Benzenamine: Human health tier II assessment

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CAS Number: 62-53-3

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.

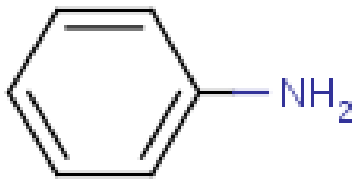
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	Aniline Aminobenzene Phenylamine Aminophen Benzidam
Structural Formula	
Molecular Formula	C6H7N
Molecular Weight (g/mol)	74.08
Appearance and Odour (where available)	Colourless to brown oily liquid with a characteristic amine odour and burning taste.
SMILES	c1(N)ccccc1

Import, Manufacture and Use

Australian

The National Pollutant Inventory (NPI) holds data for all sources of the chemical in Australia. The following use information was listed on NPI.

The chemical has reported commercial use including:

- in explosives.

The chemical has reported site-limited use including:

- in manufacturing of polyurethanes, rubber processing chemicals, diphenylamine, phenolics, fibres, dyes, pigments, and photographic chemicals; and
- in petroleum refining.

The chemical has reported non-industrial use including:

- in pharmaceuticals; and
- in the production of plant protecting products (herbicides, fungicides).

International

The following international uses have been identified through the Organisation for Economic Cooperation and Development Screening information data set International Assessment Report (OECD SIAR); Galleria Chemica; Substances and Preparations in the Nordic countries (SPIN) database; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB), and the Government of Canada (2011).

The chemical has reported site-limited use (major use) including:

- as an intermediate in the production of 4,4'-methylenedianiline (MDA). MDA is then used primarily for making polyurethane through the creation of 4,4'-methylene diphenyl diisocyanate (MDI). Lower quantities of MDA are also used as hardeners in epoxy resins and adhesives, as well as in the production of high-performance polymers;
- as an intermediate in the manufacture of dyes and pigments, especially azo dyes;
- as an intermediate in the production of series of compounds being used in the rubber industry (e.g. mercaptobenzothiazole, diphenylguanidine, diphenylamine, aniline ketone condensates etc); and
- in the synthesis of photographic chemicals (hydroquinone), explosives, petroleum refining chemicals, and phenolics.

The chemical has reported domestic use including in:

- paints, lacquers and varnishes;
- adhesives and binding agents; and
- colouring agents.

It is unclear whether these uses relate to the chemical itself or to derivatives of the chemical. The National Library of Medicine Household Products Database reports use in the USA in only one domestic product: a concrete colour additive (most colours).

The chemical has reported non-industrial use including:

- as an intermediate in the manufacture of pharmaceuticals and plant protecting products (herbicides, fungicides).

Restrictions

Australian

This chemical is listed in the Poisons Standard (Standard for the Uniform Scheduling of Medicines and Poisons—SUSMP, 2012) in Schedule 6. The Schedule 6 entry states as follows:

'ANILINE (excluding its salts and derivatives) **except** in preparations containing 1 per cent or less of aniline'.

Schedule 6 chemicals are labelled with 'Poison'. These are substances with a moderate potential for causing harm, the extent of which can be reduced by using distinctive packaging with strong warnings and safety directions on the label.

International

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

- EU Cosmetic Directive 76/768/EEC Annex II—List of substances which must not form part of the composition of 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")

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; R48/23/24/25 (Repeat dose toxicity)

Xn; R40 (Carcinogenicity Cat. 3)

Xn; R68 (Muta.Cat.3)

Xi; R41 (Irritation)

Xi; R43 (Sensitisation)

Exposure Standards

Australian

The chemical has an exposure standard of 7.6 mg/m³ (2 ppm) time weighted average (TWA).

International

The following exposure standards are identified (Galleria Chemica):

An exposure limit (TWA) of 4–19 mg/m^3 in countries such as Canada, Denmark, France, Ireland, Japan, Norway, Singapore, Spain, Sweden, Switzerland, United Kingdom, and the USA.

An exposure limit (STEL) of 8–20 mg/m^3 in countries such as Canada, South Africa, Sweden, Switzerland, and the USA.

Health Hazard Information

Toxicokinetics

The chemical has been reported to be well absorbed following oral, dermal and inhalation exposure in animals. Following oral administration, the chemical was absorbed to the extent of 89–96, 72, 80, and 56 % in rats, mice, sheep, and in pigs, respectively. A pulmonary retention of nearly 90 % has been reported for humans and a dermal absorption of up to 38 % has also been estimated for humans. Although details are not available, a biological half-life of about 3.5 hours has been reported for the chemical in humans (ECB, 2004; EC, 2010; REACH). The rate of absorption of the chemical though skin in humans has also been reported to be approximately 1000 times lower in vapour form than topically applied liquid form (REACH).

Following a single oral administration of the chemical in rats, the peak plasma radioactivity was observed at 0.5, 1 and 2 hours at 10, 30, and 100 mg/kg bw, respectively. The radioactivity decreased to less than 2 % of the peak concentration for all doses 24 hours after exposure. The distribution of the radioactivity was highest in kidney, followed by liver, plasma, lung, heart, spleen and brain for all doses. Less than 0.1 % of the administered radioactivity remained in these tissues for all doses up to 48 hours after exposure. In another study in rats treated with the chemical at 100 mg/kg bw/day orally for one day, the highest radioactivity was present in erythrocytes followed by plasma, spleen, kidney, liver, lung, heart, brain, and fat. A greater accumulation of radioactivity was observed in spleen following repeated administration of the chemical at the same dose for 10 days.

The metabolism of the chemical is the main elimination pathway for the chemical and is qualitatively similar in humans and animals. Following a single dermal application of the chemical in rats and mice, the chemical is mostly excreted within 24 hours as metabolites in urine. The chemical is metabolised primarily in the liver by three metabolic pathways: N-acetylation, aromatic ring hydroxylation, N-hydroxylation. While the N-acetylation of the chemical is catalysed by hepatic N-acetyltransferase, the cytochrome P-450 enzyme system (aniline hydroxylase) is responsible for the aromatic hydroxylation. It is believed that the N-acetylation pathway is an important route by which the chemical is detoxified, while N-hydroxylation is the principal route by which the chemical produces toxic effects through the formation of phenylhydroxylamine metabolite. Small amounts of the chemical are hydroxylated to 2- and 4-aminophenols. The methaemoglobin forming ability of the chemical is strongly based on the formation of phenylhydroxylamine metabolite, but also to some extent on the formation of 2- and 4-aminophenol metabolites. The relative in vitro (rat erythrocyte suspensions) potencies for methaemoglobin formation for phenylhydroxylamine, 2-aminophenol, and 4-aminophenol metabolites were about 10:5:1. However, the relative potencies of aminophenols for methaemoglobin formation are lower in rats after intraperitoneal injections, the ratio being 100:4:1 (phenylhydroxylamine: 2-aminophenol: 4-aminophenol).

The toxic effects of the chemical are due to the formation of methaemoglobin leading to methaemoglobinaemia, cyanosis, tremors, lacrimation and respiratory problems. Methaemoglobin is produced as a result of oxidation of thiols within erythrocytes by phenylhydronitroxide radicals, which are produced as a reaction of phenylhydroxylamine with oxyhaemoglobin. After a single oral or inhalation exposure to dogs, the formation of methaemoglobin was 1–6 times higher following oral exposure than after inhalation exposure. The maximum methaemoglobin concentration was observed in less than one hour after cessation of inhalation exposure and three hours after oral administration.

The chemical has been reported to be able to pass the placental barrier in rats following a single subcutaneous dose. The total plasma concentrations of the chemical were slightly higher (10–15 %) than the maternal plasma concentrations at 1, 2, and 4 hours after application. A similar plasma half-life of 1.5 hours was reported for foetal as well as for maternal plasma (ECB, 2004; REACH).

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Toxic if swallowed’ (T; R25) in HSIS (Safe Work Australia).

While the available animal data do not support this classification (reported LD50s were 250, 442, 780, 930 mg/kg bw in rats) (ECB, 2004; REACH; RTECS), human toxicity from oral ingestion has been reported (see **Observation in humans)**. It has also been reported that humans are more sensitive than rats to exposure to the chemical in the formation of methaemoglobin and a much lower dose is required in humans than rats to produce increased levels of methaemoglobin (HSDB). Taken all together, an amendment of this classification is not recommended.

Reported clinical signs in animals were tremors, fibrillation, hyperpnoea, cyanosis, convulsions, hypothermia, salivation and prostration. Necropsy revealed inflammation of the gastrointestinal tract in survivors, hyperaemia of lungs and haemorrhage of the gastrointestinal tract in those that died (decedents).

Dermal

The chemical is classified as hazardous with the risk phrase ‘Toxic in contact with skin’ (T; R24) in HSIS (Safe Work Australia).

While the available animal data do not support this classification (reported LD50s were 1400–1500 mg/kg bw in rats) (ECB, 2004; HSDB; REACH; RTECS), human toxicity from dermal exposure has been reported (see **Observation in humans)**. As stated above, humans are more sensitive than rats to exposure to the chemical in the formation of methaemoglobin and the chemical is also reported to be well absorbed through all exposure routes (see **Toxicokinetics**). Taken all together, an amendment of this classification is not recommended

Reported clinical signs in animals included hypoactivity, hypersensitivity and salivation. Subdermal haemorrhages, severe oedema and erythema were reported at the site of application of the chemical. Necropsy did not reveal any significant findings in survivors, but hyperaemia of liver and kidneys in decedents was observed.

Inhalation

The chemical is classified as hazardous with the risk phrase ‘Toxic by inhalation’ (T; R23) in HSIS (Safe Work Australia).

While the available animal data do not support this classification, as stated above, humans have been reported to be more sensitive than rats to exposure to the chemical in the formation of methaemoglobin (HSDB). The chemical is also reported to be well absorbed through all exposure routes and a pulmonary retention of nearly 90 % has been reported for humans (see **Toxicokinetics)**. Based on the above and that human toxicity from inhalation has been reported (see **Acute toxicity: Observation in humans**), there is insufficient evidence to support a recommendation to amend this classification. It is also noted that in a poorly reported study in 1949, an approximate LC50 value of 250 ppm (ca. 1 mg/L) was reported for a four-hour inhalation exposure (whole-body) in rats. A concentration of 250 ppm killed 2–4/6 rats in this study (ECB, 2004).

In an acute inhalation toxicity study (head-only exposure), an LC50 value of 3.27 mg/L was reported for a four-hour exposure in rats. Reported clinical signs included cyanosis, tremors, reddish brown nasal discharge, chromodachryorrhea, prostration up to 48 hours post-exposure, and corneal clouding up to 14 days post-exposure (ECB, 2004; REACH). In another acute inhalation toxicity study (whole-body exposure), an LC50 value of 1.86 mg/L was reported for a four-hour exposure in rats. Reported clinical signs included cyanosis, tremors, lacrimation, salivation up to 48 hours post-exposure, prostration and rales. Other clinical signs following conclusion of the exposure period included pallor, head and facial hair loss, reddish-brown stained mouth, nasal and perinasal areas (ECB, 2004; REACH).

Observation in humans

Acute intoxication of humans following acute exposure to the chemical by oral, dermal, and inhalation routes has been frequently reported (ECB, 2004: HSDB; REACH).

The most prominent symptoms of acute exposure to the chemical were cyanosis, lacrimation, tremors, tachypnoea, weakness, disorientation, dizziness, impaired gait, lethargy, drowsiness, convulsions, loss of consciousness, and coma (ECB, 2004; HSDB). Many of the reported adverse health effects of the chemical in humans are due to the formation of methaemoglobin, methaemoglobinaemia and accompanying anoxia, erythrocyte damage, and spleen effects. Signs and symptoms experienced are generally proportional to the percentages of methaemoglobin formation achieved in the blood. Methaemoglobin levels below 20 % generally caused no symptoms, 20–50 % methaemoglobin levels can result in dyspnoea, tachycardia, headache, and dizziness, and a methaemoglobin concentration of above 60–70 % may produce coma and death.

Accidental exposure of workers to the chemical has also resulted in symptoms of cyanosis, nausea, vomiting, dizziness, general weakness, mental disturbances, respiratory problems and heart pains. While 0.4–0.6 mg/L concentration of the chemical may be without any harm for up to one hour, a concentration of 0.1–0.25 mg/L for several hours produced mild symptoms (ECB, 2004). Inhalation of chemical vapours at 7–53 ppm caused only mild symptoms of methaemoglobinemia, while exposure to concentrations in excess of 100–160 ppm (6.91–11.06 mg/kg bw) for over one hour caused dyspnoea, tachycardia, headache, and dizziness (US EPA, 1994; HSDB). The average lethal inhalation dose for humans is reported to be 25 mg/L of air or 350–1430 mg/kg bw (ECB, 2004). Clinical adverse effects were not observed in four human volunteers exposed at the exposure standard (TWA) of 2 ppm for a complete work shift duration of eight hours. These workers wore standardised clothes typical for manufacturing industries using the chemical (REACH).

After oral treatment for three consecutive days in adult men, the no effect dose with respect to methaemoglobin formation was stated to be around 15 mg (about 0.21 mg/kg bw) (ECB, 2004; REACH). A further analysis of this study suggested an acute oral dose of 71 mg of the chemical (1 mg/kg bw/day) resulted in an adverse increase in methaemoglobinaemia formation (20 %) in humans (Government of Canada, 2011). Methaemoglobinaemia (maximal values 79.6–74.4 %) and haemolytic anaemia have been experienced by two humans following ingestion of about 4 mL of the chemical mixed with a soft drink (REACH). A dose-dependent increase in methaemoglobin formation has been reported in humans following a single oral dose of 25–65 mg of chemical (HSDB). A probable oral lethal dose in humans has been stated to be at 50–500 mg/kg bw (US EPA, 1994). A suicidal (lethal) intake of 60 mL of the chemical, equivalent to about 876 mg/kg bw, has been reported to result in death at day four (ECB, 2004; REACH). While the probable oral lethal dose for humans has been reported to be 50–500 mg/kg bw, the minimum lethal dose for humans has been reported to be approximately 150 mg/kg bw (HSDB).

Humans have been reported to be more sensitive than rats to exposure to the chemical in the formation of methaemoglobin. Following oral administration (and possibly inhalation exposure), the dose that produced increased levels of methaemoglobin was stated to be much lower for humans than for rats (US EPA, 1994; HSDB). The chemical has been reported to cross the placental barrier and induced the production of methaemoglobin in both adults and children, as the foetal liver can also N-oxygenate the chemical to produce the toxic metabolite, phenylhydroxylamine (HSDB).

Corrosion / Irritation

Skin Irritation

The chemical is reported in studies to slightly irritate skin when administered to animals. The effects were not sufficient to warrant a hazard classification. However, a 24-hour exposure period resulted in subdermal haemorrhages and severe erythema in rabbits (ECB, 2004; REACH).

Eye Irritation

The chemical is classified as hazardous with the risk phrase 'Risk of serious damage to eyes' (Xi; R41) in HSIS (Safe Work Australia). The available data support this classification (ECB, 2004; REACH).

In an eye irritation study in rabbits with the chemical (50 mg undiluted), severe corneal opacity, severe conjunctival erythema, and oedema were present. These were not reversible within eight days and liquid pannus formation was also determined at day eight of the study.

Observation in humans

There have been reports of irritation to mucous membranes in humans following exposure to the chemical from ocular and inhalation exposure. While direct contact with the skin has only resulted in mild irritation, the chemical has reportedly caused mild to severe eye irritation, corneal damage, discolouration, and possible permanent eye damage. Inhalation of the chemical can also cause irritation of the upper respiratory tract, with wheezing and coughing (HSDB).

Sensitisation

Skin Sensitisation

The chemical is classified as hazardous with the risk phrase 'May cause sensitisation by skin contact' (R43) in HSIS (Safe Work Australia). The positive results reported in several guinea pig maximisation tests support this classification (ECB, 2004; REACH).

Observation in humans

Although positive sensitisation reactions have been reported in humans, these were mainly in patients suffering from eczematous dermatitis. The positive reactions are often associated with para-group compound cross reactivity. The chemicals involved in cross sensitisation and belonging to the para-group are benzocaine, aniline, paraphenylenediamine, and diaminodiphenylmethane (ECB, 2004; REACH).

Repeated Dose Toxicity

Oral

Repeated dose toxicity studies have been mainly carried out in rats. Critical toxicological effects of the chemical in these studies have been mainly reported in blood and spleen (ECB, 2004; EC, 2010; REACH).

Repeated oral administration of the chemical in rats has resulted in toxic effects on red blood cells and the haematopoietic system, with corresponding effects on the spleen, bone marrow, liver and kidney. Clinical symptoms included cyanosis, reduced food consumption, reduced body weight gain, and premature deaths. Repeated exposure to the chemical also results in damaged erythrocytes, haemolytic anaemia, methaemoglobinaemia, and higher levels of Heinz bodies. As damaged red blood cells were scavenged predominantly in the red pulp of rat spleen, increased accumulation of haemosiderin was also noted, along with spleen congestion, dark colouration and increased spleen weight. Increased accumulation of haemosiderin was also occasionally noted in the liver and kidney. There was increased erythropoeitic activity in the bone marrow and spleen as a compensatory reaction to the haemolytic effect of the repeated administration of the chemical. Splenitis, spleen hyperplasia and fibrosis have also been reported (US EPA, 1994; ECB, 2004; EC, 2010; REACH).

A lowest observed adverse effect level (LOAEL) of 7 mg/kg bw/day, from a combined chronic/carcinogenicity study in rats that complied mostly with the OECD Test Guideline (TG 407) (see **Carcinogenicity**), has been determined for systemic effects (non-neoplastic lesions). The LOAEL was based on haematological effects (reticulocytosis; decrease of erythrocyte counts, haemoglobin and haematocrit; an increase in mean corpuscular volume—MCV), haemosiderosis and splenic haematopoiesis at the lowest administered dose of 7 mg/kg bw/day. A no observed adverse effect level (NOAEL) could not be established. In this study, the chemical (as aniline hydrochloride) was administered to Fischer 344 (F 344) rats at dietary levels of 0, 200, 600, and 2000 ppm for two years. These doses were equivalent (as aniline hydrochloride) to10, 30 100 mg/kg bw/day (equivalent to aniline doses of 7, 22, 72 mg/kg bw/day). Although other repeated dose toxicity studies have also been reported with the chemical, these were not considered here as these studies did not comply fully with the current test guidelines. A NOAEL could also not be derived in these studies (ECB, 2004; EC, 2010; REACH).

Dermal

Although no data are available, the chemical is reported to be well absorbed through all exposure routes; a dermal absorption of up to 38 % has been estimated for humans (see **Toxicokinetics**). Therefore, relevant toxic effects following dermal exposure of the chemical are expected to be similar to the those that are observed following oral administration of the chemical (ECB, 2004; EC, 2010).

Inhalation

Due to lack of properly conducted studies in animals, there are no valid data on effects of repeated inhalation exposure to the chemical. As reported above, the chemical is well absorbed through all exposure routes—a pulmonary retention of nearly 90 % has been reported for humans (see **Toxicokinetics**). Therefore, following repeated inhalation exposure in rats, reported effects on the haematopoietic system and spleen were similar to those reported following repeated oral exposures, to a lesser degree depending on the dose administered (ECB, 2004; EC, 2010 REACH).

A lowest observed adverse effect concentration (LOAEC) of ≥17 ppm (approx. 66 mg/m³), from a 14 days inhalation exposure study (exposure on 5 days/week, 6 hours/day), was determined in rats. The LOAEC was based on dose-dependent effects on spleen, reticuloendothelial system hypertrophy in the spleen, haemosiderosis, and increased haematopoiesis. In another inhalation study for 26 weeks in rats, a LOAEC of 5 ppm (19 mg/m³) was determined (exposure on 5 days/week, 6 hours/day. As no histopathology was performed in this study, the LOAEC was based on development of cyanosis (ECB, 2004; EC, 2010; REACH).

In a more recent study, Wistar rats were exposed (nose-only) to 9.2, 32.4, 96.5, and 274.9 mg/m³ of the chemical for five days/week, six hours/day, for two weeks followed by a two week post exposure observation period. While methaemoglobin formation and associated erythrocytotoxicity were the main signs of toxicity, cyanosis was also seen at concentrations ≥96.5 mg/m³ and did not progress during the exposure period. A no observed adverse effect concentration (NOAEC) of 32.4 mg/m³ was reported for erythrocytotoxicity and associated effects such as sequestration of erythrocytes, iron accumulation, and lipid peroxidation, although there is uncertainty as to the adversity of the spenic extramedullary haematopoiesis observed at this concentration (EC, 2010; Government of Canada, 2011; REACH).

Observation in humans

The chemical is classified as hazardous with the risk phrase 'Danger of serious damage to health by prolonged exposure' (Xn; R48/23/24/25) in the HSIS (Safe Work Australia). The available data, particularly the human observations, support this classification (US EPA, 1994; ECB, 2004; HSDB; REACH).

Although limited information is available on repeated exposure of humans to the chemical, it is expected that human health effects would be similar, in this case, to the human response following acute exposure to the chemical (see **Acute toxicity: Observation in humans**). In a clinical study, the chemical was administered orally to 20 humans at doses of 5, 15, or 25 mg per person on three successive days. After initial administration of the chemical at these doses, higher doses of the chemical were also given to some humans on successive days at the rate of 35 or 45 mg (five humans), 55 mg (two humans), and 65 mg (one human). At doses of 25 mg or higher, a significantly higher production of methaemoglobin formation was observed, compared with the methaemoglobin level at the 5 mg dose. Following administration of 65 mg of the chemical, a maximum methaemoglobin level of 16.1 % was reached two hours after administration. It was also concluded that, at dosages calculated to be from 0.4 mg/kg bw/day in this study, evidence of haemotoxicity (besides methaemoglobin formation) was evident (ECB, 2004; EC, 2010; REACH).

Methaemoglobinaemia (53 %) has been reported in one individual sitting in a car seat contaminated with the chemical, resulting in symptoms of cyanosis, dyspnoea, fatigue, and dizziness (HSDB). Following contact with shoes that had been dyed with a preparation containing the chemical (10–25 %), naphtha (50–100 %), and ethyl alcohol (25–50 %) in three individuals, grave methaemoglobinaemia was reported as the main symptom, requiring urgent medical assistance (ECB, 2004).

Genotoxicity

The chemical is classified as hazardous—Category 3 mutagenic substance—with the risk phrase ‘Possible risk of irreversible effects’ (Xn; R68) in HSIS (Safe Work Australia). The available data of positive findings in several in vitro and in vivo tests are sufficient to support this classification (ECB, 2004; HSDB; REACH).

The chemical was reported to be generally negative in bacterial mutation assays and negative results were also obtained for mitotic recombination in *Saccharomyces cerevisiae*. The chemical also did not induce DNA repair (unscheduled DNA synthesis-UDS) in primary human or rat hepatocytes. However, positive results were obtained in the L5178+/-mouse lymphoma gene mutation assay and in mammalian cell cultures with respect to chromosomal aberrations and sister chromatid exchange (SCE). Two metabolites of the chemical, 2-aminophenol and N-phenylhydroxylamine, also increased the frequency of SCE in human fibroblasts. Conflicting results were obtained with respect to mammalian cell gene mutation assays.

Although a negative result was obtained for the chemical in an in vivo chromosomal aberration assay with mouse bone marrow cells, a positive result was obtained with respect to the induction of SCE in in vivo mouse bone marrow cells. Following intraperitoneal administration of the chemical, induction of DNA strand breakage was noticed in the liver and kidney of rats but not in the spleen. However, this was not the case with respect to induction of DNA strand breakage in the liver, kidney and bone marrow of mice. No substantial in vivo DNA binding of the chemical was also found in rats and mice following administration of the chemical. A dominant lethal assay for germ cell mutagenicity in rats was considered to be inconclusive, due to the weakness of the effects. The chemical also had no effect on the frequency of sperm head abnormalities in male mice. Because of relatively poor sensitivities in the last two test systems, the unclear results are of limited predictive value.

The chemical has been reported to induce micronuclei in bone marrow using a mouse micronucleus test at the highest doses tested (300–380 mg/kg bw, intraperitoneally) in the presence of severe toxicity. A negative finding with respect to induction of micronuclei was noticed at relatively low doses of the chemical (125–250 mg/kg bw). As no evidence of clastogenicity was observed in another study, it was suggested that the induction of micronuclei may have arisen by a mechanism not involving direct DNA interaction. In rats, statistically significant, but small, dose-related increases in induction of micronuclei has been reported.

Carcinogenicity

The chemical is classified as hazardous—Category 3 carcinogenic substance—with the risk phrase ‘Limited evidence of carcinogenic effect’ (Xn; R40) in HSIS (Safe Work Australia). The available data support this classification (US EPA, 1994; ECB, 2004; Government of Canada, 2011; REACH). It is also noted here that the International Agency for Research on Cancer (IARC) has evaluated the chemical as not classifiable for carcinogenicity to humans (Group 3) (IARC, 1987). However, the US Environmental Protection Agency (US EPA) has determined that the chemical is a probable human carcinogen (B2) (US EPA, 1994).

The chemical was demonstrated to be carcinogenic in two strains of rats in long-term carcinogenic studies, inducing significant levels of splenic tumours at high doses in males. However, a carcinogenic effect could not be demonstrated in mice in similar long-term studies. Although the data from various in vitro or in vivo genotoxicity assays were mixed, the chemical was demonstrated to be genotoxic in vivo in rats and in mice. Although the data on carcinogenicity in humans are inadequate, it was concluded that there is no association between duration of employment where the chemical was used and increased risk of bladder cancer in chemical product workers. Therefore, as the chemical is stated to be metabolised similarly in rats and humans, a carcinogenic hazard for the chemical could not be disregarded.

In a carcinogenicity study, the chemical (as aniline hydrochloride) was administered to F344 rats at dietary levels of 0, 200, 600, and 2000 ppm for two years. These doses were equivalent to 10, 30 100 mg of aniline hydrochloride/kg bw/day (equivalent to aniline doses of 7, 22, 72 mg/kg bw/day). Male rats from the 2000 ppm group showed an increased incidence of primary splenic sarcomas. Males and females (to a lesser degree) of this group also showed stromal hyperplasia and fibrosis of the splenic red pulp, which may represent a precursor lesion of sarcoma. The majority of tumours seen in the spleen of high-dose males were fibrosarcomas and/or haemangiosarcomas. Splenic fibrosarcomas were also described as invasive with widespread extension in multiple organs of the pleural and abdominal cavities for males and females in the high dose groups (US EPA, 1994; ECB, 2004; HSDB).

In another dietary carcinogenicity study, the chemical (as aniline hydrochloride) was administered at 0, 3000 (0.3 %) or 6000 ppm (0.6 %) to F344 rats for 103 weeks. These doses were equivalent to aniline doses of 174.4 and 360.5 mg/kg bw/day in male and female rats, respectively. A statistically significant dose-related trend in the incidence of haemangiosarcomas and sarcomas or fibrosarcomas was noted in male rats. Male animals also had statistically significant increased incidences of haemangiosarcoma in the spleen; fibrosarcoma, and sarcoma (not otherwise specified) in the body cavity and spleen; and a significant, dose-related trend in the incidence of malignant phaeochromocytoma. An increased incidence of fibrosarcoma and sarcoma in multiple organs of the body cavity in female rats in the study was also noted (US EPA, 1994; ECB, 2004).

In a dietary carcinogenicity study in mice, the chemical (as aniline hydrochloride) was administered at 0, 6000 (0.6 %) or 12000 ppm (1.2 %) to B6C3F1 mice for 103 weeks. No statistically significant increase in any type of tumour in male or female mice relating to the feeding of the chemical was observed in this study (US EPA, 1994; ECB, 2004).

The mechanism of chemical-induced carcinogenicity in animals is not fully clear. It has been proposed that carcinogenicity is induced by damaged erythrocytes leading to an iron overload or oxidative damage to macromolecules (proteins, DNA or lipids) in the spleen by direct binding. Evidence for and against a genotoxic mechanism of carcinogenicity has also been proposed. It was also noted that the chemical is not considered to be a multiple-site carcinogen inducing various primary tumours, even though the tumours in several organs were observed in long-term studies. These occurrences may be caused by the invasion through the splenic capsule involving the abdominal viscera and adjacent organs directly, or by haematogenous metastasising (US EPA, 1994; ECB, 2004; Government of Canada, 2011).

As stated above, human data were limited to epidemiological studies, in which occurrence of bladder tumours among British workers in the chemical dye industry was investigated. These workers were generally exposed to aromatic amines including aniline. An association between duration of employment where the chemical was used and increased risk of bladder cancer in chemical product workers was not established. Splenic cancers were not reported (US EPA, 1994; ECB, 2004).

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.

In a developmental toxicity study (OECD TG 414), female F344 rats (21–24 animals/group) were administered the chemical by oral gavage at 7, 22, and 72 mg/kg bw/day on gestational days 7–20. A maternal NOAEL of 30 mg/kg bw/day could be determined in this study, based on decreased mean absolute body weight gain, increased methaemoglobin formation and altered haematological measures (decreased red blood cells, increased MCV) at the next higher dose of 100 mg/kg bw/day. However, a dose-dependent, statistically significant increase was also observed in the relative spleen weight of the dams during the gestation period. As increased spleen weight at the lowest tested dose of 7 mg/kg bw/day is a reflection of increased erythropoietic activity due to the haematotoxicity induced by the chemical, a NOAEL for maternal toxicity could not be derived for this study. Therefore, a LOAEL of 7 mg/kg bw/day, based on significantly increased relative spleen weights of the dams during gestation period, is derived for maternal toxicity. No treatment-related effect was noted with respect to reproductive parameters such as pregnancy rates, number of corpora lutea per dam, number of implantation sites in each dam, numbers of live foetuses in each litter, average foetal body weight, crown-rump length, relative foetal spleen weight, resorptions, or dead foetuses. Even though pregnant dams treated with the chemical induced chemical-specific haematotoxicity as well as signs of maternally related toxic effects, there was no evidence of developmental effects at the levels of the chemical that caused maternal toxicity. A developmental NOAEL of 100 mg/kg bw/day was determined in this study, based on no adverse effects noted at the highest tested dose (ECB, 2004; HSDB, REACH). A more conservative developmental NOAEL of 22 mg/kg bw/day has also been proposed, based on specific hepatotoxic effects of the chemical on prenatal foetuses and in newborns, as well as some concern about postnatal viability (ECB, 2004).

As part of the above study, additional groups of pregnant dams were also similarly treated to evaluate postnatal development, from parturition to postnatal day 60. There were no major differences in viability, growth, and development, but there were indications of increased haematopoietic activity in the pups of treated dams compared with controls.

The ability of the chemical to induce cleft palate and cardiovascular malformations in a stage-specific manner was also investigated in pregnant rats following subcutaneous injection of the chemical during specific days of gestation. While the chemical did induce cleft palate and cardiovascular malformations in a stage-specific manner in these studies, it was concluded that these effects were due to maternal hypoxia as a result of methaemoglobinemia and not by a direct teratogenic effect of the chemical (Government of Canada, 2011).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute and chronic effects (acute and repeat dose toxicity from oral/dermal/inhalation exposure) and local effects (skin sensitisation). The chemical may be a carcinogen following long-term repeated exposure and may also cause harmful effects through eye irritation.

Public Risk Characterisation

The chemical is currently listed on Schedule 6 of the SUSMP except in preparations containing 1 % or less. At concentrations greater than 1 %, a number of warning statements, first aid instructions and safety directions relating to the chemical apply. Reported uses indicated that public exposure is not expected. The current controls are considered adequate to minimise the risk to public health posed by domestic products containing the chemical. Therefore, the chemical is not considered to pose an unreasonable risk to public health.

The Government of Canada (2011) also concluded that, based on estimates of exposure to the general population, the chemical does not pose a danger to human health in Canada.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure of workers to the chemical may occur, particularly where manual or open processes are used. These may include transfer and blending activities, quality control analysis, and cleaning and maintenance of equipment. Worker exposure to the chemical at lower concentrations may 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.

Given the critical systemic long-term, acute and local health effects, the chemical may pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and inhalation exposure to the chemical 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 appropriate controls.

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. It is noted that the EC Scientific Committee on Occupational Exposure Limits (SCOEL) has recommended a lower TWA of 0.5 ppm (1.94 mg/m^3) and a lower STEL of 1.0 ppm (3.87 mg/m^3) (EC, 2010). Therefore, a Tier III assessment may be necessary to determine whether the current exposure controls are appropriate to offer adequate protection to workers.

Regulatory Control

Public Health

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

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 hazards and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]

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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)
Irritation / Corrosivity	Risk of serious eye damage (Xi; R41)*	Causes serious eye damage - Cat. 1 (H318)
Sensitisation	May cause sensitisation by skin contact (Xi; R43)*	May cause an allergic skin reaction - Cat. 1 (H317)
Repeat Dose Toxicity	Danger of serious damage to health by prolonged exposure (T; R48)*	Causes damage to organs through prolonged or repeated exposure - Cat. 1 (H372)
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 consumers

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

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal, ocular, 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 which may 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 assist with meeting 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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