Benzidine congeners: Human health tier II assessment

07 February 2014

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dichloro-	91-94-1
[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dimethoxy-	119-90-4
[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dimethyl-	119-93-7
[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dichloro-, dihydrochloride	612-83-9

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

Grouping Rationale

All members of the group are 3,3'-disubstituted benzidine congeners. The chemicals are all reasonably anticipated to be human carcinogens. The chemicals all have similar uses.

The chemicals 3,3'-dimethylbenzidine (3,3'-DMB) hydrochloride (CAS No. 612-82-8) and 3,3'-dimethoxybenzidine (3,3'-DMOB) hydrochloride (CAS No. 20325-40-0) are not listed on the Australian Inventory of Chemical Susbtances. However, data on these chemicals are considered relevant to this assessment and have been included where available.

Import, Manufacture and Use

Australian

The following Australian industrial uses were reported, for the chemical 3,3'-dimethylbenzidine (3,3'-DMB) (CAS No. 119-93-7), under previous mandatory and/or voluntary calls for information:

- textile dyeing in mills;
- wood stains and polishes;
- colour in detergents and crepe paper; and
- as identification in metal castings.

According to the previous mandatory and/or voluntary calls for information, the total volume of the chemical DMB introduced into Australia is 0.3 tonne per annum.

The use of the chemicals 3,3'-dichlorobenzidine (3,3'-DCB) (CAS No. 91-94-1) and 3,3'-dichlorobenzidine dihydrochloride (3,3'-DCB 2HCl) (CAS No. 612-83-9) is restricted in Australia (see **Restrictions: Australian**).

International

Restrictions or bans on the use of benzidine congeners in consumer goods including textiles and cosmetics are currently in place in several countries, including the EU (see **Restrictions, International**). As of 2011, these chemicals are no longer produced in large volumes in the USA but are still produced in research quantities in countries such as Germany, Hong Kong, India, Japan, the People's Republic of China, Switzerland, the Netherlands, USA, Belgium and Canada (IARC, 2010; NTP, 2011). However, the following international uses have been identified through European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH) dossiers; Galleria Chemica; Substances and Preparations in the Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and eChemPortal: OECD High Production Volume chemical program—OECD HPV, the US Environmental Protection Agency's Aggregated Computer Toxicology Resource—ACToR, and the US National Library of Medicine's Hazardous Substances Data Bank—HSDB.

The chemicals have reported commercial use including as:

- a curing agent for urethane resins; and
- a compounding agent for rubber and plastics.

The chemicals have reported site-limited use including as intermediates in the production of dyes and pigments for:

- printing inks;
- textiles and leather;
- plastics and enamels;
- paints;
- foams and rubbers;
- diisocyanates for use in adhesives; and
- various polymers.

Other varied uses of the chemicals include as reagents in laboratories, in the petroleum and food industries, and use most recently has extended in laser, liquid crystal displays, ink-jet printers and electro-optical devices (ATSDR, 1988; EPA, 2010; IARC, 2010; NTP, 2011, Government of Canada, 2013).

Restrictions

Australian

Work Health and Safety Regulations

The chemicals 3,3'-DCB (CAS No. 91-94-1) and 3,3'-DCB 2HCl (CAS No. 612-83-9) are listed in Table 10.2 under Schedule 10 as restricted carcinogens, which cannot be used at a concentration greater than 0.1 % without authorisation from the appropriate state or territory regulator (WHS, 2011).

International

Cosmetics

The chemicals are 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;

Association of South East Asian Nations (ASEAN) Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products;

Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient "Hotlist").

Other

The chemicals are also restricted by Annex XVII to REACH Regulation as follows:

- Shall not be used in substances and preparations placed on the market for sale to the general public in individual concentration equal to or greater than: either the relevant concentration specified in Annex I to Directive 67/548/EEC, or the relevant concentration specified in Directive 1999/45/EC.

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals 3,3'-DCB (CAS No. 91-94-1) and its 3,3'-dichlorobenzidine dihydrochloride (3,3'-DCB 2HCl) (CAS No. 612-83-9) are classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

Carc. Cat. 2; R45 (May cause cancer)

Xn; R21 (Acute toxicity)

R43 (Sensitisation)

The chemicals 3,3'-DMB (CAS No. 119-93-7) and 3,3'-DMOB (CAS No. 119-90-4) are classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

Carc. Cat. 2; R45 (May cause cancer)

Xn; R22 (Acute toxicity)

Exposure Standards

Australian

Whilst the chemicals have no specific exposure standard values in Australia, the chemicals 3,3'-DMB (CAS No. 119-93-7) and 3,3'-DCB (CAS No. 91-94-1) have exposure standard entries in the Hazardous Substances Information System (HSIS) (Safe Work Australia). Both chemicals are noted as carcinogen category 2 and that absorption through the skin may be a significant source of exposure.

Guidance on the Interpretation of Workplace Exposure Standards for Airborne Contaminants provides advice that exposure to carcinogens should be eliminated or minimised so far as is reasonably practicable (Safe Work Australia, 2013).

International

The following exposure standards are identified (Galleria Chemica):

3,3'-DMOB (CAS No. 119-90-4) has an exposure limit of 0.03 mg/m^3 (0.003 ppm) time weighted average (TWA) in Germany, Switzerland, and 0.01 mg/m^3 in South Korea. This chemical has a short-term exposure value of 0.12 mg/m^3 (0.012 ppm) in Austria.

3,3'-DMB (CAS No. 119-93-7) has an exposure limit of 0.03 mg/m^3 (0.003 ppm) time weighted average (TWA) in Germany, Switzerland, Austria and maximum allowable concentration (MAC) in China of 0.02 mg/m^3.

The chemical 3,3'-DCB 2HCl (CAS No. 612-83-9) has an exposure limit (TWA) of 0.03 mg/m^3 (0.003 ppm) in Germany and Austria; 0.1 mg/m^3 in the United Arab Emirates (UAE) and 100 mg/m^3 in Italy.

3,3'-DCB (CAS No. 91-94-0) has an exposure limit (TWA) of 0.03 mg/m^3 (0.003 ppm) in Switzerland, Austria and 0.12 mg/m^3 in UAE.

The American Conference of Governmental Industrial Hygienists (ACGIH) does not recommend a numerical threshold limit value for 3,3'-DCB due to the structural similarity to the known human carcinogen benzidine and the lack of relevant data for 3,3'-DCB (ACGIH, 2011).

Health Hazard Information

Toxicokinetics

Evidence from animal and human studies suggests that the chemicals are absorbed following oral, dermal and inhalation exposure. The dermal absorption rate for these chemicals is influenced by increasing temperature and relative humidity.

The chemicals are widely distributed and rapidly metabolised. Metabolism occurs mainly through N-acetylation and N-oxidation, although O-demethylation has also been observed for the chemical 3,3'-DMOB (CAS No. 119-90-4). Reactive intermediates can be formed in the bladder following hydrolysis, in weakly acidic urine, of the glucuronide conjugates. In addition, oxidative activation of the chemicals in the bladder can yield the associated diimine which is proposed to react directly with DNA to form an adduct. The metabolic products are excreted through the urine and to a greater extent the faeces. Metabolites may be excreted in the urine either free or as glucuronides. The chemicals do not appear to accumulate in the body. The half-life of these chemicals in animals was reported to be < 90 hours (Cerniglia et al., 1982; ATSDR, 1998; Sabbione and Schutze, 1998; Lee and Shin, 2002; Wiley VCHa; Wiley VCHb; Wiley VCHc; NTP, 2011; REACH).

Metabolism and distribution of the chemicals is similar to those reported for benzidine (Morgan et al., 1994; IARC, 2010).

Acute Toxicity

Oral

The data for acute toxicity of the chemicals in this group are limited.

The chemicals 3,3'-DMB and 3,3'-DMOB are classified as hazardous with the risk phrase ‘Harmful if swallowed’ (Xn; R22) in HSIS (Safe Work Australia). The available data support this classification.

The chemical 3,3'-DMB is of moderate acute toxicity in an animal test following oral exposure. The median lethal dose (LD50) in rats is 404 mg/kg bw. Rabbits fed with daily doses of 1 gram of 3,3'-DMB in aqueous solution for three days developed lethargy,

anuria and death. When autopsy was performed, discoloration of the kidneys was noted. Pathological changes in the kidneys were also observed in albino rats fed a diet containing a high 3,3'-DMB concentration (Wiley VCHc). However, this study lacked sufficient details to substantiate the findings.

The chemical 3,3'-DMOB has a reported LD50 of 1920 mg/kg bw/day (HSDB). No study details are available.

However, the available data indicate that 3,3'-DCB and 3,3'-DCB 2HCl are of low acute toxicity following oral exposure.

In a reliable acute oral toxicity study for the chemical 3,3'-DCB 2HCl, which was conducted in accordance with the OECD Test Guideline (TG) 423, female HanRcc:WIST (SPF) rats were exposed to 2000 mg/kg bodyweight of the chemical by oral gavage. All animals survived until day 15 when they were sacrificed for macroscopic examination. The results indicated that during the first 30 minutes or one hour, the animals displayed slight sedation, slightly ruffled fur and opacity of the eyes. The described clinical signs persisted up to day three. In addition, all animals showed hunched posture within the second hour of observation and two animals became cyanotic from the fifth hour of the first day. These changes continued until test day two; and thereafter, these clinical symptoms ceased. There was no significant change in bodyweights and no macroscopic findings were reported. Overall, no death was observed in female rats following a single oral treatment of 2000 mg/kg body weight of 3,3'-DCB 2HCl (REACH).

In rats, the acute-duration oral LD50 for 3,3'-DCB administered in pure olive oil was estimated to be 7070 mg/kg, while the LD50 for a 20 % suspension of the dihydrochloride salt in corn oil was 3820 mg/kg (ASTDR, 1998).

Dermal

The chemicals 3,3'-DCB and 3,3'-DCB 2HCl are classified as hazardous with the risk phrase ‘Harmful in contact with skin’ (Xn; R21) in HSIS (Safe Work Australia). Limited data are available to evaluate this classification. The reported median lethal dose (LD50) in rabbits exposed to 3,3'-DCB is >8000 mg/kg body (REACH). In addition, these chemicals are of low acute toxicity following oral exposure. Therefore, the available data do not support this classification and there is sufficient evidence to support a recommendation to remove this classification.

No data are available for the chemicals 3,3'-DMB and 3,3'-DMOB.

Inhalation

No reliable data are available.

Corrosion / Irritation

Skin Irritation

The limited data available indicate that the chemicals are not likely to be irritating to skin. Results from an acute dermal irritation study, similar OECD Test Guideline (TG) 404, showed that exposure of the New Zealand white rabbits to 3,3'-DCB-2HCl did not induce oedema. However, very slight erythema was noted in one animal but this effect was fully reversible within 72 hours (REACH).

In a study in which 3,3'-DMB was applied occlusively for 24 hours to shaved intact or shaved and scarified skin of six albino rabbits, no irritation could be detected on the intact skin and on the scarified skin only slight erythema was noted (Wiley VCHc).

Eye Irritation

Limited data are available. Whilst 3,3'-DCB did not cause any signs of irritation following instillation into rabbit eyes, 3,3'-DCB 2HCl caused erythema, chemosis and corneal opacity. An average score of 84 out of a maximum of 110 was obtained. The score was 70 after one week indicating slow recovery (ACGIH, 2011; REACH).

Although direct comparision with classification criteria is not possible, there is considered sufficient information to recommend classification of 3,3'-DCB 2HCl as at least irritating to the eyes (see **Recommendation** section).

The limited data available indicate that the free base chemicals (i.e 3,3'-DCB, 3,3'-DMB and 3,3'-DMOB) are not likely to be irritating to eyes.

Observation in humans

Upper respiratory tract infection and sore throats have been reported for workers handling 3,3'-DCB 2HCl (ACGIH, 2011).

Sensitisation

Skin Sensitisation

No data are available.

Repeated Dose Toxicity

Oral

In general, effects observed in repeat dose studies in animals were associated with cancer including multi-site tumours, preneoplastic changes and cancer-related mortality (see **Carcinogenicity** section for details).

The toxicology of both 3,3'-DMB HCl (CAS No. 612-82-8) and 3,3'-DMOB HCl (CAS No. 20325-40-0) was investigated in both two-and thirteen-week drinking water studies in rats. Non-cancer effects have been reported in the liver, kidneys, bone marrow and lymphoid organs. The actual dose received was not calculated for these studies. However, the dose conversions (ppm in drinking water to mg/kg/bw/day) in longer term studies indicated the lowest observed adverse effect levels (LOAELs) based on changes in thyroid hormones to be approximately 20-30 mg/kg bw/day for both 3,3'-DMB HCl and 3,3'-DMOB HCl.

In the two-week studies, animals received drinking water concentrations of 3,3'-DMB and 3,3'-DMOB from 600-7500 ppm and from 200-4500 ppm respectively. In the thirteen-week studies, animals received drinking water concentrations of 3,3'-DMB hydrochloride and 3,3'-DMOB hydrochloride from 300-4000 ppm and 170-2500 ppm respectively.

Dose dependent decrease in water consumption and reduced bodyweight gains were observed at higher doses in all studies. Deaths occurred in high dose groups exposed to 3,3'-DMB during the two-week study. In 3,3'-DMOB exposed animals, mortality was observed both in the two- and thirteen-week studies in animals receiving drinking water concentrations 5000 ppm and 2000 ppm, respectively. All 3,3'-DMOB hydrochloride treated rats survived the entire duration of the study.

In the thirteen week study with 3,3'-DMOB, there was an observed increase in weight gain in the liver and kidney and changes to thyroid hormones in all treated groups. 3,3'-DMOB HCl induced marked histopathological changes or lesions in the kidney and thyroid at the highest dose (2500 ppm). These were manifested as chronic nephropathy, and increased pigmentation in the follicular cells of the thyroid (Morgan et al., 1989; NTP 1990a; Wiley VCHb).

In the thirteen-week study with 3,3'-DMB, significant histopathological changes were observed in the liver, kidney, bone marrow, lymphoid organs (spleen, mandibular and mesenteric lymph nodes and thymus), pancreas, and testis of the treated rats. These were typically observed in animals receiving concentrations ≥ 2000 ppm although an increased severity of nephropathy compared with controls was observed in animals receiving 500 ppm. Histopathological changes in the majority of these organs were also observed in the two-week study in animals receiving concentrations of ≥ 5000 ppm. Changes to thyroid hormones were observed in all treated groups (Morgan et al., 1989; NTP, 1990b; Wiley VCHc).

Limited data are available for noncancer effects for 3,3'-DCB and its hydrochloride. Although in a study in which female dogs were exposed to 10.4 mg/kg/day 3,3'-DCB for 7 years, histopathological changes to kidney and liver were not detected (ASTDR,

1998). In general, effects observed in repeat dose studies in animals were cancer related including multi-site tumours, preneoplastic changes and cancer related mortality (see **Carcinogenicity** section for details).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Numerous animal studies indicate that the chemicals are genotoxic in vivo and in vitro (Prival et al., 1984; ASTDR, 1998; Morgan et. al, 1994; IARC, 2010; NTP, 1990a; NTP 1990b). The effects are considered sufficient to warrant classification (see **Recommendation** section).

The chemicals 3,3'-DCB, 3,3'-DMB, 3,3'-DMOB and/or their hydrochloride salts produced mutations in *Salmonella typhimurium* in strains TA98, TA100 and/or TA1535. The majority of positive results were in the presence of metabolic activation; although, 3,3'-DCB and 3,3'-DMOB were reported to be positive in strain TA98 without activation.

All chemicals were positive in vitro for Sister Chromatid Exchange (SCE) and 3,3'-DMB and 3,3'-DMOB were positive in vitro in the Chromosomal Aberration (CA) tests in Chinese Hamster Ovary (CHO).

The genotoxic potential of 3,3'-DMOB was also reported in the liver and urinary bladder cells of humans and rats. Following 20 hours' exposure of primary cultures of hepatocytes (rat and human) to subtoxic doses ranging from 56-180 µm 3,3'-DMOB, Martelli and colleagues (2000) demonstrated a dose-dependent frequency of DNA fragmentation. In rat hepatocytes, an increase in the frequency of micronucleated cells (indicator of chromosomal abnormalities) was observed in cells following 48 hours of treatment with 3,3'-DMOB at 100 and 180 µm doses. Similarly, results of the comet assay performed in the primary human urinary bladder mucosa indicated an increase of DNA fragmentation after 20 hours of exposure. Together, these results suggest that 3,3'-DMOB is genotoxic (Martelli et al., 2000).

In vivo, micronuclei were induced in polychromatic erythrocytes of the liver of foetal mice exposed transplacentally to 3,3'-DCB, as well as in liver cells of adult male mice treated orally with the compound at a reported maximum tolerated dose of 1,000 mg/kg. In addition, a single oral dose of 3,3'-DCB in rats and mice led to extensive binding of the chemical to tissue DNA of the liver, bladder and the small intestine.

Lethal mutations were also observed in the germ cells of *Drosophila melanogaster* following exposure to 3,3'-DMB HCl in the feed or by injection. No similar effects were observed with 3,3'-DMOB.

Carcinogenicity

All four benzidine congeners are classified as hazardous—Category 2 carcinogenic substance—with the risk phrase ‘may cause cancer’ (T; R45) in HSIS (Safe Work Australia). The available data support this classification.

The carcinogenicity of 3,3'-DCB, 3,3'-DMOB, 3,3'-DMB and 3,3'-DCB 2HCl have been extensively studied both in animal investigations and in human clinical observations. The International Agency for Research on Cancer (IARC) has reviewed and subsequently concluded that there is 'sufficient evidence of carcinogenicity' in experimental animals (IARC, 2010). The chemicals are also listed in the National Toxicology Program (NTP) Report on Carcinogens as 'reasonably anticipated to be human carcinogens' (NTP, 2011).

Evidence from several studies demonstrated that chronic exposure to the chemicals induced a variety of tumours in test animals including dogs, rats, mice and hamsters. Species differences are observed which is consistent with differences in metabolism.

In rat studies, the onset of tumours was reported to be at less than the lifetime exposure durations and was observed in several organs of males and females. In the NTP long term carcinogenicity study, rats were orally exposed to 3,3'-DMB (at doses equivalent to approximately 2-13 mg/kg/bw/day) and 3,3'-DMOB (at doses equivalent to approximately 6-24 mg/kg bw/day), for 14 and 21 months respectively. Increased incidences of multi-organ tumour development were observed. This correlated with the significant reduction survival and increased mortality of male and female rats. Exposure of the male and female rats to 3,3'-DMB and 3,3'-DMOB resulted in neoplastic lesions and tumours (adenomas and carcinomas) in the skin, Zymbal's gland, oral cavity epithelium, liver, preputial and clitoral glands and intestines. Few of these pathologies were observed in animals following nine-month interim sacrifice. When quantified, a significant percentage of malignant tumours were observed at all dose levels with 83 to 98 % of the animals from the high dose groups showing malignant tumours. Male rats had greater numbers of neoplastic lesions and tumours in the liver, skin and oral cavity epithelium than females (Morgan et al., 1994). These observations were consistent with the previous findings from the earlier NTP studies in rats which were orally exposed to 3,3'-DMB and 3,3'-DMOB through drinking water (NTP, 2011). DMOB is also reported to cause forestomach tumours in hamsters. Furthermore, subcutaneous injection of 2 mg of 3,3'-DMB in pregnant BALBc mice caused a significant increase in incidence of lung and mammary tumours and leukaemias in the pups (see "**Reproductive and Developmental Toxicity"**) (Wiley VCHc).

The induction of multi-organ tumour by 3,3'-DCB and its dihydrochloride has also been reported following dietary administration or subcutaneous injection in rodents. The findings included cancers of the mammary gland (adenoma), Zymbal's gland (carcinoma), urinary bladder (transitional-cell or papillary transitional-cell carcinoma) and liver, in the form of hepatocellular carcinoma. Other types of tumours that were identified were in the skin and also granulocytic leukaemia. Lymphoid leukaemia was also observed following prenatal exposures to 3,3'-DCB. The chemical has also been reported to cause urinary bladder cancer (transitional-cell or papillary transitional-cell carcinoma) in hamsters and in female dogs, and liver cancer (hepatocellular carcinoma) in female dogs (IARC, 2010; NTP, 2011; Wiley VCHa).

There are data to suggest that 3,3'-DCB may act synergistically with other carcinogens (ASTDR, 1998).

In general, epidemiological studies are inadequate to evaluate the relationship between human cancer and exposure specifically to the chemicals. Confounding factors include coexposure to known human carcinogens, and statistical and study design limitations (IARC, 2010; NTP, 2011). However, it is noted that the sites of tumour formation, ability to form DNA adducts and the metabolic pathways in animals, for the chemicals, are similar to benzidine which is known human carcinogen. The potencies of the chemicals in animal carcinogenicity studies are similar to that of benzidine, based on the studies reported by IARC (IARC, 2010).

Furthermore, in the long term carcinogenicity study in rats with 3,3'-DMB hydrochloride and 3,3'-DMOB hydrochloride (described above), a high percentage of the induced neoplasms contained mutations of the H-*ras* gene. This suggests that activation of cellular ras genes by point mutation may be an important step in the induction of tumours. This is consistent with effects observed with numerous carcinogens (IARC, 2010).

Therefore the chemicals can be reasonably anticipated to be human carcinogens.

Reproductive and Developmental Toxicity

The available information on the reproductive and developmental toxicity of the chemicals are limited.

However, histopathological changes of the kidney have been observed in the offspring of female BALBc mice following exposure subcutaneously to doses of approximately 93.5 mg/kg, four or five times during gestation (ATSDR, 1998). Similar effects were observed with 3,3'-DMB. Changes were observed in the embryos following subcutaneous injection of 2 mg of 3,3'-DMB in 0.1 mL sunflower oil in female BALBc mice from gestational day one to 20. These include morphological alterations in the kidneys of the embryos, specifically hyperplasia of the tubular epithelial cells. When pregnant mice were given the same amount of 3,3'-DMB four to five times during the the last week of gestation, a significant increase in tumour incidence was found (see "**Carcinogenicity**") (Wiley VCHc).

However, no malformations were observed in offspring from pregnant albino rats given doses of 1 ml of 1 % aqueous solution of 3,3'-DMB on gestation days seven to nine, with each animal receiving a total dose of 30 mg (Wiley VCHc). This observation was also consistent with the result from a study in Wistar rats. Exposure of the rats to approximately 55 mg/kg 3,3'-DMB, through subcutaneous injection, at gestational day seven did not cause foetal damage.

Risk Characterisation

Critical Health Effects

The chemicals are both genotoxic and carcinogenic in animals. Based on the similarity to the known human carcinogen benzidine the chemicals are reasonably anticipated to be potent carcinogens in humans.

The chemicals 3,3'-DMB and 3,3'-DMOB may cause sytemic acute effects (acute toxicity by the oral route of exposure).

The chemical 3,3'-DCB 2HCl may also be irritating to the eyes and cause respiratory tract irritation if inhaled.

Public Risk Characterisation

Given the uses identified for the chemical, it is unlikely that the public will be exposed to these chemicals as direct ingredients and hence further risk management for the chemicals themselves is not considered necessary for public safety.

However, the chemicals may be used to manufacture dyes and pigments. Therefore, the public may be exposed to these chemicals as a result of their release from these dyes. The risk to the public from this route of exposure will be considered in the IMAP assessment of benzidine congener dyes.

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 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.

Guidance on the Interpretation of Workplace Exposure Standards for Airborne Contaminants provides advice that exposure to carcinogens should be eliminated or minimised so far as is reasonably practicable (Safe Work Australia, 2013). The use of the chemicals 3,3'-DCB and its hydrochloride 3,3'-DCB 2HCl is restricted in Australia (see **Restrictions: Australian**). Given the similarity in toxicological profile, similar restrictions for 3,3'-DMB and 3,3'-DMOB may be appropriate.

The data available support an amendment to the hazard classification in HSIS (refer to **Recommendation section**).

NICNAS Recommendation

The use of the chemicals 3,3'-DCB and its hydrochloride 3,3'-DCB 2HCl is restricted in Australia (see **Restrictions: Australian**). Given the similarity in toxicological profile, similar restrictions for 3,3'-DMB and 3,3'-DMOB may be appropriate. Safe Work Australia should consider whether current controls are adequate for these chemicals to minimise the risk to workers.

Assessment of the chemicals 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.

However, the chemicals may be used to manufacture dyes and pigments. Recommendations for additional regulatory controls may be required to limit exposure to the chemicals as a result of the release from these dyes. This will be considered in the IMAP assessment of benzidine congener dyes.

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 hazards and environmental hazards.

NB

The acute toxicity classification is the existing classification for 3,3'-DMB and 3,3'-DMOB and only applies to these chemicals.

The chemicals 3,3'-DCB and 3,3'-DCB 2HCl are classified as hazardous with the risk phrase 'Harmful in contact with skin' (Xn; R21) in HSIS (Safe Work Australia). There is sufficient evidence to support a recommendation to remove this classification.

The irritation classification only applies to 3,3'-DCB 2HCl.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)*	Harmful if swallowed - Cat. 4 (H302)
Irritation / Corrosivity	Irritating to eyes (Xi; R36)	Causes serious eye irritation - Cat. 2A (H319)
Genotoxicity	Muta. Cat 3 - Possible risk of irreversible effects (Xn; R68)	Suspected of causing genetic defects - Cat. 2 (H341)
Carcinogenicity	Carc. Cat 2 - May cause cancer (T; R45)*	May cause cancer - Cat. 1B (H350)

[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, ocular, and inhalation exposure to the chemicals 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;
- 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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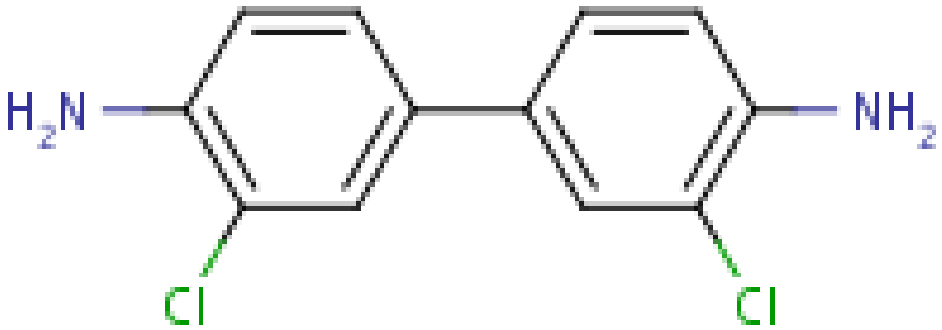
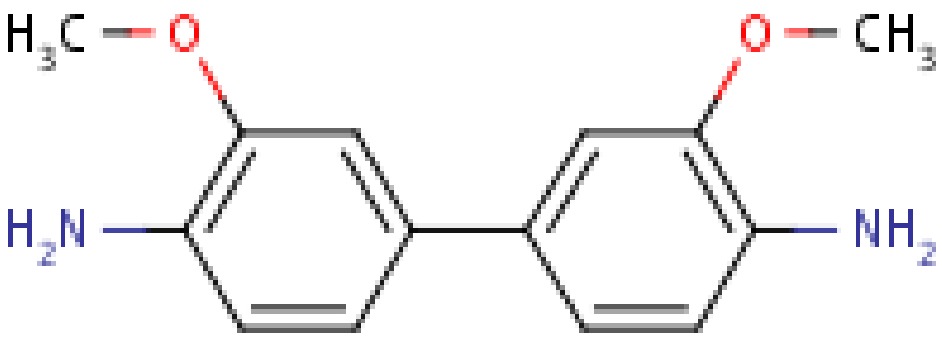
Last Update 07 February 2014

Chemical Identities

Chemical Name in the Inventory and Synonyms	**[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dichloro-** 3,3'-dichlorobenzidine 3,3'-dichloro-(1,1'-biphenyl)-4,4'-diamine 3,3'-dichloro-4,4'-biphenyldiamine 3,3'-DCB 3,3'-dichloro-p,p'-bianiline
CAS Number	91-94-1
Structural Formula	

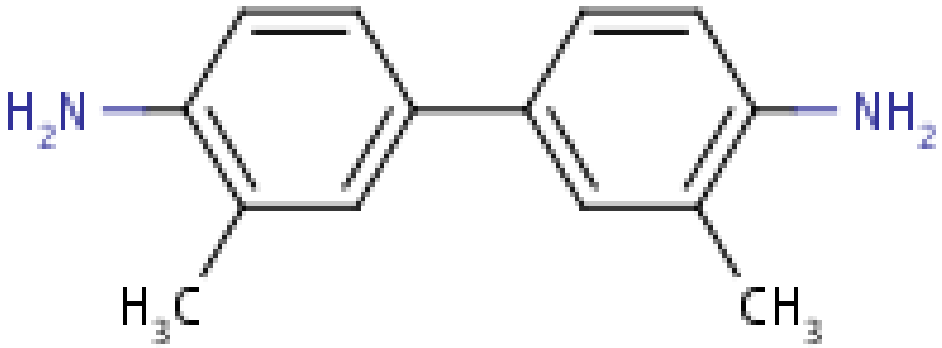
	
Molecular Formula	C12H10Cl2N2
Molecular Weight	253.13

Chemical Name in the Inventory and Synonyms	**[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dimethoxy-** 3,3'-dimethoxybenzidine 3,3'-dianisidine 3,3'-dimethoxy-4,4'-diaminobiphenyl acetamine Diazo Navy RD 3,3'-DMOB
CAS Number	119-90-4
Structural Formula	

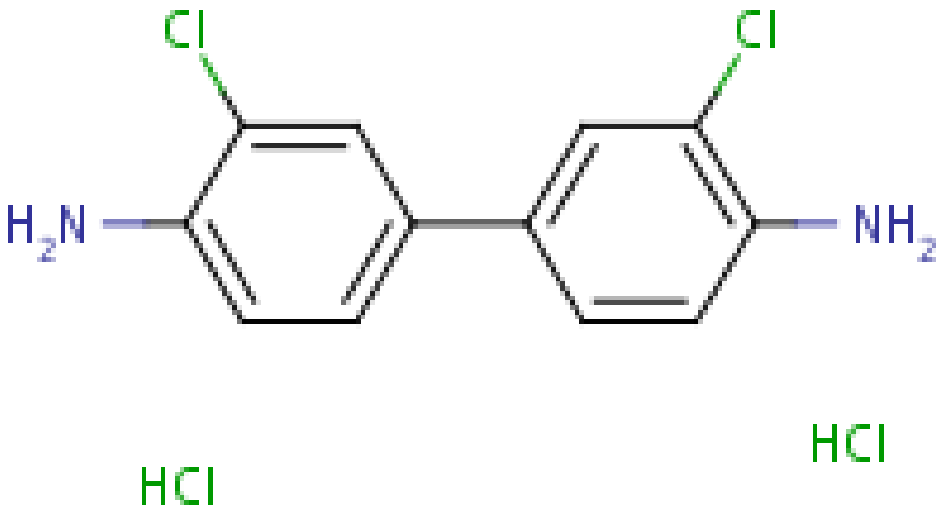


Molecular Formula	C14H16N2O2
Molecular Weight	244.29

Chemical Name in the Inventory and Synonyms	**[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dimethyl-** 3,3'-dimethylbenzidine o-tolidine 3,3'-dimethyl-4,4'-diaminobiphenyl 3,3'-DMB
CAS Number	119-93-7
Structural Formula	

	
Molecular Formula	C14H16N2
Molecular Weight	212.29

Chemical Name in the Inventory and Synonyms	**[1,1'-Biphenyl]-4,4'-diamine, 3,3'-dichloro-, dihydrochloride** 3,3-dichlorobenzidine, dihydrochloride (1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-, dihydrochloride benzidine, 3,3'-dichloro-, dihydrochloride AI3-22046 3,3'-DCB 2HCl
CAS Number	612-83-9
Structural Formula	

	
Molecular Formula	C12H10Cl2N2.2ClH
Molecular Weight	326.05

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