Benzene, nitro-: Human health tier II assessment

05 February 2016

CAS Number: 98-95-3

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.

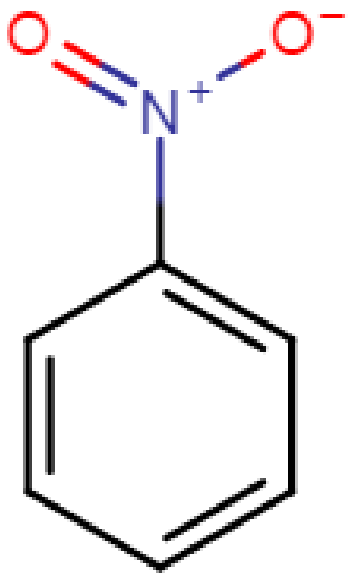
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	nitrobenzene nitrobenzol mononitrobenzene
Structural Formula	
Molecular Formula	C6H5NO2
Molecular Weight (g/mol)	123.11
Appearance and Odour (where available)	Colourless to yellow oily liquid, or greenish-yellow crystals below 5.5 degrees Celcius
SMILES	c1(N(=O)=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: the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and various international assessments (European Commission (EC), US Environmental Protection Agency (US EPA)).

The chemical has reported cosmetic use as a substitute for almond essence in the perfume industry (HSDB). However, the United Kingdom Health Protection Agency (UK HPA) stated that this use has long been discontinued due to its toxicity (UK HPA, 2014). The use of nitrobenzene in cosmetic products has been prohibited since the 1980s (EC, 2007).

The European Commission stated that there is no information of the use of consumer products containing the chemical, and no product formulations are listed. The use is almost exclusively in manufacturing aniline (EC, 2007).

The chemical has reported domestic and/or commercial uses, including:

- in reprographic agents; and
- as a masking agent in shoe and floor polishes, leather dressings, paint solvents and other materials.

The chemical has reported site-limited uses, including:

- as a precursor to aniline (primary use);
- as a solvent for cellulose ethers and acetates, in petroleum refining; and
- for manufacturing benzidine, quinoline, azobenzene and various other compounds.

The chemical has reported non-industrial uses, including:

- in the production of analgesic paracetamol; and
- as an insecticide.

Restrictions

Australian

This chemical is listed in Schedule 6 of the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP, 2015).

Schedule 6:

'NITROBENZENE **except**:

a) in solid or semi-solid polishes;

b) in soaps containing 1 per cent or less of nitrobenzene; or

c) in other preparations containing 0.1 per cent or less of nitrobenzene.'

Schedule 6 chemicals are described as 'Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label'. Schedule 6 chemicals are labelled with 'Poison' (SUSMP, 2015).

International

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

- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain; and
- Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient ‘Hotlist’).

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

- T; R23/24/25 (acute toxicity)
- T; R48/23/24 (repeat dose toxicity)
- R40 Carc. Cat 3 (carcinogenicity)
- R62 Repr. Cat 3 (reproductive toxicity)

Exposure Standards

Australian

The chemical has an exposure standard of 5 mg/m^3 (1 ppm) time weighted average (TWA).

International

The following exposure standards are identified (Galleria Chemica).

TWA:

- 4–5 mg/m^3 (0.8–1 ppm) in Canada (Alberta, British Columbia, Quebec, Saskatchewan and Yukon), Chile, Denmark, Greece, Spain, the United States of America (USA) (California, Hawaii, Minnesota, Tennessee, Vermont and Washington);
- 1–2 mg/m^3 (0.2 ppm) in China, Estonia, France, Germany, Spain, Ireland, Poland, and the United Kingdom.

Health Hazard Information

Toxicokinetics

The chemical is readily absorbed via the gastrointestinal (GI) tract, the skin and the respiratory tract. The main target tissues are the erythrocytes, spleen, liver, testes and brain. Absorption through the GI tract is estimated to be 62–69 % in rats and 43 % in mice. In both animals and humans, an administered dose of the chemical is eliminated mainly in the urine within 48 hours, and to a lesser extent in the faeces (EC, 2007; HSDB).

There are three major metabolic pathways in animals following oral administration of the chemical: reduction to aniline by intestinal microflora, reduction by hepatic microsomes and in erythrocytes, and oxidative metabolism by hepatic microsomes to form nitrophenols and aminophenols. In animals, the identified metabolites are *p*-, *m*-, and *o*-nitrophenol, *p*-hydroxyacetanilide, and *p*-aminophenol and its conjugates. The identified metabolites differ between species and strains, particularly the degree of conjugation (EC, 2007; HSDB).

In humans following oral exposure, the urinary metabolites *p*-nitrophenol and *p*-aminophenol were identified. Following a six hour inhalation exposure to the vapour of the chemical (5–30 mg/m^3) in human volunteers, pulmonary absorption was found to be around 73–87 % and dermal absorption was proportional to the concentration of the chemical in air. The urinary metabolite identified was *p*-nitrophenol. Urinary excretion of the metabolites is slow, and the elimination half-life was reported as approximately 60 hours (EC, 2007; HSDB).

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Toxic if swallowed’ (T; R25) in the HSIS (Safe Work Australia). Although the available animal data mostly suggest a lower classification, human case reports have indicated severe effects following acute oral exposure (see **Observation in Humans**). Therefore, the current classification is supported.

The median lethal dose (LD50) is 588–732 mg/kg bw in rats and 590 mg/kg bw in mice (HSDB). One rabbit died following an acute oral dose of 200 mg/kg bw and large deposits of fat in the tissues and gastrointestinal tract were observed (HSDB). The LD50 for rabbits was not reported.

Reported signs of toxicity in rats include neurotoxicity (perturbance of equilibrium, hunched posture, lateral position, piloerection, sedation and poor reflexes), cyanosis and paralysis of hind legs, bloody or closed eyes, liver and kidney effects (fatty degeneration, cerebellar lesions, hepatocellular effects), and testicular lesions restricted to the seminiferous tubules (complete destruction of spermatocytes). Hepatocellular nuclear enlargement was observed consistently at 110 mg/kg bw. Necropsy revealed blood discolouration in animals that died during exposure and hyperaemia of the parenchymatous organs. Methaemoglobin (metHb) was observed to form at 640 mg/kg bw and the intensive formation of Heinz bodies in erythrocytes was also reported (EC, 2007).

In cats, cyanosis was observed following oral administration of the chemical at 30 mg/kg bw, concurrent with the highest metHb levels. An oral administration of 120 mg/kg bw in cats caused cyanosis, apathy and mydriasis, but no mortalities occurred (EC, 2007).

Dermal

The chemical is classified as hazardous with the risk phrase ‘Toxic in contact with skin’ (T; R24) in the HSIS (Safe Work Australia). The dermal LD50 of <300 mg/kg bw in rabbits supports this classification.

Dermal LD50s of >2000 in rats and 560–760 mg/kg bw in rabbits were reported. In a Draize test in six rabbits (24 hours occlusive exposure), a dermal LD50 of <300 mg/kg bw was determined (EC, 2007).

Reported signs of toxicity in rabbits include lethargy, persistant discolouration of the skin and eyes, collapse and loss of coordination. Methaemoglobinaemia was observed in rabbits in less than 20 minutes post-application (REACH). In rats, the

toxicity symptoms reported were loss of weight, cyanosis, hyperaemia of the parenchymatous organs, elevated metHb levels (after 30 min following exposure), and intensive formation of Heinz bodies in erythrocytes (after 24 hours following exposure) (EC, 2007).

Inhalation

The chemical is classified as hazardous with the risk phrase 'Toxic by inhalation' (T; R23) in the HSIS (Safe Work Australia). Although the available animal data suggest a lower classification, human case reports have indicated severe effects following acute inhalation exposure (see **Observation in Humans**). Therefore, the current classification is supported.

The four hour median lethal concentration (LC50) is 2.8 mg/L in rats. Reported signs of toxicity include cyanosis, prostration, corneal clouding, lacrimation, tremors, laboured breathing, hyperactive or aggressive behaviour, foaming mouth and nasal discharge (EC, 2007).

Observation in humans

Many reports of nitrobenzene poisoning in humans are available. The characteristic acute toxicity symptoms of nitrobenzene exposure in humans are cyanosis, coupled with methaemoglobinaemia. Other reported symptoms are the formation of Heinz bodies in erythrocytes, effects on the bone marrow and lymphoid organs, neurotoxicity and hepatotoxicity. No causal relationship could be established between nitrobenzene exposure levels and severity of the effects, due to lack of knowledge of exposure levels or absorbed concentrations. However, babies and children were found to be particularly sensitive to the toxicity effects (EC, 2007).

In two attempted suicide case studies, two women (24 years and 19 years) who consumed nitrobenzene (12 mL and 50 mL, respectively) suffered from severe cyanosis and methaemoglobinaemia, unconsciousness, breathing difficulties and severe headache and dizziness. Cyanosis occurred after one hour and persisted for 10 days. There was also a smell of bitter almonds in the expired air. They were subjected to intensive treatments and recovered within four weeks (EC, 2007; US EPA, 2009).

Nine cases of nitrobenzene poisoning among people in Venezuela due to ingestion of bitter almond oil (containing nitrobenzene) were reported between April to July 1993. The effects observed included cyanosis (oral, distal, or general), vomiting and dizziness, respiratory depression, convulsions and general weakness. The patients also suffered from anaemia, haemolysis and elevated metHb levels (US EPA, 2009).

Case reports for inhalation exposure often include combined exposure via the dermal route. All-day occupational exposure at a threshold value of 1 ppm results in approximately 25 mg of nitrobenzene being absorbed (one-third through skin) (EC, 2007).

Several case reports in humans following dermal exposure have reported marked cyanosis and methaemoglobinaemia, rapid pulse rates, depressed respiration rates, hypoxia, neurotoxicity (nausea coma, weakness), skin rashes and bluish colouration of the skin and lips accompanied with chocolate-coloured venous blood samples. Many of these cases involved infants or children dermally exposed to the chemical through the use in disinfectants or in a laundry mark stamped on cotton mattress pads, shoe dyes, topical hair oil and almond oil. The patients usually recovered after intensive treatment (US EPA, 2009).

Corrosion / Irritation

Respiratory Irritation

The chemical may be a respiratory irritant, but available data are insufficient to classify the chemical.

Inhalation studies in animals have shown effects in the respiratory system including incidences of focal inflammation in the nasal region and severe irritation of mucous membranes following exposure to saturated vapours of the chemical (EC, 2007; US EPA, 2009).

Skin Irritation

The chemical is considered a slight skin irritant.

In a skin irritation study, the chemical (0.5 mL) was applied (occlusively) to the inner ear of New Zealand White rabbits (n = 2) for 24 hours, followed by observation for up to seven days. No signs of skin irritation were observed (REACH).

In another skin irritation study (conducted according to the US Food and Drug Administration (FDA) regulations), the chemical (0.5 mL, undiluted) was applied to the skin of Himalayan rabbits (n = 6) (occlusively) for 24 hours, with observation for up to 48 hours. Three of the animals died within two days, showing signs of cyanosis. Slight skin irritation was observed (details not reported). However, no mortalities occured in a similar study using 10 % of the chemical in sesame oil. An irritation index of 1.2, indicating mild skin irritation was reported (EC, 2007).

Eye Irritation

The chemical is a slight eye irritant.

Several poorly documented eye irritation studies are available. In one study (Draize test), the chemical (0.1 mL) was instilled in the conjunctival sac of rabbits (n = 6). Conjunctival irritation was observed one hour following instillation with an irritation index of 2 (according to FDA regulations). The substance was assessed as 'causes no conjunctival irritation'. In another Draize test, the chemical (0.05 mL) caused slight conjunctival irritation at one and 24 hours following instillation in two rabbits, but this effect resolved within 48 hours. No corneal lesions were observed (WHO, 2004; EC, 2007).

Sensitisation

Skin Sensitisation

Based on the available data, the chemical is not a skin sensitiser.

A modified local lymph node assay (LLNA) was conducted according the the OECD test guideline (TG) 429, using the chemical at concentrations of 0, 2, 10 and 50 % in acetone/olive oil. The test solutions (25 µL) were applied on the dorsal surface of the ears of NMR1 mice (n = 6/dose) on three consecutive days. None of the parameters (ear swelling, weights of the draining lymph nodes, and cell counts) of the treated animals reached or exceeded the 'positive levels', compared with controls. The cell count indices (stimulation index (SI)) for the concentrations tested were determined to be 0.96, 0.82 and 0.86, respectively. The chemical is not a skin sensitiser up to 50 % concentration (REACH).

In an ear-flank test, the chemical at 10 % concentration in dimethyl formamide was applied (0.1 mL per ear) to the ears of guinea pigs (n = 6) for three consecutive days. This was followed with a challenge exposure of 0.2 mL at a range of concentrations (not stated) of the chemical on the flank one week later. The exposed area was evaluated 24 hours following challenge exposure. No responses indicative of skin sensitisation were observed (REACH).

Repeated Dose Toxicity

Oral

Based on the available data, the chemical is considered to cause severe effects following repeated oral exposure, warranting hazard classification

The main adverse effects observed in rats and mice were in the liver, kidney, spleen and male reproductive organs, with haematotoxicity identified as one of the primary systemic effects. Neurological impairment was evident at high doses. Rats are more sensitive to the chemical than mice, with spleen and liver effects observed from around 5 mg/kg bw/day, even in a 28-day study.

In a repeat dose oral toxicity study, Fischer 344 (F344) rats were administered the chemical (gavage) at doses of 0, 5, 25 or 125 mg/kg bw/day for 28 days, with a recovery period of 14 days. One female in the high dose group died on day 27. At the highest dose, observed effects included decreased movement, pale skin, gait abnormalities, decreased body weight gains, liver and kidney effects (brown pigmentation of the liver Kupffer cells and renal tubular epithelium), and brain lesions (moderate to severe spongiotic changes and brown pigmentation in the perivascular region of the cerebellum). At ≥25 mg/kg bw/day, increased absolute weights of the liver, spleen and kidney, and decreased weights of testes, thymus and adrenals were observed. Decreased absolute testes weights correlated with severe degeneration of seminiferous tubular epithelium and seminiferous tubule atrophy in all males at 125 mg/kg bw/day. Significant changes in haematological parameters (decreased red blood cell (RBC), haemoglobin (Hb) and haematocrit (Hct) levels, and increased mean corpuscular volume (MCV)), blood biochemistry parameters (increased total cholesterol and albumin, and decreased blood urea nitrogen (BUN)) were observed at ≥25 mg/kg bw/day, indicating haemolytic anaemia. Increased liver weights in males were observed at the lowest dose (8% increase). Most of these effects decreased in severity or incidence towards the end of the recovery period. Spleen effects were severe, with congestion, increased pigmentation and increased extramedullary haematopoiesis observed in all treated groups. Increased haematopoiesis of the bone marrow occurred in all treated females and in high dose males. The lowest observed adverse effect level (LOAEL) was established as 5 mg/kg bw/day, based on increased liver weights, extramedullary haematopoiesis and spleen effects (EC, 2007; US EPA, 2009; REACH).

In a 90-day repeat dose oral toxicity study in rats conducted by the National Toxicology Program (NTP), the rats were administered the chemical at doses from 9.4–300 mg/kg bw/day. Effects similar to the 28-day study were reported. At 150 mg/kg bw/day, mortalities occurred (on day 67 in males and on day 38 in females), and neurotoxicity and enlarged spleens were observed. Histopathological lesions were observed in the brain, liver, lung, kidney and spleen (doses not specified). Dose-dependent increases in liver and kidney weights, and splenic congestion of increasing severity were reported at all dose levels. Decreased testes weights at 19–75 mg/kg bw/day and testicular atrophy at ≥75 mg/kg bw/day were observed. Haematological changes were statistically significant at ≥9.4 mg/kg bw/day. The LOAEL was determined to be 9.4 mg/kg bw/day (US EPA, 2009).

Repeat dose oral toxicity studies (14 and 90 days) in mice have shown adverse effects similar to rats, but at higher doses. The observed effects included neurotoxicity (ataxia, lethargy, hyperactivity, dyspnoea, rapid head-bobbing movements at 300 mg/kg bw/day), liver and kidney effects (increased absolute liver weights at ≥19 mg/kg bw/day in females and ≥75 mg/kg bw/day in males, increased relative kidney weights at ≥75 mg/kg bw/day in males, liver haemosiderosis and hepatocytic degeneration at 300 mg/kg bw/day), elevated absolute and relative thymus weights in females (all doses from 19 mg/kg bw/day), spleen effects (haemosiderosis, pigmentation, extramedullary haematopoiesis and congestion at ≥100 mg/kg bw/day), and anaemia at 300 mg/kg bw/day. At 300 mg/kg bw/day, decreased testes weights in males was reported, and one male had acute necrosis in the area of the vestibular nucleus in the brain. A statistically significant increase in metHb concentrations was observed from 19 mg/kg bw/day. The LOAEL in the 90-day mouse study was determined as 19 mg/kg bw/day (EC, 2007; US EPA, 2009).

Reproductive and developmental toxicity studies in rats have shown toxicity effects similar to those observed in repeat dose studies. Haematotoxicity, including anaemia due to metHb formation was evident in males from 20 mg/kg bw/day. Effects on organ weights included increased liver and spleen weights, and decreased testes and epididymidis weights at ≥60 mg/kg bw/day. Treatment-related effects were also seen in the liver, kidney, spleen, bone marrow and brain (EC, 2007).

Dermal

The chemical is classified as hazardous with the risk phrase ‘Toxic: danger of serious damage to health by prolonged exposure in contact with skin' (R48/24) in HSIS (Safe Work Australia). Reported LOAELs for rats and mice is 50 mg/kg bw/day, the lowest tested dose. However, lower doses were not tested and, therefore, the validity of the existing hazard classification cannot be confirmed.

In a range-finding study (conducted by the NTP), rats and mice were exposed to the chemical by skin painting at a dose range of 200–3200 mg/kg bw/day for 14 days. All rats and mice died or were moribund at ≥1600 mg/kg bw/day. Treated animals suffered from neurological symptoms and breathing difficulties. Mice were overall less affected than rats, in terms of histological changes in target organs (brain, liver, spleen and testes), but significantly lower weight gains were observed in all treated mice (EC, 2007).

In a 90-day repeated dose dermal study (conducted by the NTP), rats and mice (n = 10/sex/group) were exposed by skin painting with the chemical (in acetone) at 0, 50, 100, 200, 400 or 800 mg/kg bw/day. At 800 mg/kg bw/day, all rats and 17 mice (nine males and eight females) died before the end of exposure. Mice and male rats at this dose displayed acute toxicity

symptoms including inactivity, ataxia, lethargy, dyspnoea, prostration, and insensitivity to pain. Among these symptoms, only dyspnoea was observed in female rats. Brain lesions (vacuolisation of the brain or brain stem) were observed in rats at ≥100 mg/kg bw/day, in female mice at ≥400 mg/kg bw/day, and in male mice at 800 mg/kg bw/day. Brain vascular lesions were observed in rats but not in mice. For mice, localised skin inflammation of minimal to mild severity was observed at ≥400 mg/kg bw/day: inflammatory cells in the dermis, acanthosis and hyperkeratosis, crusting and necrosis extending deep into the epidermis. Additionally, liver effects (significantly increased liver weights at ≥400 mg/kg bw/day, and centrilobular liver cell heterogenecity at all doses), and thymus atrophy at 800 mg/kg bw/day were reported. Other effects observed at ≥50 mg/kg bw/day in both rats and mice were lung and spleen congestion, and fatty changes in the adrenal cortex. Both male rats and mice displayed testicular effects (atrophy, hypospermatogenesis) at ≥400 mg/kg bw/day. The LOAEL for both rats and mice was reported to be 50 mg/kg bw/day (EC, 2007; US EPA, 2009).

Inhalation

The chemical is classified as hazardous with the risk phrase ‘Toxic: danger of serious damage to health by prolonged exposure through inhalation' (R48/23) in HSIS (Safe Work Australia). The available data support this classification.

Ninety-day inhalation studies (similar to OECD TG 413) were conducted in two strains of rats (F344 and CD) and B6C3F1 mice (n = 10/sex/group), by exposing the animals to the vapour of the chemical at 0, 5, 16 or 50 ppm (0, 25, 80 or 250 mg/m^3). In rats, the liver, kidney, epididymis, spleen, bone marrow, testes and nasal turbinates were the primary target organs. Testicular toxicity consisted of marked bilateral atrophy (at ≥25 mg/m^3 in CD rats), and degeneration of tubular epithelial cells (at ≥25 mg/m^3 in F344 rats). Splenic toxicity consisted of acute sinusoidal congestion, proliferative capsular lesions and extramedullary haematopoiesis at 250 mg/m^3 in F344 rats, and increased weights at ≥80 mg/m^3 in males of both strains. Liver effects included hepatic cord disorganisation and centrilobular degeneration of hepatocytes at 250 mg/m^3. Dose-dependent increases in haemolytic anaemia (indicated by increased concentrations of serum metHb and bilirubin) were observed in all treated groups. Nasal lesions were evident in CD rats, and included lymphoid hyperplasia, inflammation and pneumonitis. Kidney effects were characterised by dose-dependent toxic nephrosis which increased in severity in both strains (EC, 2007; US EPA, 2009). In addition, nephrosis consisting of intratubular eosinophilic (hyaline) droplets was observed in F344 rats (both sexes) following 14 days' exposure and in the 90-day study at ≥5 mg/m^3, indicative of degenerative effects in renal tubular cells. This was considered a toxic effect proceeding to tumour growth (EC, 2007). In the absense of more comprehensive information and due to the appearance of these lesions in both sexes, the droplets cannot be assigned to $a_{2\mu}$-globulin-associated nephropathy (US EPA, 2009).

In mice, increased spleen and liver weights, enlarged spleens, increased metHb and elevated mean activity of alanine transaminase (ALT) were observed at ≥250 mg/m^3. Liver effects observed in males included centrilobular hyperplasia, basophilic cytoplasm, and enlarged hyperchromatic nuclei. In females, less severe centrilobular hyperplasia and hypertrophy resulting in some normal cord disorganisation was observed. Both sexes had mild bronchial epithelial hyperplasia and generalised bone marrow hyperplasia at the highest dose. No signs of anaemia were present. Females at 25 mg/mg^3 had cellular vacuolisation of the zona reticularis of the adrenal, which increased in severity at higher doses. The lowest observed adverse effect concentration (LOAEC) for systemic effects was 5 ppm (25 mg/m^3), and the no observed adverse effect concentration (NOAEC) for localised respiratory effects was 16 ppm (80 mg/m^3) (EC, 2007; US EPA, 2009).

In a two-year chronic inhalation study, F344 and CD rats were exposed to the chemical at 0, 1, 5, or 25 ppm, and B6C3F1 mice at 0, 5, 25, or 50 ppm. The study was terminated at 15 months, for some rats, for interim evaluations. At the highest doses, the mice and F344 rats showed statistically significant reductions in RBCs, Hct and Hb concentrations. Statistically significant increases in metHb levels were observed in all treated CD rat groups after 15 months exposure. Other non-neoplastic lesions observed in mice following two year exposure were significantly increased incidences of lung hyperplasia and bronchiolisation, increased thyroid follicular cell hyperplasia, increased liver centrilobular hepatocytomegaly and multinucleated hepatocytes. Effects observed in rats at the end of exposure were in the liver (increased eosinophilic foci and centrilobular hepatocytomegaly), thyroid (follicular cell hyperplasia), kidneys (tubular hyperplasia and progressive nephropathy), spleen (extramedullary haematopoiesis, congestion, stromal hyperplasia), nasal turbinates (inflammatory exudate and epithelial hyperplasia) and testes. Most of these effects were observed from the lowest dose, and increased in severity with dose (US EPA, 2009).

Genotoxicity

The available data indicate mixed results for genotoxicity, and are not sufficient to derive a conclusion on the genotoxic potential of the chemical. The US EPA report (2009) stated that, 'Nitrobenzene appears to be at most weakly genotoxic'.

Negative results were reported in the in vitro assays listed below (EC, 2007; US EPA, 2009):

- bacterial reverse mutation assays with several strains of *Salmonella typhimurium* at concentrations up to 3000 µg, with or without metabolic activation;
- a chromosomal aberration test in Chinese hamster lung (CHL) cells up to 0.5 mg/mL, without metabolic activation; and
- an unscheduled DNA synthesis (UDS) assay using primary human hepatocytes at concentrations 0.01–1.0 mmol (1.23–123 µg/mL).

Most of the in vitro studies in mammalian cells were not conducted according to the OECD test guidelines. Positive findings for chromosomal aberrations were reported in cultured human lymphocytes. The chemical caused a statistically significant increase in DNA damage in rat primary kidney cells (0.125–0.5 mM) and human kidney cells (obtained from patients with kidney cancer) (0.062–0.25 mM) following a 20 hour incubation. However, there were some methodical errors which made the results ambiguous. Overall, the studies were either inconclusive, methodically inadequate or contained insufficient data (EC, 2007).

Negative results were reported for the following in vivo studies (EC, 2007; US EPA, 2009):

- the chemical did not induce sister chromatid exchanges (SCE) or chromosomal aberrations in lymphocytes of isolated spleen or peripheral blood from rats exposed to the chemical up to 260 mg/m^3 per day for 29 days, via inhalation; and
- the chemical did not increase micronucleated polychromatic erythrocytes in a bone marrow micronucleus test in mice administered the chemical intraperitoneally (i.p.) up to 250 mg/kg bw (OECD TG 474); and
- in a DNA repair test, no increase in UDS was seen in the rat liver following administration of the chemical by gavage at 200 or 500 mg/kg bw.

In a non-guideline study, a statistically significant increase in DNA damage (comet assay) and broken or detached chromosomes (micronucleus assay) were observed in male Sprague-Dawley (SD) rats when administered (gavage) a single dose of the chemical at 300 mg/kg bw (US EPA, 2009). The study lacked detailed descriptions and was considered by the country rapporteur as being methodologically inadequate (EC, 2007).

Carcinogenicity

The chemical is classified as hazardous—Category 3 carcinogenic substance—with the risk phrase ‘Limited evidence of carcinogenic effect’ (Xn; R40) in the HSIS (Safe Work Australia). The available data support this classification.

The International Agency for Research on Cancer (IARC) has classified the chemical as ‘*possibly carcinogenic to humans*’ (Group 2B), based on inadequate evidence for carcinogenicity in humans but sufficient evidence in experimental animals (IARC, 1996).

Combined chronic/carcinogenicity inhalation studies in B6C3F1 mice and two strains of rats (F344 and CD) are available. The animals were exposed to air containing specific concentrations of the chemical in inhalation chambers for 24 months. The primary target organs for tumours were the liver and kidneys.

In a carcinogenicity study (OECD TG 453), B6C3F1 mice (n = 70/sex/dose) were exposed (whole body) to the chemical at concentrations of 0, 5, 25 or 50 ppm (0, 25, 125 or 250 mg/m^3), six hours/day, five days/week for two years. In males, significantly increased incidences of alveolar-bronchiolar neoplasms (adenomas and carcinomas) at ≥5 ppm, and thyroid follicular-cell adenomas at 25 ppm were observed. In females, significantly increased incidences of mammary gland adenocarcinomas and marginally increased hepatocellular adenomas at 50 ppm were observed (IARC, 1996; REACH).

In another carcinogenicity study, F344 rats (n = 70/sex/dose) were exposed (whole body) to the chemical at concentrations of 0, 1, 5 or 25 ppm (0, 5, 25 or 125 mg/m^3) for two years. In males, significantly increased incidences of hepatocellular neoplasms (adenomas and carcinomas) and renal tubular cell adenomas at 25 ppm, and marginally increased incidences of thyroid follicular neoplasms (adenoma or adenocarcinomas) were observed. In females, increased (but not statistically significantly) hepatocellular neoplasms (adenomas or carcinomas) and endometrial stromal polyps were observed at 25 ppm (IARC, 1996; REACH).

Groups of male CD rats (n = 70/sex/dose) were exposed to the chemical at the same concentrations as F344 rats. Increased incidence of hepatocellular adenomas (similarly to F344 rats) were observed in all treated groups. However, the incidence of hepatocellular carcinomas was not different from controls (EC, 2007; REACH).

Reproductive and Developmental Toxicity

The chemical is classified as hazardous—Category 3 substance toxic to reproduction—with the risk phrase 'Possible risk of impaired fertility' (Xn; R62) in the HSIS (Safe Work Australia). The available data support this classification.

Many reproductive studies are available in rats, with a large number designed specifically to investigate adverse effects of the chemical on the male reproductive system. Persistent effects on male reproductive organs and spermatogenesis lead to reduced fertility. Haematotoxicity is also present in reproductive toxicity studies but this is discussed in the repeat dose toxicity section (see **Repeat Dose Toxicity**).

In a two-generation reproductive and developmental toxicity study, groups of SD rats (n = 30/sex/dose) were exposed (whole body) to the vapour of the chemical at concentrations of 0, 1, 10 or 40 ppm (5.1, 51.2 or 204.8 mg/m^3), six hours/day, five days/week. All animals were exposed during the premating period for 10 weeks, followed by a mating period of two weeks, and F0 females were further continuously exposed through to gestation day (GD) 19, and from postnatal day (PND) 5 to 21 (dams only). The duration of exposure for the F1 generation was identical to F0, with the addition of a nine-week recovery period for high-dose F1 males after the mating period. No treatment-related clinical signs or mortality were observed for F0, F1 and the recovery group. The gestational parameters (implantations, resorptions and postimplantation losses) were not affected in matings that resulted in live offspring. Significant reductions in testes and epididymis weights were observed in F0 males at 40 ppm after 12 weeks exposure, and in the F1 recovery group. The epididymis of high-dose F0 and F1 males showed degenerative spermatocytes and decreased numbers of spermatids. Marked or severe seminiferous tubule atrophy in the high-dose F0 males (14/30) and F1 males (21/30) were also observed. No histopathological changes in the reproductive organs of female rats were observed at this concentration. A NOAEC of 10 ppm for reproductive toxicity was determined based on reduced fertility and male reproductive organ toxicity at 40 ppm (EC, 2007; US EPA, 2009).

In a reproductive toxicity study (OECD TG 422), SD rats (n = 10/sex/dose) were orally administered the chemical in sesame oil at doses of 0, 20, 60 or 100 mg/kg bw/day, during the premating period for 14 days, during mating, gestation, and until day three of lactation. Neurotoxicity in parental animals was evident at the highest dose (see **Neurotoxicity**). No statistically significant differences in the copulation, fertility and implantation indices compared with controls were observed at all doses. However, the mortality rate was high in dams at 100 mg/kg bw/day. No abnormalities in the gestation period and delivery conditions in the remaining females were observed. Effects on the testes included seminiferous tubule atrophy, Leydig cell hyperplasia and loss of intraluminal sperm in the epididymis at ≥20 mg/kg bw/day. In the offspring, statistically significant decreases in pup body weights were observed at day 0 for both males and females treated at ≥60 mg/kg bw/day, and on day 4 in males at ≥20 mg/kg. The survival rate of the pups was significantly decreased in the high dose groups. None of the pups showed external or visceral malformations. A LOAEL of 20 mg/kg bw/day for reproductive toxicity (fertility) was determined, based on seminiferous tubule atrophy (US EPA, 2009; REACH).

A follow-up reproductive study on male rat fertility was conducted to determine the affected spermatogenic endpoints. Male SD rats were exposed via gavage to 60 mg/kg bw/day, and mated with non-treated females at specific timepoints up to 70 days. Significant and pronounced decreases in testicular and epididymal weights, sperm count, and sperm motility were observed on day 14 and onwards. Significant decreases in sperm viability and increased abnormal sperm rates were observed from day 21. Sperm counts were reduced to less than 10 %, compared with the control values on day 21. Histopathology of the testes revealed elongated spermatids and multi-nucleated giant cells on day 14. All females in the control groups were fertilised. Significant decreases in fertility index was observed in the treated groups on day 21, and there were no pregnant animals in the groups treated for 28 days or longer (EC, 2007).

Developmental toxicity

Exposure through inhalation in rats and in rabbits did not show any evidence of developmental or teratogenic effects. Any observed effects were likely to be secondary to maternal toxicity.

In a developmental toxicity study (OECD TG 414), groups of pregnant SD rats (n = 26/sex/dose) were exposed (whole body) via inhalation to the vapour of the chemical at 0, 1, 10 or 40 ppm (0, 5.1, 51.2 or 204.8 mg/m^3), six hours/day on GD 6–15. The effects observed in parental animals included transiently reduced maternal weight gain at 40 ppm and increased absolute and relative spleen weights at =10 ppm. No maternal deaths, early deliveries or abortions were observed. Gestational parameters, such as the number of resorptions, live or dead foetuses per litter, pre- or post-implantation loss (as percentage), sex ratio or foetal body weights per litter did not differ from controls. There were no significant increases in the number of litters or foetal malformations. The incidence of skeletal variations did not indicate foetal toxicity. The NOAEC of ≥40 ppm (≥204.8 mg/m^3) was determined for developmental toxicity (EC, 2007; REACH).

In another developmental toxicity study, pregnant New Zealand White rabbits were exposed (whole body) to the vapour of the chemical at 0, 10, 40 or 100 ppm (0, 67, 302 or 660 mg/m^3) on GD 7–19. No adverse effects were observed on pregnancy rate, early deliveries or abortions. At the highest dose, slightly elevated resorption parameters were observed but were not statistically significant. Observed maternal effects included increased mean liver weights and metHb levels at ≥40 ppm. For the offspring, no adverse effects were apparent for foetal weight, crown-rump distance and sex ratio, and there were no increased incidences of malformations or variations. A NOAEC of 40 ppm for developmental toxicity was derived, based on increased resorptions. The NOAEC for maternal toxicity was 10 ppm (EC, 2007; REACH).

Other Health Effects

Neurotoxicity

Neurological symptoms were evident in rats when exposed to the chemical at high doses or concentrations, irrespective of the route of administration (oral, dermal and inhalation). Neurotoxicity in humans following exposure to the chemical has been reported with symptoms such as severe headache, nausea, dizziness, generalised weakness and convulsions. Long term exposure (>3 months) may cause degenerative changes in the peripheral nerves and multiple neuritis (US EPA, 2009).

Acute and repeated dose toxicity studies in animals have reported neurological symptoms including piloerection, sedation, hyperactivity, ataxia at ≥150 mg/kg bw/day (see **Acute** and **Repeat Dose Toxicity**). Reproductive studies in rats have shown piloerection, salivation and emaciation at oral doses of ≥60 mg/kg bw/day (see **Reproductive & Developmental Toxicity**).

Several neurotoxicity studies in animals with limited documentation are available. Four Wistar rats were exposed to a constant flow of the chemical vapour at a concentration of $5.4x10^{-11}$ mol/L ($6.6x10^{-6}$ mg/L) for five or 10 weeks. Degeneration of the mitral cell layer (representing principal relay neurones) in the olfactory bulb was observed, with the most severe degeneration in the ventral region. Male F344 rats administered the chemical at a single oral dose of 550 mg/kg bw were euthanised at six, 24 and 48 hours later. Effects including petechial haemorrhages in the brain stem and cerebellum, and bilaterally symmetric degeneration in the cerebellum, and cerebellar peduncles developed within 48 hours following administration (REACH).

In another neurotoxicity study, rabbits were injected with the chemical via the ear vein or by topical application on the back (doses not stated). Acutely, the neurotoxic effect of convulsion was reported, and chronic intoxication resulted in paralysis of the limbs, and elevated sensitivity (details not available). A large number of well-defined round vacuoles were found in the medulla, and these became more pronounced in the high dose group and in the animals treated dermally for a longer term (duration not stated) (US EPA, 2009).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include:

- systemic long-term effects (carcinogenicity and reproductive toxicity);
- systemic acute effects (acute toxicity from oral, dermal and inhalation exposure); and
- systemic effects including neurotoxicity following repeated exposure through oral, dermal and inhalation exposure.

Public Risk Characterisation

The international uses indicate that the chemical is used as a masking agent in shoe and floor polishes. The chemical is listed on Schedule 6 of the SUSMP for preparations containing more than 0.1 % of the chemical. A number of warning statements, first aid instructions and safety directions apply for any preparations containing the chemical, except for solid and semi-solid polishes and, soaps containing ≤1 % of the chemical. Indications are that consumer uses of the chemical have been decreasing over time.

The current controls are considered adequate to minimise the risk to public health posed by any products containing the chemical. Therefore, the chemical is not considered to pose an unreasonable risk to public health.

Occupational Risk Characterisation

During product formulation, oral, dermal and inhalation exposure may occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic long-term and systemic acute health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal and inhalation 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 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

Public Health

Products containing the chemical should be labelled in accordance with state and territory legislation (SUSMP, 2015).

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]
Acute Toxicity	Toxic if swallowed (T; R25)* Toxic in contact with skin (T; R24)* Toxic by inhalation (T; R23)*	Toxic if swallowed - Cat. 3 (H301) Toxic in contact with skin - Cat. 3 (H311) Toxic if inhaled - Cat. 3 (H331)
Repeat Dose Toxicity	Toxic: Danger of serious damage to health by prolonged exposure in contact with skin (T; R48/24)* Toxic: danger of serious damage to health by prolonged exposure through inhalation (T; R48/23)* Toxic: Danger of serious damage to health by prolonged exposure if swallowed (T; R48/25)	Causes damage to organs through prolonged or repeated exposure through the dermal route - Cat. 1 (H372) Causes damage to organs through prolonged or repeated exposure through inhalation - Cat. 1 (H372) Causes damage to organs through prolonged or repeated exposure if swallowed - Cat. 1 (H372)
Carcinogenicity	Carc. Cat 3 - Limited evidence of a carcinogenic effect (Xn; R40)*	Suspected of causing cancer - Cat. 2 (H351)
Reproductive and Developmental Toxicity	Repro. Cat 3 - Possible risk of impaired fertility (Xn; R62)*	Suspected of damaging fertility - Cat. 2 (H361f)

[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 consumers

Products containing the chemical should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal and inhalation 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;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- 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;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- 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.

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Last update 05 February 2016