Benzene, 1-chloro-4-nitro-: Human health tier II assessment

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CAS Number: 100-00-5

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.

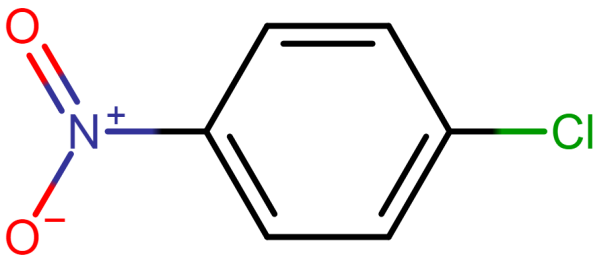
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	1-chloro-4-nitrobenzene p-chloronitrobenzene p-nitrochlorobenzene
Structural Formula	
Molecular Formula	C6H4ClNO2
Molecular Weight (g/mol)	157.56
Appearance and Odour (where available)	Light yellow solid.
SMILES	c1(Cl)ccc(N(=O)=O)cc1

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; the Organisation for Economic Co-operation and Development (OECD) Screening information data set International Assessment Report (SIAR) (OECD, 2005); Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the OECD High Production Volume chemical program (OECD HPV); the US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR); the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and other international sources (Nair et al., 1986; Rickert & Held, 1989; IARC, 1996; Li et al., 1998 and Li et al., 1999).

The chemical has reported site-limited uses, including:

- in the manufacture of dyes;
- in the manufacture of gasoline additives and corrosion inhibitors;
- in the rubber industry to produce antioxidants; and
- as an intermediate in the manufacture of other chemicals.

The chemical has non-industrial uses including in:

- the manufacture of agriculture chemicals, antioxidants and oil additives;
- insecticides; and
- the manufacture of dapsone (an antimalarial drug).

Restrictions

Australian

No known restrictions have been identified.

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;
- Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient ‘Hotlist’); and
- ASEAN Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products.

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) based on the EU's harmonised classification and labelling:

- T; R23/24/25 (acute toxicity);

- Xn; R48/R20/R21/R22 (repeat dose toxicity);
- R40 Carc. Cat 3 (carcinogenicity); and
- R68 Mut. Cat 3 (mutagenicity).

Exposure Standards

Australian

The chemical has an exposure standard of 0.64 mg/m^3 (0.1 ppm) time weighted average (TWA) (HSIS).

International

The following exposure standards are identified (Galleria):

An exposure limit of 0.5-1.0 mg/m^3 (0.1-0.6 ppm) TWA and 1.5-2.0 mg/m^3 short-term exposure limit (STEL)/MAK/occupational exposure limit (OEL) in different countries such as Canada (Quebec, Yukon), Colombia, Denmark, Hungary, Italy, Japan, Korea, Malaysia, Mexico, New Zealand (NZ), Nicaragua, Peru, South Africa, Taiwan, Uruguacy and the USA (Alaska, Hawaii, Idaho, Michigan, Tennessee, Washington,Wyoming).

Health Hazard Information

The chemical is refered to as *para*-chloronitrobenzene (p-CNB) in this assessment.

Toxicokinetics

Para-chloronitrobenzene (p-CNB) is rapidly absorbed into the skin, gastrointestinal tract (GIT) and respiratory tract and distributed into the tissue (fat, blood cells, skeletal muscle, liver and kidney). The chemical undergoes three major biotransformations including nitro-reduction, displacement of the chlorine with glutathione conjugation, and hydroxylation of the ring. The metabolites include 2-chloro-5-nitrophenol, N-acetyl-S-(4-nitrophenyl)-l-cysteine, 4-chloroaniline and 4-chloroformanilide. The major route of excretion is via the urine (OECD, 2005).

Several human studies have been conducted with mixed exposure to p-CNB and nitrobenzene. The chemical was determined to be rapidly absorbed through the skin and respiratory tract. Signs of intoxication include methaemoglobinaemia, vomiting, headache and, in severe cases, collapse (OECD, 2005). Exposure to p-CNB caused methaemoglobinaemia in humans and animals; and examination of the blood after exposure showed increased levels of Heinz bodies, reticulocytes and methaemoglobin and decreased haematocrit (Yoshindi, 1993).

In a study conducted in male Sprague Dawley (SD) rats (n=15), radiolabelled chemical was administered intravenously at doses 30, 100 or 333 mg/kg bodyweight (bw). Plasma concentrations were reported to be proportional to the dose concentration; and the radioactivity clearance rates decreased with increasing doses. Following collection of blood and urine, the metabolites, 2-chloro-5-nitrophenol (16 %), N-acetyl-S-(4-nitrophenyl)-l-cysteine (48 %) and other aromatic amino acids (4-chloroaniline and 4-chloroformanilide) (38 %) were determined by high performance liquid chromatography (HPLC). A non-linear decrease in the plasma concentration of p-CNB was observed. The volume of distribution was determined to be 4180 mL/kg. The results indicated the chemical to be distributed throughout the tissue (Yoshindi, 1993).

Male rats (n=3; strain not specified) were dermally exposed to the chemical at doses of 0, 0.65, 5.6 and 65 mg/kg bw/day for 72 hours (h). The dermal absorption of the chemical was found to be dose-dependent and ranged from 51 to 62 % (REACH).

In a study conducted in rats, 78 % of the chemical was orally absorbed and 62 % was dermally absorbed. The highest concentrations of the chemical were distributed in the fat, blood cells, skeletal muscles, liver and kidney 24 h post treatment. The applied dose was excreted in the urine (78 %) and in the faeces (12 %), after 72 h. Excretion was delayed at larger doses. There were no signs of accumulation (OECD, 2005).

In a study conducted in Wistar rats (number/sex/dose not specified), the chemical was intraperitoneally (i.p.) administered at a dose of 150 mg/kg bw. The maximum blood concentration was determined to be 25.9 µg/mL (MAK, 2012).

The chemical was orally administered to rabbits (number and strain not specified) at a single dose of 200 mg/kg bw. After 48 h, the applied dose was excreted in the urine as sulfates (21 %), glucuronides (19 %), mercapturic acids (7 %), free p-chloroaniline (9 %) and conjugated p-chloroaniline (4 %) (MAK, 2012).

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Toxic if swallowed’ (T; R25) in the HSIS. The available data (median lethal dose—LD50 of 294-600 mg/kg bw in rats) support this classification.

In a study conducted on male Wistar rats (n=10), the chemical in polyethylene glycol (PEG) was administered by gavage at doses of 100, 200, 300, 350, 400, 500 or 600 mg/kg bw and the subjects were observed for 14 days. Mortalities reported were: 3/10 at 200 mg/kg bw; 5/10 at 300 mg/kg bw; 5/10 at 350 mg/kg bw; 8/10 at 500 mg/kg bw; and 10/10 at 600 mg/kg bw. Clinical signs of toxicity included cyanosis, diarrhoea, increased urination and "reduced general condition." The LD50 was determined to be 294 mg/kg bw (OECD, 2005; REACH).

In another study conducted on female Wistar rats (n=10/dose) the chemical in sesame oil was administered by gavage as single doses of 250, 400, 500, 560, 630, 1000 or 1600 mg/kg bw. Animals were observed for 14 days. Mortalities reported were: 1/10 at 500 mg/kg bw; 3/10 at 560 mg/kg bw; and 10/10 at 630, 1000 and 1600 mg/kg bw. Clinical signs of toxicity included imbalance and cyanosis. The LD50 was determined to be 565 mg/kg bw (OECD, 2005; REACH).

In a study conducted on rats (n=3-5/sex/dose; strain not specified), the chemical was administered as single doses of 80, 100, 200, 400 and 600 mg/kg bw. Mortalities reported were 1/3 at 400 mg/kg bw dose; and 5/5 at 600 mg/kg bw dose. An LD50 was not reported (REACH).

In a study conducted in female rats (strain and number not specified), the chemical was administered at doses of 120-2100 mg/kg bw as a 10 % solution in gummi arabicum in water. Mortalities were not reported, but the LD50 was determined to be 420 mg/kg bw. Clinical signs of toxicity included decreased motility, sedation, atony and urinary stammering (REACH).

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 available data (LD50— 750-1722 mg/kg bw in rats) support an amendment to this classification (refer to **Recommendation** section).

In a study conducted according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 402, male Wistar rats (n=10/dose) were administered the chemical in polyethylene glycol (PEG) at doses of 500, 600, 700, 900, 1000 or 1200 mg/kg bw, semiocclusively, for 24 h. Mortalities reported were: 2/10 at 600 mg/kg bw; 5/10 at 700 mg/kg bw; 8/10 at 900 and 1000 mg/kg bw; and 10/10 at 1200 mg/kg bw. The LD50 was determined to be 750 mg/kg bw. Clinical signs of toxicity included sedation, cyanotic appearance, unkempt fur, palm spasm and low body temperature (REACH).

In a study conducted according to OECD TG 402, the chemical was applied to the shaved backs of Wistar rats (n=6/sex/dose) at doses of 1000, 1250, 1600 or 2000 mg/kg bw, for 24 h. A 14-day observation period followed. Mortality was observed at 1250 mg/kg bw (2/6), 1600 mg/kg bw (2/6) and 2000 mg/kg bw (4/6). Signs of toxicity include poor general condition, cyanosis and brown coloured urine (REACH).

The LD50s in male and female rats (strain and number not specified) were reported as 750 and 1722 mg/kg bw, respectively, when the chemical was applied in the skin in PEG solution (males) or in sesame oil (females). Clinical signs of toxicity included weakness, collapse and death, which occurred within 4-6 days. Areas of the lungs, liver and kidney were discoloured, the spleen was darkened and inflammation occurred in the gastrointestinal tract (GIT) (OECD, 2005).

In a study conducted according to the OECD TG 402, undiluted chemical was applied to the skin of New Zealand White (NZW) rabbits (n=2/sex/dose) at doses of 2000, 2510, 3160, 3980 or 5010 mg/kg bw once for 24 h. The animals were observed for 14 days post-treatment. Lethargy, muscle weakness, collapse and mortality were observed. The LD50 was determined to be 3020 mg/kg bw (REACH).

The chemical was applied, undiluted, to the skin of rabbits (n=2/sex/dose) for 24 h. The LD50 values were reported as 3550 mg/kg and 2510 mg/kg for males and females, respectively (OECD, 2005).

Inhalation

The chemical is classified as hazardous with the risk phrase ‘Toxic by inhalation’ (T; R23) in the HSIS. There were no available studies to determine the median lethal concentration (LC50) of the chemical. The lowest lethal concentration (LCLo) was reported at 16.1 mg/L, supporting amendment to this classification (refer to **Recommendation** section).

In a study conducted similarly to OECD TG 403, male Crl:CD rats (n=10/dose) were exposed to the chemical vapour (head-only) at doses of 2.63, 2.84, 3.27, 3.35, 3.73, 6.12, 9.47 or 16.1 mg/L for 4 h. The LCLo was determined to be 16.l mg/L, with 1/10 reported mortality after three days. Clinical signs of toxicity observed within two weeks include: cyanosis, corneal opacity, abnormal arched-back posture, lethargy, reddish-brown nasal and frothy mouth discharges, tachypnoea and semi-prostration (OECD, 2005; REACH).

In a study conducted in Wistar rats (n=6/sex/dose), the chemical vapour was administered (nose-only) for 7 h. The exposure concentration increased analytically: 53 mg/m^3 air (50 mins), 74 mg/m^3 air (170 mins) and 77 mg/m^3 air (290 mins). Rats were observed for 14 days after the treatment. Signs of toxicity included narrowed palpebral fissures and tachypnoea; behaviour returned to normal after 14 days. No mortality was reported (OECD, 2005; REACH).

In a study conducted on Crl:CD SD BR rats (n=10 male/dose), the chemical was administered via head-only inhalation for 4 h at doses of 402, 435, 500, 513, 571, 936, 1449 or 2463 mL/m^3. The rats were observed for 14 days. The lowest observed adverse effect concentration (LOAEC) was determined to be 402 mL/m^3 (488 mg/L). Cyanosis was observed at 936 mL/m^3 (1137 mg/L) and hind leg ataxia, abnormal arched-back posture, cyanosis, corneal opacity, tachypnoea at 2463 mL/m^3 with weakness, a stained perineal area on days 1-2, dermal irritation on the ears until day 13 and corneal opacity in one rat until day seven. In the highest dosing group, one rat died on day three (OECD, 2005; MAK, 2012).

Corrosion / Irritation

Skin Irritation

The chemical is reported to slightly irritate skin in animal studies. The effects were not sufficient to warrant hazard classification.

In a study conducted according to OECD TG 404, the chemical (500 mg moistened with 1-2 drops of water) was applied occlusively to the shaved skin of NZW rabbits (six animals) for 24 h and observations were recorded after 24 h, 72 h and eight days. The mean oedema scores after 24 h and 72 h were 2.17 and 1.0, respectively. The chemical was found to be slightly irritating (OECD, 2005; REACH).

In a study conducted according to OECD TG 404, the chemical (500 mg; undiluted) was occlusively applied to abraded and intact skin of six Himalayan rabbits for 24 hours. The intact skin showed no erythema and slight oedema (score 2.17/4), while the abraded skin had slight erythema (score 0.17/4) and slight oedema (score 1.67/4). The reactions mostly resolved by 72 hours post application. The chemical was determined to be slightly irritating (OECD, 2005; REACH).

In a study conducted in rabbits (strain not specified), 20 mg of the chemical was applied to the skin for 24 h with 96 h observation. The chemical was found to be non-irritating (OECD, 2005; REACH).

In a study conducted in rabbits (n=6; strain not specified), 500 mg of the chemical moistened with water was applied to intact and abraded skin and covered with occlusive dressing for 24 h. Slight oedema (2.17/4) was observed in the intact skin; slight oedema (1.67/4) and erythema (0.17/4) were observed in the abraded skin and cyanosis was observed in one rabbit. The effects were reversible within 72 h.The chemical was reported to be slightly irritating (OECD, 2005).

Eye Irritation

Based on available information, the chemical is not irritating to the eye.

In a study conducted similarly to OECD TG 405, NZW rabbits (n=6) were administered the chemical (0.1 mL). An irritation score of 2/110 was calculated after 24 h. Irritation was fully reversed after eight days. The chemical was determined to be non-irritating (OECD, 2005; REACH).

In a study conducted equivalent to OECD TG 405, rabbits (n=2) were administered the chemical (20 mg) and observed for 1, 24 and 96 h. Slight irritation was observed (OECD, 2005; REACH).

In a study conducted similarly to OECD TG 405, Himalayan rabbits (n=6) were exposed to the chemical (100 mg) in the conjunctival sac of one eye, for 24 h. Observations were recorded at 1, 7, 24, 48, 72 h post-treatment and scored based on the Draize method. After 24 h, an irritation index of 6/110 was reported and the chemical was determined to be non-irritating (OECD, 2005; REACH).

In a study conducted in rabbits (n=2), the chemical (10 mg undissolved) was instilled into the conjunctival sac of the right eye. After 20 seconds, the treated eye of one rabbit was washed. Rabbits were observed at 1 and 4 h post-treatment. Slight corneal cloudiness was observed in the washed eye, which disappeared 4 h after treatment. No corneal, iridial or conjunctival effects were observed in the unwashed eye. The chemical was determined to be slightly irritating (OECD, 2005; REACH).

Sensitisation

Respiratory Sensitisation

Positive respiratory reactions to the chemical were observed in a study where rats (n=25) were exposed to the chemical via inhalation at concentration 0.008 mg/m^3 for five months (OECD, 2005). No further details are available.

Skin Sensitisation

Positive and negative skin reactions were reported from the available information on the skin sensitisation potential of the chemical. However, the animal studies were poorly reported, not conducted in accordance with any acceptable national or international test guidelines, and predated the principles of good laboratory practice (GLP). The OECD (OECD, 2005) concluded that the skin sensitisation potential of the chemical cannot be determined.

The chemical and its metabolites do not contain functional alerting groups for binding to DNA as indicated in the profilers of the OECD Quantitative Structure-Activity Relationship (QSAR) Application Toolbox v.3.3.

Repeated Dose Toxicity

Oral

The chemical is classified as hazardous with the risk phrase 'Harmful: danger of serious damage to health by prolonged exposure if swallowed' (Xn; R48/22) in HSIS (Safe Work Australia). The available data support this classification.

In a study conducted according to OECD TG 408, SD rats (n=60/sex/dose) were orally administered the chemical at concentrations of 0, 3, 10 or 30 mg/kg bw/d for 90 days. General paleness was observed in the 30 mg/kg bw/d group (males and females) and the 10 mg/kg bw/d group (females) immediately after treatment. There were significant increases in the levels of methaemoglobin and significant dose-dependent decreases in the erythrocyte count at the 3, 10 and 30 mg/kg bw/d doses. The liver at 30 mg/kg bw/d, spleen at all doses, and kidneys at 30 mg/kg bw/d were enlarged. Excessive haemopoiesis was observed at all doses. Heart enlargement in females and hyperplasia of bone marrow in both sexes were reported at 30 mg/kg bw/d. The lowest observed adverse effect level (LOAEL) was established to be 3 mg/kg bw/d. A no observed adverse effect level (NOAEL) could not be determined (OECD, 2005; REACH).

Albino rats (n=42) were administered the chemical at doses of 0.0025, 0.005, 0.025 or 5.0 mg/kg bw/d for 6 months. Administration of the chemical at the three highest doses caused increased levels of methaemoglobin, increased reticulocyte count and increased Heinz bodies in the erythrocytes. Alkaline phosphatase activity in blood and urine was increased in the highest dose. No effects were observed at the lowest dose (MAK, 2012).

In a study conducted according to OECD TG 453 (combined repeat dose/carcinogenicity study) with some deviations, Sprague Dawley (SD) rats (n=60/sex/dose) were orally administered the chemical (in corn oil) by gavage at doses of 0, 0.1, 0.7 or 5 mg/kg bw/d, daily for 24 months. Survival rates for males and females were 33-43 % and 48-60 %, respectively. Physical abnormalities were seen in all groups including the control group. At all doses (but with slightly higher incidences in the high dosed rats during the second year), yellow staining of the anogenital area was observed in males (week 60 and 104) and excessive lacrimation, chromodacryorrhoea, alopecia in females (during the last month). Significant increases in methaemoglobin levels were observed in males and females at 0.7 mg/kg bw/d (1.9 % after 6 months; 1.5 % after 24 months) and at 5.0 mg/kg bw/d (3.9 – 4 % after 6 months; 5.6 - 6 % after 24 months). At the highest dose, slight anaemia was observed. Pathological examinations showed significant increases in absolute and relative spleen weights at the highest dose in both sexes. The LOAEL was determined to be 0.7 mg/kg bw/d. The NOAEL was evaluated to be 0.1 mg/kg bw; however, due to the methodological limitations of this study, the value could not be clarified (OECD, 2005; REACH).

In several other studies conducted in rats, the LOAEL values were determined to range from 1-5 mg/kg bw/d and the NOAEL was recorded as 0.05 mg/kg bw/d. Significant effects include increases in the aerobic and anaerobic metabolic rates (adenosine triphosphate (ATP) and creatine phosphate concentrations in the brain and liver); increased red cell counts; changes in the differential white blood cell counts; and increased serum sulfhydryl (SH) groups (MAK, 2012).

In a study conducted on Swiss CD-1 mice (n=8/sex/dose) the chemical in corn oil was orally administered by gavage at doses of 0, 40, 80, 160, 320 or 640 mg/kg bw/d, daily for 14 days. At 160 and 320 mg/kg bw/d, cyanosis was observed and water consumption was significantly decreased. The maximum tolerated dose (MTD) was recorded at 250 mg/kg bw/d (REACH).

Dermal

The chemical is classified as hazardous with the risk phrase 'Harmful: danger of serious damage to health by prolonged exposure in contact with skin ' (Xn; R48/21) in HSIS (Safe Work Australia). Based on the observed dermal bioavailability (see **Toxicokinetics** section) and the repeat dose oral toxicity results, this classification is considered appropriate.

Inhalation

The chemical is classified as hazardous with the risk phrase 'Harmful: danger of serious damage to health by prolonged exposure through inhalation' (Xn; R48/20) in HSIS (Safe Work Australia). The available data support this classification.

In a 4 and 13 week repeated dose inhalation toxicity study in male and female SD rats, the lowest observed adverse effect concentration (LOAEC) for the chemical after 4 weeks was reported to be 5 mg/m^3 and, after 13 weeks, 9.81 mg/m^3, based on methaemoglobinaemia. A NOEAC could not be determined (Nair et al., 1986; OECD, 2005).

A group of SD rats (n=10/sex/dose) was exposed to the chemical (0.33, 1.1, 3.3 % w/v in ethylene glycol monoethyl ether) as an aerosol at doses of 5, 15 or 45 mg/m^3, six h/d, five days/week for four weeks. The animals were observed two times a day. The chemical caused concentration-dependent cyanosis, significant methaemoglobinaemia, significant reductions in RBC count and an increase in spleen weight. The LOAEC was determined at 5 mg/m^3 based on methaemoglobinaemia. A NOAEC could not be determined (Nair et al., 1986; OECD, 2005).

A study was conducted in Fischer 344 (F344)/N rats (n=10/sex) and B6C3F1 (n=10/sex) mice via whole body inhalation for six h/day, five per week for 13 weeks. The animals were exposed to the chemical at concentrations of 1.5, 3, 6, 12 or 24 ppm (9.6 to 154 mg/m^3). In rats, the reported effects include methaemoglobinaemia; increased nucleated erythrocytes and leucocytes; decreased RBC, haemoglobin and haematocrit; and an increase in spleen, liver and kidney weights. Histopathological examination identified haematopoietic cell proliferation. In mice, the reported effects include increases in spleen, liver and right kidney weights; and haematopoiesis. The severity observed was concentration-dependent. The LOAEC for rats was determined to be 1.5 ppm (1500 mg/m^3) and the NOAEC > 6 ppm (6000 mg/m^3) in mice (Travlos et al., 1995; REACH).

SD rats (number not specified) were exposed to the chemical vapour (doses were not reported) for four weeks. Methaemoglobinaemia and changes in blood count were observed. A NOAEL was not reported (MAK, 2012).

Observation in humans

In a case study, a male worker was identified as having decreased blood haemoglobin levels after exposure to the chemical via inhalation. Three months after exposure, the worker was diagnosed with reparative polycythaemia, and he recovered after two years (MAK, 2012).

In another case, four workers exposed to the chemical by inhalation had discoloured skin and lips; the conjunctivae and fingernails appeared cyanotic. The workers had severe pain in the head and neck. Haemoglobin levels were reduced for several days after exposure to the chemical (MAK, 2012).

Genotoxicity

The chemical is classified as hazardous—Category 3 mutagenic substance—with the risk phrase 'Possible risk of irreversible effect' (R68) in the HSIS (Safe Work Australia). The available data support the classification.

In vitro studies

The chemical gave positive results in the following in vitro tests:

- Ames test (OECD TG 471) conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 with and without metabolic activation at doses up to 10000 µg/plate (OECD, 2005; MAK, 2012; REACH);
- mammalian chromosome aberration test (MCAT) (OECD TG 473), conducted in Chinese hamster ovary (CHO) cells with and without metabolic activation at doses up to 900 µg/mL (REACH);
- micronucleus assay in mouse bone marrow (OECD TG 474) with and without metabolic activation at doses up to 500 mg/kg bw (OECD, 2005);
- mouse lymphoma assay (OECD TG 476) in mouse lymphoma cells L5178Y TK+/- at concentrations up to 600 µg/mL with and without metabolic activation; cytotoxicity was reported at = 300 µg/mL (OECD, 2005);
- sister chromatid exchange (SCE) assay (OECD TG 479) in CHO cells with and without metabolic activation at doses up to 500 µg/mL (REACH).

The chemical gave negative results in the following in vitro tests:

- Ames test conducted in *S. typhimurium* strains TA98, TA100, TA1530, TA1535, TA1537, TA1538, TA1532, TA1950, TA1975, TA1978, G46 with and without metabolic activation at doses up to 2000 µg/plate (MAK, 2012; REACH);

- hypoxanthine phosphoribosyl transferase (HPRT) assay (OECD TG 476) in CHO cells with and without metabolic activation at doses up to 2.38 mM (OECD, 2005);
- spondyloocular syndrome (SOS) chromosome test conducted in *Escherichia coli* (*E. Coli*) PQ 37 with and without metabolic activation; dosages were not reported (MAK, 2012; REACH);
- DNA damage and/or repair (OECD TG 482) in rat hepatocytes without metabolic activation at doses up to 500 µg/mL (REACH);
- SOS induction assay (UMU) test conducted in *S. typhimurium* (strain TA1535/pSK1002) with and without metabolic activation at test concentration 100 µg/mL (REACH); and
- chromosome aberation test in human peripheral lymphocytes at 8-158 µg/mL (Health Council of the Netherlands, 2002).

In vivo studies

In a DNA strand break test conducted in Swiss CD1 mice (n=12/dose), the chemical was injected at doses of 30, 60, 80 or 100 mg/kg bw. There was a dose-dependent increase in DNA strand breakages in the brain, liver and kidney (MAK, 2012).

In a chromosome aberration assay conducted according to OECD TG 475, SD rats were administered the chemical via gavage at doses of 0, 30, 100, 300 mg/kg bw/day. Negative results were reported in the bone marrow cells with and without metabolic activation (OECD, 2005).

Negative results were reported in a *Drosophila melanogaster* sex-linked recessive lethal (SLRL) test by oral and intraperitoneal (i.p.) injection routes (IARC, 1996).

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 the classification.

In a previously described study (see **Repeated dose toxicity: Oral**), unilateral and bilateral interstitial cell tumors of the testes were observed at the following frequencies: 1.7 % in controls, 6.8 % at the low dose, 8.3 % at the mid dose, and 10 % at the high dose (OECD, 2005; REACH).

In another study conducted in CD rats (n=25 males/dose) and CD1 HaM/ICR mice (n=25/sex/dose), the chemical was orally administered at doses of 0, 150 or 300 mg/kg bw/day and 0, 450 or 900 mg/kg bw/d respectively, for 18 months. After three months, the doses were reduced to 18.75 and 37.5 mg/kg bw/d in rats due to toxicity. No tumours were found in the rats. Increased incidences of vascular tumours were recorded in both sexes of mice after three months: 2/14 at the low dose and 4/14 at the high dose (males) and 2/20 at the low dose and 7/18 at the high dose (females). Hepatomas were also found in males (OECD, 2005; REACH). This study was not considered adequate for the evaluation of the carcinogenicity potential of the chemical by the International Agency for Research on Cancer (IARC) (1996).

Groups of SD rats (n=60/sex/dose) were administered the chemical (in corn oil) at doses of 0, 0.1, 0.7 or 5 mg/kg bw by gavage over 24 months. The tumour incidence was not reported in females. Interstitial cell tumours of the testes were reported in 1/60 control animals, 1/60 at 0.1 mg/kg bw, 4/59 for 0.7 mg/kg bw and 6/60 at 5 mg/kg bw. The increases were not statistically significant (MAK, 2012).

The positive results obtained in the studies conducted on the chemical could be from the presence of 4-chloroaniline, a p-CNB metabolite produced in humans, rats and rabbits, in the blood. This chemical has been found to cause tumours in the spleen, subcutaneous tissues, kidney, adrenal gland, liver and blood (NICNAS).

Reproductive and Developmental Toxicity

The chemical does not show specific reproductive or developmental toxicity. Any reproductive and developmental effects were only observed secondary to maternal toxicity.

Reproductive Toxicity

In a study conducted according to OECD TG 416, CD rats (n=15 male and n=30 female/dose) were orally administered 0, 0.1, 0.7 or 5.0 mg/kg bw/d via gavage for 14 weeks prior to mating, throughout gestation and lactation. The first flial (F1) generation was given the same doses 18 weeks before mating and then selected to produce the second flial (F2) generation. No males died in the study. Mortalities were recorded in females in the control group (1/20), the 0.7 mg/kg bw/d group (3/30), and the 5 mg/kg bw/d group (2/30). Mating indices for the parental (F0) generation were 93.3 %, 86.7 %, 80 % and 93.3 % for the control to high dose groups respectively. Litter survival indices were slightly lower in the mid and high dose groups compared with controls. No malformations or tissue changes in organs were seen. Mortality rates of the F1 adults were not dose-dependent. In the F0 males in the 5 mg/kg bw/d dose group, changes in the testes (bilateral degeneration/atrophy of epithelium in 2/15 animals, and bilateral maturation arrest of the germinal epithelium in 1/15 animals) were reported. These effects were not examined in other dose groups. In the F1 generation, extramedullary haematopoiesis and reticuloendothelial cells containing brown pigment in the spleen of all rats in all treated groups were observed, but the effects were more pronounced in the high dose group. There was no impairment of fertility up to 5 mg/kg bw/d (OECD, 2005).

In a study conducted in rats (n=5/sex/dose) and mice (n=5/sex/dose), the chemical (vapour) was administered at 0, 1.5, 3, 6, 12 or 24 ppm (equivalent to 0, 1.5, 3, 6, 12 or 24 mg/kg bw/d), 6 h/d, 5 times/week over 13 weeks. In rats, an increase in the oestrous cycle length in females was observed at =6 ppm. Effects in males at 24 ppm included decreased left caudal epididymal and testicular weights, epididymal spermatozoa count per gram of caudal tissue, and total spermatid head count per testes. In mice, the oestrous cycle length was increased in females at 24 ppm (24 mg/kg bw/d). No findings were reported in males (OECD, 2005).

In another study, no testicular toxicity was found in five male F344 rats orally administered the chemical at a single dose of 250 mg/kg bw/d (MAK, 2012).

In a study conducted in female SD rats (n=5/dose), the chemical (in corn oil) was administered by gavage at doses of 0, 2, 9, 35, 135 or 550 mg/kg bw/d on gestation days (GD) 6-19. On day 20, the foetuses were delivered by caesarean section. Mortality was recorded after nine days at 550 mg/kg bw/d (5/5) and 135 mg/kg bw/d (2/5). Cyanosis was seen in all the dose groups; spleen enlargement was only seen at doses ³ 9 mg/kg bw/d. Resorption was increased at doses ³ 35 mg/kg bw/d (MAK, 2012).

In a continuous breeding study, breeding pairs of Swiss CD mice (n=10/sex/dose) were exposed to the chemical (in corn oil) by gavage at doses of 0, 62.5, 125 or 250 mg/kg bw/d for seven days prior to mating, followed by 98 days of continuous breeding. After the weaning of the last litter in the F1 generation, the same procedures were followed to produce the F2 generation. In the F0 generation, 3/10, 3/10, 1/10, 4/10 mortalities were recorded for the control to high dose groups, respectively. Each breeding pair delivered four litters. Pup delivery was not affected by the administration of the chemical. In the 62.5, 125 and 250 mg/kg bw/d dose groups of the F1 generation, reduced pup live weights were seen. Pup delivery in the F2 generation was significantly reduced in the 250 mg/kg bw/d dose group. The frequency of still-born pups was increased at this dose. At mating, the F1 animals were cyanotic in the highest dose. Additional effects in males observed at the highest dose include increased liver and spleen weights, darkened spleens, and decreased seminal vesicle weights. The NOAEL for fertility was determined at 125 mg/kg bw/d and NOAEL for general toxicity of offspring is 62.5 mg/kg bw/d (OECD, 2005). The relevance of the results in this study is limited since only the effects in the offspring were examined.

Developmental toxicity

In a study conducted according to OECD TG 414, mated female CD rats (n=24 dose) were administered the chemical (in corn oil) via gavage at doses of 0, 5, 15 or 45 mg/kg bw/d during GD 6-19. Terminal body weight was significantly reduced and the number of resorptions was increased in high dose females. Post-mortem observations revealed significantly higher spleen weights. The NOAEL for maternal toxicity was established at 5 mg/kg bw/d. At the high dose, there was a significant increase in the incidence of skeletal malformations. The NOAEL for developmental toxicity was determined to be 15 mg/kg bw/d (OECD, 2005).

In another study, the chemical (in corn oil) was administered to NZW rabbits via gavage at doses of 0, 5, 15 or 40 mg/kg bw/day during GD 7-19. The reported mortalities from control to high-dose were 1/18, 1/18, 1/18 and 8/18, respectively. Anogenital staining was observed in the 15 mg/kg bw/d dose. In the 40 mg/kg bw/d dose, greyish/pale eyes were noted at days 10, 15 and 19. Skeletal malformations were not dose-dependent and are historically seen at a low incidence in this particular strain of rabbit. The NOAEL for maternal and developmental toxicity was determined at 5 mg/kg bw/d (OECD, 2005).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects (carcinogenicity and mutagenicity) and systemic acute effects (acute toxicity from oral, dermal, and inhalation exposure). The chemical causes harmful effects following repeated exposure through oral, dermal and inhalation routes.

Public Risk Characterisation

Given the site-limited use identified for the chemical, it is unlikely that the public will be exposed. Although the public could come into contact with articles/coated surfaces containing the chemical, it is expected that the chemical will be bound within the article/coated surface; and, hence will not be bioavailable. Therefore, the chemical is not considered to pose an unreasonable risk to public health.

Occupational Risk Characterisation

During product formulation, dermal, ocular 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 chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

NICNAS Recommendation

Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory. No further assessment is required.

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]
Acute Toxicity	Toxic if swallowed (T; R25)* Harmful in contact with skin (Xn; R21) Harmful by inhalation (Xn; R20)	Toxic if swallowed - Cat. 3 (H301) Harmful in contact with skin - Cat. 4 (H312) Harmful if inhaled - Cat. 4 (H332)
Repeat Dose Toxicity	Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed (Xn; R48/20/21/22)*	Causes damage to organs through prolonged or repeated exposure - Cat. 1 (H372)

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Genotoxicity	Muta. Cat 3 - Possible risk of irreversible effects (Xn; R68)*	Suspected of causing genetic defects - Cat. 2 (H341)
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 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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