Benzenamine, N-nitroso-N-phenyl-: Human health tier II assessment

25 November 2016

CAS Number: 86-30-6

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

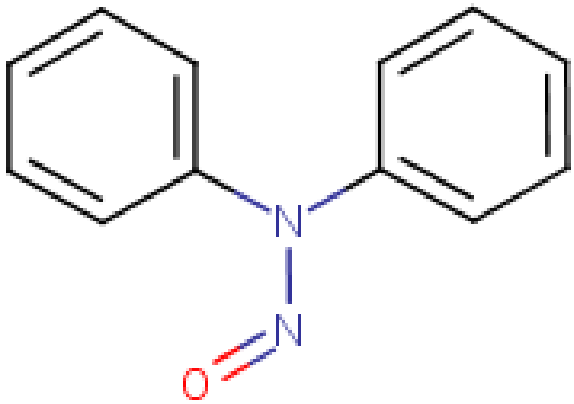
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:www.nicnas.gov.au

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	nitrosodiphenylamine N,N-di(phenyl)nitrous amide N-nitrosodiphenylamine diphenylnitrosamine
Structural Formula	
Molecular Formula	C12H10N2O
Molecular Weight (g/mol)	198.22
Appearance and Odour (where available)	Yellow flakes.
SMILES	c1(N(c2ccccc2)N=O)ccccc1

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through Galleria Chemica; the United States (US) National Library of Medicine's Hazardous Substances Data Bank (HSDB); the US Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile report and the International Agency for Research on Cancer (IARC) Monograph.

Nitrosodiphenylamine has reported site-limited use as a rubber chemical and an intermediate in the manufacture of para-nitrosodiphenylamine.

Small amounts are produced as a side reaction during some manufacturing processes, as a contaminant in some herbicides, and during the manufacture of some rubber products. In the early 1980s most US rubber manufacturers replaced nitrosodiphenylamine with more efficient chemicals.

Restrictions

Australian

No known restrictions have been identified.

International

The chemical is listed on the following (Galleria Chemica):

Existing Work Health and Safety Controls

Hazard Classification

This chemical is not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific international exposure standards are available.

Health Hazard Information

Toxicokinetics

The appearance of metabolites in the urine of rats and in the serum of guinea pigs following oral administration provides indirect evidence of gastrointestinal absorption of the chemical. Furthermore, the occurrence of systemic effects in rats and mice in oral carcinogenicity studies suggests that the chemical is absorbed through the gastrointestinal tract in these animals. The appearance of lung adenomas in a dermal carcinogenicity study with mice is possible indirect evidence of dermal absorption of nitrosodiphenylamine (ATSDR, 1993).

Animal studies provide evidence that the first step in the metabolic activation of the chemical is the denitrosation to diphenylamine and nitric oxide. A single dose of the chemical in corn oil (1000 mg/kg) was administered to female Wistar rats. Nitrate was identified as the major urinary metabolite, while nitrite, diphenylamine, and a monohydroxydiphenylamine were found in smaller amounts. The conclusion is that the chemical is denitrosated to diphenylamine and nitric oxide. The nitric oxide is then converted into nitrite and nitrate. Nitrite is oxidised in substantial amounts to nitrate (ATSDR, 1993).

Following oral administration of a single 1000 mg/kg dose of the chemical to female Wistar rats, the maximum urinary excretion of nitrate and nitrite was found 24–48 hours after administration. Within 36 hours of administration, 24.8% and 1.4% of the administered dose of nitrosodiphenylamine was excreted as nitrate and nitrite respectively. Ninety-six hours after administration, about 30% of the administered dose had been eliminated as nitrite and nitrate. The chemical is more rapidly eliminated after intraperitoneal administration compared to oral dosing. Results from a study of rats, rabbits, and guinea pigs receiving 50 mg/kg of nitrosodiphenylamine through intraperitoneal injection suggested that the rate of excretion of the chemical into the bile and elimination of the chemical from the bile varies among species (ATSDR, 1993).

Acute Toxicity

Oral

The chemical has low acute toxicity based on the limited results available from two animal tests following oral exposure, both containing limited or no information on experimental detail or methodologies used. Median lethal dose (LD50) values of 3000 mg/kg bw and 3850 mg/kg bw were determined for rats and mice respectively (ATSDR, 1993).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Animal studies of poor quality found that the chemical can irritate the skin (ATSDR, 1993).

Eye Irritation

No data are available.

Sensitisation

Respiratory Sensitisation

No data are available.

Skin Sensitisation

No data are available.

Repeated Dose Toxicity

Oral

The chemical does not have high repeat dose toxicity via the oral route. The effects observed were not sufficient to warrant classification. Studies in rats and mice showed that the urinary bladder is the target organ following chronic oral exposure to the chemical. Reductions in weight gain were also observed in some studies. Effects on kidneys and lungs were reported but these studies had significant limitations.

In subchronic studies, Fischer 344 (F344) rats and B6C3F$_1$ mice were fed diets containing up to 46000 mg/kg of the chemical (estimated to be 2300 and 6900 mg/kg bw/day for rats and mice respectively) for eight or 11 weeks. Female rats did not survive concentrations greater than 16000 mg/kg of diet (estimated to be 800 mg/kg bw/day); female mice survived higher concentrations. Male rats and male mice survived the highest levels tested (10000 and 22000 mg/kg of diet, respectively; estimated to be 500 mg/kg bw/day for rats and 3300 mg/kg bw/day for mice). Reductions in weight gain ranged from 37% in female rats fed 16000 mg/kg to 14% in female mice fed 46000 mg/kg of diet (IARC, 1982).

In a carcinogenicity study, male B6C3F$_1$ mice were administered 10000 or 20000 ppm (estimated to be 1500 and 3000 mg/kg bw/day) in feed for 101 weeks. Females initially received one of two concentrations, 5000 or 10000 ppm (approximately 750 and 1500 mg/kg bw/day respectively) and for 38 weeks. Due to the excessive reduction in the amount of mean body weight gained in the treated groups, the dietary concentrations for the females were then reduced to 1000 ppm and 4000 ppm respectively (approximately 150 and 600 mg/kg bw/day), and administration at the lowered levels was continued for 60 weeks (see the **Carcinogenicity section** for more study details and for information on the neoplastic effects of the chemical on the urinary bladders of rats). Chronic submucosal inflammation in the urinary bladder was observed in low dose males (12/49), high dose males (31/46), low dose females (31/47) and high dose females (30/38) (NCI, 1979). This lesion was not seen in controls. The inflammation was associated with connective tissue degeneration in the mucosa (Cardy et al, 1979).

Dermal

Limited data are available for the chemical.

Mice (strain and number unspecified) were painted with 0.1 mL of a 0.1% solution (33 mg/kg bw/week) of the chemical in acetone on the intrascapular region once per week for 20 weeks. All painted animals were reported to have small skin ulcerations and scarring. The study did not report whether these data included the control animals painted with the solvent or only the animals treated with the test substance. Only one dose was used in the study (ATSDR, 1993).

Inhalation

Limited data are available for the chemical.

In a subchronic study rats (strain unspecified) were exposed to 350–400 mg/m^3 nitrosodiphenylamine dust for two hours per day for 20 weeks. Observations included catarrhal bronchitis, reduced phagocytic activity of leukocytes and a 'lengthening of the chronaxie of the extensors of the rear extremities' (neurological effects). Interpretation of the results of this study is not possible because of what was considered to be 'severe limitations in the experimental procedure and reporting of data' by the ATSDR (1993).

Genotoxicity

The available data indicate mostly negative results for genotoxicity, and the weight of evidence indicates that the chemical is not genotoxic. The positive in vitro responses generally occurred in studies using exogenous metabolic activation. Based on mostly negative responses reported in in vivo studies, the chemical does not appear to be genotoxic (ATSDR, 1993).

In vitro studies

Numerous gene mutation test results were summarised in the ATSDR Toxicological Profile (ATSDR, 1993; ATSDR, 2010) with limited data regarding metabolic activation and no information on test concentrations. The majority of gene mutation studies conducted in *Salmonella typhimurium*, *Escherichia coli*, mouse lymphoma cells, Chinese hamster V79 cells and rat embryo cells were negative. However, two *Salmonella* assays detected gene mutations after exposure to metabolically activated nitrosodiphenylamine (ATSDR, 1993). One study found positive results when tested with high concentrations (ATSDR, 2010).

Mixed results were reported (ATSDR, 1993) in in vitro studies investigating chromosomal damage:

- the chemical exhibited no effect on mitotic crossing-over and gene conversion in *Saccharomyces cerevisiae*;
- chromosomal aberration assays for Chinese hamster fibroblasts and Chinese hamster Don cells were inconclusive;
- sister chromatid exchange was unaffected in Chinese hamster ovary cells exposed to nitrosodiphenylamine, but a positive response was noted in Chinese hamster Don cells after exposure to the chemical without metabolic activation; and
- tests for DNA damage have produced mixed results (mostly negative) among prokaryotes and fungi.

However, tests for DNA damage using mammalian hepatocytes were positive. One out of three non-standard tests for DNA damage and repair reported DNA damage following exposure of human fibroblasts to metabolically activated nitrosodiphenylamine.

In vivo studies

Negative results were mostly reported in the following in vivo studies:

- studies in mice and rats consistently showed negative results for DNA damage, micronuclei, DNA synthesis inhibition, and abnormal sperm morphology;
- a recessive lethal study involving *Drosophila melanogaster* was negative; and
- a positive response for DNA damage was observed in a host-mediated assay in *Escherichia coli* that were injected along with the chemical into the abdomen of male *Drosophila melanogaster*.

Carcinogenicity

A number of carcinogenicity studies have been conducted in rats and mice. The only neoplastic lesion that was significantly correlated with exposure to the chemical was an increase in bladder transitional cell carcinoma in rats in one study. The difference was only significant at the higher of the two doses tested. Increases in other neoplastic lesions, including cancers of the integumentary system and liver, were found in orally exposed rats and mice, but the increases were not statistically significant. Some early studies reported no treatment-related tumours in orally exposed rats. However, the bladder was not routinely examined in these studies. A non-significant increase in reticulum cell sarcomas was reported in mice subcutaneously injected with the chemical (ATSDR, 1993).

In a carcinogenicity study nitrosodiphenylamine was administered in the diet of F344 rats (50/sex) at concentrations of either 4000 or 1000 ppm (estimated to be a maximum of 240 and 320 mg/kg bw/day for males and females respectively) for 100 weeks. Matched control groups consisted of 20

untreated animals of each sex. Significant increases in the incidences of transitional cell carcinomas in the urinary bladder occurred in rats receiving the highest concentration (an increase of 36% in males and 82% in females) compared to the controls. In these animals, 'the entire spectrum, from transitional cell hyperplasia to papillary transitional cell carcinoma and transitional cell carcinoma, was observed in the urinary bladder' in both males and females (Cardy et al, 1979). The possible mechanism by which these tumours were induced, such as calculi formation in the bladder or nitrosation of amines present in feed to a carcinogenic nitrosamine, is unknown. A non-statistically significant and dose-related increase in fibromas of the integumentary system (i.e. the subcutis and the skin) occurred in males (NCI, 1979).

In a carcinogenicity study B6C3F$_1$ mice (50/sex/group) were fed nitrosodiphenylamine with the matched control groups consisting of 20 untreated mice of each sex. Males were fed diets containing the chemical at one of two concentrations, either 10000 or 20000 ppm (estimated to be 1500 and 3000 mg/kg bw/day respectively) for 101 weeks. Females were initially fed at one of two concentrations, either 5000 or 10000 ppm (estimated to be 750 and 1500 mg/kg bw/day respectively) for 38 weeks. However, because of an excessive depression in mean weight gain, dietary levels were reduced to 1000 and 4000 ppm (estimated to be 150 and 600 mg/kg bw/day), respectively and administration at the lowered levels were continued for 60 weeks (NCI, 1979). Consistent with the NCI rat study reported above, the primary organ affected by nitrosodiphenylamine was the urinary bladder. In mice the lesion of greatest significance was inflammation. For more information on non-neoplastic effects, see the **Repeat Dose** section. Transitional cell carcinomas of the bladder were reported in a low-dose male and female, as well as transitional cell papillomas in a low and high-dose male. However, there were no statistically significant increases in tumour incidence in the treated animals (ATSDR, 1993). Epithelial hyperplasia of urinary bladder mucosa occurred in low-dose males (2/49), high-dose males (7/46), low-dose females(3/47) and high-dose females (6/38). These lesions were not seen in the controls (IARC, 1982).

Twenty-five male Wistar rats were given 1070 micrograms of the chemical in 1 mL of 1% aqueous methylcellulose daily by gavage five days a week for 45 weeks (total dose, 240 mg/rat). All 25 rats survived to 53 weeks, when the experiment was terminated. Autopsies were conducted and various organs were examined histologically. No tumours were observed (IARC, 1982).

Nitrosodiphenylamine was administered to 20 BD rats of unspecified sex in drinking water at a daily dose of 120 mg/kg bw and a total dose of 65,000 mg/kg bw. Histopathological examinations consisting of gross evaluation of the liver, brain, and unspecified organs were conducted after 700 days, but there was no evidence of tumours in the treated animals. The study lacked important details (IARC, 1982).

Groups of 18 male and 18 female (C57BL/6XC3H/Anf)F$_1$ mice and (C57BL/6XAKR)F$_1$ mice received nitrosodiphenylamine according to the following schedule: 1000 mg/kg bw in dimethyl sulfoxide at seven days of age by stomach tube and the same absolute amount (without adjustment for increasing body weight) daily up to four weeks of age; subsequently, the mice were fed 3769 mg/kg in the diet until they reached 79 weeks of age. The dose was the maximum tolerated dose for infant and young mice. No statistically significant increases in the incidences of tumours were found (IARC, 1982).

Hairless hr/hr Oslo strain mice (16 male and 24 female) were treated with single applications of nitrosodiphenylamine (0.1 mL applications of a 1% solution in acetone, 33 mg/kg/week) on the intrascapular region each week for 20 weeks. Gross and histological examinations were performed on the lungs and palpable lesions of surviving animals (14 males and 24 females) following 80 weeks of observation. Lung adenomas were detected in three of the treated males. Histopathological examinations were limited in this study and control data were not available (ATSDR, 1993).

A group of 24 male CB stock rats, six to seven weeks of age, received intraperitoneal injections of the chemical (25 mg per animal) in polythethylene glycol 400 once a week for six months (total dose, 325 mg/rat). A group of 24 rats injected with only the vehicle served as controls. The experiment was terminated after two years. By 18–24 months, 10 controls and five treated rats were alive. Scattered neoplasms were found in treated rats: one with a hepatoma and one with a pituitary adenomas. One of the controls had a hepatoma (IARC, 1982).

The International Agency for Research on Cancer (IARC) has stated 'there is limited evidence for the carcinogenicity of N-nitrosodiphenylamine in experimental animals. No evaluation of the carcinogenicity of N-nitrosodiphenylamine to humans could be made.' (IARC, 1982).

Reproductive and Developmental Toxicity

No data are available regarding developmental toxicity.

No treatment-related effects of the testes, prostate, uterus, or ovaries were reported in a chronic study of rats and mice treated with the chemical in their food. No functional studies were performed (ATSDR, 1993).

Other Health Effects

Neurotoxicity

No treatment-related histological effects were reported in the brains of rats and mice chronically exposed to nitrosodiphenylamine in their food. No functional studies were performed that might provide data supporting the histological evidence (ATSDR, 1993).

Risk Characterisation

Critical Health Effects

The critical health effect for risk characterisation is carcinogenicity. The chemical can also cause harmful effects to the urinary bladder following high level repeated oral exposure.

Public Risk Characterisation

Given the uses identified for the chemical, it is unlikely that the public will be exposed. Hence, the public risk from this chemical is not considered to be unreasonable.

Occupational Risk Characterisation

Given the critical systemic long-term health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise exposure are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the Hazardous Substances Information System (HSIS) (Safe Work Australia) (refer to **Recommendation** section.

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Carcinogenicity	Carc. Cat 3 - Limited evidence of a carcinogenic effect (Xn; R40)	Suspected of causing cancer - Cat. 2 (H351)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

References

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