Dichloroanilines: Human health tier II assessment

10 March 2017

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Benzenamine, 3,4-dichloro-	95-76-1
Benzenamine, 2,5-dichloro-	95-82-9
Benzenamine, 2,4-dichloro-	554-00-7
Benzenamine, 2,3-dichloro-	608-27-5
Benzenamine, 2,6-dichloro-	608-31-1
Benzenamine, 3,5-dichloro-	626-43-7

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**

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ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals in this group are the six isomers of dichloroaniline. The chemicals are:

- benzenamine, 3,4-dichloro- (3,4-DCA) (CAS No 95-76-1);
- benzenamine, 2,5-dichloro- (2,5-DCA) (CAS No 95-82-9);
- benzenamine, 2,4-dichloro- (2,4-DCA) (CAS No 554-00-7);
- benzenamine, 2,3-dichloro- (2,3-DCA) (CAS No 608-27-5);
- benzenamine, 2,6-dichloro- (2,6-DCA) (CAS No 608-31-1); and
- benzenamine, 3,5-dichloro- (3,5-DCA) (CAS No 626-43-7).

The following considerations justify the inclusion of these chemicals into a group:

- functional group structural similarity including two chlorine and one amino group attached directly to the benzene ring;
- similarity of the physico-chemical properties including melting point, boiling point and log Kow; and

- similarity in most toxicologically relevant human health effects, where available, including acute toxicity, repeated dose toxicity, and genotoxicity.

Import, Manufacture and Use

Australian

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

International

The chemicals have reported international site-limited use as intermediates in the production of pesticides, dyes (including azo dyes) and antimicrobials identified through the following sources: the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Galleria Chemica (Galleria); Government of Canada (2016); the European Chemicals Bureau (ECB, 2006); the United States National Toxicology Program (US NTP); the Substances and Preparations in Nordic countries (SPIN) database; the US Environmental Protection Agency's (EPA) Aggregated Computer Toxicology Resource (ACToR); the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and Otutu (2012).

Restrictions

Australian

No known industrial restrictions have been identified.

The maximum residue limits of linuron (urea, N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl-; CAS No. 330-55-2) plus 3,4-DCA in parsnip, chia and vegetables (except celeriac, celery, leek) of 0.05 mg/kg have been established in Schedule 20 of the Australia New Zealand Food Standards Code (FSANZ, 2017).

International

The chemicals are listed in Health Canada’s Cosmetic Ingredient Hotlist (under 'Aniline (CAS RN 62-53-3), its salts and its halogenated and sulfonated derivatives') (Government of Canada, 2016).

Existing Worker Health and Safety Controls

Hazard Classification

The chemical, 3,4-DCA (CAS no. 95-76-1) is classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemical Information System (HCIS) (Safe Work Australia):

Acute toxicity – category 3; H331 (Toxic if inhaled) H311 (Toxic in contact with skin) H301 (Toxic if swallowed)

Eye damage – category 1; H318 (Causes serious eye damage)

Skin sensitisation – category 1; H317 (May cause an allergic skin reaction)

The other chemicals in this group are not listed by their CAS numbers on the HCIS but are classified as hazardous under the general category 'chloroanilines, with exception of those specified elsewhere in this database', with the following hazard

categories and hazard statements for human healthh in the HCIS (Safe Work Australia):

- Acute toxicity – category 3; H331 (Toxic if inhaled) H311 (Toxic in contact with skin) H301 (Toxic if swallowed)
- Specific target organ toxicity – category 2; H373 (May cause damage to organs through prolonged or repeated exposure)

Exposure Standards

Australian

No specific exposure standards are available.

International

The exposure standards are mostly for a general group of chemicals under 'aniline & homologues'. Time weighted average (TWA) exposure standards in Bulgaria, Belgium, Egypt, Korea, New Zealand, South Africa and United States varies between 0.5 - 19 mg/m^3 for 8 hours (Galleria).

Health Hazard Information

Limited hazard information is available for the individual dichloroaniline (DCA) isomers. The majority of available data are for the 3,4-DCA and 2,3-DCA isomers and some isomers have little or no data. Read-across has been used for all six DCA isomers to assess hazard, although the read across is of low reliability given the likely significant isomeric differences.

While microbial dechlorination may occur under different environmental conditions (Okutman Tas & Pavlostathis, 2013), the potential for dechlorination has not been demonstrated in mammalian cells (see Toxicokinetics). Therefore, aniline or monochlorinated anilines are not used for read-across.

Toxicokinetics

Limited data are available for the DCA isomers. Based on the available information, the chemicals are expected to be rapidly absorbed, distributed, and excreted following oral exposure. Absorption in the skin is low. Hydroxylated and acetylated metabolites of 3,4-DCA were reported from in vitro studies.

Absorption and distribution

In an excretion study, 5.04 µg of ^{14}C-3,4-DCA was orally administered to male Wistar rats (n=2). Approximately 80 % of the radioactivity was excreted in the urine within 24 hours after administration. After 72 hours, the liver, kidney, muscle, and blood contained = 1% and adrenals, thyroid, and spleen = 0.1% of the radiolabelled dose (Worobey and Shields, 1991; ECB, 2006).

In an in vivo study, rats (strain not specified; n=3/group) were treated with 40 µL of 3,4-DCA or 3,5-DCA solution (30 % in methanol), applied topically to the shaved dorsal skin or administered the same dose by gavage before urine sampling. The urine samples were collected for a period of 24 hours after treatment and the excretion of 3,4-DCA and 3,5-DCA was investigated using gas chromatography mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) methods after urine extraction. The study reported unchanged 3,4-DCA or 3,5-DCA and some metabolites (not identified) were excreted 24 hours after administration (REACHf).

The rate of dermal absorption of 3,4-DCA was estimated in an Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) study “Skin absorption: In Vitro method”. The epidermal side of the human abdominal skin was treated with ^{14}C-3,4-DCA (10 µg in PEG-400 or vaseline or 11.1 µg in liquid soap) in a diffusion cell. The calculated dermal absorption extents in 24 hours were 0.018 % from polyethylene glycol (PEG)-400, 0.55 % from Vaseline and 0.33 % from liquid

soap. Removal of the stratum corneum increased the permeability constant (approximately 140 times in PEG-400) suggesting that the permeability of 3,4-DCA through intact skin is poor but that it increases markedly in compromised skin (HSDB).

In another in vitro percutaneous absorption study, the epidermal side of the dorsal skin of nude female rats was treated with 2 mg/cm^2 3,4-DCA (20 % in chlorobenzene) in a Franz diffusion cell. The movement through the barrier proceeded slowly with little absorption measurable in the first 4 h and reached a measurable concentration after 10 h (19.7 % of the applied dose was absorbed). A steady state was not achieved. In the same study, the penetration of 3,5-DCA was shown to be less than that of 3,4-DCA (HSDB; REACHf).

Metabolism

In in vitro studies, both ring hydroxylated (2- and 6-hydroxy-3,4-DCA) and N-hydroxylated (N-hydroxy-3,4-DCA) and N-acylated (N-(3,4-dichlorophenyl) acetamide and N-(3,4-dichlorophenyl) formamide metabolites of 3,4-DCA were detected (ECB, 2006).

Elimination

Following oral administration of ^{14}C-3,4-DCA (5.04 µg/rat as described above), 81 % of the radioactivity was detected in urine and 26 % in faeces with the majority of the radioactivity excreted within the first 24 hours after administration. After 72 hours of administration, the chemical had been excreted completely (ECB, 2006; Worobey and Shields, 1991).

Acute Toxicity

Oral

The chemicals are classified as hazardous with hazard category 'Acute toxicity – category 3 (oral)' and hazard statement ‘Toxic if swallowed’ (H301) in HCIS (Safe Work Australia). The reported median lethal dose (LD50) values for DCA isomers in rats range from 340 to 3110 mg/kg bw, but are mainly below 1000 mg/kg bw, supporting this classification. Observed sub-lethal effects include acute symptoms of methaemoglobinemia (cyanosis), fatigue, dyspnoea, and muscle weakness.

The primary toxic effect of DCAs is expected to be methaemoglobin formation (Valentovic et al., 1995; Valentovic et al., 1997; ECB, 2006). In addition, acute administration of DCAs alters renal function in rats (Lo et al., 1990). The order of decreasing nephrotoxic potential was 3,5-DCA > 2,5-DCA > 2,4-DCA, 2,6-DCA, 3,4-DCA > 2,3-DCA (Lo et al.,1990).

In an OECD TG 401 'Acute Oral Toxicity' study, Wistar rats (n=5/sex/dose) were treated with 2000, 2500, 3150, and 4000 mg/kg bw of 2,4-DCA (in 2 % aequous potato starch) via oral gavage. A calculated LD50 value of 3110 mg/kg bw was reported for male and female rats. Clinical signs included cyanosis (up to 4 days post application), irregular respiration, blood-coloured secretions of noses and eyes, clonic convulsions and miosis, and nonspecific symptoms of the musculoskeletal system. All symptoms disappeared within 6 days (REACHc).

In a study similar to OECD TG 401, male and female Wistar rats were treated with 3,4-DCA (in 1 % carboxymethylcellulose) via oral gavage. The calculated LD50s were 880 mg/kg bw for males and 530 mg/kg bw for females. Observed signs of toxicity were agitation, difficult breathing and cyanosis (REACHa).

In a study similar to OECD TG 401, male Wistar TNO W74 rats (n=10/dose) were treated with 300, 500, 600, 700 or 800 mg/kg bw of 3,4-DCA via oral gavage. No toxicity was evident in rats treated with 300 mg/kg bw. All other groups showed increasing signs of toxicity including diarrhoea, chromodacryorrhea, bloody snout, face down position, reduced spontaneous activity, narcosis, paralysis of hind extremities. The calculated LD50 was 570 mg/kg bw (REACHa).

In a study similar to OECD TG 401, male and female NMRI mice were treated with 3,4-DCA (in 1 % carboxymethylcellulose) via oral gavage. No further study details were available. The calculated LD50 values for mice were 510 mg/kg bw for males and 470 mg/kg bw for females. Observed signs of toxicity were agitation, difficult breathing and cyanosis (REACHa).

In a non-guideline study, Sprague-Dawley rats (5/dose/sex) received a single oral (gavage) dose of 631, 794, 1000, 1260, 1580 mg/kg bw of 2,3-DCA. The calculated LD50 value was 940 mg/kg bw. The clinical signs of toxicity included reduced appetite and activity (one to two days in survivors), increasing weakness and ocular discharge (REACHd).

In a non-guideline study, Wistar rats (15/dose/sex) received a single oral (gavage) dose of 250-5000 mg/kg bw (males) or 500-3500 mg/kg bw (females) of 2,3-DCA (in polyethylene glycol). The calculated LD50 values were 2635 mg/kg bw (males) and 2489 mg/kg bw (females). The clinical signs of toxicity included difficulties in breathing, tremor, cyanotic discolouration of skin and visible mucous membranes (REACHd).

Dermal

The chemicals are classified as hazardous with hazard category 'Acute toxicity – category 3 (dermal)' and hazard statement 'Toxic in contact with skin' (H311) in HCIS (Safe Work Australia). The reported LD50 values in rabbits are <1000 mg/kg bw supporting this classification. Observed sub-lethal effects included cyanosis, salivation, lacrimation, prostration and ataxia.

Male albino rabbits (n=1/dose) were treated with 130, 200, 300, 450, 670, 1,000 or 1,500 mg/kg bw of 3,4-DCA in acetone (50% wt/vol) applied to the backs and covered with elastic and with adhesive bandage. After 24 hours, the bandages were uncovered, skin washed and observed for 14 days, or until death. Doses of 300 mg/kg bw and higher were lethal. The LD50 was not determined. The chemical caused kidney and liver pathology (not specified) as well as lung and liver congestion (ECB, 2006). The clinical signs included cyanosis, salivation, lacrimation, prostration and ataxia.

In a non-guideline study, 3,4-DCA was administered on the skin of rabbits (no other study details available) at a dose range of 400 to 2,500 mg/kg bw. The animals died at doses 1,000 and 2,500 mg/kg bw. The dermal LD50 was regarded to be 631 < LD50 < 1,000 mg/kg bw (ECB, 2006).

In a non-guideline 14 day toxicity study, Wistar rats received 500, 750, 1000 µL/kg bw (males) or 100, 250, 500, 1000 µL/kg bw (females) of undiluted 2,3-DCA on the shaved skin for 24 hours. The calculated LD50 was 934 µL/kg bw (estimated as 934 mg/kg bw; males) and >1000 µL/kg bw (>1000 mg/kg bw; females). Clinical signs included reduced general condition, sedation difficulties in breathing, cramps, and inflammation at the application sides (REACHd).

Inhalation

The chemicals are classified as hazardous with hazard category 'Acute toxicity – category 3 (inhalation)' and hazard statement 'Toxic if inhaled' (H331) in the HCIS (Safe Work Australia). The reported median lethal concentrations (LC50) for 3,4-DCA is 3300 mg/m^3/4 hours (3.3 mg/L/4 hours) supporting this classification. Observed sub-lethal effects included methaemoglobinemia (cyanosis), lethargy, and staggering.

In a OECD TG 403 "Acute Inhalation Toxicity" study in rats, the LC50 value was found to be greater than the highest tested concentration of 631 mg/m³/4 hours following aerosol exposure to 3,4-DCA (ECB, 2008).

In a non-guideline study, the gaseous or aerosolised form of 3,4-DCA was lethal in 4-10 out of 10 rats at concentrations 2800 to 4700 mg/m³ for 4 hours. This corresponded to a LC50 value of 3300 mg/m³/4 hours. No lethality was seen at concentrations of 47 to 2200 mg/m³/4 hours. Rats exposed to 840, 2200 and 2800 mg/m³/4 hours were lethargic and/or limp and were staggering. The surviving rats had methaemoglobin values of approximately 28% while deaths occurred at methaemoglobin values of 47-62 %. The average percentage of methaemoglobin had returned to baseline by approximately nine days post exposure (ECB, 2006).

In an OECD TG 403 study, Wistar rats (10/sex/dose) were exposed to 0, 809, 8047 mg/m³ or air (control) of 2,3-DCA (nose/head only) for 4 hours. No deaths occurred in the study resulting in LC50 value >8047 mg/m³/4 hours.

Corrosion / Irritation

Skin Irritation

The chemical 2,3-DCA was shown to be highly irritating to the skin of rabbits. Based on this, hazard classification is warranted for the 2,3-DCA. Minimal skin irritation in rabbits was reported for 2,4-DCA and 3,4-DCA, while no information is available for the

rest of the chemicals in the group. Therefore, based on the available information, the classification should not be applied to other chemicals in the group.

In an OECD TG 404 'Acute Dermal Irritation / Corrosion' study, New Zealand White (NZW) rabbits (n=3) received the undiluted chemical 2,3-DCA on shaved skin (semiocclusive) for 4 hours. Erythema (mean score 2.9 out of 4) was observed in treated skin of all rabbits up to 14 days post exposure. Oedema (mean score 1.9 out of 4) was observed in treated skin of all rabbits (mean score 1.9) and was fully reversed by 14 days post exposure. Eschar formation occurred in treated skin of all rabbits up to 14 days post exposure. Treated skin was fissured in all treated rabbits up to day 7, but was reversible within 14 days. Hardened skin was observed in the treated skin of one rabbit on day 7 and whitish squamous coat on the treated skin of all rabbits on day 14 (REACHd).

In an OECD TG 404 study, NZW rabbits (n=3) received 500 mg of 2,4-DCA moistened with PEG-400 to shaved skin under semi-occlusive dressing for 4 hours. Afterwards the area was washed with lukewarm water and regularly observed for up to 7 days. The treated area showed very slight redness (mean score: 0.8) and very slight oedema (mean score 0.1) which disappeared within the 7 day observation period suggesting very slight irritation (REACHc).

In a non-guideline study, with 24 hour treatment with 2,4-DCA under occlusive conditions, the chemical was reported as moderately irritating to rabbits (REACHc). No other study details were provided.

In a Draize skin test, 3,4-DCA was slightly irritating to rabbit skin. Slight reversible erythema (grade 1, reversible in two days) and no oedema were reported (ECB, 2006).

In a previously described acute dermal toxicity test (see **Acute toxicity: Dermal**), the chemical 3,4-DCA in acetone (50 % wt/vol) applied to the backs of male albino rabbits. All the treated skin was slightly red and swollen 24 hours after application of the chemical. No signs of acne was seen on skin of the rabbits at 14 days after application of the chemical (ECB, 2006).

Eye Irritation

The chemical 3,4-DCA is classified as hazardous with hazard category 'Eye damage – category 1' and hazard statement ‘Causes severe eye damage’ (H318) in HCIS (Safe Work Australia). The data available for 3,4-DCA support this classification. Additionally, 2,3-DCA and 2,4-DCA are reportedly irritating to rabbit eyes. Based on these findings, hazard classification should be applied to all the chemicals in this group (see **Recommendation** section).

In an eye irritation study in rabbits (Draize eye test according to OECD TG 405), moderate reversible irritation of conjunctivae, iris and cornea (reversible within 13 days) were observed after application of neat 3,4-DCA. Serious damage of the eye, observed as vascularisation of the cornea, was seen in 2 out of 3 rabbits beginning at day 7 and this was still present at day 14 (ECB, 2006).

In an eye irritation study in rabbits (Draize eye test according to OECD TG 405), mild but reversible irritation of the conjunctivae, iris and cornea, conjunctival oedema, and corneal opacity were reported after application of neat 3,4-DCA. Vascularisation of the cornea was observed in 1 out of 3 animals, beginning 7 days after application of the chemical and was still present after 14 days (ECB, 2006).

In an eye irritation study in rabbits (Draize eye test according to OECD TG 405), 100 mg of neat 2,4-DCA was applied into the conjunctival sac of the left eye of each of the 3 rabbits. The respective right eye served as the control. The ocular reactions were observed at 1, 24, 48, 72 hours and 7 and 14 days after application. The eyes were rinsed at 24 hours post application. Mean scores for cornea (0.7 of max 4), conjunctivae (1.6 of max 3), chemosis (1.2 of max 4), and iris (0.6 of max 2) were reported up to 72 hours. Reactions were reversible within 14 days (REACHc).

In an eye irritation study in rabbits (Draize eye test according to OECD TG 405), 100 mg of neat 2,3-DCA was applied into the conjunctival sac of the left eye of 3 rabbits. One hour after application, the treated corneas of the rabbits were swollen and red and clear discharges were evident. Mild irritation was observed in treated eyes in all rabbits up to 7 days and vascularisation of the cornea was observed in one animal as late as 7 days post application. Due to the vascularisation of the cornea, 2,3-DCA is considered irritating (REACHd).

Observation in humans

Cases of chloracne have been reported in workers exposed to industrial grade 3,4-DCA, but the skin effects were attributed to contamination with 3,3',4,4'-tetrachloroazobenzene or 3,3',4,4'-tetrachloroazoxybenzene. This is supported by findings that since the introduction of impurity-free 3,4-DCA, no cases of chloracne have been reported (ECB, 2006; Toxline).

Sensitisation

Skin Sensitisation

The chemical 3,4-DCA is classified as hazardous with hazard category 'Skin sensitisation – category 1' and hazard statement 'May cause an allergic skin reaction' (H317) in HCIS (Safe Work Australia). The data available for 3,4-DCA support this classification. Additionally, 2,5-DCA is a potential skin sensitiser. Based on these findings, hazard classification should be applied to all the chemicals in this group (see **Recommendation** section).

In a Magnusson Kligman maximisation test, 20 male guinea pigs received an intradermal injection of 2.5 % and a topical application of 50 % chemical 3,4-DCA (in vehicle propylene glycol). Ten guinea pigs were used as controls. A first challenge was conducted at 50 % and a second challenge at 5 % and 25 % of the chemical in vehicle. The dermal reactions were evaluated 48 and 72 hours after challenge. While some control animals had dermal reactions, the frequency and intensity of the dermal reactions observed in test animals demonstrated a sensitisation potential with up to 75 % (15/20) of the test animals showing a positive reaction to the challenge (ECM, 2006).

A maximisation test on the guinea pigs indicated that 2,5-DCA has skin sensitising potential (HSDB).

Repeated Dose Toxicity

Oral

The chemicals (except 3,4-DCA) are classified as hazardous with hazard category 'Specific target organ toxicity (repeated exposure) – category 2' and hazard statement 'Causes damage to organs' (H372). Due to the potential for dichloroanilines to induce haematological changes via all routes, this classification is warranted for all chemicals in the group (see **Recommendation** section).

Similar to effects observed in acute oral toxicity studies (see **Acute Toxicity: Oral, Dermal and Inhalation**), haematological changes are evident after repeated application of dichloroanilines in rats (HSDB).

In a non-guideline study, Wistar rats were orally administered 30, 150 or 750 mg/kg bw/day of 2,5-DCA for 28 days. Haemolytic anaemia with increased medullary and extramedullary erythropoietic activity and hemosiderin deposits in the spleen was reported at dose levels of 150 mg/kg bw/day and higher. In this study, a no observed adverse effect level (NOAEL) of 30 mg/kg bw/day was established (ECM, 2006).

In a non-guideline study, rats (sex or strain not specified) were orally administered (intermittent basis) either 6373 mg/kg bw/day of 3,4-DCA during a 13-week study period. Changes in spleen weight and erythrocyte (red blood cell) count were reported (REACHg).

Dermal

The chemicals are classified as hazardous with hazard category 'Specific target organ toxicity (repeated exposure) – category 2' and hazard statement 'Causes damage to organs' (H372). Due to the potential for dichloroanilines to induce haematological changes via all routes, this classification is warranted.

Haematological changes were observed in acute toxicity studies (see **Acute Toxicity: Oral, Dermal and Inhalation**).

In a skin absorption subacute test, male rabbits (10 animals/group) were applied 60 mg/kg bw/day of 3,4-DCA (99.9 % in 10 % solution in acetone) on the dorsal skin for 6 hours a day for 10 consecutive days. The control group received the vehicle only.

Five animals in each group were terminated on day one post-treatment and the remaining five rabbits thirteen days post-treatment. Blood was obtained from each animal prior to the start of test, on day five and day ten of applications. An increase in methaemoglobin concentrations were observed at days 5 and 10 of treatment. On day one post-treatment, dark brown spleen was observed in all five rabbits of the treated group; two rabbits showed enlarged swollen and heavy spleens (ECB, 2006).

Inhalation

The chemicals are classified as hazardous with hazard category 'Specific target organ toxicity (repeated exposure) – category 2' and hazard statement 'Causes damage to organs' (H372). Due to the potential for dichloroanilines to induce haematological changes via all routes, this classification is warranted.

Haematological changes were observed in acute toxicity studies (see **Acute Toxicity: Oral, Dermal and Inhalation**).

In a 14-day inhalation study with 10, 45, or 200 mg/m^3 of 3,4-DCA (atmospheres containing both vapour and solid particles) to male Crl:CD BR rats (OECD TG 412, 6 hours/day, 5 days/week) a lowest observed adverse effect concentration (LOAEC) of 10 mg/m^3 was derived based on significant increase of methaemoglobin levels (SIAM, 2005; ECB, 2006).

Observation in humans

Methaemoglobinemia was reported in pesticide plant workers following exposure to 3,4-DCA (HSDB).

Genotoxicity

Based on the available information, the chemicals are not genotoxic. While some mutagenic potential has been reported from in vitro studies, the mutagenic potential is unlikely to be expressed in vivo.

In vitro studies

Negative results were obtained in the following in vitro assays (ECB, 2006; REACH; CCRIS):

- Bacterial mutation tests with *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 were negative with and without S9-mix for doses up to 2,000 µg/plate of 3,4-DCA;
- In OECD TG 471 'Bacterial Reverse Mutation Assay' with *S. typhimurium* strains TA97, TA98, TA100 and TA1535 were negative with and without S9-mix for 2,4-DCA;.
- Bacterial mutation tests with *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 were negative with and without S9-mix for doses up to 1,000 µg/plate of 2,5-DCA;
- Bacterial mutation tests with *S. typhimurium* strains TA97, TA98, TA100, and TA1535 were negative with and without S9-mix for doses up to 3,333 µg/plate of 3,4-DCA;
- A mammalian cell gene mutation test with Chinese hamster ovary (CHO) cells was negative for doses up to 250 µg/ml of 3,4-DCA with and without S-9 mix;
- A chromosome aberration (CA) test with human lymphocytes was negative for concentrations up to 1 mmol/L of 3,4-DCA with and without S-9 mix; and
- Unscheduled DNA synthesis (UDS test) with rat liver cells were negative for doses up to 10 µg/ml of 3,4-DCA and up to 1 mmol/L of 2,5-DCA or 3,5-DCA. Another UDS test had an equivocal result because of inconsistent findings for doses ranging from 0.16 to 47.7 µg/ml of 3,4-DCA.

Positive or weakly positive results were obtained in the following in vitro assays (ECB, 2006; REACH):

- In OECD TG 471 'Bacterial Reverse Mutation Assay' with *S. typhimurium* strain TA100 was weakly positive with S9 mix for doses up to 666 µg/plate of 2,4-DCA, but the chemical was negative in another bacterial reverse mutation assay (REACHc).

- Sister chromatid exchange (SCE) test with human lymphocytes was weakly positive with S-9 mix at 0.125 mmol/l of 3,4-DCA and above and the effect was dose-dependent. The test was negative without S9 mix for doses up to 1.0 mmol/l;
- A mitotic spindle damage test with V79 cells was positive after 3-hour exposure to 0.25 and 0.5 mmol/L of 3,4-DCA; the main effect was an induction of monopolar metaphases;
- A CA test with Chinese hamster lung cells was positive (Structural changes and ploidy) with and without S9 mix for 3,5-DCA; and

In vivo studies

Negative results were obtained with 3,4-DCA in micronucleus tests in bone marrow cells of mice after oral gavage of 980 mg/kg bw and in bone marrow cells of mice after intraperitoneal (i.p.) injection of doses up to 200 mg/kg bw (two injections, 24 hours apart) (ECB, 2006).

Carcinogenicity

No data are available for the carcinogenic potential of the chemicals.

In general, there is evidence that methaemoglobin-producing anilines may have non-genotoxic mechanism for the induction of haemangio- and fibrosarcomas in the spleen of rats (US EPA, 1988). This is supported by findings in a related monochlorinated 4-chloroaniline (CAS No 106-47-8) which is classified as Carcinogenicity - category 1B with a hazard statement of ‘May cause cancer’ (HCIS). One of the chemicals in this group, the 3,4-DCA, is also suggested to have carcinogenicity concern (Bruschweiler et al., 2014). The relative potency of dichloroanilines compared to monochlorinated anilines is not known but is assumed to be lower due to the fact that the additional chlorine atom in the benzene ring of dichloroanilines decreases the methaemoglobin-forming activity (ECM, 2006). Following oral administration of 81 mg (0.5 mmol) 3,4-DCA/kg bw for female rats, the haemoglobin binding index (in mmol/mol Hb/dose (mmol/kg bw)) was nine for the assessed chemical 3,4-DCA, compared to 569 for 4-chloroaniline (ECB, 2006).

Reproductive and Developmental Toxicity

Limited data are available for the reproductive and developmental toxicity of the chemicals. However, based on available data, there is no information to indicate that the chemicals are teratogenic.

No data is available to assess reproductive toxicity of the chemicals. Only limited information on potential effects on male reproductive organs can be derived from the 14 day repeated dose inhalation toxicity study of 3,4-DCA in rats (see **Repeat Dose Toxicity: Inhalation**). No changes in absolute and relative testes weights or histopathological appearance of testes and epididymides were reported (ECB, 2006).

In a teratology study, pregnant Charles River Crl:CD BR rats (n=28/dose) were orally administered (gavage) 0, 5, 25, or 125 mg/kg bw/day of 3,4-DCA (as a suspension in aqueous carboxymethylcellulose/Tween 80 vehicle) during gestation days (GD)s 6-15. Maternal toxicity as shown by significantly reduced food consumption and reduced body weight gains was observed at doses 25 and 125 mg/kg bw/day. Borderline developmental toxicity occurred at the maternally toxic high dose level (125 mg/kg bw/day) as indicated by slight and non-significant increase in resorptions and consequently post-implantation loss. No significant or toxicologically relevant effects were reported with doses up to 25 mg/kg bw/day. Based on these findings a NOEAL for maternal toxicity is considered to be 5 mg/kg bw/day and for developmental toxicity 25 mg/kg bw/day.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation for all the chemicals include systemic acute and chronic effects (acute and chronic toxicity from oral/dermal/inhalation exposure and possible carcinogenicity) and local effects (eye irritation and skin sensitisation). Additionally, the chemical 2,3-DCA is irritating to the skin.

Public Risk Characterisation

The chemicals could be used as intermediates in the manufacture of azo dyes and pigments (see **International use** section) which may be used in tattoo inks and textile dyes, and it may then be regenerated by reductive cleavage of the azo dyes. The chemical 3,4-DCA was indicated as a potential aromatic amine cleavage product of concern from azo dyes (Bruschweiler et al., 2014). As such, further regulatory controls for public health may be determined as part of a Tier III assessment for ‘Azo dyes that cleave to aromatic amines of potential toxicological concern’ (NICNAS).

Occupational Risk Characterisation

Given the critical health effects (acute and chronic toxicity, eye irritation and skin sensitisation), the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise exposure to the chemicals are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the Hazardous Chemical Information System (HCIS) (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

Assessment of these chemicals are 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.

The chemicals are recommended for a Tier III assessment as part of the assessment of 'Azo dyes that cleave to aromatic amines of potential toxicological concern' (NICNAS).

Regulatory Control

Public Health

The need for regulatory control for public health will be determined as part of the Tier III assessment.

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

The recommended classification for skin irritation only applies for the chemical 2,3-DCA (CAS No. 608-27-5). All other classifications apply to all chemicals in the group.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

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

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Toxic if swallowed - Cat. 3 (H301)* Toxic in contact with skin - Cat. 3 (H311)* Toxic if inhaled - Cat. 3 (H331)*
Irritation / Corrosivity	Not Applicable	Causes serious eye damage - Cat. 1 (H318) Causes skin irritation - Cat. 2 (H315)
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1 (H317)
Repeat Dose Toxicity	Not Applicable	Causes damage to organs through prolonged or repeated exposure - Cat. 1 (H372)

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

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

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

Advice for consumers

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

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal, 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 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 chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

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

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

Obligations under workplace health and safety legislation

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

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals 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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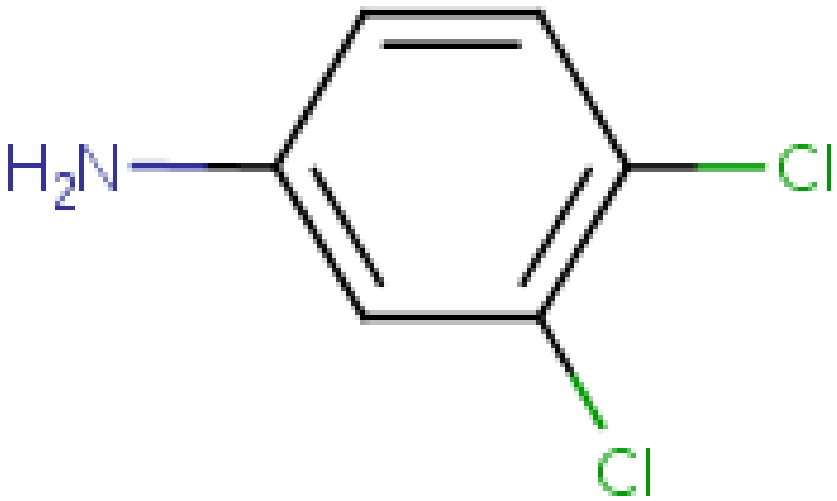
Last Update 10 March 2017

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Benzenamine, 3,4-dichloro-** aniline, 3,4-dichloro- 3,4-dichloroaniline 3,4-DCA 1-amino-3,4-dichlorobenzene
CAS Number	95-76-1
Structural Formula	

	
Molecular Formula	C6H5Cl2N
Molecular Weight	162

Chemical Name in the Inventory and Synonyms	**Benzenamine, 2,5-dichloro-** 2,5-dichloroaniline 2,5-DCA 2,5-dichloro-1-aminobenzene 2,5-dichlorobenzenamine
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	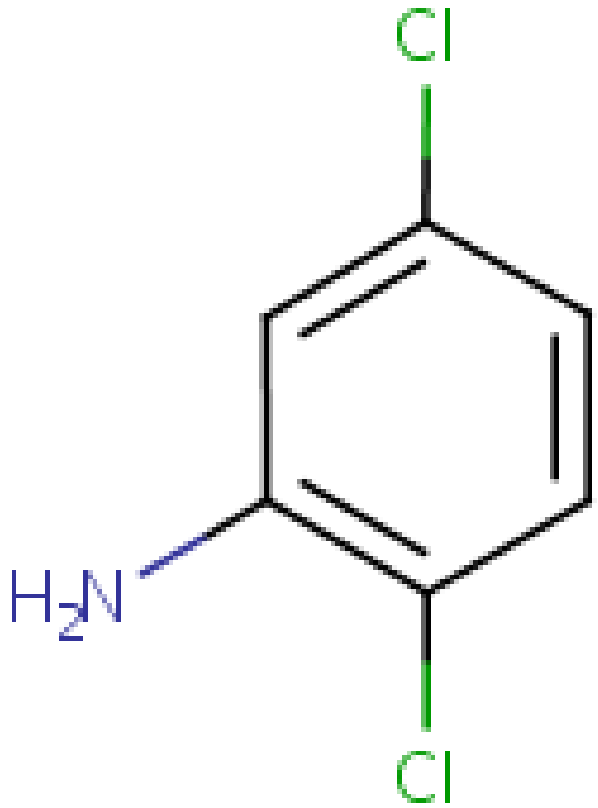
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Molecular Weight	162

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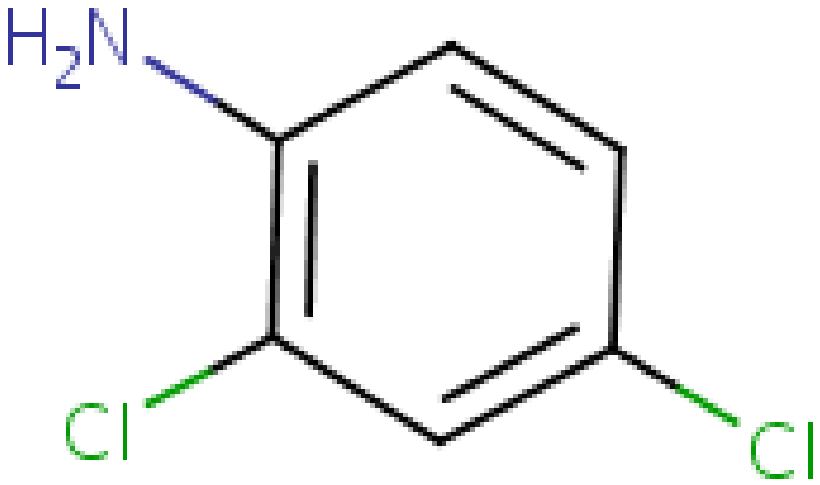
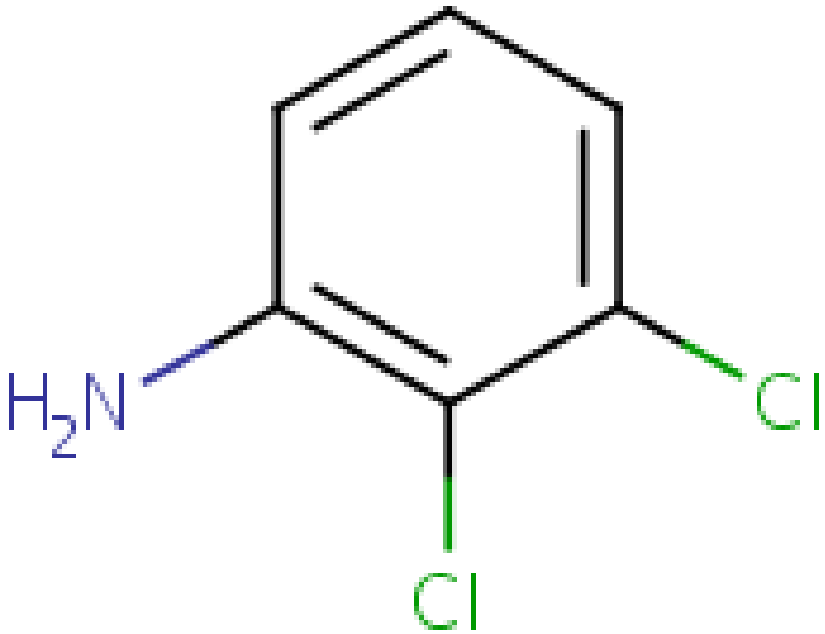
	
Molecular Formula	C6H5Cl2N
Molecular Weight	162

Chemical Name in the Inventory and Synonyms	**Benzenamine, 2,3-dichloro-** 2,3-dichloroaniline 2,3-DCA 2,3-dichlorobenzenamine 2,3-dichlorobenzene-1-amino
CAS Number	608-27-5
Structural Formula	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Molecular Weight	162

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	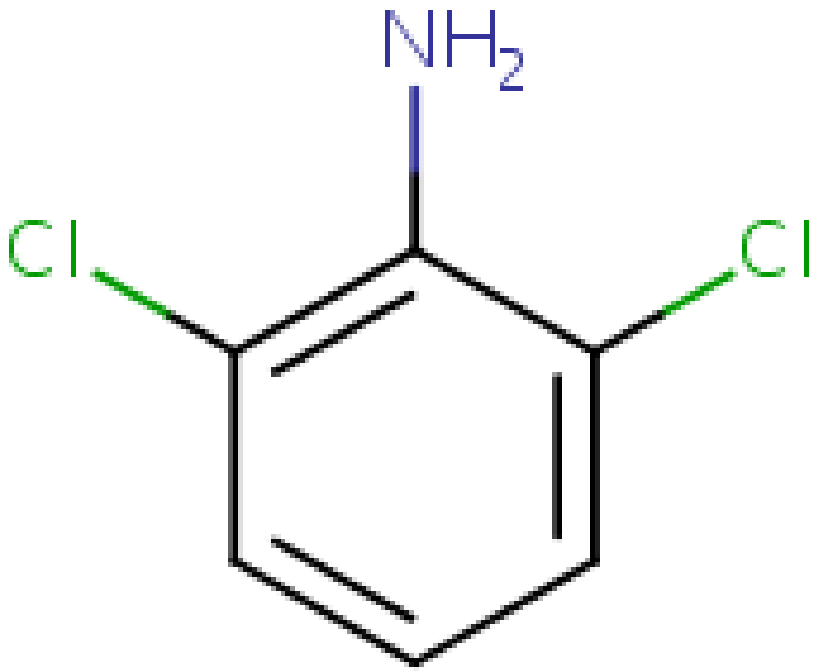
Molecular Formula	C6H5Cl2N
Molecular Weight	162

Chemical Name in the Inventory and Synonyms	**Benzenamine, 3,5-dichloro-** 3,5-dichloroaniline 3,5-DCA 3,5-dichlorobenzenamine 3,5-dichlorobenzene-1-amino
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	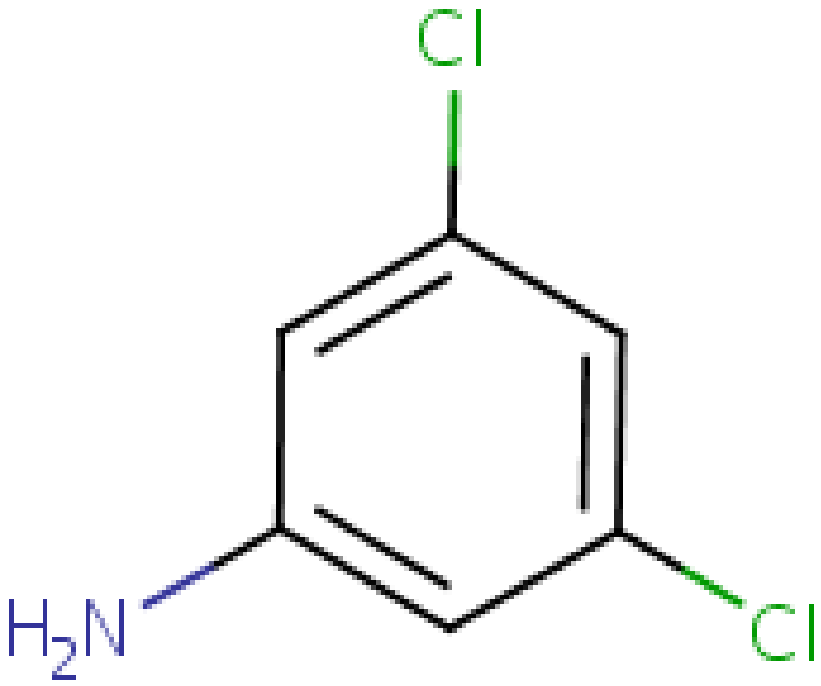
Molecular Formula	C6H5Cl2N
Molecular Weight	162

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