Draft Screening Assessment for the Challenge

2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*''-bis(4-chlorophenyl)-3,12-diimino-, diacetate (Chlorhexidine acetate)

Chemical Abstracts Service Registry Number
56-95-1

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on 2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*''-bis(4-chlorophenyl)-3,12-diimino-, diacetate, Chemical Abstracts Service Registry Number[1] 56-95-1; this substance will be referred to by its common name, chlorhexidine acetate, in the assessment. This substance was identified as a high priority for screening assessment and included in the Challenge initiative under the Chemicals Management Plan because it had been found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms and is believed to be in commerce in Canada.

Chlorhexidine acetate was not considered to be a high priority for assessment of potential risks to human health, based upon application of the simple exposure and hazard tools developed for categorization of substances on the Domestic Substances List.

Chlorhexidine acetate is an organic substance that is used primarily as a disinfectant and antibacterial agent. The substance does not naturally occur in the environment. As a result of industry surveys conducted pursuant to section 71 of CEPA 1999, chlorhexidine acetate was not reported to be manufactured in Canada in 2005 or 2006. One company reported importing a total of 600 kg into the country in 2006.

Based on reported industrial and commercial/consumer use patterns and certain assumptions, chlorhexidine acetate is predicted to be released in wastewater (before treatment; about 1% from industrial use), to surface water (up to 43% from consumer/commercial uses), to soil (up to 43% via the application of biosolids and manure), and through waste disposal (incineration and landfill; ~10%). Chlorhexidine acetate is a salt and dissociates in water to produce the acetate counterion and chlorhexidine. Chlorhexidine is a strong base and is expected to protonate in water at pH 6 to 9, such that virtually all (~99%) of the substance will exist with two of its amine groups positively charged.

Experimental and predicted data suggest that chlorhexidine acetate will persist in water, soil and sediment. Its physical and chemical properties suggest that chlorhexidine acetate has a low bioaccumulation potential. Experimental acute toxicity data for chlorhexidine acetate and chlorhexidine show that they have the potential to cause acute harm to aquatic organisms at low concentrations.

For this draft screening assessment, a realistic worst case exposure scenario was selected in which an industrial operation discharges chlorhexidine acetate into the aquatic

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environment through a wastewater treatment plant. Release and exposure levels of chlorhexidine acetate were estimated based on quantities imported in 2006. The predicted environmental concentration in water of this substance was above the predicted no-effect concentration for sensitive aquatic organisms.

Based on the ecological information available, it is proposed that chlorhexidine acetate meets the criteria in paragraph 64*(a)* of CEPA 1999, as it is entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. However, chlorhexidine acetate does not meet the criteria under paragraph 64*(b)* of CEPA 1999, as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends. It is also proposed that this substance meets the persistence criteria but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*.

General population exposure from environmental media is expected to be low. Exposure from the diet is not expected. Exposure to the general population can occur from use of products containing this substance. No evidence of carcinogenicity or genotoxicity were observed in the available health effects data on chlorhexidine acetate and chlorhexidine gluconate, and a threshold approach is used to characterize risk to human health. The margins between upper-bounding estimates of exposure from environmental media and from use of consumer products and levels associated with effects in experimental animals are considered adequate to address uncertainties in the health effects and exposure databases. Based on available information for human health considerations, it is proposed that chlorhexidine acetate does not meet the criteria in paragraph 64*(c)* of CEPA 1999, as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on the information available, it is proposed that chlorhexidine acetate meets one or more criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. Where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006a, 2006b), that challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance, 2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*''-bis(4-chlorophenyl)-3,12-diimino-, diacetate (or chlorhexidine acetate), had been identified as a high priority for assessment of ecological risk as it had been found to be persistent, bioaccumulative and inherently toxic to aquatic organisms and is believed to be in commerce in Canada. The Challenge for this substance was published in the *Canada Gazette* on December 26, 2009 (Canada 2009a, 2009b). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information pertaining to the properties, uses, persistence and bioaccumulation potential of the substance were received.

Although chlorhexidine acetate was determined to be a high priority for assessment with respect to the environment, it did not meet the criteria for GPE or IPE and high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine scientific

information and develop conclusions by incorporating a weight-of-evidence approach and precaution.[2]

This draft screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to September 2010. Key studies were critically evaluated, along with modelling results, to reach conclusions.

When available and relevant, information presented in hazard assessments from other jurisdictions was considered. The draft screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

This draft screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological portions of this assessment have also undergone external written peer review/consultation. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

The critical information and considerations upon which the draft assessment is based are summarized below.

[2] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Controlled Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA 1999 does not preclude actions being undertaken under other sections of CEPA or other Acts.

Substance Identity

Substance Name

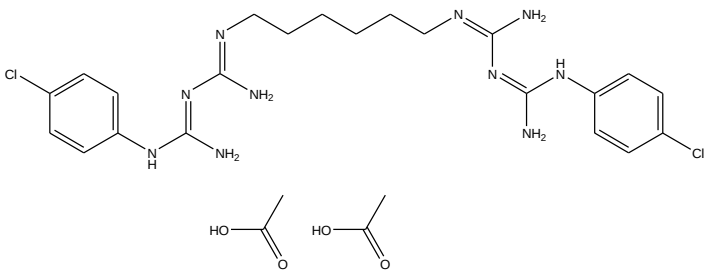
For the purposes of this document, this substance will be referred to as chlorhexidine acetate. Relevant data for the parent compound chlorhexidine and its other salts (i.e., gluconate and hydrochloride) were considered in this assessment in characterizing the hazard of chlorhexidine acetate, but it is not the subject of this assessment.

Table 1. Substance identity for chlorhexidine acetate

Chemical Abstracts Service Registry Number (CAS RN)	**56-95-1**
DSL name[3]	**2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*''-bis(4-chlorophenyl)-3,12-diimino-, diacetate**
National Chemical Inventories (NCI) names[4]	*2,4,11,13-Tetraazatetradecanediimidamide, N,N''-bis(4-chlorophenyl)-3,12-diimino-, diacetate* (ENCS, ASIA-PAC, NZIoC, AICS, SWISS) *chlorhexidine di(acetate)* (EINECS)
Other names	*1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide] diacetate* *1,6-Bis(p-chlorophenylbiguanido)hexane diacetate* *Arlacide A* *Bactigras* *Biguanide, 1,1'-hexamethylenebis[5-(p-chlorophenyl)-, diacetate* *Chlorasept 2000* *Chlorhexidine acetate* *Chlorhexidine diacetate* *Chlorzoin* *Dosisepsine* *EC 40* *EC 40 (antibacterial)* *Hibitane diacetate* *Jie-Yin Liquid Disinfectant* *NSC 526936*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Low-molecular carbo-monocyclic organic compounds

[3] DSL (Domestic Substances List)

[4] National Chemical Inventories (NCI). 2007: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); NZIoC (New Zealand Inventory of Chemicals); SWISS (Swiss Giftliste 1 and Inventory of Notified New Substances).

Major chemical sub-class	Guanidines, anilines, secondary aromatic amines, aliphatic amines
Chemical formula	$C_{22}H_{30}Cl_2N_{10}\cdot 2(C_2H_4O_2)$
Chemical structure	
SMILES[5]	Chlorhexidine acetate: c1(Cl)ccc(NC(=N)NC(NCCCCCCNC(NC(=N)Nc2ccc(Cl)cc2)=NOC(C)=O)=NOC(C)=O)cc1
	Chlorhexidine: Clc1ccc(NC(=N)NC(=N)NCCCCCCNC(=N)NC(=N)Nc2ccc(Cl)cc2)cc1
Molecular mass	625.56 g/mol

[5] Simplified Molecular Input Line Entry System. The SMILES of chlorhexidine is used for QSAR modelling, as these models only accept the neutral form of a chemical as input.

Physical and Chemical Properties

Table 2 contains experimental and modelled data on the physical and chemical properties of chlorhexidine acetate that are relevant to its environmental fate. The Study Submission (2010) reporting experimental data on the octanol-water partition coefficient for this substance was critically evaluated and this review (as a robust study summary) is found in Appendix I.

Table 2. Physical and chemical properties for chlorhexidine acetate

Property	Type	Value[a]	Descriptor	Reference
Physical form	White to pale yellow powder			Chemicalland21 2010
Melting point (ºC)	Experimental	154–155[b]		PhysProp 2006
Density (kg/m^3)	Experimental	1.2×10^3 ($1.2\ g/cm^3$)		US EPA 1996
Vapour pressure (Pa)	Modelled (neutral form)	1.5×10^{-12} (modified Grain method)	25°C	MPBPVP 2008
Henry's Law constant ($Pa \cdot m^3/mol$)	Modelled (neutral form)	1.2×10^{-25} (bond estimation)	25°C	HENRYWIN 2008
	Calculated[c]	2.4×10^{-13}	20–25°C	HENRYWIN 2008
Log D[d] (Distribution coefficient) (dimensionless)	Experimental (Log K_{ow}; octanol-water)	-1.1[b]	19°C pH 6.7	Study Submission 2010
	Modelled (Log D_{ow}; octanol-water)	1.6	pH 6–9	ACD/PhysChem Suite 2009
	Modelled (Log D_{oc}; organic carbon-water)	0.9 1.6–3.9	pH 6–9 pH 10–14	ACD/PhysChem Suite 2009

Property	Type	Value[a]	Descriptor	Reference
Water solubility (mg/L)	Experimental	1.0×10^4 6.9×10^3 3.6×10^3 3.3×10^3	pH = 4 pH = 5 pH = 6 pH = 7 [b]	Anusavice et al. 2006
		1.9×10^4	20°C (pH unknown)	O'Neil 2001
Other solubilities	Soluble in alcohol, glycerol, propylene glycol, polyethylene glycol			O'Neil 2001
	6.7×10^4 (in ethanol); slightly soluble in glycerol and propane			US EPA 1996

[a] Values in parentheses represent the original ones as reported by the authors or as estimated by the models.
[b] Values selected in modelling with EPIsuite (2008). The SMILES for chlorhexidine is used in this model (as it only accepts the neutral form of a chemical as input) along with the experimental water solubility and log K_{ow} values shown here. These user inputs provide some correction for the ionizing characteristics of this substance.
[c] Henry's Law constant was calculated using the modelled vapour pressure and selected experimental water solubility indicated, and is therefore different from the modelled Henry's Law constant, which is based on inputs for chlorhexidine (HENRYWIN 2008).
[d] The distribution coefficient or log D takes into account the presence of the ionic species; it represents the net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH.

Models based on quantitative structure-activity relationships (QSARs) were used to generate data for the vapour pressure and Henry's Law constant of chlorhexidine acetate. These models are mainly based on fragment addition methods (i.e., they rely on the structure of the chemical) and accept only the neutral (i.e., un-ionized) form of a chemical as input (in SMILES form).

Chlorhexidine acetate is a salt and dissociates in water to produce the acetate counterion and chlorhexidine (CAS RN 55-56-1; 505.46 g/mol). Chlorhexidine is a strong base and is predicted to ionize in water as a base in seven steps whereby protons are attracted to the amine groups (ACD/PhysChem Suite 2009). Therefore, chlorhexidine is expected to protonate in water at pH 6 to 9, such that virtually all (~99%) of the substance will exist with two of its amine groups positively charged. In the aquatic environment, chlorhexidine will exist in equilibrium with the acetate salt and the acetate counterion.

The ionic nature of chlorhexidine acetate is an important consideration in interpreting its physical and chemical properties as they relate to its environmental fate and behaviour (see the Environmental Fate section for further discussion). This substance is very soluble in water (Anusavice et al. 2006; O'Neil 2001), as is the parent compound chlorhexidine (800 mg/L; O'Neil 2001). The experimental log K_{ow} value for chlorhexidine acetate (-1.1) and the

predicted log D_{ow} value (1.6) account for the ionizing characteristics of the substance (although the predicted value is higher than the observed value).

Sources

Chlorhexidine acetate does not naturally occur in the environment.

No companies reported manufacturing chlorhexidine acetate above the 100 kg/year threshold in either 2005 or 2006 (Environment Canada 2006; Environment Canada 2010a). Fewer than four companies each imported a total of between 100 and 1000 kg of this substance into Canada in 2005, and one company reported importing a total of 600 kg in 2006. Fewer than 10 companies identified themselves as having a stakeholder interest in this substance in 2005.

During the DSL nomination, the total quantity reported to be manufactured, imported or in commerce in Canada during 1986 was 2200 kg (Environment Canada 1988).

Chlorhexidine acetate has not been identified as a high production volume (HPV) chemical in the United States (US EPA 2006), but this substance has been used in that country since as early as 1955 for use as a farm premise disinfectant/virucide and is registered in two products (each containing 2% chlorhexidine acetate) for use as hard surface-treatment disinfectants/virucides (US EPA 1996, 2011). Chlorhexidine acetate is listed in the European Inventory of Existing Commercial Chemical Substances (EINECS) and has been reported as a low production volume chemical (ESIS c1995–2009), meaning that it is placed on the market in quantities between 10 and 1000 tonnes per year per producer or importer. The substance was also used in Nordic countries, including Sweden from 1999 to 2008, Finland from 2001 to 2008, and in Norway in 2004 (SPIN 2010).

Uses

Chlorhexidine and its salt forms (acetate, gluconate, and hydrochloride) are broad-spectrum antiseptics used for sterilization, cleaning skin and hands, and disinfecting wounds, and are generally effective against a wide variety of bacteria and yeasts (Chemicalland21 2010).

In Canada, chlorhexidine acetate is listed in the Drug Products Database (DPD) as an active ingredient in more than 30 drugs (as of April 2011) for human or veterinary use and hard-surface disinfectants (DPD 2011). Three of the products are drugs for human use: over-the-counter gauze dressing for skin loss wounds (DPD 2011; Smith & Nephew 2010); over-the-counter spray for minor burns and sunburns (DPD 2011); and anti-cavity dental varnish requiring a prescription (DPD 2011; CHX Technologies Inc. 2004). Two of the products are for hard-surface disinfection: disinfectant of animal accommodations for veterinary use only (DPD 2011) and floor cleaner of an all-in-one floor mopping system (DPD 2011). The remainder of the products are drugs for veterinary use only and include bovine udder wash (DPD 2011; Westagro Canada 2009a); bovine teat dips (DPD 2011; Westagro Canada 2009b); anti-infective skin ointment (DPD 2011; Partnar Animal Health 2010); and oral rinse for cats

and dogs (DPD 2011; Pfizer Canada Inc. 2010). Chlorhexidine acetate is listed in the Natural Health Products Ingredient Database (NHPID) as a non-natural health product because it is not a naturally occurring substance included in Schedule 1 of the *Natural Health Products Regulations* (NHPID 2010). It is not listed in the Licensed Natural Health Products Database (LNHPD); therefore, no current licensed natural health products contain this substance as a medicinal ingredient or as a non-medicinal ingredient (LNHPD 2010).

Chlorhexidine acetate is not listed as an approved food additive under Division 16 of the *Food and Drug Regulations* (Canada 1978), nor has it been identified as being used/present in formulations of incidental additives or used in food packaging materials (November 2010 email from Food Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

Chlorhexidine acetate is on the Cosmetic Ingredient Hotlist, Health Canada's administrative list of ingredients that are intended to be prohibited or restricted for use in cosmetics in Canada, and is permitted in cosmetics in concentrations equal to or less than 0.19% (Health Canada 2010a). One cosmetic product (aftershave) containing chlorhexidine acetate has been identified in the Cosmetic Notification System database (CNS 2010).

Chlorhexidine acetate is not currently present in Canada as a formulant in pest control products, as it is not listed in the Pest Management Regulatory Agency List of Formulants (Health Canada 2010b).

Chlorhexidine acetate has also been identified as a component of foot baths for farm visitors (OMAFR 2009).

This substance has been used in Finland and Sweden in non-agricultural pesticides, preservatives and other (undefined) products in the areas of health/social work and the manufacture of chemicals/chemical products; however, use patterns were reported as confidential (SPIN 2010).

Releases to the Environment

A method has been developed by Environment Canada to estimate a substance's losses during different stages of its life cycle, including its fate within a finished product or article (Environment Canada 2008). This method, referred to as Mass Flow, consists of a life cycle analysis and a spreadsheet tool that integrates information on the manufacturing, importation and use data available for the substance. Starting with an identified mass of the substance, each life cycle stage is subsequently evaluated until all of the mass is accounted for. Relevant factors are considered, uncertainties recognized and assumptions may be made during each stage, depending on information available. The estimated losses represent the complete mass balance of the substance over the life cycle of the substance and include releases to wastewater and other receiving compartments (land, air), chemical transformation, transfer to recycling activities and transfer to waste disposal sites (landfill, incineration). However, unless specific

information is available on the rate or potential for release of the substance from landfills and incinerators, the method does not quantitatively account for releases to the environment from disposal.

In general, releases of a substance to the environment depend upon various losses from its manufacture, industrial use and consumer/commercial use. These losses can be grouped into seven types: (1) discharge to wastewater; (2) emission to air; (3) loss to land; (4) chemical transformation; (5) disposal to landfill; (6) loss to incineration; and (7) disposal through recycling (i.e., recycling is deemed a loss and not considered further). They are estimated using regulatory survey data, industry data and data published by different organizations. The discharge to wastewater refers to raw wastewater prior to any treatment, whether it be on-site industrial wastewater treatment or off-site municipal wastewater treatment. In a similar manner, the loss via chemical transformation refers to changes in a substance's identity that may occur within the manufacture, industrial use, and consumer/commercial use stages, but excludes those during waste management operations such as incineration and wastewater treatment. The loss to land includes unintentional transfer or leakage to soil or paved/unpaved surfaces during the substance's use and service life (e.g., from the use of agricultural machinery or automobiles). The loss to land, however, does not include transfers subsequent to a substance's use and service life (e.g., land application of biosolids and atmospheric deposition).

The losses estimated for chlorhexidine acetate over its life cycle (estimated under a realistic worst-case scenario) are presented in Table 3 (Environment Canada 2010b). Chlorhexidine acetate is not manufactured in Canada above reporting thresholds, so estimated losses are based on import quantities reported in 2006.

Table 3. Estimated losses of chlorhexidine acetate during its life cycle using the Mass Flow Tool

Type of loss	Proportion (%)	Pertinent life cycle stages
Wastewater (before treatment)	~1	Industrial use
Surface water	up to 43	Consumer/commercial use
Land	up to 43	Consumer/commercial use
Air emission	0	
Chemical transformation	0	
Incineration	< 1	Industrial use and consumer/commercial use

Landfill	9	Industrial use and consumer/commercial use
Residue on hard surfaces	3	Consumer/commercial use
Total	100	

Chlorhexidine acetate is estimated to be released in wastewater (before treatment; about 1% from industrial use), to surface water (up to 43% from consumer/commercial uses), to land (soil; up to 43% via the application of biosolids and manure), and through waste disposal (incineration and landfill; < 10%). Residue on hard surfaces as a result of consumer and commercial uses is estimated to be 3%.

Assumptions were made to estimate releases associated with the production of veterinary drugs and products (i.e., industrial use), and are based on cosmetics manufacturing processes. Assumptions also include losses during container handling. Approximately 6 kg per year are estimated to be discharged into wastewater from the manufacturing of veterinary drugs and products containing chlorhexidine acetate. With the potential for chlorhexidine acetate to be released in wastewater, and depending on the degree to which it may subsequently partition to sludge during effluent treatment, there is also the potential for it to be applied to soil through the land application of biosolids (treated sludge)

Specifically regarding its use as an antibacterial product on cattle (i.e., dairy farms), it is assumed that the substance would end up in manure pits, lagoons, or silos, with subsequent application of manure to land and possible runoff to surface water. Depending on product formulation and use, chlorhexidine acetate, which is typically used in products at a concentration of up to 0.55%, could be applied to cows in farms across the country as a standard of care in preventing mastitis infection. However, given the very low concentration of chlorhexidine acetate in the antibacterial products and the limited quantities used at farms across Canada, the losses at any one particular farm are expected to be lower than the estimated quantity released at an industrial site.

Environmental Fate

Based on its physical and chemical properties (Table 2), its ionic characteristics, uses and Mass Flow Tool loss estimates, chlorhexidine acetate is expected to be found mainly in water and/or soil as a result of releases to the environment.

If released to the aquatic environment, chlorhexidine acetate is expected to be found mostly in the water column, as the substance is highly soluble. This substance dissociates in water to produce the acetate counterion and chlorhexidine, with the majority of the chlorhexidine existing in a protonated form (~99%) at environmentally relevant pH of 6 to 9—that is, with two of its amine groups carrying a positive charge. Adsorption characteristics would be influenced by its ionic nature, and thus this substance would have an affinity for suspended solids having a negative charge (e.g., humic and fulvic acids, clay materials) through electrostatic interactions. Therefore, adsorption to suspended solids could result in some settling to bed sediments.

Similarly, if released to soil, this substance would have an affinity for organic matter, which in general has a negative charge, and may or may not be mobile depending on the moisture content and soil type (e.g., it would likely be less mobile in soils with high organic matter or high clay content).

Chlorhexidine acetate is not expected to be released to air given its intended uses and physical and chemical properties. Its very low vapour pressure, negligible Henry's Law constant, high water solubility, and existence in a protonated form in the environment indicate that volatilization would be negligible from either dry or moist soil surfaces, or surface waters.

Persistence and Bioaccumulation Potential

Environmental Persistence

Given that chlorhexidine acetate dissociates in water to produce chlorhexidine and the acetate counterion, information on chlorhexidine is also considered in the following assessment of persistence and bioaccumulation potential.

Limited experimental biodegradation data relevant to the persistence of chlorhexidine acetate are available. However, relevant data on other salts of chlorhexidine were also considered. An activated sludge die-away experiment was conducted using freshly collected activated sludge dosed with 50 μg/L ^{14}C chlorhexidine dihydrochloride (CAS RN 3697-42-5)(Study Submission 2010). In the initial test, there was a lack of degradation observed, which was thought to be due to the absence of competent degraders due to the very low environmental exposure concentration in activated sludge compared with the test concentration of the substance. Therefore, a second test was conducted using acclimated activated sludge, acclimation being conducted with activated sludge continuously exposed to wastewater amended with 200 μg/L chlorhexidine dihydrochloride for a 31-day period. Both tests were conducted according to the test procedures of OECD 314B (for determining rates of primary and ultimate degradation rates), and used test concentrations of 50 μg/L ^{14}C chlorhexidine dihydrochloride and a biosolids concentration of 2500 mg/L. The results from both die-away experiments showed no significant primary degradation of the test material based on high-performance liquid chromatography (HPLC) analysis of the solvent extracts, and there was no significant increase in $^{14}CO_2$ or non-extractable radioactivity associated with biomass over time (Study Submission 2010).

The following studies were cited in HSDB (1983–2010) with limited study details. A closed bottle test using an activated sludge inoculum (1.5 mg/L) and chlorhexidine at a concentration of 5.35 ppm resulted in 0% chemical oxygen demand (COD) after 28 days (De Waart and Van der Most 1986). In another test, ^{14}C-labelled chlorhexidine was incubated at 0.05 ppm in an activated sludge for 5 days, with results of 0.1% CO_2 evolution, 94.3% non-extractable residues (amount retained in sludge), and 0.2% volatilization (Freitag et al. 1982). No degradation was observed after 21 days in an OECD minimal media test for detergents, which studied the potential biodegradation of chlorhexidine (12 ppm) in wastewater (Voets et al. 1976).

The results of two more recent studies appear to conflict with the above results. Tanaka et al. (2005, 2006) have reported microbial degradation of chlorhexidine gluconate (CAS RN 18472-51-0), which was applied to a variety of bacterial strains under laboratory conditions. Although degradation was not quantified, the authors reported "significant" degradation of chlorhexidine within 7 days based on the results of the HPLC chromatograms. These findings indicated a possible resistance mechanism of some bacterial strains to disinfectants via biodegradation. However, environmental conditions would be quite different compared to these laboratory designs (i.e., lower bacterial concentrations, varying temperatures, and other environmental conditions); thus microbial degradation is not anticipated to be a dominant degradation pathway for chlorhexidine acetate in the environment.

Abiotic degradation of chlorhexidine acetate is not expected to play a significant role in the environmental fate and persistence of this substance. Chlorhexidine acetate does not contain functional groups expected to undergo hydrolysis (HYDROWIN 2008; Table 4). Although chlorhexidine acetate is not expected to be released to air, reactions with hydroxyl radicals will be the most important fate process in the atmosphere (estimated half-life of 25 minutes; Table 4). The substance is not expected to react appreciably with other photo-oxidative species in the atmosphere (such as O_3; Table 4). The substance contains chromophores that absorb at wavelengths greater than 290 nm, and thus may be susceptible to direct photolysis (Freitag et al. 1985). Therefore, this substance is not likely to be persistent in air.

Although limited experimental data are available, a QSAR-based weight-of-evidence approach (Environment Canada 2007) was also applied using the degradation models summarized in Table 4 below. These models are based on chemical structure, and results are consistent with the available empirical information.

Table 4. Modelled data for degradation of chlorhexidine

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Degradation			
Atmospheric oxidation	AOPWIN 2008[a]	$t_{1/2}$ ~ 25 minutes	< 2
Ozone reaction	AOPWIN 2008[a]	n/a[b]	
Hydrolysis	HYDROWIN 2008[a]	n/a[b]	
Primary biodegradation – water			
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 4: Expert Survey (qualitative results)	2.57[c] "does not biodegrade fast"	≥ 182
Ultimate biodegradation – water			
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 3: Expert Survey (qualitative results)	1.40[c] "biodegrades slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 5: MITI linear probability	-0.73[d] "biodegrades very slowly"	≥ 182
Biodegradation	BIOWIN 2008[a]	0[d]	≥ 182

(aerobic)	Sub-model 6: MITI non-linear probability	"biodegrades very slowly"	
Biodegradation (aerobic)	CATABOL 2004–2008 % BOD (biological oxygen demand)	20 "biodegrades slowly"	≥ 182

[a] From EPIsuite (2008), using SMILES notation in Table 1 for chlorhexidine.
[b] Model does not provide an estimate for this type of chemical structure.
[c] Output is a numerical score from 0 to 5 related to a predicted biodegradation rate.
[d] Output is a probability score.

Results for both the primary biodegradation model (BIOWIN Sub-model 4) and the three ultimate biodegradation models (BIOWIN Sub-models 3, 5 and 6) indicate that biodegradation is slow and that the half-life in water would be more than 182 days. In addition, the ultimate degradation prediction from CPOPs (CPOPS 2008; CATABOL 2004–2008) also indicates a very slow rate of biodegradation. Given the agreement among these results, the half-life of chlorohexidine and chlorhexidine acetate in water is expected to be more than 182 days.

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al. 1995), the model-estimated ultimate biodegradation half-life in water is used to extrapolate the half-lives in other environmental media. The ultimate degradation half-life in aerobic soil is also expected to be ≥ 182 days and the half-life in aerobic sediment is expected to be ≥ 365 days.

Based on the experimental and predicted information on chlorhexidine and its salts, it is proposed that chlorhexidine acetate meets the persistence criteria in water, soil and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

There are no empirical bioaccumulation data available for chlorhexidine acetate. The experimental log K_{ow} of -1.1 (which is essentially a log D_{ow} and takes into account the ionic species at a given pH) indicates that this chemical has a low potential to bioaccumulate.

One study evaluated the bioconcentration potential of the parent compound, chlorhexidine, in golden eye (*Leuciscus idus melanotus*). Fish were exposed to chlorhexidine at 0.05 µg/L for 3 days (Freitag et al. 1985). Evaluation of the concentration of chlorhexidine in fish compared with the concentration in water resulted in a bioconcentration factor (BCF) of 40, indicating a low bioconcentration potential. Details of the methods used were limited in this study, and 3 days is insufficient to achieve a steady state; however, these results are consistent with what would be expected given the very low experimental log K_{ow} of chlorhexidine acetate.

Given the limited BCF data relevant to this substance, a predictive approach was applied using available BCF and bioaccumulation factor (BAF) models as shown in Table 5.

Table 5. Modelled bioaccumulation data for chlorhexidine acetate

Test organism	Endpoint	Wet weight (L/kg)	Reference
Fish	BAF	1	Arnot and Gobas 2003 (Arnot-Gobas middle trophic level)
	BCF	1	
Fish	BCF	4	CPOPs 2008
Fish	BCF	3	BCFBAF 2008

According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF is ≥ 5000. All estimated BCFs and BAFs are much lower than the 5000 threshold. The Arnot and Gobas model (2003) and the BCFBAF model (2008) were run using EPIsuite (2008), and user-defined values for water solubility and log K_{ow} for chlorhexidine and chlorhexidine acetate were used in all models.

Biotransformation will not play a significant role, as the log K_{ow} for chlorhexidine acetate is very low; this is demonstrated by the Arnot-Gobas model results where BCF and BAF values are similar both with and without consideration of biotransformation (EPIsuite 2008). The middle trophic level fish in the Arnot-Gobas model was used to represent overall model output, as suggested by the model developer, as it is most representative of fish weight likely to be consumed by an avian or terrestrial piscivore. Also, the BCF of 3 for the BCFBAF model is the default value for an ionic chemical. The model outcomes indicate that chlorhexidine acetate has a low potential to bioaccumulate in fish. This assumption is supported by the physical and chemical properties of the substance.

Therefore, considering the physical and chemical properties of this substance (high water solubility, low experimental log K_{ow} and predicted log D_{ow}, large molecule), its dissociation in water and resulting ionizing characteristics of chorhexidine, as well as the experimental BCF study, it is proposed that chlorhexidine acetate does not meet the bioaccumulation criteria (BCF or BAF ≥ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

In the Aquatic Compartment

There are experimental studies available that investigate ecological effects of both chlorhexidine acetate and chlorhexidine. Given that chlorhexidine acetate dissociates in water to produce chlorhexidine and the acetate counterion, aquatic organisms will be exposed to chlorhexidine. Data are summarized in Table 6a below.

Table 6a. Empirical data for aquatic toxicity of chlorhexidine acetate and chlorhexidine

Test organism	Type of test	Endpoint	Value	Reference

			(mg/L)	
Chlorhexidine acetate				
Rainbow trout (*Oncorhynchus mykiss*)	Acute (96 hours)	LC_{50}	1.9	Murphy and Smith 1991a
Bluegill sunfish (*Lepomis macrochirus*)	Acute (96 hours)	LC_{50}	0.6	Murphy and Smith 1991b
Daphnia magna	Acute (48 hours)	EC_{50} (immobilization)	0.06	Murphy and Smith 1991c
Chlorhexidine				
Zebrafish *(Brachydanio rerio)*	Acute (96 hours)	LC_{50}	1.4	European Commission 2000

LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
EC_{50} – The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
* Critical toxicity value

According to the experimental acute toxicity data on bluegill sunfish (LC_{50}=0.6 mg/L) and on *Daphnia magna* (EC_{50}=0.06 mg/L), chlorhexidine acetate is expected to be highly hazardous to aquatic organisms (acute LC_{50}s or EC_{50}s < 1.0 mg/L). These data were submitted to the U.S. EPA in support of the reregistration of chlorhexidine acetate as a pesticide active ingredient (US EPA 1996); the original studies were not available for review. Additional empirical evidence on the parent compound indicates that chlorhexidine exposure results in acute effects in zebrafish at 1.4 mg/L. Thus, the acute study on *D. magna* reporting an EC_{50} of 0.06 mg/L was identified as the critical toxicity value to be used in deriving the predicted no-effects concentration (PNEC) described later in this report. The empirical evidence suggests that chlorhexidine acetate and chlorhexidine could cause harm to aquatic organisms at low concentrations.

The weight of evidence regarding experimental data for chlorhexidine acetate and chlorhexidine indicates that chlorhexidine acetate is expected to cause acute harm to aquatic organisms at low concentrations (acute LC_{50}s and EC_{50}s are < 1.0 mg/L).

In Other Environmental Compartments

There are some toxicological data on terrestrial organisms for chlorhexidine acetate, and ecological effects are summarized in Table 6b below. The U.S. EPA review of the toxicology data (US EPA1996) concluded that chlorhexidine acetate is mildly to moderately toxic when administered by inhalation, oral and dermal routes.

Table 6b. Toxicity on terrestrial animals for chlorhexidine acetate

Test organism	Type of test	Endpoint	Value	Reference
Northern Bobwhite	Acute single-dose oral (14 days)	LD_{50}	2 013 mg/kg	Campbell et al. 1991
	Subacute dietary (8 days)	LC_{50}	> 5 620 ppm	Long et al. 1991a
Mallard	Subacute dietary (8 days)	LC_{50}	> 5 620 ppm	Long et al. 1991b

LD_{50} – The dose of a substance that is estimated to be lethal to 50% of the test organisms.
LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.

Ecological Exposure Assessment

No data concerning concentrations of chlorhexidine acetate in water in Canada have been identified; therefore, environmental concentrations are estimated from available information, including substance quantities in commerce, estimated release rates, and size of receiving water bodies. The focus of this exposure assessment is on the aquatic environment given that the quantity estimated to be released at the industrial site is assumed to be higher than what may be released as a result of its use on a dairy farm. Given the very low concentration of chlorhexidine acetate in antibacterial products and the limited quantities used at farms across Canada, the losses at any one particular farm are expected to be lower than the quantity released at an industrial site.

Industrial Release

The aquatic exposure of organisms to chlorhexidine acetate is expected if the substance is released from industrial use to a wastewater treatment plant and the treatment plant discharges its effluent to a receiving water body. The concentration of the substance in the receiving water near the discharge point of the wastewater treatment plant is used as the predicted environmental concentration (PEC) in evaluating the aquatic risk of the substance. It can be calculated using the equation

$$C_{water-ind} = \frac{1000 \times Q \times L \times (1-R)}{N \times F \times D}$$

where

$C_{water-ind}$:	aquatic concentration resulting from industrial releases, mg/L
Q:	total substance quantity used annually at an industrial site, kg/yr
L:	loss to wastewater, fraction
R:	wastewater treatment plant removal rate, fraction
N:	number of annual release days, days/yr
F:	wastewater treatment plant effluent flow, m^3/day
D:	receiving water dilution factor, dimensionless

A site-specific exposure analysis was conducted for the aquatic compartment at the industrial site where chlorhexidine acetate was used to produce antibacterial veterinary products (Environment Canada 2010c). This site was identified from the CEPA section 71 survey (Environment Canada 2010a). The quantity of the substance used at this site was 550 kg per year.

The PEC in the receiving water was estimated based on the concentration in the wastewater treatment effluent and by applying a dilution factor limited to a maximum of 10. The

concentration in the wastewater treatment effluent was estimated based on a fraction of the substance lost from the facility to a local municipal wastewater treatment plant, a wastewater treatment plant removal rate and its effluent flow. The fraction lost to wastewater from the production processes was estimated at 1% (i.e., resulting from the cleaning of chemical containers and process equipment relevant to the facilities under consideration) over a release period of 250 days per year. The removal rate by a local wastewater treatment plant is estimated by computer model at 54% for secondary treatment (SimpleTreat 1997).

Based on the above assumptions, the PEC has been estimated to be 0.0011 mg/L (Environment Canada 2010c).

Consumer Release

Chlorhexidine acetate is found in various commercial/consumer products, including specialty products and pharmaceuticals used in veterinary applications, products used as dressings or topical anti-infectives, disinfectant products for barns, domestic cleaners, and a dental product used only in dental offices (see Uses section). Although the substance may be released to municipal wastewater treatment plants through the use of some of these consumer products, the majority of it is expected to be sent to landfill. Therefore, a consumer release scenario was not developed.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine the supporting information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a risk quotient calculation, as well as information on persistence, bioaccumulation, inherent toxicity or ecotoxicity, sources, and fate of the substance.

Chlorhexidine acetate is expected to be highly persistent in water, soil and sediment. It is expected to have a low bioaccumulation potential. The quantity of this substance imported into Canada, along with information on its uses, indicate potential for release into the Canadian environment. Once released into the environment, it is expected to be found mainly in water and soil, although depending on the environmental conditions, may also adsorb to suspended solids and settle in bed sediments or biosolids. With respect to terrestrial organisms, the toxicological data suggest that exposure to chlorhexidine acetate may result in low toxicity to avian species. The available aquatic toxicity data demonstrate that it is highly toxic to aquatic organisms.

A risk quotient analysis was performed by integrating realistic worst-case estimates of exposure with aquatic toxicity information to determine whether there is potential for ecological harm in Canada. The site-specific industrial scenario yielded a PEC of 0.0011 mg/L. A predicted no-effect concentration (PNEC) for chlorhexidine acetate was derived from the acute EC_{50} toxicity value of 0.06 mg/L (the most sensitive valid experimental value) for *D. magna*, by dividing this value by an assessment factor of 100 (to account for interspecies and

intraspecies variability in sensitivity, to estimate a long-term no-effects concentration, and to extrapolate from lab to field studies). The resulting PNEC value is 0.0006 mg/L. The resulting risk quotient (PEC/PNEC) is 2.

Given the inherent toxicity of this substance, its persistence in water, soil and sediment, and a risk quotient indicating the potential for risk, harm to aquatic organisms is possible.

Uncertainties in Evaluation of Ecological Risk

One empirical study was available to support the assessment of the bioaccumulation potential of chlorhexidine acetate. The bioaccumulation assessment also relied on the interpretation of physical and chemical properties (i.e., the water solubility and log D) and the results from model predictions. Although all predictions have some degree of uncertainty, generally these models are relatively reliable, and model outputs confirmed the physical and chemical property indications that chlorhexidine acetate is expected to have a low bioaccumulation potential.

Aquatic toxicity studies on chlorhexidine acetate and chlorhexidine (the dissociation product in water that is of main concern) were available. An assessment factor was used to address additional uncertainties of interspecies and intraspecies variability in sensitivity, to estimate a long-term no-effects concentration and to extrapolate from lab to field studies. Given the potential for this substance to partition to sediments or sludge during wastewater treatment (and hence potentially end up being applied to land as a soil amendment), the significance of soil and sediment as media of exposure is not well addressed by the effects data available.

There is no information on environmental concentrations (e.g., monitoring data) of chlorhexidine acetate in the Canadian environment. This situation necessitated the estimate of concentrations in water using substance quantities in commerce, estimated release rates, and size of receiving water bodies. The quantitative estimate of risk was based on predicted concentrations in water near an industrial point source discharge. Releases from waste disposal sites are possible but difficult to quantify. However, it is expected that the industrial release scenario would provide a realistic worst-case exposure scenario.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media

Empirical data on concentrations of chlorhexidine acetate in environmental media or diet in Canada or elsewhere were not identified. In Canada, chlorhexidine acetate is not expected to be found in the diet based upon the current use pattern. ChemCAN v6.00, a Canada-specific level III fugacity model, was used to estimate potential concentrations of chlorhexidine acetate in various environmental media (ChemCAN 2003). This model is used for the purpose of estimating potential exposure of the general population to environmental concentrations, and therefore differs from the point-source models used in the ecological assessment.

Based on the information submitted in response to a notice published under section 71 of CEPA 1999, the total quantity in commerce was reported to be in the range of 100 to 1 000 kg in 2006 (Environment Canada 2010a). Loss percentages predicted by the Mass Flow Tool (see Table 3) were applied to the upper value of the range (1000 kg) of chlorhexidine acetate quantities in commerce in Canada in 2006. Based on this, annual releases are conservatively estimated to be approximately 455 kg to water from loss to wastewater, surface water and half the loss as a residue on hard surfaces and 535 kg to soil from losses to land, landfill (assuming all the chemical is leached to soil, as a worst-case scenario) and half the loss as a residue on hard surfaces.

The estimated environmental concentrations are presented in Appendix II. Conservative upper-bounding estimates of daily intakes of chlorhexidine acetate for the general population in Canada were derived based on the estimated environmental concentrations (see Appendix III), resulting in a maximum total intake from all routes of environmental exposure of 0.05 μg/kg-bw (kilogram of body weight) per day for formula-fed infants (0–6 months), deriving predominantly from the estimated concentration in surface water (ChemCAN 2003). The estimated surface water concentration was used as a surrogate for drinking water data and is considered to result in a very conservative intake estimate as water treatment was not considered.

Consumer Products

Potential exposure from use of aftershave containing chlorhexidine acetate was estimated using ConsExpo v4.1 (ConsExpo 2006). Typically, aftershave is applied daily. A daily dermal applied dose of 0.0322 mg/kg-bw per day was estimated based on a concentration of chlorhexidine acetate at 0.19% weight/volume (w/v) (Health Canada 2010a). The concentration of 0.19% w/v is the maximum permissible level of chlorhexidine acetate in personal care products as specified on the Cosmetic Ingredient Hotlist (Health Canada 2010a), and is within the concentration reporting range of 0.1-0.3% w/v for aftershave in the Cosmetic Notification System database (CNS 2010). Assumptions used in modelling exposure are presented in Appendix IV.

The liquid cleaner of a floor mopping product available for residential use in Canada contains chlorhexidine acetate at 0.01% w/v (DPD 2011). The cleaner is sprayed onto the floor then mopped with a disposable cleaning pad; inhalation may occur during spraying while dermal contact may occur when the user removes a finished cleaning pad (RIVM 2006b). Exposure estimates were generated using ConsExpo v4.1 for a 4-hour cleaning period (RIVM 2006b). Dermal exposure was estimated to be low with a dermal applied dose of 3.53×10^{-4} mg/kg-bw per event, while inhalation exposure was considered to be negligible (see Appendix IV).

Direct exposure from use of chlorhexidine acetate in therapeutic products (e.g., anti-cavity dental varnish, burn spray and medicated paraffin gauze dressing) is addressed under the *Food and Drug Regulations* (Canada 1978) and is not considered further in this screening assessment.

Health Effects Assessment

Health effects information on chlorhexidine acetate, its parent compound chlorhexidine and another salt, chlorhexidine gluconate, was taken into consideration in the assessment of health effects of chlorhexidine acetate. The empirical health effects data used in this assessment are summarized in Appendix V. The structures of chlorhexidine and chlorhexidine gluconate are presented in Appendix VI.

No classification of the health effects of chlorhexidine acetate by international regulatory agencies was identified. A carcinogenicity study on chlorhexidine gluconate did not identify any increase in neoplasms in rats that were exposed to chlorhexidine gluconate at 5, 25 or 40 mg/kg-bw per day in drinking water (ICI 1992).

In vitro and *in vivo* genotoxicity data for chlorhexidine acetate and chlorhexidine gluconate were predominantly negative. *In vivo* genotoxicity data on chlorhexidine gluconate were negative in a mouse dominant lethal assay that exposed male Swiss mice to two applications of 10, 20 or 30 mg/kg-bw chlorhexidine gluconate at 24-hour intervals and a hamster cytogenetic test at the highest dose of 250 mg/kg-bw (COLIPA 1984; McEvoy 2010). In another *in vivo* genotoxicity study that exposed male Wistar rats to 0.5 mL of 0.12% chlorhexidine gluconate in drinking water, positive DNA damage was observed in leukocytes and oral mucosal cells, but micronucleus assay of erythrocytes from peripheral blood cells was negative (Ribeiro et al.

2004). *In vitro* genotoxicity data for chlorhexidine acetate in a mouse lymphoma cell assay, a chromosomal aberration assay in Chinese hamster ovary cells and a DNA damage assay in rat hepatocyte cultures were all negative (Farrow 1983; Myhr 1983; Cifone 1984).

The acute toxicity of chlorhexidine acetate following inhalation, dermal and oral exposure is considered to be low based on the lowest oral mean lethal dose (LD_{50}) of 1180 mg/kg-bw in rats, the lowest dermal LD_{50} of greater than 2000 mg/kg-bw in rabbits and the lowest inhalation mean lethal concentration (LC_{50}) of 300 mg/m^3 in rats (Miller 1993a, 1993b; Shapiro 1993). Chlorhexidine acetate caused peritonitis and death in Sprague-Dawley rats at a dose of 20 mg/kg-bw following intraperitoneal administration for 5 consecutive days (Greener et al. 1985).

Chlorhexidine acetate is minimally or marginally irritating to the skin and eyes of rabbits (Greener et al. 1985). Several cases of sensitization were reported in humans in patch or prick tests with chlorhexidine acetate (Reynolds and Harman 1990; Evans 1992; Wong et al. 1990; Leow and Goh 1999), and its analogue, chlorhexidine gluconate (Roberts et al. 1981; Bechgaard et al. 1985; Bergovist-Karlsson 1988; Okano et al. 1989; Osmundsen 1982). However, these sensitization cases were mostly observed in individuals with pre-existing skin disorders or when applied to mucous membrane. Garvey et al. (2003) investigated the prevalence of sensitization and allergy to chlorhexidine in health care workers. None of the 104 doctors, nurses and auxiliary staff had any reactions to skin patches containing chlorhexidine acetate (1%) and chlorhexidine gluconate (1%) in water (Garvey et al. 2003).

Following subchronic dermal exposure, skin irritation and decreased enzyme activity coupled with degenerative changes in the liver were observed in New Zealand White rabbits dosed at 250 mg/kg-bw per day for 13 weeks (Henwood 1988). Lung effects were observed in 2 of 4 beagle dogs exposed to fog with an unknown concentration of chlorhexidine acetate repeatedly for 30 days (Andrews and Paul 1977).

A reproductive study that tested a number of related compounds reported that chlorhexidine reduced the number of litters by half in mice that were exposed to the test substances at 400 mg/kg-bw per day for a week (information on maternal toxicity of chlorhexidine was not provided) (Cutting et al. 1964). No empirical data on reproductive toxicity for chlorhexidine acetate were identified.

A developmental study on chlorhexidine acetate did not identify adverse developmental effects in rats that were exposed orally to 0, 15.63, 31.26 or 62.5 mg/kg-bw per day on gestation day 6 through 15 (Lamb 1991). Maternal toxicity including dose-related reduced body weight gain, rales and increased salivation were observed at 31.25 mg/kg-bw per day (Lamb 1991). Similarly, a developmental study on chlorhexidine did not identify an adverse effect in the fetuses of pregnant rats that were exposed to a dose of 68.5 mg/kg-bw per day via gastric intubation on gestation days 6-15 (Gilman and De Salva 1979).

Empirical data in humans on dermal absorption of chlorhexidine acetate were identified and a low absorption potential was reported. Using a 4% handwash or 5% aqueous solution of radio-labelled chlorhexidine (the parent compound of chlorhexidine acetate), investigators

administered the compound for 3 hours to the intact forearm skin of adults and did not observe significant dermal absorption, i.e., 96–98% of radio-labelled chlorhexidine was recovered from the skin and no radio-labelled chlorhexidine was detected in blood or urine (Case 1980). In another study presented in Case (1980), chlorhexidine was not detected in the blood of human volunteers at any sampling time throughout the period of an exaggerated surgical scrub routine using 4% chlorhexidine five times daily for three 5-day weeks, each treatment lasting 6 minutes (detection limit of 0.01-0.05 mg/L). In addition, blood samples of hospital staff who had used the same 4% chlorhexidine solution for surgical hand disinfection for at least 6 months had no detectable chlorhexidine (Case 1980). Dermal absorption of chlorhexidine gluconate, another salt of chlorhexidine, in humans, is also reported to be low, if it occurs at all, based on a method detection limit of 0.005 mg/L (RMOL 2004). A study of dermal absorption of a 5% solution of chlorhexidine gluconate through the abdominal skin of female hairless rats resulted in only 0.01% of the initial quantity diffused through intact skin after 48 h of exposure (Lafforgue et al. 1997). Lastly, no detectable levels of chlorhexidine were detected in the blood or urine when a skin cleanser containing 8% w/v chlorhexidine digluconate was administered to the skin of five neonatal rhesus,monkeys, representing a total surface area exposed of roughly 500-600 cm^2, for 90–92 days, with a daily 5-minute washing period (detection limit of 11 ng/mL). The author considered that chlorhexidine digluconate was bound in the cutaneous layer of the skin and would diffuse into vascular layers only at a very low rate. However, trace levels of chlorhexidine digluconate were found in some samples of adipose tissue (detection limit of 11 ng/g), kidneys (detection limit of 6 ng/g) and liver (detection limit of 6 ng/g), indicating potentially low levels of dermal absorption or possible oral ingestion from monkey grooming habits, despite a rinsing protocol after the 5-minute washing period, or contamination of samples (Gongwer et al. 1980).

Characterization of Risk to Human Health

In the available empirical data on genotoxicity of chlorhexidine acetate and carcinogenicity and genotoxicity of chlorhexidine gluconate, no evidence for carcinogenicity or genotoxicity were observed. Therefore, characterization of risk in this assessment is based on non-cancer effects of chlorohexidine acetate.

The lowest lowest-observed-effect level (LOEL) following subchronic dermal exposure of chlorhexidine acetate was 250 mg/kg-bw per day based on skin irritation and liver effects observed in New Zealand White rabbits. The lowest LOEL following short-term repeated oral exposure was 31.25 mg/kg-bw per day based on dose-related reduced body weight gain, rales and increased salivation observed in female rats in a developmental study. Reproductive effects were observed at a higher dose in mice after one-week oral exposure to chlorhexidine, the parent compound of chlorhexidine acetate.

The principal source of exposure to chlorhexidine acetate for the general population is expected to be through the use of consumer products (aftershave and cleaning products), predominantly via the dermal route. Based on this, a dermal LOEL from a 13-week study was considered to be the most relevant endpoint for use for risk characterization. Comparison of the applied dermal doses of chlorhexidine acetate from use of aftershave (0.0322 mg/kg-bw per day) and floor mopping system (3.53×10^{-4} mg/kg-bw per event) to the lowest subchronic

dermal LOEL for dermal irritation and liver effects in rabbits (250 mg/kg-bw per day) results in margins of exposure of 7800 to 708 000. These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases.

Comparison of the upper-bounding intake from environmental media (0.05 μg/kg-bw per day for formula-fed infants) and the lowest oral LOEL for dose-related reduced body weight gain, rales and increased salivation in female rats (31.25 mg/kg-bw per day) results in a margin of exposure of 625 000. This margin of exposure is considered adequate to address uncertainties in the health effects and exposure databases.

Uncertainties in Evaluation of Risk to Human Health

There is a significant level of uncertainty associated with the environmental exposure estimate for chlorhexidine acetate. Data in the literature were not identified for concentrations of this substance in environmental media; therefore, modelling with ChemCAN v6.00 was conducted. There were uncertainties with the modelling assumptions such as using estimated physical and chemical properties, using loss percentages from the Mass Flow Tool as surrogates for release quantities and using the maximum value of the quantity in commerce range instead of the actual quantity in commerce. Uncertainties associated with the consumer product exposure estimates are moderate. Consumer uses in Canada were identified from responses to a notice issued under section 71 of CEPA 1999 in addition to literature searches and information received from other groups within Health Canada. Consumer exposure estimates were modelled using default scenarios available in ConsExpo v4.1 based upon reasonably conservative assumptions.

The modes of action of chlorhexidine acetate for the induction of the health effects observed in animals were not fully elucidated. Empirical data on acute, short-term, subchronic, developmental toxicity and *in vitro* genotoxicity for chlorhexidine acetate were available. The parent compound, chlorhexidine, and another salt of chorhexidine, chlorhexidine gluconate, were considered in this assessment.

Conclusion

Based on the information presented in this draft screening assessment, it is proposed that chlorhexidine acetate meets the criteria in paragraph 64*(a)* of CEPA 1999, as it is entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. However, chlorhexidine acetate does not meet the criteria under paragraph 64*(b)* of CEPA 1999 as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends. Additionally, chlorhexidine acetate meets the criteria for persistence but does not meet the criteria for bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Based on the information currently available on its potential to cause harm to human health, it is proposed that chorhexidine acetate does not meet the criteria in paragraph 64*(c)* of CEPA 1999, as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed that chlorhexidine acetate meets one or more criteria under section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

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Appendix I – Robust Study Summary

Determination of the partition coefficient for chlorhexidine acetate

Item	Weight	Response	Mark
Reference: Determination of the partition coefficient for chlorhexidine acetate (Study Submission 2010)			
Test substance: CAS RN:			
Could you repeat the experiment with available information?	5		5
Is a clear objective stated?	1		1
Is water quality characterized or identified (distilled or deionized)?	2		2
Are the results presented in detail, clearly and understandably?	3		3
Are the data from a primary source and not from a referenced article?	3		3
Was the chemical tested at concentrations below its water solubility?	5		5
Were particulates absent?	2		1
Was a reference chemical of known constant tested?	3		2
Were other fate processes considered?	5	n/a	
Was a control (blank) run?	3	n/a	
Was temperature kept constant?	5		5
Was the experiment done near room temperature (15–30^{o}C)?	3		3
Is the purity of the test chemical reported (> 98%)?	3		3
Was the chemical's identity proven?	3		3
Is the source of the chemical reported?	1		1
Results:			
Score:	37/39		
Degree of reliability	Satisfactory		

N/A – not applicable

Appendix II – Estimated Concentrations of Chlorhexidine Acetate in Environmental Media Using ChemCAN Version 6.00 (ChemCAN 2003)[1]

Medium[2]	Estimated concentration
Ambient air[3]	5.58×10^{-3} ng/m^3
Surface water[4]	438 ng/L
Soil[4]	1.28 ng/g solids
Sediment[4]	0.477 ng/g solids

[1]The concentrations were estimated for the area of southern Ontario.
[2]Default inflow concentrations of 2 ng/m^3 in air and 3 ng/L in water were specified by ChemCAN.
[3]The atmospheric oxidation half-life in air was assumed to be approximately 25 minutes (AOPWIN 2008).
[4] The degradation half-lives in the aquatic, soil and sediment compartments were assumed to be indefinite.

Appendix III – Upper-bounding Estimates of Daily Intakes of Chlorhexidine Acetate for Various Age Groups

Route of exposure	Estimated intake (µg/kg-bw per day) of chlorhexidine acetate by various age groups							
	0–0.5 years[1,2,3]			0.5–4 years[4]	5–11 years[5]	12–19 years[6]	20–59 years[7]	60+ years[8]
	Breast milk fed	Formula fed	Not formula fed					
Air[9]	1.6×10^{-6}	1.6×10^{-6}	1.6×10^{-6}	3.3×10^{-6}	2.6×10^{-6}	1.5×10^{-6}	1.3×10^{-6}	1.1×10^{-6}
Drinking water[10]	N/A	4.7×10^{-2}	1.8×10^{-2}	2.9×10^{-2}	1.6×10^{-2}	8.9×10^{-3}	9.3×10^{-3}	9.7×10^{-3}
Food and beverages[11]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Soil[12]	5.1×10^{-6}	5.1×10^{-6}	5.1×10^{-6}	8.3×10^{-6}	2.7×10^{-6}	6.5×10^{-7}	5.4×10^{-7}	5.3×10^{-7}
Total intake	6.7×10^{-6}	4.7×10^{-2}	1.8×10^{-2}	2.0×10^{-2}	1.6×10^{-2}	8.9×10^{-3}	9.2×10^{-3}	9.7×10^{-3}
Maximum total intake from all routes of exposure: 0.05 µg/kg-bw per day								

N/A – not available

[1] No quantitative data were identified for concentrations of chlorhexidine acetate in breast milk.

[2] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).

[3] For exclusively formula-fed infants, intake from water is synonymous with intake from food. No quantitative data on concentrations of chlorhexidine acetate in drinking water or formula were identified. The concentration of chlorhexidine acetate in drinking water was estimated using ChemCAN v6.00 at 438 ng/L (ChemCAN 2003). For non-formula-fed infants, approximately 50% are introduced to solid foods by four months of age and 90% by six months of age (NHW 1990).

[4] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998).

[5] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998).

[6] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[7] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[8] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[9] No quantitative data were identified for concentrations of chlorhexidine acetate in air. The concentration of chlorhexidine acetate in air was estimated using ChemCAN v6.00 at 5.58×10^{-3} ng/m^3 (ChemCAN 2003).

[10] No quantitative data were identified for concentrations of chlorhexidine acetate in drinking water. The concentration of chlorhexidine acetate in drinking water was estimated using ChemCAN v6.00 at 438 ng/L (ChemCAN 2003).

[11] No quantitative data were identified for concentrations of chlorhexidine acetate in food or beverages.

[12] No quantitative data were identified for concentrations of chlorhexidine acetate in soil. The concentration of chlorhexidine acetate in soil was estimated using ChemCAN v6.00 at 1.28 ng/g solids (ChemCAN 2003).

Appendix IV – Dermal and Inhalation Exposure Estimates for Aftershave and Floor Mopping System

Product	Assumptions	Exposure estimate
Aftershave[1]	Used ConsExpo v4.1 for modelling (ConsExpo 2006). - Maximum weight percent: 0.19% w/v (Health Canada 2010a) - Exposure frequency: 365/yr (RIVM 2006a) - Exposed area: 318.75 cm^2 (¼ area of adult head) (Health Canada 1995) - Applied amount: 1.2 g (RIVM 2006a) - Exposure duration : constant (24 h/day) (RIVM 2006a)	Applied dermal dose: 0.0322 mg/kg-bw per day
Floor Mopping System[1]	Used ConsExpo v4.1 default scenario for floor mopping system: Dermal exposure calculation: - Concentration: 0.01% w/v (DPD 2011) - Application procedure: cleaning pad is applied to mop head, floor area is sprayed with liquid cleaner then mopped (RIVM 2006b) - Exposure frequency: 104/yr (RIVM 2006b) - Floor area: 22 m^2 (living room) (RIVM 2006b) - Product amount: 245 g (RIVM 2006b) - Exposure duration: 3 min (dermal contact when removing cleaning pad) (RIVM 2006b) - Assumption of 10% (i.e. 24.5 g) of cleaner remains in the pad and 1% of this amount contacts skin, therefore dermal load of 0.25 g (RIVM 2006b) - Skin area exposed: 227.5 cm^2 (¼ area of hands) (Health Canada 1995) Inhalation exposure calculation (evaporation from increasing area at a linear rate): - Exposure duration: 240 min (RIVM 2006b) - Product amount: 245 g (RIVM 2006b) - Room volume: 58 m^3 (living room) (RIVM 2006b) - Ventilation rate: 0.5/hr (living room) (RIVM 2006b) - Application duration: 30 min (RIVM 2006b) - Molecular weight matrix: 22 g/mol (RIVM 2006b) - Mass transfer rate: 1.5×10^3 m/min (Langmuir's method) (RIVM 2006b) - Uptake fraction: 1	Applied dermal dose: 3.53×10^{-4} mg/kg-bw per event Inhalation mean event concentration: 1.33×10^{-15} mg/m^3 Inhalation acute internal dose: 5.07×10^{-17} mg/kg-bw per event

1 The adult body weight and inhalation rate were assumed to be 70.9 kg and 16.2 m^3/day respectively (Health Canada 1998).

Appendix V: Summary of Health Effects Information on Chlorhexidine Acetate and Its Analogues (Chlorhexidine and Chlorhexidine Gluconate)

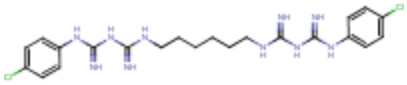
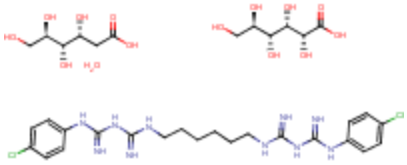
Endpoint	LD_{50}/LC_{50} or Lowest/no effect levels[1]/results
Acute toxicity	**Lowest inhalation LC_{50}** = 300 mg/m^3 in male rats (Shapiro 1993; cited in US EPA 1996). **Other inhalation LC_{50}** = 430 mg/m^3 in female rats (Shapiro 1993; cited in US EPA 1996). [No additional acute inhalation studies identified] **Lowest oral LD_{50}** = 1180 mg/kg-bw in male rats (Miller 1993a; cited in US EPA 1996). **Other oral LD_{50}** = 1710 mg/kg-bw in female rats (Miller 1993a; cited in US EPA 1996). [Other acute oral studies: Davies et al. 1954] **Lowest dermal LD_{50}** > 2000 mg/kg-bw in rabbits (Miller 1993b; cited in US EPA 1996) [No additional acute dermal studies identified] [Additional acute studies: Greener et al. 1985; Ostad and Gard 2000]
Short-term repeated-dose toxicity	**Inhalation**: groups of 4 beagle dogs, exposed to fog (concentration unknown) created from three ounces of chlorhexidine acetate and one gallon of water in a Fog Master Tri-Jet, twice daily for 30 days. Multifocal consolidation of the lungs was noted in 4 treated dogs and 2 controls. Several scattered focal accumulations of polymorphonuclear and lymphoid inflammatory cells were present in the submucosa of one treated dog (Andrews and Paul 1977).
Subchronic toxicity	**Lowest dermal LOEL**= 250 mg/kg-bw per day, based on minimal dermal irritation (erythema, edema, desquamation and/or fissuring), decreased liver enzyme activity, coupled with microscopically observed degenerative changes in the liver (indicative of a hepatic effect) in female New Zealand White rabbits (group size and sex ratio unknown) treated topically at doses of 0, 250, 500 or 1000 mg/kg/day for 13 weeks (Henwood 1988; cited in US EPA 1996)
Chronic toxicity/ carcinogenicity	**Chlorhexidine gluconate (CAS RN 18472-51-0)** Groups of 112 male and 112 female Wistar-derived specific pathogen-free rats were given chlorhexidine gluconate-dosed drinking water at 5, 25 and 40 mg/kg-bw per day. Chlorhexidine gluconate did not induce an increase in neoplasms. No information on non-neoplastic effects was provided (ICI 1992; cited in Willis 1993).
Developmental toxicity	**NOAEL**(developmental toxicity) = 62.5 mg/kg-bw per day, **LOEL** (maternal toxicity) = 31.25 mg/kg per day, based on dose-related reduced body weight gain, rales, and increased salivation in Sprague-Dawley rats dosed by gavage at 0, 15.63, 31.25 or 62.5 mg/kg-bw per day, on gestation days 6 through 15. No malformations or developmental toxicity were observed at any dose level tested (Lamb 1991; cited in US EPA 1996). **Chlorhexidine (CAS RN 55-56-1)** **Oral NOAEL** = 68.5 mg/kg-bw per day, based on no adverse effects observed in

	pregnant rats that were exposed to chlorhexidine (free base) via gastric intubation at 68.5 mg/kg-bw per day on gestation days 6 through 15. Rats were killed on day 20 and the fetuses examined (Gilman and De Salva 1979; cited in Willis 1993).
Reproductive toxicity	**Chlorhexidine (CAS RN 55-56-1)** **Oral LOAEL** = 400 mg/kg-bw per day (0.2% in drinking water, converted based on bw = 0.03 kg, water consumption = 0.006 L/day [Calabrese and Kenyon 1991], based on reduced number of litters in a study that exposed male and female mice (strain, group size unknown) to chlorhexidine (free base) in drinking water at concentration of 0.2% for 1 week. The sexes were mixed and the litters counted. Chlorhexidine reduced the number of litters by half, but did not influence the number of mice in each litter (Cutting et al. 1964; cited in Willis 1993).
Genotoxicity and related endpoints: *in vivo*	**Chlorhexidine gluconate (CAS RN 18472-51-0)** **Mouse dominant lethal assay** **Negative**: 3 groups of 10 male Swiss mice exposed to two applications of 10, 20 and 30 mg/kg-bw chlorhexidine gluconate in a dimethyl sulfoxide vehicle for 2 applications at 24-hour intervals (COLIPA 1984; cited in Willis 1993) **Hamster cytogenetic test** **Negative**: hamster (group size, sex, strain not specified), 250 mg/kg (detailed dose regime not specified) (McEvoy 2010) **Comet assay (DNA damage)** **Positive**: Groups of 30 male Wistar rats exposed to 0 or 0.5 mL of 0.12% chlorhexidine gluconate, or 0.5g/L of 4-nitroquinoline 1-oxide (as positive control) in drinking water, twice daily for 8 days, peripheral leukocytes and oral mucosal cells were examined (Ribeiro et al. 2004). **Miconucleus assay** **Negative**: Groups of 30 male Wistar rats exposed to 0 or 0.5 mL of 0.12% chlorhexidine gluconate, or 0.5g/L of 4-nitroquinoline 1-oxide (as positive control) in drinking water, twice daily for 8 days peripheral blood erythrocytes were examined (Ribeiro et al. 2004).
Genotoxicity and related endpoints: *in vitro*	**Gene mutation in mammalian cell assay** **Negative**: mouse lymphoma cell, up to cytotoxic levels (6 µg/mL in non-activated assays and 15–16 µg/mL in activated assays) (Cifone 1984; cited in US EPA 1996). **Chromosomal aberrations** **Negative:** Chinese hamster ovary cells, with and without activation at test levels up to 10 µg/mL (reduced cell growth 30% of control) (Farrow 1983; cited in US EPA 1996). **Unscheduled DNA synthesis assay** **Negative**: rat hepatocyte cultures, at levels up to 2.42 µg/mL (increased net nuclear counts at 18 hours) (Myhr 1983; cited in US EPA 1996)
Irritation	**Dermal irritation** **Irritating (marginally):** groups of 3 male and 3 female rabbits, at 24 hours after treatment, irritation index of 0.417 ± 0.563 (Greener et al. 1985). **Ocular irritation** **Irritating (minimal):** groups of 6 male and 6 female rabbits, at 3 hours after treatment, irritation index of 0.67 ± 1.03, but not at 1 to 7 days after treatment (Greener et al. 1985).
Experience with human exposure	***Chlorhexidine acetate*** **Sensitization** **Sensitizing:** a 74-year-old man, patch testing to 0.5% and 0.05% chlorhexidine acetate swab. 20 controls were also patch tested to chlorhexidine acetate with negative results

	(Reynolds and Harman 1990). **Sensitizing**: a 19-year-old man, cleaned and dressed with a tulle gras dressing containing 0.5% chlorhexidine acetate BP. The patient complained of pruritus and feeling lightheaded. Erythematous rash, periorbital edema, and mild angio-edema were observed through examination (Evans 1992). **Sensitizing**: a 37-year-old Chinese patient, prick test with 0.5% aqueous solution, resulted in wheals of 7 mm, with flares, after 20 minutes. 5 controls had negative prick tests to chlorhexidine acetate (Wong et al. 1990; Leow and Goh 1999). **Not sensitizing**: a 37-year-old Chinese patient, patch test with 0.5% aqueous solution (Wong et al. 1990). **Sensitizing:** 1063 eczema patients. Original test showed positive reactions in 52 patients (4.9%). 29 were retested. The following numbers are for the retested patients: 7–16 patients (0.66%–1.5%) tested positive for 0.05%–0.5% chlorhexidine acetate. 10–19 patients (0.94%–1.8%) tested positive for 0.1%–1.0% chlorhexidine gluconate (Andersen and Brandrup 1985). **Not sensitizing:** 104 health workers (with daily exposure to chlorhexidine) had any reactions in patch tests with 1% chlorhexidine acetate or 1% chlorhexidine gluconate. These individuals were not super humans; 33.7% of them had other verified allergies (pollen, fur animals, dustmites, veggies/fruits, nickel, latex, formaldehyde) (Garvey et al. 2003). ***Chlorhexidine gluconate*** **Sensitizing:** 66-year-old man, positive reaction with 0.5% aq. chlorhexidine digluconate (Bergovist-Karlsson 1988). **Sensitizing:** 6 cases of sensitization to concentration of chlorhexidine gluconate 0.05–1.0%. Reactions observed when chlorhexidine gluconate was applied to the skin or on a mucous membrane (Okano et al. 1989). **Sensitizing:** 2 new cases (also discusses 2 previous cases). Contact sensitivity to chlorhexidine digluconate 0.04% aq., or greater (Roberts et al. 1981). **Sensitizing:** 14 out of 551 patients (2.5%) showed strong positive reactions to patch tests with chlorhexidine gluconate 1% in water. Of those 14 patients, 10 had eczema of the legs, while 4 had eczema of the face and scalp. (Osmundsen 1982). **Sensitizing**: 2061 eczema patients. 48 patients (2.3%) showed positive reactions to the first patch test (chlorhexidine gluconate 1.0% aq.). 14 patients were retested, and only 1 showed a positive reaction on this 2nd test. The authors propose that the "apparent loss of sensitivity may be due to irritable skin at the initial testing, the so-called excited skin syndrome" (Bechgaard et al. 1985).

[1]LD_{50}/LC_{50} = median lethal dose/median lethal concentration; LOEL/LOEC = lowest-observed-effect level/concentration; LOAEL/LOAEC = lowest-observed-adverse-effect level/concentration; NOAEL/NOAEC = no-observed-adverse-effect level/concentration.

Appendix VI – Structure and Molecular Weight of Chlorhexidine and Chlorhexidine Gluconate

Name / CAS RN	Structure	Molecular weight (g/mol)
Chlorhexidine 55-56-1		505.46
Chlorhexidine gluconate 18472-51-0		899.78