. CASRN_s 924-42-5 and 1852-16-0 were not mutagenic in bacteria, but induced chromosomal aberrations in mammalian cells <i>in vitro</i>. CASRN 924-42-5 did not induce micronuclei <i>in vivo</i>. CASRN 924-42-5 was not carcinogenic when tested in rats, but was carcinogenic in mice. CASRN 924-42-5 was neurotoxic to both rats and mice. Both CASRN_s are irritating to rabbit skin. CASRN 924-42-5 is not irritating rabbit eyes. CASRN 1852-16-0 is irritating to rabbit eyes.</p>	

For N-(methyl)-acrylamides category, the 96-hour LC₅₀ to fish ranges from 75 to >100 mg/L. No experimental data are available for acute toxicity to aquatic invertebrates and toxicity to aquatic plants.

Data gaps were identified for acute toxicity to aquatic invertebrates and toxicity to aquatic plants under the HPV Challenge Program.

The sponsor, the NMA/NBMA Association, submitted a Test Plan and Robust Summaries to EPA for the *N*-(methyl)-acrylamides category on September 7, 2001. EPA posted the submission on the ChemRTK HPV Challenge website on October 15, 2001 (<http://www.epa.gov/oppt/chemrtk/pubs/summaries/nmac/c13168tc.htm>). EPA comments on the original submission were posted to the website on June 4, 2002. Public comments were also received and posted to the website. The sponsor submitted revised documents on October 8, 2002, which were posted to the ChemRTK website on October 18, 2002.

Category Justification

The sponsor proposed that NMA and NBMA can be grouped together because of their “close structural similarities” (both have the acrylamide moiety) and “relatively minor” structural differences (variation in the N-substituent: -CH₂OH for NMA; -CH₂OCH₂CH₂CH₂CH₃ for NBMA). While both compounds have an acrylamide moiety and the potential to release formaldehyde from their respective hydroxymethyl and butoxymethyl groups, formaldehyde is more likely to be released with greater efficiency from the hydroxymethyl group, especially under relatively acidic conditions. As such, NMA is expected to be more toxic than NBMA for human health effects endpoints. Therefore, for this hazard characterization, EPA supports a read-across strategy from NMA to NBMA, with NMA representing a worst-case scenario. For environmental effects, the sponsor’s proposed category is acceptable.

Justification for Supporting Chemical

In the Test Plan, the sponsor did not indicate acrylamide (AMD, CASRN 79-06-1) as a member of the category, but proposed that AMD could be grouped together with the other chemicals because of their “close structural similarities” (i.e., the presence of the acrylamide moiety). However, AMD does not have the potential to produce formaldehyde, since its N-substituent does not contain a methyl group. Moreover, EPA requested that the sponsor provide discussion on whether the parent compound or metabolites are responsible for AMD-induced neuropathies, lesions in male reproductive organs, genotoxic actions and/or carcinogenicity. In its revised submission, the sponsor did not adequately address the question regarding the contribution of metabolism to the toxic effects observed with AMD. Without such information, it was not possible to determine the relevance of AMD data to the sponsored chemicals. Additionally, the supporting chemical, AMD, is not an appropriate analog to address ecotoxicity for this category. Therefore, data on AMD were not used in this hazard characterization.

1. Chemical Identity

1.1 Identification and Purity

The following description is taken from the 2001 Test Plan:

NMA and NBMA are precursor monomers for manufacturing polymers, which are used in a variety of commercial applications. The structural difference between these compounds results from a substitution on acrylamide; when one of the hydrogens on the nitrogen atom is replaced with either a methylol (-CH₂OH) or butoxymethyl (-CH₂OCH₂CH₂CH₂CH₃) group, NMA or NBMA are formed, respectively. NMA is derived from acrylamide by reaction with formaldehyde at alkaline pH. NMA is unstable at neutral pH conditions and undergoes demethylation to acrylamide and formalin. Alkylation of NMA with butanol (large excess) under acidic conditions yields NBMA. Dilute aqueous solutions of NBMA undergo hydrolysis slowly to give a mixture of NMA, AMD and formaldehyde.

Although not mentioned in the test plan, several robust summaries indicate that the purity of CASRN 924-42-5 and CASRN 1852-16-0 was >98% and 80%, respectively.

1.2 Physical-Chemical Properties

The physical-chemical properties of the *N*-(methyl)-acrylamides category are summarized in Table 1. NMA is a solid and NBMA is a liquid. Both chemicals have moderate vapor pressure and high water solubility.

Property	2-Propenamide, <i>N</i>-(Hydroxymethyl)-	2-Propenamide, <i>N</i>-(Butoxymethyl)-
CASRN	924-42-5	1852-16-0
Molecular Weight	101.11	157.24
Physical State	Crystalline solid ²	Liquid (based on melting point)
Melting Point	74–75°C (measured)	-9 to -6°C (measured) ³
Boiling Point	276.5°C (estimated)	125–128°C at 0.5 mm Hg (measured) ³ ; 323–327°C at 760 mm Hg (estimated) ⁴ ; 121–124°C at 0.1 mm Hg (measured) ³ ; 353–357°C at 760 mm Hg (estimated) ⁴ ;
Vapor Pressure	2.1×10^{-4} mmHg at 25°C (estimated) ⁵	1.8×10^{-4} - 2.2×10^{-4} mm Hg at 25°C (estimated) ⁴ ; 2.3×10^{-5} - 3.1×10^{-5} mm Hg at 25°C (estimated) ⁴ 2.3×10^{-4} mm Hg at 25°C (estimated) ⁵
Water Solubility	1,220,000 mg/L at 10°C (measured); 1,880,000 mg/L at 20°C (measured); 3,540,000 mg/L at 40°C (measured); 7,550,000 mg/L at 60°C (measured)	32,870 mg/L at 25°C (estimated) ⁵
Dissociation Constant (pK _a)	Not applicable	Not applicable
Henry's Law Constant	9.45×10^{-12} atm·m ³ /mole (estimated) ⁵	7.1×10^{-9} atm·m ³ /mole (estimated) ⁵
Log K _{ow}	-1.81 (estimated) ⁵	0.92 (estimated) ⁵

¹ NMA/NBMA Association, SRA International, Inc. September 7, 2001. Revised Robust Summary and Test Plan for *N*-(Methyl)-Acrylamides Category. Available online from: <http://www.epa.gov/chemrtk/pubs/summaries/nmac/c13168tc.htm> as of April 8, 2010.

² Lide, D.R. 2008. CRC Handbook of Chemistry and Physics 89th edition. CRC Press.

³ Beilstein online database. Searched online from: <https://www.reaxys.com/> as of April 8, 2010.

⁴ NOMO5. 1987. Programs to Enhance PC-Gems Estimates of Physical Properties for Organic Compounds. The Mitre Corp.

⁵ 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/episuitedi.htm> as of April 8, 2010.

2. General Information on Exposure

2.1 Production Volume and Exposure

According to the 2006 IUR submissions, the *N*-(methyl)-acrylamides category chemicals had an aggregated production and/or import volume in the United States between 10.5 and 51 million pounds.

- CASRN 924-42-5: 10 to <50 million pounds; and
- CASRN 1852-16-0: 500,000 to <1 million pounds.

CASRN 924-42-5

Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include adhesive manufacturing as adhesives and other binding agents; and other basic organic chemical manufacturing. Non-confidential commercial and consumer uses of this chemical include “other”.

CASRN 1852-16-0

Industrial processing and use information, as well as commercial and consumer uses for the chemical were claimed confidential.

2.2 Environmental Exposure and Fate

The environmental fate properties are provided in Table 2.

N-(Methyl)-acrylamides are expected to have a high mobility in soil. CASRN 924-42-5 was shown to be not readily biodegradable using the ready biodegradability closed bottle test (OECD 301D), but inherently biodegradable using the MITI-II test (OECD TG 302C). CASRN 1852-16-0 was shown to be readily biodegradable using the ready biodegradability closed bottle test (OECD 301D) and the MITI-I test (OECD TG 301C). Volatilization is considered low based on the estimated Henry’s Law constants. The rate of hydrolysis under environmental conditions is slow to negligible. The rate of atmospheric photooxidation is considered moderate. *N*-(Methyl)-acrylamides are expected to have low persistence (P1) and low bioaccumulation potential (B1).

Property	2-Propenamide, <i>N</i>-(Hydroxymethyl)-	2-Propenamide, <i>N</i>-(Butoxymethyl)-
CASRN	924-42-5	1852-16-0
Photodegradation Half-life	4.5 hours (estimated) ²	2.5 hours (estimated) ²
Hydrolysis Half-life	Dilute aqueous solutions are unstable at neutral pH conditions and undergo demethylolation to acrylamide and formalin; Commercial substance is sold at 48% conc. in water, and remains stable under mildly acidic conditions (pH's of 5.5-7) ³	Dilute solutions of test substance will hydrolyze slowly, presumably at neutral pH, to yield acrylamide, formaldehyde and n-butanol
Biodegradation	51.9% biodegradation in 28 days (not readily biodegradable); 99–100% biodegradation in 28 days (inherently biodegradable) ⁴	79.6% biodegradation in 28 days (readily biodegradable); 76% biodegradation in 28 days (readily biodegradable) ⁴
Bioaccumulation Factor	BAF = 0.9 (estimated) ²	BAF = 1.3 (estimated) ²
Log K _{oc}	0 (estimated) ²	1.3 (estimated) ²
Fugacity (Level III Model) ²		
Air (%)	<0.1	0.1
Water (%)	37.7	29.1
Soil (%)	62.2	70.8
Sediment (%)	0.07	0.08
Persistence ⁵	P1 (low)	P1 (low)
Bioaccumulation ⁵	B1 (low)	B1 (low)

¹NMA/NBMA Association, SRA International, Inc. September 7, 2001. Revised Robust Summary and Test Plan for *N*-(Methyl)-Acrylamides Category. Available online from:

<http://www.epa.gov/chemrtk/pubs/summaries/nmac/c13168tc.htm> as of April 8, 2010.

²U.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/episuitedi.htm> as of April 8, 2010. Estimates were performed using measured melting point and water solubility at 20°C for 2-propenamide, *N*-(hydroxymethyl)- and melting point for 2-propenamide, *N*-(butoxymethyl)-.

³Cytec Industries, Inc.; Product Bulletin: CYLINK NMA Monomer *N*-Methylol Acrylamide (PRT-707-B), West Paterson, NJ, 1995. Available online from: <http://www.cytec.com/specialty-chemicals/downloads/PRT%20707-C%20CYLINK%20NMA.pdf> As of April, 8 2010.

⁴National 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 April 8, 2010.

⁵Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

Conclusion: The N-(methyl)-acrylamides category contains a solid CASRN 924-42-5 (2-propenamide, N-(hydroxymethyl)-) and a liquid CASRN 1852-16-0 (2-propenamide, N-(butoxymethyl)-), both with moderate vapor pressures and high water solubility. These chemicals are expected to have high mobility in soil. Volatilization is considered low based on the estimated Henry's Law constants. The rate of hydrolysis is considered negligible to slow under environmental conditions. The rate of atmospheric photooxidation is considered moderate. N-(methyl)-acrylamides category chemicals are expected to have low persistence (P1) 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 tested category member are read-across (RA) to the untested member of the category.

Acute Oral Toxicity

NMA (CASRN 924-42-5)

(1) Fischer 344 rats (5/sex/dose) were administered NMA via gavage (in water) at 50, 100, 200, 400 or 800 mg/kg and observed for 14 days following dosing. Mortality occurred at 400 mg/kg and above. All rats in the 800 mg/kg group died on or before day 2. Four males and three females in the 400 mg/kg group died during the first week of observation.

LD₅₀ = 400 mg/kg

(2) B6C3F1 mice (5/sex/dose) were administered NMA via gavage (in water) at 50, 100, 200, 400 or 800 mg/kg and observed for 14 days following dosing. Mortality occurred at 400 mg/kg and above. All male mice died by day 2 from the 400 and 800 mg/kg groups. All female mice in the 800 mg/kg group died on or before day 2. One female mouse in the 400 mg/kg group died on day 2.

LD₅₀ = 400 mg/kg

NBMA (CASRN 1852-16-0)

Male Harlan-Wistar rats (3 groups of 5 and 1 group of 2) were administered NBMA at doses of 0.625, 1.25, 2.5 or 10.0 mL/kg via oral gavage (approximately 566, 1133, 2265 and 9060 mg/kg, respectively). The observation period was not indicated. All rats from the 2265 and 9060 mg/kg groups (5 and 2 rats, respectively) died. The mortality in the 566 and 1133 mg/kg groups was 0 and 80%, respectively. All deaths occurred by Day 2.

LD₅₀ = 924 mg/kg

Acute Dermal Toxicity

NMA (CASRN 924-42-5)

Albino rabbits (4/dose) were administered NMA topically on to the intact skin at 2000, 4000, 8000 or 16,000 mg/kg-bw under occluded conditions for 24 hours and observed for 5 – 7 days. One animal in the high dose group died shortly after 24 hours. No other mortality occurred during exposure and observation periods.

LD₅₀ > 16,000 mg/kg-bw

NBMA (CASRN 1852-16-0)

Male albino rabbits (2 groups of 4 and 2 groups of 2) were administered NBMA topically on to the skin at doses of 0.625, 1.25, 2.5 or 10.0 mL/kg-bw (approximately 566, 1133, 2265 and 9060 mg/kg-bw, respectively). The observation period was not indicated. All rabbits from the 2265 and 9060 mg/kg-bw (2/2 rabbits each) died. The mortality in the 566 and 1133 mg/kg-bw groups was 25% (1/4 rabbits) and 75% (3/4 rabbits), respectively.

LD₅₀ = 801 mg/kg-bw

Repeated-Dose Toxicity

NMA (CASRN 924-42-5)

(1) In a repeated-dose toxicity study, male albino rats (10/dose) were administered NMA via the diet at 0, 4, 46 or 247 mg/kg-bw/day for 28 days. The 247 mg/kg-bw/day group started out at a higher dosage (not reported), but after 1 week was reduced to this level due to marked weight loss and signs of toxicity including tremors, slow righting reflexes and hyperexcitability.

Animals at this dose also showed growth retardation, decreased food consumption, hindlimb weakness and swaying movements. Five rats from this group were sacrificed at the end of the 4-week treatment period and the remaining five rats were on the control diet for a 4-week recovery period. The recovery group animals increased their food consumption and body weight gain and showed decreased severity of signs of toxicity. Rats receiving 46 mg/kg-bw/day showed signs of growth retardation and decreased food consumption. Three animals in this group were sacrificed at the end of 4 weeks; four animals continued on the 46 mg/kg-bw/day diet for four additional weeks and the remaining three were on the control diet for four weeks following 4-weeks of the treatment diet. From weeks 4 to 8 at 46 mg/kg-bw/day, animals showed more severe signs including hindlimb weakness. Two of these animals appeared slightly ataxic. The condition of the rats that switched to the control diet improved during the recovery period. Rats receiving 4 mg/kg-bw/day showed no treatment-related effects, and their food consumption and weight gain were comparable to the controls. No gross pathological findings were found at necropsy in any group.

LOAEL = 46 mg/kg-bw/day (based on growth retardation and hindlimb weakness)

NOAEL = 4 mg/kg-bw/day

(2) In a repeated-dose study conducted by the National Toxicity Program (NTP), F344/N rats (10/sex/dose) were administered NMA via gavage at 0, 12.5, 25, 50, 100 or 200 mg/kg-day for 5 days/week for 13 weeks. All rats that received 100 or 200 mg/kg-day NMA died before the end of the study. These rats had hind limb ataxia, which progressed to hind limb paralysis. The final mean body weight of rats that received 25 or 50 mg/kg was 8% or 16% lower than that of the controls for males and 6% or 10% lower for females. At 50 mg/kg-day, the relative testis weight for males ($p < 0.001$) and kidney weight for females ($p < 0.05$) were significantly greater than those for the control animals. Rats that received 50 mg/kg-day had hind limb ataxia beginning at week 8, which progressed to hind limb paresis by week 11. Decreased hindlimb and forelimb grip strength was seen at week 6 at 25 and 50 mg/kg-day and at week 13 at doses as low as 25 mg/kg for female rats and at 12.5 mg/kg for male rats. A decreased startle response at 6 weeks was seen for females given 100 mg/kg and at 13 weeks at doses as low as 25 mg/kg-day. The landing foot spread was significantly increased at 6 weeks for male and female rats at 50 mg/kg-day. Motor activity was not consistently affected by NMA dosing, although it appeared reduced at 6 weeks in female rats given 100 mg/kg-day. Axon filament and myelin sheath degeneration

of the brain stem, spinal cord, and/or peripheral nerves was seen at increased incidences at 25 mg/kg and higher. At 200 mg/kg-day, brain lesions in rats consisted primarily of degeneration and cellular necrosis in the granular cell layer of the cerebellum. Spinal cord lesions were limited to the white matter and consisted of shrunken or dilated axons, many of which were missing the axon filament. Peripheral nerve lesions consisted of degenerative changes of varying degrees in the myelin. Inflammation and/or hemorrhage and edema of the mucosal cells lining the urinary bladder were seen at and above 25 mg/kg-day. These lesions were seen in rats whose urinary bladders appeared distended upon gross examination. [http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchhome]

LOAEL = 12.5 mg/kg-day (based on decreased forelimb and/or hindlimb grip strength in males)

NOAEL = Not established

(3) In a repeated-dose study conducted by NTP, B6C3F1 mice (10/sex/dose) were administered NMA via gavage at 0, 12.5, 25, 50, 100 or 200 mg/kg-day for 90 days (5 days/week for 13 weeks). All mice that received 200 mg/kg died within 5 weeks, and exhibited hind leg paresis starting in the second week. Final mean body weights of dosed and vehicle control mice were similar. Decreased relative testes weights were observed in male mice at 12.5 mg/kg-day and above. The relative kidney weights for male mice at 50 and 100 mg/kg were significantly greater ($p < 0.05$ and $p < 0.001$, respectively) than that for the vehicle controls. Dose-related decreases in forelimb grip strength were seen at weeks 6 and 13 in male and female mice given doses as low as 25 mg/kg, and decreases in hind limb grip strength were also seen in males and females at week 13 at 25 mg/kg-day. An exaggerated startle response was seen at week 13 for female mice receiving 100 mg/kg-day, but changes at other doses and times were inconsistent. A reduction in rotarod performance was seen at week 6 for male and female mice receiving 100 mg/kg and for male mice receiving 25 mg/kg. Performance at 13 weeks was not significantly reduced for dosed mice compared with that for vehicle controls. Motor activity measures were not significantly different for dosed and vehicle control mice. At 200 mg/kg-day, hepatocellular necrosis and thymic lymphocytic necrosis was seen in males and females and hemorrhage, necrosis, and mineralization of the zona reticularis of the adrenal gland was seen in female mice. At 100 mg/kg-day, female mice had cytoplasmic vacuolization of the adrenal cortex.

Vacuolization was also observed at lower doses and in vehicle controls. The lesion decreased in severity and incidence with decreasing dose. [http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchhome]

LOAEL = 12.5 mg/kg-day (based on decreased relative testicular weight)

NOAEL = Not established

(4) In a repeated-dose study, male Wistar rats (4/dose) were administered NMA via drinking water at 0, 3.36, 5.41, 8.65 or 13.8 mM (equivalent to ~ 0, 33.9, 54.6, 87.4 or 139.4 mg/kg-bw/day). Reduction in body weight gain was observed among all treated animals (6% at lowest dose and 43% at highest). At day 90, impaired rotarod performance was observed at the two highest doses. Other clinical signs of toxicity included weakness, tendency toward spreading and dragging hindlimbs and urinary incontinence among more severely affected animals, although information was not provided on the doses at which these changes were observed.

Light microscopy examination showed moderate to severe changes, including shrinkage and loss of myelinated fibers, myelin retraction and corrugation of myelin sheath. A 50% reduction of the colchicine-binding was also detected in the spinal cord of both cervical and lumbar regions at 13.8 mM when compared with the control. This reduction was not seen in the brain.

LOAEL = 33.9 mg/L/day (based on reduced body weight gain)

NOAEL = Not established

NBMA (CASRN 1852-16-0)

Charles River albino rats (5/sex/concentration) were administered NBMA (commercial product containing approx. 5% AMD and 5% NMA) in the diet at 0, 0.062, 0.125 or 0.25% (0, approx. 72, 140 or 306 mg/kg-bw/day in males and 0, approx. 76, 157 or 294 mg/kg-bw/day in females) for 42 days. Animals were observed daily for signs of toxicity and possible changes in appearance, behavior, gait and excretory function. Food intake and body weight were measured weekly. All animals were necropsied. No mortalities were observed. Ataxia was observed in animals at 294-306 mg/kg-bw/day beginning at week 4, with males showing some improvement by week 5 and females showing improvement at week 6. There was a trend toward lower body weight as the study progressed. Food consumption was decreased in all treatment groups.

LOAEL = 294-306 mg/kg-bw/day (based on ataxia)

NOAEL = 140-157 mg/kg-bw/day

Reproductive Toxicity

NMA (CASRN 924-42-5)

(1) In a modified reproductive assessment by continuous breeding, CD-1 mice (20 breeding pairs/dose) were tested for reproductive toxicity, neurotoxicity, and dominant lethal effects by administering NMA in drinking water at 0, 60, 180 or 360 ppm (equivalent to 0, approx. 13, 37 and 68 mg/kg for males and 17, 47 and 101 mg/kg for females) daily for 27 weeks. The mice were housed as breeding pairs for 98 days, following 7 days of pre-mating exposure to NMA while housed singly. At the end of 98 days, the pairs were separated and housed individually with continued dosing. Assessment of the F₁ generation was conducted using offspring from all four dose groups. Twenty F₁ offspring/sex/dose were selected to receive the same doses as their F₀ parents (beginning at weaning). When the animals were approximately 74 days of age, they were cohabited within treatment groups for mating in order to produce an F₂. The F₁ adult mice were more susceptible to the neuromuscular effects of NMA at all doses than F₀ adult mice, as evidenced by decreased grip strength in both limbs in both sexes. F₁ mice had lowered body weights and increased liver and kidney/adrenal weight at all doses. At the end of the continuous cohabitation phase, a dominant lethal test was conducted in which treated males of all dose groups were mated with untreated females. There were 50 and 300% increases in total post-implantation loss at 180 and 360 ppm, respectively; and a concomitant 22% decrease in live fetuses at 360 ppm only (approximately three pups per litter). Decreased pups per litter (by 26%) were seen at 360 ppm in F₀ mice and at 60 ppm and above in F₁ mice. There was a 14% reduction in testes weights at 360 ppm, and a similar decrease in epididymal sperm concentration. The survival of the second generation was not affected by consumption of the test substance, but by the week of mating (74±10 days of age), male F₁ body weights were reduced by 10, 8, and 8% at 60, 180 and 360 ppm, respectively. The F₁ generation produced 19, 18, and 55% fewer live F₂ pups per litter at 60, 180 and 360 ppm, respectively. Terminal male body weights were decreased at all doses by 12, 11 and 9% for the F₁ males (at 60, 180 and 360 ppm,

respectively) with a corresponding increase in the relative kidney and liver weights. Seminal vesicle weight was reduced by 12 and 22% at 180 and 360 ppm, respectively. In F₁ mice there was a 10 to 15% reduction in grip strength during maturation, but treatment-related differences were gone by the week of mating. There were no treatment-related microscopic alterations in the nerves or testes of treated F₁ mice. Overall, NMA produced significant dominant lethality in the presence of mild and variable neurotoxicity and no effects on body weight in the F₀ mice.

[http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchhome]

LOAEL (parental systemic toxicity) = Not established (based on limited information in the study summary)

LOAEL (reproductive toxicity/males) ~ 68 mg/kg-bw/day (based on decreases in testes weight and epididymal sperm concentrations)

NOAEL (reproductive toxicity/males) ~ 37 mg/kg-bw/day

LOAEL (reproductive toxicity/females) ~ 13 mg/kg-bw/day (based on decreased F₂ pups per litter and decreased live F₂ pups per litter in F₁ females)

NOAEL (reproductive toxicity/females) = Not established

(2) DdY mice (14 males and 24 females/dose) were administered NMA at doses of 0 or 4.3 mM in drinking water (~ 86.9 mg/kg-day) for 6 weeks. On completion of the dosing period, half of the treated males and all of the females were mated with untreated controls of the opposite sex. Uterine contents were examined on day 13 of gestation for implants and resorptions except for half of the females at the highest exposure level, which were permitted to complete their gestation period. Delivered pups were examined for a further 4 weeks for any abnormalities. After the 6-week exposure period, the males not used for mating were killed and weights of liver, testes, seminal vesicles were measured, and sperm count and sperm cell morphology were determined. Slight signs of hindlimb weakness were observed among treated females after 6 weeks of exposure. Body weight and food and water consumption were unaffected. The fertility rate, measured on day 13 and on day of delivery, was affected only when treated males were mated with control females. No abnormalities in body weight, body weight gain or behavior were observed in offspring up to 4 weeks postpartum. At week 6, there were decreases in relative weights of the testis and a reduction in seminal vesicles of males not used for mating. Epididymal sperm count was also reduced and there was an increase in head and tail abnormalities in sperm.

(3) DdY mice (5 – 7 males/dose) were administered NMA in saline by gavage at doses of 0 or 292 mg/kg-day, 2 times/week for 8 weeks (16 doses). Testicular weight was taken at necropsy. Relative testicular weight was reduced (55% of control value) in treated mice. A reduced number of spermatids and spermatocytes were observed in the epithelium when compared with controls. Reduction in spermatozoa and the presence of multinucleate giant cells were also observed in treated mice. Sertoli cells and interstitial cells were not affected.

Developmental Toxicity

NMA (CASRN 924-42-5)

(1) In the modified protocol reproductive toxicity study described previously under Reproductive Toxicity (study #1), no effects were observed in growth or survival in the F₁ generation during the preweaning phase (before the F₁ mice directly received the test substance). However, F₁ mice had lowered body weights during the post-weaning phase of the study (when they received the test substance in the same concentrations as the F₀ generation). Increased liver and kidney/adrenal weights were also seen at all doses, also presumably during the post-weaning phase. F₁ mice were more susceptible to the neuromuscular effects of the test substance during post-weaning development at all doses compared with the F₀ generation, as evidenced by decreased grip strength in both limbs in both sexes.

LOAEL (maternal toxicity) = Not established (based on limited information in the study summary)

LOAEL (developmental toxicity)~ 13 mg/kg-bw/day (based on decreased F₂ pups per litter and decreased live F₂ pups per litter)

NOAEL (developmental toxicity) = Not established

(2) In the reproductive toxicity study described previously in DdY mice (study #2), uterine contents were examined on day 13 of gestation for implants and resorptions in half of the dosed females. The other dams delivered their pups, which were evaluated for abnormalities for 4 weeks. No abnormalities in body weight, body weight gain or behavior were observed in the offspring after delivery at the single dose tested.

Genetic Toxicity – Gene Mutation

In vitro

NMA (CASRN 924-42-5)

(1) In an NTP study, *Salmonella typhimurium* strains TA97, TA98, TA100 and TA 1535 were exposed to NMA at concentrations up to 10,000 µg/plate with and without metabolic activation. Negative and positive controls responded appropriately. No cytotoxicity was seen up to a concentration of 10,000 µg/Plate. Results were negative in all strains with and without metabolic activation.

NMA was not mutagenic in this assay.

(2) *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to NMA at concentrations up to 5000 µg/plate with and without metabolic activation. Negative and positive controls were used; however, their response was not reported. The submission did not include information on cytotoxicity. Results were negative in all strains with and without metabolic activation.

NMA was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro

NMA (CASRN 924-42-5)

(1) In a chromosomal aberration assay conducted by NTP, Chinese hamster ovary (CHO) cells were exposed to NMA in DMSO at doses of 250, 375, 437.5 and 500 µg/mL without metabolic activation and at 2500, 3750 and 5000 µg/mL with metabolic activation. Exposures were approximately 20 hours both with and without activation. Negative and positive controls were tested concurrently and responded appropriately.

NMA induced chromosomal aberrations in this assay.

(2) In a sister chromatid exchange assay conducted by NTP, Chinese hamster ovary (CHO) cells were exposed to NMA in DMSO at doses of 16.7, 50, 125, 166.7, 250, 375 and 500 µg/mL without metabolic activation and at 166.7, 500, 1700 and 5000 µg/mL with metabolic activation. Negative and positive controls were tested concurrently and responded appropriately.

NMA induced chromosomal aberrations in this assay.

In vivo

NMA (CASRN 924-42-5)

In a micronucleus test conducted by NTP, male B6C3F1 mice (5/dose) were exposed to NMA in corn oil by i.p. injection using two injections at 24 hour intervals, at doses of 37.5, 75 or 150 mg/kg-bw. Bone marrow smears were prepared 24 hours after the second injection and 2000 polychromatic erythrocytes (PCEs) were scored for the incidence of micronuclei. Negative and positive controls responded appropriately. No increase in micronucleated polychromatic erythrocytes (PCEs) was observed.

NMA did not induce micronuclei in this assay.

Additional Information

Skin Irritation

NMA (CASRN 924-42-5)

Albino rabbits (4/dose) were administered NMA dermally at 2000, 4000, 8000 or 16,000 mg/kg-bw under occluded conditions for 24 hours with animals observed for 5 – 7 days following administration. One animal in the high-dose group died shortly after the exposure period. Tremors and hindlimb impairment were observed prior to death. Some incidences of skin irritation at all doses were observed, including erythema, edema and desquamation. The high dose produced caustic burns.

NMA was irritating to rabbit skin in this assay.

NBMA (CASRN 1852-16-0)

Six rabbits (strain not specified) were administered NBMA dermally at 0.1 mL to one intact and one abraded site under occluded conditions for 72 hours. Erythema and edema were scored at 24 and 72 hours using the Draize method. Very slight to well-defined erythema was observed in intact skin at 24 hours. Edema ranged from very slight to slight in abraded skin. The Primary Irritation Index was 1.8.

NBMA was mildly irritating to rabbit skin in this assay.

Eye Irritation

NMA (CASRN 924-42-5)

Albino rabbits (3/dose) were administered NMA in the left eye at 3 mg and were observed for 5 days. The right eye was not treated. The eyes remained unwashed. Mild irritation was noted immediately following application. Within 1 hour of application, no irritation was observed.

NMA was not irritating to rabbit eyes in this assay.

NBMA (CASRN 1852-16-0)

Two groups of six rabbits (strain not specified) were administered NBMA (80% purity) at a dose of 0.5 mL into the eyes. No other details were provided. Corneal opacity, iritis and conjunctival irritation were scored at 24, 48 and 72 hours. Three of six rabbits in each group had corneal damage.

NBMA was mildly irritating to rabbit eyes in this assay.

Carcinogenicity

NMA (CASRN 924-42-5)

(1) In an NTP study, Fischer 344 rats (50/sex/dose) were administered NMA by gavage at doses of 0, 6 or 12 mg/kg-day, 5 days/week for 103 weeks. Necropsy was performed on all animals and all organs and tissues were examined for grossly visible lesions. Complete histological exams were performed on all high-dose and control animals and on low-dose animals dying through month 21 of the study. Histopathological exams were also performed on all grossly visible lesions in all dose groups. Mean body weight of dosed animals was within 6% of controls. Survival of female rats in the low-dose group was lower than controls after day 550. Survival of females at the 12 mg/kg- day dose did not differ from controls. No other differences in survival were seen in any other group. No biologically important non-neoplastic or neoplastic changes were attributed to treatment. [http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchhome]

NMA was not carcinogenic in this study.

(2) In an NTP study, B6C3F1 mice (50/sex) were administered NMA (~ 98% purity) by gavage at doses of 0, 25 or 50 mg/kg- day, 5 days/week for 103 weeks. Necropsy was performed on all animals and all organs and tissues were examined for grossly visible lesions. Complete histological exams were performed on all high-dose and control animals and on low-dose animals dying through month 21 of the study. Histopathological exams were also performed on all grossly visible lesions in all dose groups. Mean body weight of females was as much as 25% greater than controls, while mean body weight of males was up to 13% greater than controls. No differences in survival were noted. Incidence of adenomas of the Harderian gland was increased in the high-dose males and females and in low-dose males. Incidences of hepatocellular adenomas were increased in males and females in the high-dose group, and hepatocellular carcinomas were marginally increased in males at both doses. Treated females exhibited increased incidence of ovarian atrophy and benign granulosa cell tumors. Both males and females in the high-dose group had increased incidence of alveolar/bronchiolar adenomas and carcinomas. [http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchhome]

NMA was carcinogenic in this study.

Neurotoxicity

NMA (CASRN 924-42-5)

(1) In the NTP repeated-dose study in rats described previously, all animals died in the 100 and 200 mg/kg-day groups. Animals at 50 mg/kg-day and above developed hindlimb ataxia, which progressed to hindlimb paralysis. Decreased forelimb and/or hindlimb grip strength was seen at 100 mg/kg-day at 6 weeks and at doses down to 25 mg/kg-day for females and 12.5 mg/kg-day for males after 13 weeks. Landing foot spread was increased at 6 weeks only for rats in the 50 mg/kg-day group. Degeneration of peripheral nerves was observed at doses \geq 50 mg/kg-day using light microscopy and \geq 25 mg/kg-day using electron microscopy.

NMA was neurotoxic to rats in this assay.

(2) In the NTP repeated-dose study in mice described previously, all animals in the high-dose group developed hindlimb paralysis and died. Behavioral tests performed at 6 and 13 weeks of treatment showed dose-related decreases in forelimb grip strength in both sexes at 25 mg/kg-day and above. Motor activity was not significantly different in any dose group. Rotarod performance and startle response did not show consistent dose-related changes. No nervous system lesions were observed using light microscopy.

NMA was neurotoxic to mice in this assay.

(3) In the repeated-dose study described previously (study #4), male Wistar rats (4/dose) were administered NMA via drinking water at 0, 3.36, 5.41, 8.65 or 13.8 mM (equivalent to \sim 0, 33.9, 54.6, 87.4 or 139.4 mg/kg-bw/day) for 90 days. Impaired rotarod performance was observed at the two highest doses at day 90. Other clinical signs of toxicity included weakness, tendency toward spreading and dragging hindlimbs and urinary incontinence among more severely affected animals. Light microscopy examination showed moderate to severe changes, including shrinkage and loss of myelinated fibers, myelin retraction and corrugation of myelin sheath. A reduction of the colchicines-binding was also detected in the spinal cord of both cervical and lumbar regions. This reduction was not seen in the brain. Information was not provided on the doses at which most of the changes in this study were observed.

NMA was neurotoxic to rats in this assay.

Conclusion: The acute oral toxicity of CASRN 924-42-5 is moderate in rats and mice, and for CASRN 1852-16-0, it is low in rats. The acute dermal toxicity of CASRN 924-42-5 is low in rabbits and for CASRN 1852-16-0 it is moderate. Repeated-dose, 90-day subchronic toxicity studies with CASRN 924-42-5 via gavage in rats and mice showed neurotoxic and neurobehavioral effects at 12.5 mg/kg-day (the lowest dose tested); the NOAEL for systemic toxicity is not established. A repeated-dose, 28-day toxicity study with CASRN 924-42-5 via the diet in rats showed growth retardation and hindlimb weakness at 46 mg/kg-bw/day; the NOAEL for systemic toxicity is 4 mg/kg-bw/day. In a 4-week drinking water study in rats, similar neurotoxic effects (as with those observed in the gavage studies) were seen at 33.9 mg/kg-bw/day, the lowest dose tested; the NOAEL is not established. Limited information in a 42-day dietary study in rats with CASRN 1852-16-0 indicated ataxia in both sexes at approximately 300 mg/kg-bw/day (highest dose); the NOAEL for systemic toxicity is approximately 148 mg/kg-bw/day.

In a modified reproductive continuous breeding study in mice with CASRN 924-42-5 via drinking water, decreases in pups per litter and in number of live pups per litter were observed in F2 pups at 17 mg/kg-bw/day, the lowest dose tested; the NOAEL for both reproductive (in females) and developmental toxicity is not established. In males, decreases in testes weight and epididymal sperm concentrations were observed at 68 mg/kg-bw/day; the NOAEL for reproductive toxicity is 37 mg/kg-bw/day. CASRNs 924-42-5 and 1852-16-0 were not mutagenic in bacteria, but induced chromosomal aberrations in mammalian cells *in vitro*. CASRN 924-42-5 did not induce micronuclei *in vivo*. CASRN 924-42-5 was not carcinogenic when tested in rats, but was carcinogenic in mice. CASRN 924-42-5 was neurotoxic to both rats and mice. Both CASRNs are irritating to rabbit skin. CASRN 924-42-5 is not irritating rabbit eyes. CASRN 1852-16-0 is irritating to rabbit eyes.

Table 3. Summary of Human Health Data		
Endpoints	NMA (924-42-5)	NBMA (1852-16-0)
Acute Oral Toxicity LD ₅₀ (mg/kg-bw)	400	924
Acute Dermal Toxicity LD ₅₀ (mg/kg-bw)	>16,000	80
Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	NOAEL = Not established LOAEL = 12.5	NOAEL = 148 LOAEL = 300
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	Females: NOAEL = Not established LOAEL ~ 17 Males: NOAEL ~ 68 LOAEL ~ 37	No Data Females; NOAEL = Not established LOAEL ~ 17 (RA) Males: NOAEL ~ 68 LOAEL ~ 37
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) Maternal Toxicity Developmental Toxicity	NOAEL/LOAEL = Not established NOAEL = Not established LOAEL ~ 17	No Data NOAEL/LOAEL = Not established LOAEL ~ 17 (RA)
Genetic Toxicity – Gene Mutation <i>In vitro</i>	Negative	No Data Negative (RA)
Genetic Toxicity – Chromosomal Aberrations <i>In vitro</i>	Positive	No Data Positive (RA)
Genetic Toxicity – Chromosomal Aberrations <i>In vivo</i>	Negative	No Data Negative (RA)
Additional Information Skin Irritation Eye Irritation Carcinogenicity Neurotoxicity	Irritating Not irritating Positive Neurotoxic	Mildly irritating Mildly irritating – –

Measured data in bold text; (RA) = Read Across; – indicates that endpoint was not evaluated for this substance

4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4.

Acute Toxicity to Fish

NMA (CASRN 924-42-5)

(1) Rainbow trout (*Oncorhynchus mykiss*) were exposed to NMA at nominal concentrations of 0, 12.5, 25, 50 or 100 mg/L under static conditions for 96 hours. Water temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. No information was provided on measured test concentrations. No mortalities were observed.

96-h LC₅₀ > 100 mg/L

NBMA (CASRN 1852-16-0)

Rainbow trout (*Oncorhynchus mykiss*) were exposed to NBMA at nominal concentrations of 0, 62.5, 125, 250, 500 or 1000 mg/L under static conditions for 96 hours. Water temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Temperature was maintained at 15 °C. No information was provided on measured test concentrations. All fish died at ≥ 125 mg/L. Ten percent mortality was observed in the 62.5 mg/L group.

96-h LC₅₀ = 75 mg/L

96-h LC₅₀ = 43.5 mg/L (ECOSAR v. 1.00a)

Acute Toxicity to Aquatic Invertebrates

No measured data were provided for the sponsored chemicals. Testing is recommended for at least NBMA.

(1) ECOSAR v. 1.00a was used to estimate toxicity to CASRN 1852-16-0

48-h EC₅₀ = 34.5 mg/L (ECOSAR v. 1.00a)

Toxicity to Aquatic Plants

No data were provided for the sponsored chemicals. Since ECOSAR (ECOSAR v. 1.00a) values for aquatic plants are not available for the chemical class “acrylamides”, testing is recommended for at least NBMA.

Conclusion: For N-(methyl)-acrylamides category, the 96-hour LC₅₀ to fish ranges from 75 to >100 mg/L. No experimental data are available for aquatic invertebrates and aquatic plants for this category.

Data gaps were identified for acute toxicity to aquatic invertebrates and toxicity to aquatic plants for the category N-(Methyl)-Acrylamides under the HPV Challenge Program.

Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program –Aquatic Toxicity Data		
Endpoints	NMA (924-42-5)	NBMA (1852-16-0)
Fish 96-h LC₅₀ (mg/L)	>100 (m) (498) (e)	75 (m) 43.5 (e)
Aquatic Invertebrates 48-h EC₅₀ (mg/L)	No data	No data 34.5 (e)
Aquatic Plants 72-h IC₅₀ (mg/L)	No data	No data

(m) = measured data (derived from testing); (e) = estimated data (derived from modeling, ECOSAR v. 1.00a)

The sponsor provided the following additional repeated-dose toxicity studies in its robust summaries: CR albino rats (5/sex/group) were administered NBMA at 0, 0.062, 0.125 or 0.25% in the diet (~ 0, 0.072, 0.140 or 0.306 mg/kg-bw/day in males and 0, 0.076, 0.157 or 0.294 mg/kg-bw/day in females) for 42 days. The commercial product contained approximately 5% AMD and 5% NMA. Animals were observed daily for signs of toxicity, as well as possible changes in appearance, behavior, gait and excretory function. Food intake and body weight were measured weekly. All animals were necropsied. No mortalities were observed. Ataxia was observed in the high-dose group beginning at week 4, with males showing some improvement by week 5 and females showing improvement at week 6. No changes in body weight were observed. Food consumption was decreased in all treatment groups. By the end of the second week, the 0.062% group was eating approximately the same amount as the control group. However, EPA considers the study to be inadequate because it did not include histopathological and clinical pathology examinations. Therefore, this study was not included in the hazard characterization.