Chlorhexidine: Human health tier II assessment

25 November 2016

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-	55-56-1
2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-, diacetate	56-95-1
2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride	3697-42-5
D-Gluconic acid, compound with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide (2:1)	18472-51-0
D-Gluconic acid, compd. with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide	98474-48-7

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**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals chlorhexidine diacetate (CAS No. 56-95-1), chlorhexidine dihydrochloride (CAS No. 3697-42-5) and chlorhexidine digluconate (CAS Numbers 18472-51-0 and 98474-48-7) are salts of chlorhexidine (CAS No. 55-56-1) with two acetic, hydrochloric and gluconic acids respectively. The speciation of the chlorhexidine moiety in biological fluids will be dependent on pH, but independent of the original form. The sodium salts of acetate, chloride and gluconate are considered to be of low concern and pose no unreasonable risk to human health (NICNAS). Therefore, the chlorhexidine moiety is expected to be responsible for the toxicity of the chemicals.

Import, Manufacture and Use

Australian

No specific Australian industrial use, import, or manufacturing information has been identified.

Non-industrial uses have been identified in Australia. The Australian Register of Therapeutic Goods listed the chemicals as ingredients in a large number of therapeutic goods, including antiseptic wipes, creams and disinfectant lotions (ARTG, 2013).

International

The following international uses have been identified through the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (EU REACH) dossiers; Galleria Chemica; the European Commission Cosmetic Ingredients and Substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) dictionary; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB), the US Department of Health and Human Services Household Products Database (HHPD) and various international assessments (CIR, 1993; EMEA, 1996; VKM, 2010, Government of Canada, 2013).

Chlorhexidine and its salts have reported use in cosmetics with the identified functions as preservatives, antimicrobial and oral care agents. The major reported cosmetic use is in hair treatment applications. Other products include make-up and makeup removers, skin care products, hair colouring products, shampoos, aftershave and mouthwashes and breath fresheners. The concentration in cosmetic products is restricted in several countries (see **International restrictions**). Typical reported concentrations in products are below 0.1 %.

These chemicals have reported domestic uses as hard surface disinfectants and all-purpose antibacterial cleaning agents.

Other reported non-industrial uses of the chemicals include:

- in dental and surgical applications;
- in veterinary applications;
- in topical antiseptics; and
- in sunscreen products.

Restrictions

Australian

The chemicals are listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedules 5, 6 and 7 (SUSMP, 2016).

Schedule 7:

'CHLORHEXIDINE except:

(a) when included in Schedule 5 or 6;

(b) in preparations containing 1 per cent or less of chlorhexidine; or

(c) when in solid preparations.'

Schedule 6:

'CHLORHEXIDINE in preparations containing 7 per cent or less of chlorhexidine except:

(a) when included in Schedule 5;

(b) in preparations containing 1 per cent or less of chlorhexidine; or

(c) when in solid preparations.'

Schedule 5:

'CHLORHEXIDINE in preparations containing 3 per cent or less of chlorhexidine except:

(a) in preparations containing 1 per cent or less of chlorhexidine; or

(b) when in solid preparations.'

Schedule 7 chemicals are described as 'Dangerous Poison – Substances with a high potential for causing harm at low exposure and which require special precautions during manufacture, handling or use. These poisons should be available only to specialised or authorised users who have the skills necessary to handle them safely. Special regulations restricting their availability, possession, storage or use may apply'. Schedule 7 chemicals are labelled with 'Dangerous Poison' (SUSMP, 2016).

Schedule 6 chemicals are described as 'Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label'. Schedule 6 chemicals are labelled with 'Poison' (SUSMP, 2016).

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2016).

International

The chemicals are listed on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex V—List of preservatives allowed in cosmetics;
- New Zealand Cosmetic Products Group Standard—Schedule 7: Preservatives cosmetic products may contain with restrictions—Table 1; and
- Association of South East Nations (ASEAN) Cosmetic Directive Annex VI Part 1: List of preservatives allowed for use in cosmetic products.

For the above, the chemicals are restricted to 0.3 % (as chlorhexidine).

The chemicals are listed on the Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient "Hotlist"). 'The chemicals are permitted at concentrations equal to or less than 0.14 %, calculated as chlorhexidine (CAS No. 55-56-1) free base; 0.19 %, calculated as chlorhexidine diacetate (CAS No. 56-95-1); 0.20 %, calculated as chlorhexidine digluconate (CAS No. 18472-51-0); and 0.16 %, calculated as chlorhexidine dihydrochloride (CAS No. 3697-42-5)'.

The US Cosmetic Ingredient Review (CIR) published the following safety assessment conclusions 'Chlorhexidine and its salts are safe for use in cosmetic products at concentrations up to: 0.14% calculated as Chlorhexidine free base; 0.19% as chlorhexidine diacetate; 0.20% as chlorhexidine digluconate; and 0.16% as chlorhexidine dihydrochloride' (CIR, 1993; CIR; 1999).

The chemicals, with the exception of chlorhexidine acetate, are listed in the Japanese *Standards for Cosmetics*. The chemicals are restricted in a range of cosmetic products in the concentration range 0.001–0.1 % (MHLW).

The chemicals CAS No. 55-56-1; CAS No. 56-91-5 and CAS No. 18472-51-0 are also listed in the *Thailand Cosmetic Act—Prohibited Substances*.

Whilst CAS No. 98474-48-7 is not specifically listed as a CAS number in the international restrictions listed above for chlorhexidine digluconate, these restrictions are considered relevant.

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals are not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

The chemicals are known to degrade into p-chloroaniline (PCA) (CAS No. 106-47-8) with prolonged storage (CIR, 1993). The decomposition process is accelerated by low pH and high temperatures. The PCA concentrations are not expected to be at levels where acute effects occur, but toxicological data for low exposure, repeated dose effects such as carcinogenicity, are considered in this assessment.

Toxicokinetics

The toxicokinetics of the chemicals have been investigated in humans and in a number of laboratory animals (CIR, 1993). Owing to their cationic nature, the chemicals bind strongly to skin and mucosa; thus, they are poorly absorbed through the skin and the gastrointestinal tract (EMEA, 1996; US FDA, 2004).

In laboratory animals (rats, dogs, marmosets and rhesus monkeys), ^{14}C-labelled chlorhexidine was orally administered in gelatine capsules with the radiolabel in either the ring or side chain portion of the chemical. The doses tested were 0.05–50 mg/kg bodyweight (bw). The results showed that 90 % of the administered dose in animals was recovered in the faeces, 2 % in the bile and 0.2–1.3 % in the urine. The main compound identified was the unmetabolised form of chlorhexidine. The oral bioavailability was reported to be only approximately 1 % (CIR, 1993; EMEA, 1996).

In newborn rhesus monkeys bathed daily for 90 days with 8 % of chlorhexidine digluconate in a cleanser, traces of radioactive residues were found in the fat, kidneys, liver and an appreciable amount in the skin (CIR, 1993; EMEA, 1996). Chlorhexidine gluconate has been reported to rapidly bind to protein (CIR, 1993).

In humans, the chemicals were not detected in the blood in several studies where the chemicals were topically applied. Small amounts (0.3 %) have been detected in the urine (CIR, 1993; EMEA, 1996; Government of Canada 2013). Following exposure to radiolabelled chlorhexidine or chlorhexidine gluconate in mouthwash, these chemicals were found to be significantly retained within the oral cavity and were slowly released (CIR, 1993).

By contrast, PCA, the breakdown product of the chemicals being assessed, is readily absorbed by the skin and rapidly absorbed in the gastrointestinal tract. It is widely distributed throughout the body including to the muscle, fat, skin, blood, liver, spleen and kidneys. It is also rapidly metabolised in the liver and eliminated through urinary, faecal and biliary excretion. The metabolic pathways for PCA are C-, N- hydroxylation, N-oxidation, and N-acetylation. The breakdown product, PCA is also reported to bind to haemoglobin and to kidney and liver proteins (NICNAS).

Acute Toxicity

Oral

The chemicals had low to moderate acute toxicity in animal tests following oral exposure. Based on the results from well-documented studies performed according to the Organisation for Economic Co-operation and Development Test Guideline (OECD TG) 401, classification is not considered to be warranted.

In a study performed according to OECD TG guideline 401, groups of Wistar rats (five males/five females) were dosed orally via gavage with chlorhexidine. The median lethal dose (LD50) was estimated to be in the range of 5000 mg/kg bw. At the highest administered dose (5110 mg/kg bw), clinical signs such as tremors, convulsions, prone position, disturbed startle reflexes, diarrhoea, and laboured breathing were observed (REACHa).

In a guideline-compliant study in Wistar rats, the LD50 values for chlorhexidine gluconate were 2270 mg/kg bw for males and 2000 mg/kg bw for females. Mortalities were noted six days after exposure. Psychomotor depression, ataxia, depressed respiratory tract, sporadic incidence of ptosis (drooping eyelid), chromodacryorrhoea (bloody tears), epistaxis (nasal bleeding) and diarrhoea were observed in treated rats (EMEA, 1996; REACHb). In another study, the LD50 was reported to be approximately 2500 mg/kg bw in mice and >3000 mg/kg bw in rats (CIR, 1993; EMEA, 1996). In poorly-documented mouse studies, chlorhexidine gluconate has reported LD50 values of 1260–1800 mg/kg bw (REACHb).

The LD50 value for chlorhexidine diacetate is 1180 mg/kg bw in rats. No study details were available.

Dermal

Based on the available data for chlorhexidine digluconate and chlorhexidine diacetate, the chemicals are considered to have low acute dermal toxicity.

In a dermal acute toxicity study in rabbits (EPA guideline-compliant), exposure to 5000 mg/kg of chlorhexidine gluconate caused increased blood flow (hyperaemia) and skin irritation including eschar (scab) formation. Thickening of the skin was also reported. With the exception of one animal, these observations were reversed within a week. No mortality was observed (REACHa; REACHb).

The dermal LD50 for chlorhexidine diacetate was reported be >2000 mg/kg bw in rabbits (Government of Canada, 2013).

Inhalation

Limited data are available. The median lethal concentration (LC50) in rats for chlorhexidine diacetate was reported to be 300 mg/m^3 (Government of Canada, 2013). The duration of exposure is unknown. No further details were provided.

Observation in humans

Chlorhexidine has been reported to induce bradycardia (slow heart beat) with associated cyanotic spells in a newborn female. This was caused by using chlorhexidine spray on the mother's breast to prevent mastitis from the third feed, when the baby was 12 hours old. Episodes of bradycardia occurred less frequently after spraying stopped (Quinn & Bini, 1989).

Chlorhexidine gluconate has been suggested to induce acute respiratory distress syndrome (ARDS). Accidental ingestion of 200 mL of chlorhexidine gluconate by an 80-year-old female was fatal. Clinical signs observed before death (within 12 hours of ingestion) included hypotension, rapid deterioration of consciousness, progressively deteriorating arterial oxygen and eventually death from ARDS. In another case report, a patient developed ARDS after an intravenous injection of 0.8 mg of the chemical (no further details provided) (REACHb).

Cyanosis and methaemoglobinaemia have also been observed in incubated premature infants exposed to small amounts of PCA resulting from the breakdown of chlorhexidine gluconate in incubators (NICNAS).

Corrosion / Irritation

Skin Irritation

The chemicals are reported to slightly irritate skin in animal studies, particularly following repeated exposure (refer **Repeated dose toxicity** section). The effects were not sufficient to warrant a hazard classification.

In OECD TG 404-compliant studies, chlorhexidine and chlorhexidine digluconate (500 mg/animal) were applied to the shaved skin of New Zealand White (NZW) and Himalayan rabbits for four hours under occlusive and semi-occlusive conditions respectively. Reversible slight erythema was observed (REACHa; REACHb).

Chlorhexidine acetate (concentration approximately 0.04 %) was reported to be marginally irritating to skin in rabbits with a reported primary irritation index of 0.4 (Greener et al., 1985; Government of Canada, 2013).

In a chamber scarification test in humans (closed Duhring chamber), chlorhexidine caused a slight irritation to the skin (see **Irritation: Observation in humans**) (REACHa).

Eye Irritation

Irreversible damage to corneal tissue and corrosion of the conjunctivae and eyelids have been observed following exposure to the chemicals. The available data support recommendation for classification for all the chemicals in this group (see **Recommendation** section).

Results from an OECD TG 405-compliant study demonstrated that a single application of chlorhexidine (0.1 g) to albino rabbits caused irreversible damage to the cornea and iris. After one hour of treatment, partial or total clouding of the cornea were observed, increasing with time. Other effects included conjuntival hyperaemia (increased blood flow) and hypersecretion (REACHa). Irreversible damage to the rabbit eye was observed following a single application of a 20 % aqueous solution of chlorhexidine digluconate (CIR, 1993; REACHb).

Ocular exposure of NZW rabbits to 0.1 mL solution containing 5 % chlorhexidine digluconate resulted in irritation of the cornea, iris and conjunctivae. The eyes of 3/9 animals tested were rinsed with distilled water five seconds after treatment and followed by a two-minute wash. The chemical was reported to be severely irritating in the unrinsed eyes, although effects appeared to be partially reversible by the end of the seven day observation period. Reversible mild irritation was reported in the rinsed eyes (REACHb). In a similar study with a 4 % solution of chlorhexidine gluconate, no irritation of the cornea and iris was observed. Irritation of the conjunctivae was observed, but the effect was reversible and barely detectable after one week (unrinsed eyes) (REACHb). Minimal eye irritant effects have been observed in rabbits following exposure to products containing 0.04–0.05 % chlorhexidine digluconate (CIR, 1993).

In another study, the corneas of cats and NZW rabbits were exposed to chlorhexidine digluconate for 30–40 minutes. Examination of the corneas by scanning electron microscopy (SEM) showed progressive corneal damage between 0.001 % to 0.01 % with minimal damage at concentrations up to 0.005 % (Burnstein, 1980).

Chlorhexidine acetate (concentration approximately 0.04 %) was reported to be marginally irritating to eyes in rabbits with a reported primary irritation index of 0.67 (Greener et al., 1985; Government of Canada, 2013).

Observation in humans

In a chamber scarification test, healthy human volunteers were exposed to 100 µL of 0.5 % chlorhexidine (in water) applied in the forearm skin once daily for three days in a closed Duhring chamber. Scarification was carried out using a needle. The results indicated that chlorhexidine is slightly irritating to the skin (REACHa).

Application of 1 % chlorhexidine with 75 % alcohol as a disinfectant has been reported to be used during blood collection to reduce the bacterial counts in the skin. However, a number of donors reported skin irritation at the application site with itchiness, with or without erythema (Magee, 2007).

Chlorhexidine, as eye drops, has been used to treat acanthamoeba keratitis. Whilst it is considered safe and non-toxic, treatment-related progressive ulcerative keratitis and a number of cases of cataract and iris atrophy have been reported (Magee, 2007).

Sensitisation

Respiratory Sensitisation

Limited data are available for the chemicals. However, two cases of allergic asthma reactions due to exposure to chlorhexidine gluconate in an aerosol were reported (see **Sensitisation: Observation in humans**) (CIR, 1993). The chemical has been reported to cause acute respiratory distress syndrome and eventually death in an elderly female by accidental ingestion (see **Acute toxicity: Observation in humans**).

Skin Sensitisation

There is a lack of strong evidence to support the dermal sensitisation potential of the chemicals in animals in OECD TG 406-compliant studies. Effects observed were not sufficient to warrant classification. However, a number of cases of contact dermatitis in humans were reported following exposure to the chemicals (see **Sensitisation: Observation in humans**). The available data support the recommendation for classification (see **Recommendation** section).

In a guinea pig maximisation test (Dunkin-Hartley), chlorhexidine gluconate was found to be a weak sensitiser at induction concentrations of 2.5 % (intradermal) and 25 % (topical), with a challenge concentration of 12.5 % (CIR, 1993; REACHb). In a split adjuvant test in guinea pigs (inbred from DNCB-sensitive strain), the chemical was reported to be a very weak sensitiser at 2.5 % intradermal induction and 12.5 % challenge concentration. Responses were observed in <30 % of animals. Results from a Buehler test in Dunkin-Hartley guinea pigs were equivocal (REACHb).

Chlorhexidine gluconate was tested in a popliteal lymph node assay in female A/J mice. In this assay, 0.2 mg (20 %) of the chemical was subcutaneously injected into the right hind footpad. Effects were evaluated after seven days by removing the popliteal lymph nodes and counting the dissociated cells. The results showed a significant increase in cellularity index, indicating sensitisation (REACHb).

The primary antibody response to the haptens chlorhexidine digluconate and its N-chloro derivative (generated from the addition of 2 mM chlorine water to chlorhexidine digluconate) was tested in female Balb/c mice via intraperitoneal (i.p.) injection of 25 µg or 100 µg of the compounds. The results demonstrated that when bound to a protein carrier, chlorhexidine digluconate induced low concentrations of the IgG antibody, while the N-chloro derivative induced a dose-dependent increase in both IgG and IgE. Without the protein carrier, the chemicals did not elicit an immune response (CIR, 1993).

The breakdown product of the chemicals being assessed, PCA, is classified as hazardous with the risk phrase ‘May cause sensitisation by skin contact’ (R43) in HSIS (Safe Work Australia). The positive results in a guinea pig maximisation test and mouse local lymph node assay (LLNA) support this classification (NICNAS).

Observation in humans

The chemicals have been reported as causative agents of contact dermatitis, hives (urticaria), shortness of breath (dyspnoea), and anaphylactic shock (CIR, 1993). Several cases of sensitisation were reported in humans in patch or prick tests with the chemicals (CIR, 1993; Government of Canada, 2013). However, the majority of positive reactions were in individuals with pre-existing skin disorders or when applied to a mucous membrane.

Results from a patch test showed positive reaction to 1.0 % of chlorhexidine digluconate or diacetate in a number of volunteers, including patients with eczema (positive reactions were observed in 2–10 % of participants). An overall greater percentage of positive results for patients with eczema was noted. (CIR, 1993; Government of Canada, 2013). At a 0.5 % concentration, chlorhexidine digluconate induced contact dermatitis and photoallergenic reaction (photocontact dermatitis) in <1 % of volunteers from a photopatch study. Sensitisation was not observed in patch tests with 0.05 % chlorhexidine digluconate (CIR, 1993; REACH). Reactions to the chemical were increased after irradiating the skin with ultraviolet A (UVA) from 20 J/cm^2 dose (Hasan & Jansen, 1996). Analysis of the medical histories and epicutaneous patch test results for 5202 patients indicated that only a small number of patients (15) showed contact dermatitis from the chemical chlorhexidine digluconate (REACHb).

The prevalence of sensitisation and allergy to chlorhexidine was investigated in 104 health care workers. No reactions to skin patches containing chlorhexidine acetate (1 %) and chlorhexidine gluconate (1 %) were observed (Government of Canada, 2013).

Observed sensitisation reactions are considered to be indicative of an IgE (Immunoglobulin E)-mediated allergic reaction (CIR, 1999). The sera of non-chlorhexidine and chlorhexidine-sensitive patients were tested for IgG (Immunoglobulin G) and IgE antibodies. The results showed that only IgE (an antibody associated with allergic response) was identified in the chlorhexidine-sensitive patients (CIR, 1993).

Occupational allergic asthma reactions (two cases) followed exposure to an aerosol disinfectant containing chlorhexidine digluconate and alcohol. In both cases the affected individuals displayed coughing, wheezing and tightness of the chest within minutes of exposure. When a challenge test using the disinfectant was performed, the forced expiratory volume of the lungs was reduced by 20 % (CIR, 1993: REACHb).

Repeated Dose Toxicity

Oral

Based on the available data, hepatic damage from repeated oral exposure to the chemicals cannot be ruled out. However, data are not sufficient to warrant classification.

In a combined 6- and 12-month studies (OECD TG 452-compliant), male and female Beagle dogs were orally dosed with 0, 0.5, 5 and 40 mg/kg bw/day (as a chlorhexidine base) of chlorhexidine digluconate in gelatine capsules (EMEA, 1996; REACHb). At 40 mg/kg bw/day, animals showed decreased body weight, increased vomiting, hair loss and staining of hair. Bronchopneumonia was observed in two dogs, a condition that was suggested to be exacerbated by the chemical. These animals were subsequently euthanised due to their poor health. Due to these effects, the highest dose was reduced to 25 mg/kg bw/day after 29 weeks. The chemical also induced liver damage. The serum levels of liver enzymes, alanine aminotransferase and aspartate aminotransferase, were significantly elevated, especially at the highest dose (40 mg/kg bw/day). The following histopathological findings in the liver (in animals exposed to 5–40 mg/kg bw/day) were reported:

- focal degeneration, irregular areas of liver necrosis and loss of hepatocytes (40 mg/kg bw/day); and
- centrilobular fibrosis (5 mg/kg bw/day).

These chemically-induced alterations were not found in the animals at the lowest dose of 0.5 mg/kg bw/day; therefore, this was established as the no observed adverse effect level (NOAEL) (REACHb).

There was no evidence of liver toxicity in long-term studies in rats and mice (see **Carcinogenicity** section for details). Significant systemic effects were not observed at doses below 400 mg/kg bw/day in mice. Reversible histopathological changes in the mesenteric lymph nodes were observed in rats with a low observed effect level of 5 mg/kg bw/day (as a chlorhexidine base) (CIR, 1993; REACHb).

In animal studies, the breakdown product of the chemicals, PCA, is reported to cause damage to health from prolonged exposure. The target organs are the blood, liver, spleen and kidneys. The lowest observed-effect levels (LOELs) reported in rats and mice were 5–7.5 mg/kg bw/day. Beagle dogs exposed to 5–15 mg/kg bw of the chemical for 90 days showed cyanosis and blood-related disorders (NICNAS).

Dermal

Based on the limited data available, the chemicals are not considered to cause serious damage to health from repeated dermal exposure.

In a 13-week subchronic dermal study in rabbits, a NOAEL for systemic effects was established as 250 mg/kg bw/day. Changes in liver enzyme activity and minimal liver necrosis was observed at higher doses (≥500 mg/kg bw/day). Minimal skin irritation such as erythema, oedema, desquamation and/or fissuring was observed at all doses (Government of Canada, 2013; REACHb).

Administration of a skin cleanser containing 8 % chlorhexidine digluconate to newborn rhesus monkeys for a period of three months did not result in any significant treatment-related effects (REACHb).

Daily topical application of 2 % chlorhexidine in hamster cheek pouches for three weeks resulted in discrete white lesions being formed, and hyperplastic areas of the epithelium with inflammatory cells (CIR, 1993).

Inhalation

Limited data are available.

The subchronic inhalation toxicity of 13 different products containing 0.20–0.25% chlorhexidine digluconate was investigated in four separate studies using rats. In a controlled environment, selected doses (conforming to anticipated human use concentrations) were discharged from aerosol cans for 2–8 seconds every five minutes, four hours/day, five days/week, for 65 days. No significant treatment-related effects were reported (CIR, 1993).

In another study, 'lung effects were observed in 2 of 4 Beagle dogs exposed to fog with an unknown concentration of chlorhexidine acetate repeatedly for 30 days' (Government of Canada, 2013).

Observation in humans

Significant treatment-related effects were not reported in long-term studies on mouthwashes containing up to 0.2 % chlorhexidine digluconate (CIR, 1993).

Genotoxicity

Based on the weight of evidence from the available data the chemicals are not considered to be genotoxic.

Positive results were reported for chlorhexidine digluconate in a non-guideline bacterial reverse mutation assay (Ames test) in *Salmonella typhimurium* strains TA1535 and TA1538, with or without metabolic activation. However, the chemical was negative in a more recent Ames test conducted according to OECD TG 471 and in an umu test in *S. typhimurium* TA 1535. Negative results were observed in several other in vitro assays (CIR, 1993; Government of Canada, 2013; REACHb), including:

- gene mutation in Chinese hamster lung cells (CHL V79) (with and without metabolic activation);
- chromosomal aberration (CA) in Syrian hamster embryo (SHE) cells (with and without metabolic activation); and
- sister chromatid exchange in SHE cells (without activation).

Negative results were observed for chlorhexidine acetate in several in vitro assays (Government of Canada, 2013):

- gene mutation in mouse lymphoma cells (with and without metabolic activation);
- CA in Chinese hamster ovary (CHO) cells (with and without metabolic activation); and
- unscheduled DNA synthesis in rat hepatocytes.

Chlorhexidine digluconate produced DNA damage in peripheral leukocytes and oral mucosal cells in Wistar rats exposed to drinking water containing 0.5 mL of 0.12 % of the chemical (Comet assay) (Government of Canada, 2013). However, the chemical produced negative results in the following in vivo assays:

- rat micronucleus assay;
- hamster cytogenetic test; and
- mouse dominant lethal assay (CIR, 1993; Government of Canada, 2013).

The breakdown product of the chemicals, PCA, tested positive in several in vitro assays. At the 300 mg/kg bw dose, the chemical caused a significant increase in micronucleus frequency in B6C3F1 mice. It has also been reported to be genotoxic in a sex-linked recessive lethal assay in *Drosophila melanogaster* (NICNAS).

Carcinogenicity

Chlorhexidine digluconate has been tested in the following long-term studies;

- Wistar rats were exposed to 0, 5, 25 or 50 mg/kg bw/day (as a chlorhexidine base) in the diet for 105 weeks;
- Wistar rats were exposed to 0, 5, 25 or 40 mg/kg bw/day (as a chlorhexidine base) in drinking water for two years; and
- C57BL/10J mice were exposed to 0, 100, 200, 400 and 800 mg/kg bw/d (as a chlorhexidine base) in the diet for 78 weeks.

In the drinking water study, animals treated with 40 mg/kg bw/day were also exposed to approximately 0.178 mg/kg/day (500 ppm) of the breakdown chemical, PCA.

No carcinogenic effects were observed in any of these investigations (CIR, 1993; REACHb).

The breakdown product of the chemicals, PCA, is classified as hazardous (Category 2 carcinogenic substance) with the risk phrase ‘May cause cancer’ (T; R45) in HSIS (Safe Work Australia). The available studies in which a number of chemically-induced tumours, primarily in the spleen, subcutaneous tissues, kidneys, adrenal gland, liver and blood were reported, support this classification (NICNAS).

Reproductive and Developmental Toxicity

Based on the weight of evidence of the available data, the chemicals are not considered to cause specific reproductive or developmental toxicity. Any developmental effects were only observed secondary to maternal toxicity.

Chlorhexidine digluconate was administered by gavage to pregnant rats from day 15 of pregnancy and through lactation (0–5 mg/kg bw/day) and to their offspring (0–25 mg/kg bw/day) from weaning for periods up to six months. There were no treatment-related effects on the number and size of the F1-generation, on the sex ratios or growth rate. There were no treatment-related embryotoxic effects. Mild to moderate histiocytosis in the mesenteric lymph nodes was noted in dams and foetuses. The severity of findings increased with duration of exposure, with pups appearing to be less susceptible than the dams (REACHb).

Chlorhexidine (free base) has been reported to reduce the number of litters (but not litter size) in mice. Mice of both sexes were administered a concentration of 0.2 % for one week in drinking water. Although overt toxicity was not observed, minor toxicity was not ruled out (CIR, 1993).

In a developmental toxicity study in rats (via gavage from gestation days (GD) 6–15), chlorhexidine diacetate did not cause malformations or significant developmental toxicity at doses up to 62.5 mg/kg bw/day. However effects such as reduced body weight gain, rales, and increased salivation were observed. These effects were dose-related. The reported developmental and maternal toxicity no observed effect level (NOEL) values are equal to or greater than 62.5 mg/kg bw/day and 15.63 mg/kg bw/day respectively (US EPA, 1996). Additionally, exposure of pregnant female rats to 0, 10 or 50 mg/kg bw/day of chlorhexidine daily from GD15 to postnatal day 21, only resulted in irritability or excitability of the dams at the highest dose (EMEA, 1996).

Signs of maternal toxicity were reported in CD/Crl:CD (SD) rats exposed to 0, 10, 30 or 100 mg/kg bw/day of chlorhexidine digluconate via gavage (OECD TG 414-compliant study) from GD 6–19. At 30 mg/kg bw/day, laboured breathing was observed. The animals dosed with 100 mg/kg bw/day displayed piloerection, nosebleeding, and reduced mobility. At the maternally toxic dose of 100 mg/kg bw/day, significant increases in early, late and total resorptions were noted, along with a reduction in the number of foetuses. Significant increases in foetal or litter incidences for skeletal retardations were also reported (REACHb).

Other Health Effects

Neurotoxicity

Several reports have indicated that exposure to the chemicals in this group causes neurotoxicity. The use of chlorhexidine as a preoperative disinfectant has been linked to sensorial hearing or otoxicity in patients after myringoplasty operations to repair perforated eardrums (Bicknell, 1971). In animals, 0.05 % of chlorhexidine was also ototoxic in guinea pigs after the chemical was introduced into the cavity of the middle ear (CIR, 1993). Furthermore, ocular injection of chlorhexidine (0.25–7.5 µg) to albino rats produced dose-dependent degeneration of adrenergic nerves (Henschen & Olson, 1984).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include local effects (serious eye damage, skin sensitisation, and respiratory sensitisation). The chemicals could also cause harmful systemic effects following a single exposure through inhalation exposure.

The breakdown product of the chemicals, PCA, is a probable human carcinogen following long-term repeated exposure. A genotoxic mode of action cannot be excluded (NICNAS).

The critical health effects for risk characterisation also include systemic chronic effects by all routes of exposure (refer to **Repeated dose toxicity** sections).

Public Risk Characterisation

Although the use in cosmetic and domestic products in Australia is not known, the major reported international cosmetic use is in hair treatment applications. Typical reported concentrations in products are below 0.1 % (see **Import, manufacture and use** section).

At these concentrations, minimal eye irritation effects are expected and the risk of sensitisation is considered to be low.

The chemicals are known to degrade into PCA following prolonged storage. The decomposition process is accelerated with low pH and high temperatures. The stability of a formulation containing 20 % chlorhexidine digluconate was investigated in a 156-week study. In this study, elevated levels of PCA were reported at the first sample time (12 weeks). The highest level found throughout the study was 492 ppm (CIR, 1993). Based on typical usage levels, the concentration of PCA in products is estimated to be approximately 2.5 ppm. There was no evidence of carcinogenicity in a two-year study in rats receiving a chlorhexidine digluconate solution that also contained 500 ppm PCA.

The chemicals are currently listed in Schedules, 5, 6 and 7 of the SUSMP, except in preparations containing 1 % or less of chlorhexidine or in solid preparations. Preparations containing >7 % of the chlorhexidine are available only to specialised or authorised users. Preparations containing 1–7 % are required to be labelled with the appropriate signal words. Other specific warning statements and safety directions do not apply (SUSMP, 2016).

Controls applying to chlorhexidine in cosmetic products overseas are stricter than the controls in the SUSMP. However, specific health-based rationales for the controls were not provided and therapeutic products with higher concentrations are available (CIR, 1993). It is likely that the concentrations were chosen as being the maximum in use for cosmetic purposes.

Overall, the public risk from these chemicals is not considered to be unreasonable.

Occupational Risk Characterisation

Occupational exposure to the chemicals can occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise ocular, dermal and inhalation exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking at a workplace (such as an employer) has adequate information to determine appropriate controls. The data available support an amendment to the hazard classification in HSIS (refer to **Recommendation** section).

The data available support an amendment to the hazard classification in the Hazardous Substances Information System (HSIS) (Safe Work Australia) (refer to **Recommendation** section.

NICNAS Recommendation

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.

Regulatory Control

Work Health and Safety

The chemicals are 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]

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Irritation / Corrosivity	Risk of serious eye damage (Xi; R41)	Causes serious eye damage - Cat. 1 (H318)
Sensitisation	May cause sensitisation by inhalation (Xn, R42) May cause sensitisation by skin contact (Xi; R43)	May cause allergy or asthma symptoms or breathing difficulties if inhaled - Cat. 1 (H334) May cause an allergic skin reaction - Cat. 1 (H317)

[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 dermal, ocular and inhalation exposure to these 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 hazardous chemicals depend on the physical form and the manner in which the chemicals are used. Examples of control measures which can 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 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 chemicals have not been undertaken as part of this assessment.

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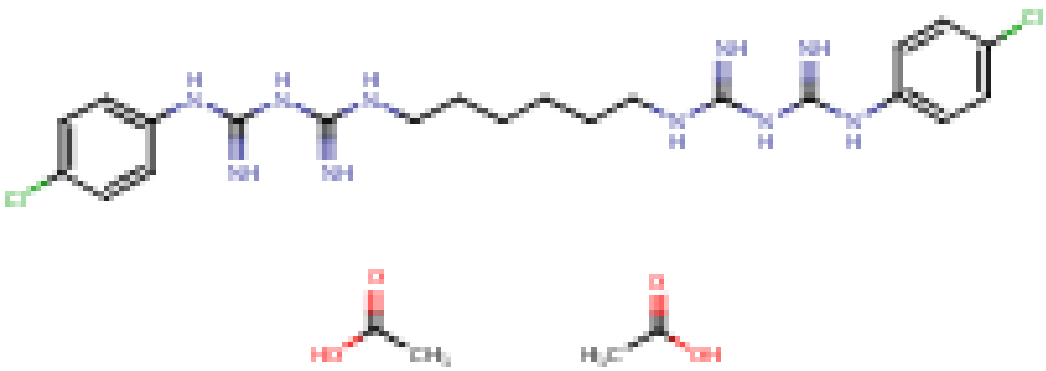
Last Update 25 November 2016

Chemical Identities

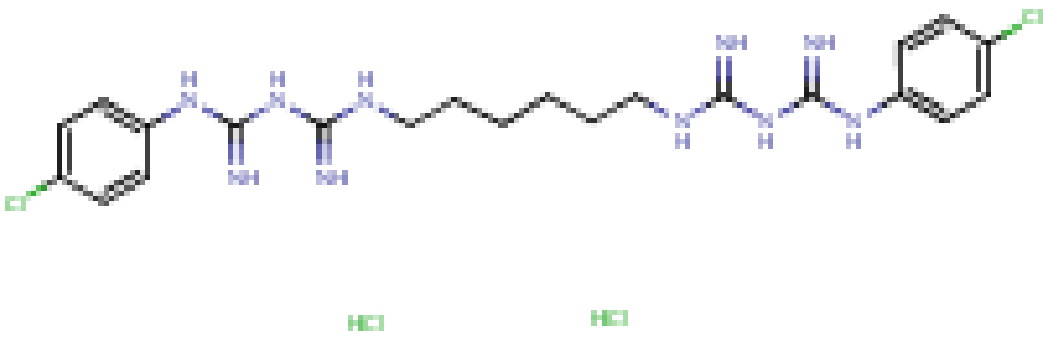
Chemical Name in the Inventory and Synonyms	**2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-** Chlorhexidine 1,1'-hexamethylenebis(5-(p-chlorophenyl)biguanide) 1,6-Bis(5-(p-chlorophenyl)biguandino)hexane Hexadol
CAS Number	55-56-1
Structural Formula	
Molecular Formula	C22H30Cl2N10
Molecular Weight	505.45

Chemical Name in the Inventory and Synonyms	**2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-, diacetate** Chlorhexidine acetate Chlorhexidine diacetate 1,6-bis(5-(p-chlorophenyl)biguandino)hexane diacetate 1,1-hexamethylene-bis[5-(4-chlorophenyl)biguanide
CAS Number	56-95-1
Structural Formula	

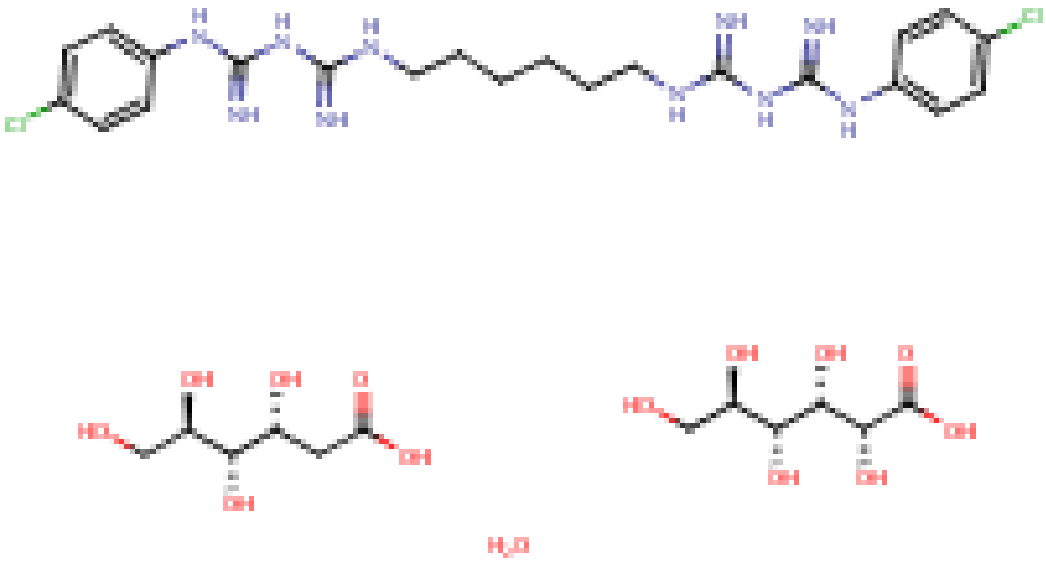


Molecular Formula	C22H30Cl2N10.2C2H4O2
Molecular Weight	625.55

Chemical Name in the Inventory and Synonyms	**2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride** Chlorhexidine hydrochloride 1,1'-hexamethylenebis(5-(p-chlorophenyl)biguanide) dihydrochloride Arlacide H biguanide,1,1'-hexamethylenebis(5-(p-chlorophenyl)-, dihydrochloride
CAS Number	3697-42-5
Structural Formula	

	
Molecular Formula	C22H30Cl2N10.2ClH
Molecular Weight	578.37

Chemical Name in the Inventory and Synonyms	**D-Gluconic acid, compound with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide (2:1)** Chlorhexidine gluconate Chlorhexidine digluconate D-gluconic acid, compd. with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecane diimidamide (2:1) 1,1'-hexamethylene bis(5-(p-chlorophenyl)biguanide), digluconate Abacil
CAS Number	18472-51-0
Structural Formula	



Molecular Formula	C22H30Cl2N10.2C6H12O7
Molecular Weight	899.77

Chemical Name in the Inventory and Synonyms	**D-Gluconic acid, compd. with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide**
CAS Number	98474-48-7
Structural Formula	**No Structural Diagram Available**

Molecular Formula	C22H30Cl2N10.xC6H12O7
Molecular Weight	Unspecified

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