

SCREENING-LEVEL HAZARD CHARACTERIZATION Mononitroanilines Category

2-Nitrobenzenamine (CASRN 88-74-4)

4-Nitrobenzenamine (CASRN 100-01-6)

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.

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>.

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)	88-74-4 100-01-6
Chemical Abstract Index Name	Benzenamine, 2-nitro- Benzenamine, 4-nitro-
Structural Formula	See Section 1

Summary

The mononitroanilines are solid substances. CASRN 100-01-6 has a low vapor pressure and moderate water solubility and CASRN 88-74-4 has a moderate vapor pressure and high water solubility. Both members of the category are expected to have high mobility in soil. Volatilization for both category members is considered low based on their Henry's Law constant. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered moderate. Mononitroanilines are expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

Acute toxicity of the category chemicals via oral (rats), inhalation (rats) and dermal (rabbits) routes is low. Repeated-dose studies with CASRN 100-01-6 in rats via gavage and inhalation showed effects on spleen and hematological parameters at 3 mg/kg/day and 0.01 mg/L, respectively. NOAELs were not established in these studies. Repeated-dose studies with CASRN 88-74-4 in rats via inhalation showed hematological effects at 0.093 mg/L; the NOAEL was 0.0098 mg/L. In an oral two-generation reproductive toxicity study in rats with CASRN 100-01-6, a statistically significant reduction in pregnancy rate was observed at 9 mg/kg/day; the NOAEL for reproductive toxicity was 1.5 mg/kg/day. In the same study, effects on the spleen were observed at 9 mg/kg/day in F1 rats; the NOAEL for maternal toxicity was 1.5 mg/kg/day, and no pre- and postnatal developmental toxicity was reported at 9 mg/kg/day (the highest dose tested). An oral prenatal developmental toxicity study in rats with CASRN 88-74-4 showed reduced maternal body weight gain at 300 mg/kg/day and no developmental toxicity at the highest dose (600 mg/kg/day) tested. The NOAEL for maternal toxicity was 100 mg/kg/day. An oral prenatal developmental toxicity study in rats with CASRN 100-01-6 showed significantly increased mean maternal spleen weights and reduced mean fetal weights at 85 mg/kg/day. The NOAEL for maternal and developmental toxicity was 25 mg/kg/day. An oral prenatal developmental toxicity study in rabbits with CASRN 100-01-6 showed significant maternal mortality and body weight loss at 125 mg/kg/day; the NOAEL for maternal toxicity was 75 mg/kg/day. No developmental toxicity was observed at the highest dose (125 mg/kg/day) tested. *In vitro* and *in vivo* genotoxicity data are predominantly negative, suggesting that these chemicals are probably non-genotoxic.

For the acute hazard of mononitroanilines category chemicals, based on CASRN 88-7-4, the measured 96-hour LC₅₀ to fish is 19.5 mg/L, the measured 48-hour EC₅₀ to aquatic invertebrates is 14.5 mg/L, and the estimated 96-hour EC₅₀ to aquatic plants is 6.47 mg/L

No data gaps were identified in the HPV Challenge Program.

The sponsor, Solutia, Inc., submitted a Test Plan and Robust Summaries to EPA for the Mononitroanilines Category on November 15, 2002. EPA posted the submission on the ChemRTK HPV Challenge website on April 21, 2003 (<http://www.epa.gov/chemrtk/pubs/summaries/mntranlc/c14391tc.htm>). EPA comments on the original submission were posted to the website on August 26, 2003. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on April 14, 2004, which were posted to the ChemRTK website on August 24, 2004. The mononitroanilines category consists of two chemicals as shown in Table 1.

Category Justification

The mononitroanilines category is based on the structural similarities of the members and the demonstrated similarities in their physicochemical and environmental fate properties, and toxicological and ecotoxicological effects. EPA agreed with the grouping of these chemicals for the HPV Challenge Program.

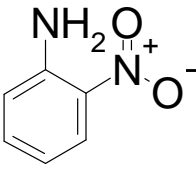
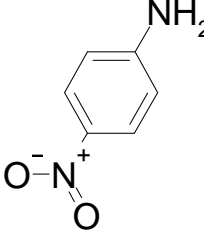
1 Chemical Identity

1.1 Identification and Purity

The following description is taken from the final Test Plan (2004):

The two chemicals selected for inclusion in this category are isomeric forms of the same base chemical, nitroaniline. Each of these amino compounds are aromatic hydrocarbons for which one benzene ring hydrogen has been replaced by a nitro (NO₂) radical and one benzene ring hydrogen further replaced with an amino (NH₂) group; the position (either *ortho* to or *para* to the nitro grouping) of the ring placement of the amino grouping is the only structural difference between these two chemicals.

The chemical structures of the two chemicals in this category are listed in Table 1.

Chemical Name	CASRN	Structure
2-Nitrobenzenamine	88-74-4	
4-Nitrobenzenamine	100-01-6	

1.2 Physical-Chemical Properties

The physical-chemical properties of the monoaniline category are summarized in Table 2. The mononitroanilines are solid substances. 4-Nitrobenzenamine has a low vapor pressure and moderate water solubility and 2-nitrobenzenamine has a moderate vapor pressure and high water solubility.

Property	4-Nitrobenzenamine	2-Nitrobenzenamine
CASRN	100-01-6	88-74-4
Molecular Weight	138.13	138.13
Physical State	Solid	Solid
Melting Point	146°C (measured)	71.5°C (measured)
Boiling Point	332°C (measured)	284°C (measured)
Vapor Pressure	3.2×10 ⁻⁶ mm Hg at 25°C (extrapolated)	8.6×10 ⁻⁴ mm Hg at 25°C (extrapolated) ²
Water Solubility	724 mg/L at 25°C (measured)	1,470 mg/L at 25°C (measured)
Log K _{ow}	1.39 (measured) ⁴	1.85 (measured) ⁴
Dissociation Constant (pK _a)	1.00 (measured) ³	-0.28 (measured) ³
Henry's Law Constant	1.26×10 ⁻⁹ atm·m ³ /mole (measured) ³	5.90×10 ⁻⁸ atm·m ³ /mole (measured) ³

¹Solutia, Inc. April 14, 2004. Revised Robust Summary for Mononitroanilines Category.

<http://www.epa.gov/chemrtk/pubs/summaries/mntranlc/c14391tc.htm>.

²The vapor pressure value was reported in the Revised Robust Summaries as 1.15×10⁻³ hPa (8.6×10⁻³ mm Hg). The mm Hg value seems to be a conversion error and should be 8.6×10⁻⁴ mm Hg as calculated by the reviewer.

³SRC. The Physical Properties Database (PHYSPROP). Syracuse, NY: Syracuse Research Corporation. Available from <http://srcinc.com/what-we-do/dataforms.aspx?id=386> as of September 15, 2008.

⁴The log K_{ow} values were reported in the Revised Robust Summaries as calculated, but the source cited by the submitter indicates that they are experimental values.

2 General Information on Exposure

2.1 Production Volume and Use Pattern

The two chemicals in the mononitroanilines category have an aggregated production and/or imported volume in the United States of 1.5 million to 11 million pounds.

Non-confidential information in the IUR for CASRN 100-01-6 indicated that the industrial processing and uses of the chemical include intermediates in the manufacturing of other basic organic chemicals. The HSDB states that CASRN 100-01-6 is used as an intermediate in the manufacture of dyes, antioxidants, pharmaceuticals and pesticides, and as an intermediate to produce p-phenylenediamine. CASRN 88-74-4 is primarily used as a chemical intermediate for dyes and pigments and in the synthesis of photographic antifogging agent. The HPV submission for the mononitroanilines category states that both chemicals are intermediates which serve as basic building blocks for the ultimate manufacture of numerous industrial chemicals. CASRN 100-01-6 is utilized in preparation of antioxidants, antiozonants, and dyes and pigments while CASRN 88-74-4 is converted to polymer additives, veterinary pharmaceuticals and water-treatment chemicals.

2.2 Environmental Exposure and Fate

There is release information from the Toxics Release Inventory for CASRN 100-01-6. The 2007 TRI shows releases of CASRN 100-01-6 to air and landfills. Release information from the 2007 TRI reporting year follows.

- CASRN 100-01-6
 - Total releases: 362 lbs.
 - Off-site Water Releases (POTW Transfers): 102 lbs.
 - Deep-well injection/off-site landfills: 260 lbs.

The environmental fate properties are provided in Table 3. Both members of the category are expected to have high mobility in soil. Volatilization for both category members is considered low based on their Henry's Law constant. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered moderate. Mononitroanilines are expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

Table 3. Environmental Fate Characteristics of Mononitroanilines¹

Property	4-Nitrobenzenamine	2-Nitrobenzenamine
CASRN	100-01-6	88-74-4
Photodegradation Half-life	9.5 hours (estimated)	9.5 hours (estimated); Direct photolysis = 16% after 3 hours (measured)
Hydrolysis Half-life	Stable	Stable
Biodegradation	30% removal during weeks 1–13; 82% mean loss between weeks 16–33; 19% mean loss after week 33 (measured; 10 month SCAS test); 50 mg/L test sample biodegraded after 20 day lag period in aerobic sewage; Half-life = 3.8 days in river water (monitoring study) (biodegradable under optimal conditions); 0% after 14 days (not readily biodegradable) ²	7% mean loss after 10 month SCAS test (measured) (no significant biodegradation); 0% after 14 days (not readily biodegradable) ²
Bioconcentration	BCF = 2.9–3.6 (measured in carp 0.5 ppm test concentration) ² ; BCF <10 (measured in carp; 0.05 ppm test concentration) ²	BCF = 2.1–4.9 (measured in carp; 0.5 ppm test concentration) ² ; BCF <10 (measured in carp; 0.05 ppm test concentration) ²
Log K _{oc}	1.71 (estimated) ³	1.72 (estimated) ³
Fugacity (Level III Model)	Air = <0.01% Water = 99.8% Soil = <0.01% Sediment = <1%	Air = <0.01% Water = 99.6% Soil = <0.01% Sediment = <1%
Persistence ⁴	P2 (moderate)	P2 (moderate)
Bioaccumulation ⁴	B1 (low)	B1 (low)

¹Solutia, Inc. April 14, 2004. Revised Robust Summary for Mononitroanilines Category.

<http://www.epa.gov/hpv/pubs/summaries/mntranlc/c14391tc.htm>.

²National Institute of Technology and Evaluation. 2002. Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law.

http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

³U.S. EPA. 2008. Estimation Programs Interface Suite™ for Microsoft® Windows, v 3.20. United States Environmental Protection Agency, Washington, DC, USA.

<http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>.

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

3. Human Health Effects

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

2-Nitrobenzeneamine (CASRN 88-74-4)

Sprague-Dawley rats (5/mixed sex/dose) were administered 2-nitrobenzeneamine as a 10% corn oil solution via gavage at doses of 1260, 1580, 2000 and 2510 mg/kg-bw and observed for 14 days.

LD₅₀ = 2050 mg/kg-bw

4-Nitrobenzeneamine (CASR N 100-01-6)

Sprague-Dawley rats (5/mixed sex/dose) administered 4-nitrobenzeneamine as a 20% solution-suspension in corn oil at doses of 794, 1000, 1260, 1580 and 2000 mg/kg-bw and observed for 14 days.

LD₅₀ = 1400 mg/kg-bw

Acute Inhalation Toxicity

2-Nitrobenzeneamine (CASR N 88-74-4)

Ten Wistar rats (5/sex/dose) were exposed (nose-only) to a single aerosol concentration of 2-nitrobenzeneamine solution in polyethylene glycol at the maximum achievable concentration of 2529 mg/m³ (2.53 mg/L) for 4 hours. A vehicle control group of rats was exposed to polyethylene glycol/acetone. The observation period was 14 days. No mortality was observed.

LC₅₀ > 2.53 mg/L

Acute Dermal Toxicity

2-Nitrobenzeneamine (CASR N 88-74-4)

New Zealand White rabbits were exposed dermally (intact skin) to 5010 (1 animal) or 7940 mg/kg-bw (2 animals) of 2-nitrobenzeneamine as 40% solution-suspension in corn oil under occluded conditions for 24 hours and observed for 14 days. There were no deaths.

LD₅₀ > 7940 mg/kg-bw

4-Nitrobenzeneamine (CASR N 100-01-6)

Three New Zealand White rabbits were exposed dermally (intact skin) to 5010 (1 animal) or 7940 mg/kg-bw (2 animals) of 4-nitrobenzeneamine as 40% solution-suspension in corn oil under occluded conditions for 24 hours and observed for 14 days. There were no deaths.

LD₅₀ > 7940 mg/kg-bw

Repeated-Dose Toxicity

2-Nitrobenzeneamine (CASR N 88-74-4)

In a 28-day study, male Sprague-Dawley rats (10/dose) were exposed to 2-nitrobenzeneamine via inhalation (whole-body exposure) at 0, 9.8 or 93 mg/m³ (0, 0.0098 or 0.093 mg/L) for 6 hours/day, 5 days/week for 4 weeks. Concurrent negative controls were used. Body, brain and testicular weights were recorded, hematological parameters were measured, and testes and epididymides were examined macroscopically and microscopically. Changes in some hematological parameters (increases in methemoglobin and red blood cell counts and decreases in total leukocytes and segmented neutrophils) were seen at 0.093 mg/L.

LOAEL = 0.093 mg/L (based on effects on some hematological parameters)

NOAEL = 0.0098 mg/L

4-Nitrobenzeneamine (CASR N 100-01-6)

(1) In a 90-day study, Sprague-Dawley rats (20/sex/dose) were administered 4-nitrobenzeneamine in corn oil via gavage at 0, 3, 10 or 30 mg/kg-bw/day. At 30 mg/kg-bw/day, pale appearance around ears and statistically significant increases in urinary urobilinogen were seen. Also at this dose, significant increases in methemoglobin levels, red blood cell counts and hemoglobin levels were also reported. All animals from this group had discolored spleens at necropsy, significant increased spleen weights, splenomegaly and excessive splenic hemosiderin deposition. At 10 mg/kg-bw/day, significant increased methemoglobin, and decreased red blood cell counts and hemoglobin concentrations were noted in female rats. All animals at this dose had splenomegaly, significant elevated splenic weights, discolored spleens and excessive hemosiderin deposits. At 3 mg/kg-bw/day, methemoglobin levels were significantly increased and microscopic changes were seen in the spleen of both males and females.

LOAEL = 3 mg/kg-bw/day (based on effects on the spleen and changes in some hematological parameters)

NOAEL = Not established

(2) In a 28-day study, Sprague-Dawley rats (10/sex/group) were exposed to 4-nitrobenzeneamine aerosol via inhalation (whole-body) at 0, 10, 32 and 80 mg/m³/day (0, 0.01, 0.032 and 0.08 mg/L/day) for 6 hours/day, 5 day/week for 4 weeks. At 80 mg/m³/day, statistically significant increases in methemoglobin were seen in both males and females and higher incidence of polychromasia and anisocytosis were seen in females. Significantly increased absolute and relative spleen weights, iron deposits in the spleen and extramedullary hematopoiesis in spleen (males and females) and liver (females) were seen. At 32 mg/m³/day, effects observed included significantly increased levels of methemoglobin, higher incidence of polychromasia (both sexes) and anisocytosis (females), and significantly increased spleen weight (males) and increased iron deposits and extramedullary hematopoiesis in the spleen. At 10 mg/m³/day, elevated methemoglobin, and significantly increased spleen weights and iron deposits and extramedullary hematopoiesis in the spleen were seen in both sexes.

LOAEL = 0.01 mg/L/day (based on effects on the spleen and increased levels of methemoglobin)

NOAEL = Not established

Reproductive Toxicity

4-Nitrobenzeneamine (CASR N 100-01-6)

In a two-generation study, F0 and F1 generations (15 males and 30 females/generation) of Sprague-Dawley rats were administered 4-nitrobenzeneamine via gavage at 0, 0.25, 1.5 and 9 mg/kg-bw/day during a pre-mating (14 weeks for F0 and 18 weeks for F1) growth period, and through the ensuing mating, gestation and lactation intervals. F1 rats were continued on treatment during a post-weaning period of 30 days. No mortality or adverse effects on body weights or food consumption or in-life evaluations were observed in either F0 or F1 adults. Mating indices were comparable to controls for both F0 and F1 animals. A statistically significant reduction in pregnancy rate was observed in the 9 mg/kg-bw/day F0 group. The male fertility index was slightly lower at 9 mg/kg-bw/day in F0 animals. No adverse effects were observed in mean length of gestation, number of live and dead pups, pup weights during lactation or pup and litter survival. In the longest exposure to the test substance in F1 animals, changes in methemoglobin were not reported, but there was evidence of "brown pigment" in the spleen at the highest dose.

LOAEL (systemic toxicity) = 9 mg/kg-bw/day (based on effects on spleen)

NOAEL (systemic toxicity) = 1.5 mg/kg-bw/day

LOAEL (reproductive toxicity) = 9 mg/kg-bw/day (based on reduced pregnancy rate)

NOAEL (reproductive toxicity) = 1.5 mg/kg-bw/day

Developmental Toxicity

2-Nitrobenzeneamine (CASR N 88-74-4)

Pregnant Sprague-Dawley rats (25 females/group) were administered 2-nitrobenzeneamine in corn oil via gavage at doses of 0, 100, 300 and 600 mg/kg-bw/day from gestation days 6 to 15. Daily clinical signs were observed on gestation days 6 – 21. Maternal toxicity was evidenced by reduced body weight gains, reduced food consumption and piloerection in 300 and 600 mg/kg-bw/day groups. No effects were noted on pregnancy rates, mean number of live and dead pups, resorptions, nidations or corpora lutea. The mean fetal weights were slightly (but not statistically) lower than control in the 600 mg/kg-bw/day group. No effects were seen on number of litters, fetuses or malformations.

LOAEL (maternal toxicity) = 300 mg/kg-bw/day (based on reduced body weight gain)

NOAEL (maternal toxicity) = 100 mg/kg-bw/day

NOAEL (developmental toxicity) = 600 mg/kg-bw/day (based on no effects at highest dose tested)

4-Nitrobenzeneamine (CASR N 100-01-6)

(1) Pregnant Sprague-Dawley rats (25 females/group) were administered 4-nitrobenzeneamine in corn oil via gavage at doses of 25, 85 and 250 mg/kg-bw/day during gestation days 6 – 19. There was no maternal toxicity, embryotoxicity or fetotoxicity at 25 mg/kg-bw/day. At 85 mg/kg-bw/day, significantly increased mean

maternal spleen weights and reduced mean fetal weights (both sexes) were observed. At 250 mg/kg-bw/day, maternal toxicity was evidenced by reduced body weight gains, pale eye coloration and occasional convulsions after dosing, significantly increased mean number of resorptions and percent resorptions and significantly increased mean spleen weights (absolute and relative). Significant lower mean fetal weights (both sexes), increase in number of fetuses with delayed or no ossification and increase in number of fetuses with external, soft tissue or skeletal malformations (predominantly kinked or shortened tail, absence of kidneys or ureter and fused ribs) were seen at 250 mg/kg-bw/day.

LOAEL (maternal toxicity) = 85 mg/kg-bw/day (based on increased spleen weights)

NOAEL (maternal toxicity) = 25 mg/kg-bw/day

LOAEL (developmental toxicity) = 85 mg/kg-bw/day (based on reduced fetal body weights)

NOAEL (developmental toxicity) = 25 mg/kg-bw/day

(2) In a two-generation study, F0 and F1 generations (15 males and 30 females/generation) of Sprague-Dawley rats were administered 4-nitrobenzeneamine via oral gavage at 0, 0.25, 1.5 and 9 mg/kg-bw/day during a pre-mating (14 weeks for F0 and 18 weeks for F1) growth period, and through the ensuing mating, gestation and lactation intervals. F1 rats were continued on treatment during a post-weaning period of 30 days. No developmental toxicity was reported.

NOAEL (developmental toxicity) = 9 mg/kg-bw/day (based on no effects at the highest dose tested)

(3) Pregnant New Zealand White rabbits (18 females/group) were administered 4-nitrobenzeneamine in corn oil via gavage at 0, 15, 75 and 125 mg/kg-bw/day during gestation days 7 – 19. At 125 mg/kg-bw/day, 7/18 females died. Maternal toxicity was evidenced by grayish appearing eyes and body weight loss. No differences were observed in mean number of implantations, resorptions or variable fetuses or mean fetal weights. At 75 mg/kg-bw/day, the only observed effect was grayish eyes; there were no other effects on measured maternal, embryo or fetal parameters.

LOAEL (maternal toxicity) = 125 mg/kg-bw/day (based on mortality and body weight loss among survivors)

NOAEL (maternal toxicity) = 75 mg/kg-bw/day

NOAEL (developmental toxicity) = 125 mg/kg-bw/day (based on no effects at the highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro

2-Nitrobenzeneamine (CASR N 88-74-4)

In an Ames assay, *Salmonella typhimurium* strains (TA98, TA100, TA1535 and 1537) were exposed to 2-nitrobenzeneamine up to 1500 µg/plate in the presence and absence of metabolic activation. 2-Nitrobenzeneamine did not show any marked increases in mutagenic activity for any of the four tested strains. Sponsors noted that the positive controls gave an expected response.

2-Nitrobenzeneamine was not mutagenic in this assay.

4-Nitrobenzeneamine (CASR N 100-01-6)

In an Ames assay, *S. typhimurium* strains (TA98, TA100, TA1535, and/or 1537) were exposed to 4-nitrobenzeneamine up to 10 mg/plate in the presence and absence of metabolic activation. Positive controls were used. There were no marked increases in mutagenic activity for any of the four tested strains up to maximum concentration of 25 mg/spot in the spot test. However, in the plate incorporation assay, mutagenic activity was elevated in the TA98 strain with metabolic activation (not statistically significant) and without activation (statistically significant).

4-Nitrobenzeneamine gave equivocal results in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vivo

2-Nitrobenzeneamine (CASR N 88-74-4)

In a micronucleus assay male/female CD-1 mice were administered two single doses of 2-nitrobenzeneamine via intraperitoneal injection at 0, 50, 250 and 500 mg/kg-bw, 24 hours apart. Bone marrow cells were evaluated for the presence of micronuclei in erythrocytes. No increases in micronuclei were observed at any dose level. Positive controls responded appropriately. Animals exhibited signs of listlessness and unresponsive behavior at all doses. Females at 500 mg/kg-bw/day showed marked body weight loss.

2-Nitrobenzeneamine did not induce chromosomal aberrations in this assay.

4-Nitrobenzeneamine (CASR N 100-01-6)

In a micronucleus assay male/female CD-1 mice were administered two single doses of 4-nitrobenzeneamine via intraperitoneal injection at 80, 400 and 800 mg/kg-bw, 24 hours apart. Bone marrow cells were evaluated for the presence of micronuclei in erythrocytes. No increases in micronuclei were observed at any dose level. Positive controls responded appropriately. One death and clear signs of toxicity (unresponsiveness and tremors up to 4 hours after dosing) were noted at 800 mg/kg-bw; at 400 mg/kg-bw, listlessness and some tremors were seen occasionally after dosing and at 80 mg/kg-bw, listlessness immediately after dosing was noted. No effects on body weight were observed at any test level.

4-Nitrobenzeneamine did not induce chromosomal aberrations in this assay.

Conclusion: Acute toxicity of the category chemicals via oral (rats), inhalation (rats) and dermal (rabbits) routes is low. Repeated-dose studies with CASRN 100-01-6 in rats via gavage and inhalation showed effects on spleen and hematological parameters at 3 mg/kg/day and 0.01 mg/L, respectively. NOAELs were not established in these studies. Repeated-dose studies with CASRN 88-74-4 in rats via inhalation showed hematological effects at 0.093 mg/L; the NOAEL was 0.0098 mg/L. In an oral two-generation reproductive toxicity study in rats with CASRN 100-01-6, a statistically significant reduction in pregnancy rate was observed at 9 mg/kg/day; the NOAEL for reproductive toxicity was 1.5 mg/kg/day. In the same study, effects on the spleen were observed at 9

mg/kg-bw/day in F1 rats; the NOAEL for maternal toxicity was 1.5 mg/kg/day, and no pre- and postnatal developmental toxicity was reported at 9 mg/kg/day (the highest dose tested). An oral prenatal developmental toxicity study in rats with CASRN 88-74-4 showed reduced maternal body weight gain at 300 mg/kg/day and no developmental toxicity at the highest dose (600 mg/kg/day) tested. The NOAEL for maternal toxicity was 100 mg/kg/day. An oral prenatal developmental toxicity study in rats with CASRN 100-01-6 showed significantly increased mean maternal spleen weights and reduced mean fetal weights at 85 mg/kg/day. The NOAEL for maternal and developmental toxicity was 25 mg/kg/day. An oral prenatal developmental toxicity study in rabbits with CASRN 100-01-6 showed significant maternal mortality and body weight loss at 125 mg/kg/day; the NOAEL for maternal toxicity was 75 mg/kg/day. No developmental toxicity was observed at the highest dose (125 mg/kg/day) tested. *In vitro* and *in vivo* genotoxicity data are predominantly negative, suggesting that these chemicals are probably non-genotoxic.

Table 4. Summary of Human Health Data

Endpoints	2-Nitrobenzeneamine (CASRN 88-74-4)	4-Nitrobenzeneamine (CASRN 100-01-6)
Acute Oral Toxicity LD₅₀ (mg/kg-bw)	2050	1400
Acute Inhalation Toxicity LC₅₀ (mg/L)	> 2.53 (4-h)	No Data > 2.53 (4-h) (RA)
Acute Dermal Toxicity LD₅₀ (mg/kg-bw)	> 7940	> 7940
Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	No Data NOAEL = Not established LOAEL = 3 (RA)	NOAEL = Not established LOAEL = 3
Repeated-Dose Toxicity NOAEL/LOAEL Inhalation (mg/L)	NOAEL = 0.098 LOAEL = 0.093	NOAEL = Not established LOAEL = 0.01
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) Systemic Toxicity	No Data NOAEL = 1.5 LOAEL = 9	NOAEL = 1.5 LOAEL = 9
Reproductive Toxicity	NOAEL = 1.5	NOAEL = 1.5

	LOAEL = 9 (RA)	LOAEL = 9
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) Maternal Toxicity	(Rat) NOAEL = 100 LOAEL = 300	(Rat) NOAEL = 25 LOAEL = 85 (Rabbit) NOAEL = 75 LOAEL = 125
Developmental Toxicity	(Rat) NOAEL = 600	(Rat) NOAEL = 25 LOAEL = 85 (Rabbit) NOAEL = 125 LOAEL = 125
Genetic Toxicity – Gene Mutation <i>In vitro</i>	Negative	Equivocal
Genetic Toxicity – Chromosomal Aberrations <i>In vivo</i>	Negative	Negative

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

4 Hazards 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

2-Nitrobenzeneamine (CASRN 88-74-4)

Zebrafish (*Brachydanio rerio*) were exposed to measured concentrations of 2-nitrobenzeneamine (test concentration values were not provided) of under semi-static conditions for 96 hours.

96-h LC₅₀ = 19.5 mg/L

4-Nitrobenzeneamine (CASR N 100-01-6)

Rainbow trout (*Salmo gairdneri*) were exposed to nominal concentrations of 4-nitrobenzeneamine ranging from 5.6 to 100 mg/L under static conditions for 96 hours. No deaths were seen at concentrations up to 32 mg/L. At 56 mg/L, mortality was 80%

after 24 hours and 90% after 48 and 96 hours. Mortality was 100% at 100 mg/L at all three time points.

96-h LC₅₀ = 45 mg/L

Acute Toxicity to Aquatic Invertebrates

2-Nitrobenzeneamine (CASR N 88-74-4)

Daphnia magna were exposed to 2-nitrobenzeneamine at concentrations of 6.25, 12.5, 25, 50 and 100mg/L under static conditions for 48 hours.

48-h EC₅₀ =14.5 mg/L

4-Nitrobenzeneamine (CASR N 100-01-6)

Daphnia magna were exposed to 4-nitrobenzeneamine for 48 hours under static conditions using nominal concentrations. The test concentrations ranged between 3.2 and 32 mg/L, spaced logarithmically.

48-h LC₅₀ = 20 mg/L

Toxicity to Aquatic Plants

2-Nitrobenzeneamine (CASRN 88-74-4)

Green algae (*Scenedesmus sp.*) was exposed to 2-nitrobenzeneamine at five nominal concentrations under static conditions for 48 hours. The 48-hour EC₅₀ for growth rate inhibition was 64.5 mg/L. The submitted study duration deviates from the standard 72- or 96-hour algal toxicity study duration. The 96-hour EC₅₀ value of 6.47 mg/L for this endpoint was estimated using ECOSAR (v 1.00).

48-h EC₅₀ (growth) = 64.5 mg/L

96-h EC₅₀ (growth) = 6.47 mg/L (estimated)

4-Nitrobenzeneamine (CASR N 100-01-6)

Green algae (*Scenedesmus sp.*) was exposed to 4-nitrobenzeneamine at five nominal concentrations under static conditions for 48 hours. The 48-hour EC₅₀ for growth rate inhibition was 54.9 mg/L. The submitted study duration deviates from the standard 72- or 96-hour algal toxicity study duration. The 96-hour EC₅₀ value of 7.29 mg/L for this endpoint was estimated using ECOSAR (v1.00).

48-h EC₅₀ (growth) = 54.9 mg/L

96-h EC₅₀ (growth) = 7.29 mg/L (estimated)

Conclusion: For the acute hazard of mononitroanilines category chemicals, based on CASRN 88-7-4, the measured 96-hour LC₅₀ to fish is 19.5 mg/L, the measured 48-hour EC₅₀ to aquatic invertebrates is 14.5 mg/L, and the estimated 96-hour EC₅₀ to aquatic plants is 6.47 mg/L.

Table 5. Summary of Environmental Effects – Aquatic Toxicity Data		
Endpoints	2-Nitrobenzeneamine CASRN 88-74-4	4-Nitrobenzeneamine CASRN 100-01-6
Fish 96-h LC₅₀ (mg/L)	19.5 (m)	45 (m)
Aquatic Invertebrates 48-h EC₅₀ (mg/L)	14.5 (m)	20.0 (m)
Aquatic Plants 96-h EC₅₀ (mg/L) (growth)	64.5 (48-h) (m) 6.47 (96-h) (e)	54.9 (48-h) (m) 7.29 (96-h) (e)

(m) = measured data (i.e., derived from testing); (e) = estimated data (i.e., derived from modeling)