Screening Assessment

Cyclosporin A and Cyclosporin E

Chemical Abstracts Service Registry Numbers

59865-13-3 and 63798-73-2

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 of cyclosporin A and cyclosporin E, Chemical Abstracts Service Registry Numbers 59865-13-3 and 63798-73-2, respectively. Substances in this grouping were identified as priorities for assessment as they met categorization criteria under subsection 73(1) of CEPA 1999 and/or were considered as a priority based on other human health concerns. Cyclosporin A was identified as a priority for assessment because it had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity. Cyclosporin E has no such classification but is structurally very similar to cyclosporin A. Both cyclosporin A and cyclosporin E met the categorization criteria for persistence and inherent toxicity to aquatic organisms.

Drugs containing cyclosporin A as an ingredient are assessed under the *Food and Drugs Act* (F&DA) with respect to their safety, effectiveness and quality. This assessment focused on uses and exposures that were not covered as part of the F&DA assessment, specifically the risks posed by the residues resulting from manufacture, formulation and disposal after use.

Cyclosporins are naturally occurring organic substances in the environment, produced by certain species of fungi. Cyclosporins may be released by the fungi as toxins or to impair immune responses within the infected organisms (e.g., insects), thereby facilitating fungal development.

Cyclosporin A is used in Canada as an active pharmaceutical ingredient in human and veterinary drugs. It is a therapeutic and immunosuppressant agent, commonly used in humans to prevent the rejection of allograft/organ transplants and to treat rheumatoid arthritis and psoriasis. Data were available to estimate that 622, 548 and 544 kg of cyclosporin A were sold to hospitals and pharmacies across Canada in 2007, 2011 and 2012, respectively. There are no registered uses for cyclosporin E as a pharmaceutical in Canada, and no other uses were identified, therefore cyclosporin E is not believed to be in commerce in Canada.

Cyclosporin E is considered to be structurally and chemically similar to cyclosporin A, such that any differences would not significantly impact the functionality or toxicity of the substance. As such, the available modelled and experimental data for cyclosporin A were used directly as read-across data for cyclosporin E.

Based on their physical and chemical properties [water solubility, volatility and octanol–water partition coefficient (log K_{ow})], cyclosporin A and cyclosporin E are expected to reside in air, water and soil, depending on the compartment of release. Modelled data suggest that cyclosporin A and cyclosporin E have the

potential to persist in water, soil and sediment. Cyclosporin A and cyclosporin E have low bioaccumulation potential based on modelled data, their physical and chemical properties (i.e., high molecular weight, low log K_{ow}) and the high potential for fish to metabolize cyclosporin A.

Cyclosporin A can make its way to surface waters through release from manufacturing or formulation sites and/or release of the unmetabolized substance in feces or urine from consumers using this substance. Given these potential releases, the main source of ecological exposure to cyclosporin A is through water. Because no information was available regarding actual releases of this substance in Canada, realistic conservative exposure scenarios, selected for a site-specific industrial operation and for down-the-drain releases through prescribed use of cyclosporin A, were developed to estimate discharges of cyclosporin A into the aquatic environment. As cyclosporin E is not registered for pharmaceutical use in Canada, there are no known releases to or exposures in the Canadian environment. Cyclosporin A and cyclosporin E are considered to have moderate to high acute aquatic toxicity. A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The conservative industrial and consumer use scenarios yielded risk quotients well below 1 (e.g, 0.015). Therefore, harm to aquatic organisms is unlikely from industrial use or the consumption of pharmaceutical products that contain cyclosporin. This information suggests that cyclosporin A and cyclosporin E do not have the potential to cause ecological harm in Canada.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from these substances. It is concluded that cyclosporin A and cyclosporin E do not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as they are not 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 or that constitute or may constitute a danger to the environment on which life depends.

In terms of general population exposure, the principal potential source of exposure is drinking water. The exposure to cyclosporin A present in drinking water is significantly smaller than the exposure to cyclopsorin A through its use as a pharmaceutical.

For this assessment, conservative assumptions were used when estimating the potential indirect exposure of the general population to cyclosporin A. No measured concentrations were identified in any media in Canada or elsewhere. For the purposes of this assessment, modelled concentrations in surface water in Canada were used as conservative proxies for drinking water concentrations.

In regard to potential general population exposure, upper-bounding estimated intakes of cyclosporin A from environmental media were low. Based on these low exposures, risks from this substance are not expected. To further support this risk characterization, the upper-bounding estimated indirect exposures of the general population were compared with the lowest therapeutic dose (LTD) identified for the substance. The margin of exposure was large (20 000).

Since cyclosporin E is not identified to be in commerce in Canada, exposure—and hence risk—is not expected.

Based on the adequacy of the margins of exposure, it is concluded that cyclosporin A and cyclosporin E do not meet the criteria under paragraph 64(*c*) of CEPA 1999, as they are 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.

Conclusion

It is concluded that these substances do not meet any of the criteria set out in section 64 of CEPA 1999.

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1. Introduction

Pursuant to sections 68 and 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999), the Minister of the Environment and the Minister of Health conduct screening assessments of substances to determine whether these substances present or may present a risk to the environment or to human health.

A screening assessment was undertaken on the substances cyclosporin A and cyclosporin E, Chemical Abstracts Service Registry Numbers (CAS RNs) 59865-13-3 and 63798-73-2, respectively, as they were identified during the categorization of substances on the Domestic Substances List (DSL) as meeting the criteria for persistence and inherent toxicity to aquatic organisms. They did not meet the criteria for bioaccumulation potential. Cyclosporin A had also been identified as posing a potential high hazard to human health based on classifications by other national or international agencies for carcinogenicity.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999). Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.[1]

This assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposure, including additional information submitted by stakeholders. Relevant data were identified up to March 2013. Empirical data from key studies as well as some results from models were used to reach conclusions. When available and relevant, information presented in risk and hazard assessments from other jurisdictions was considered.

The 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.

[1] 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 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the Controlled Products Regulations, which are part of the regulatory framework for the Workplace Hazardous Materials Information System 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 taken under other sections of CEPA 1999 or other Acts.

Drugs containing cyclosporin A as an ingredient were previously assessed under the *Food and Drugs Act* (F&DA) (Canada 1985) with respect to their safety, effectiveness and quality. This assessment focused on uses and exposures that were not covered as part of the F&DA assessment, specifically the risks posed by the residues resulting from manufacture, formulation and disposal after use.

The 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 and human health portions of this assessment have undergone external written peer review and/or consultation. Comments on the technical portions relevant to the environment were received from Chris Metcalfe, Trent University and Vance Trudeau, University of Ottawa. Comments on the approach used to assess the substance with respect to human health were received from Warren Foster, McMaster University, Sam Kacew, McLaughlin Centre for Population Health Risk Assessment, and Beate Escher, University of Queensland. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada.

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

2. Substance Identity

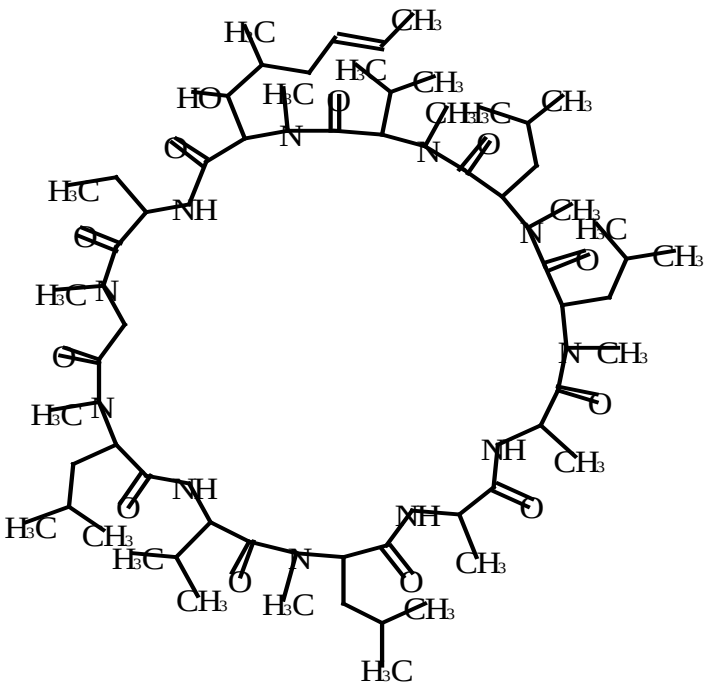
Cyclosporin A can be manufactured as chemical grade for use in research and development (Sigma-Aldrich 2010) or as pharmaceutical grade. For the purpose of this screening assessment, information from both the pharmaceutical and the chemical grades of cyclosporin A is treated equally and interchangeably.

Cyclosporin A and cyclosporin E are cyclic oligopeptides composed of amino acid residues. Cyclosporin A is considered to have neutral (Ran et al. 2001), lipophilic (Podsiadlowski et al. 1998) and hydrophobic properties (Weiser and Matha 1988; Vilcinskas et al. 1999; Sigma-Aldrich 2010).

Substance identity information on cyclosporin A and cyclosporin E is given in Tables 2-1 and 2-2, respectively.

Table 2-1. Substance identity: cyclosporin A

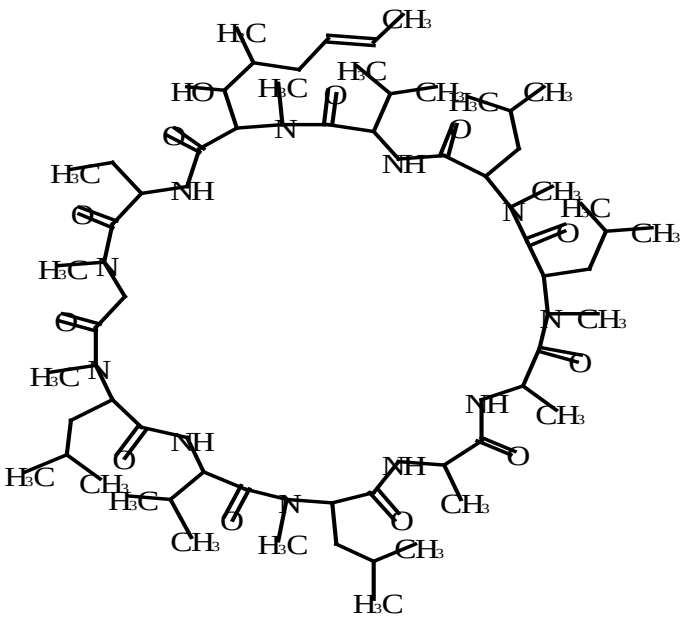
CAS RN	59865-13-3
DSL name[1]	Cyclosporin A
NCI names[1]	Cyclosporin A (ASIA-PAC, NZIoC)

Other names	Antibiotic S 7481F; Arpimune ME; Cicloral; Cicloral antibiotic; Ciclosporin; Cipol N; Consupren; Cyclosporin; Cyclosporine; Cyclosporine A; Cyclo[L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-(3*R*,4*R*,6*E*)-6,7-didehydro-3-hydroxy-*N*,4-dimethyl-L-2-aminooctanoyl-L-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl]; Debio088; Equoral; Gengraf; Neoplanta; Neoral; NSC 290193; OL 27-400; Papilock Mini; Ramihyphin A; Restasis; S-Neoral; Sandimmun; Sandimmun Neoral; Sandimmune; Sandimmune Neoral; Sang-35; SangCyA; SDZ-OXL 400; Sigmasporin Microoral; Zinograf M
Major chemical class or use	Amino acid, cyclic peptide and protein
Major chemical subclass	Cyclic undecapeptide (oligopeptide)
Chemical formula	$C_{62}H_{111}N_{11}O_{12}$
Chemical structure	
SMILES	CN1C(=O)C(C)NC(=O)C(C)NC(=O)C(CC(C)C)N(C)C(=O)C(C(C)C)NC(=O)C(CC(C)C)N(C)C(=O)CN(C)C(=O)C(CC)NC(=O)C(C(O)C(C)CC=CC)N(C)C(=O)C(C(C)C)N(C)C(=O)C(CC(C)C)N(C)C(=O)C1CC(C)C
Molecular mass	1202.61 g/mol

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; NCI, National Chemical Inventories; NZIoC, New Zealand Inventory of Chemicals; SMILES, simplified molecular input line entry system

[1] NCI (2007)

Table 2-2: Substance identity: cyclosporin E

CAS RN	63798-73-2
DSL name[1]	Cyclosporin E
NCI names[1]	Cyclosporin E (DSL)
Other names	11-Demethylcyclosporin A; 5-L-Valine-cyclo[L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-L-valyl-(3*R*,4*R*,6*E*)-6,7-didehydro-3-hydroxy-*N*,4-dimethyl-L-2-aminooctanoyl-L-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl]
Major chemical class or use	Amino acid, cyclic peptide and protein
Major chemical subclass	Cyclic undecapeptide (oligopeptide)
Chemical formula	$C_{61}H_{109}N_{11}O_{12}$
Chemical structure	
SMILES	CN1C(C(NC(C(NC(C(N(C(C(NC(C(N(C(CN(C(C(NC(C(N(C(C(NC(C(N(C(C1CC(C)C)=O)C)CC(C)C)=O)C(C)C)=O)C)C(C(C/C=C/C)C)O)=O)CC)=O)C)=O)C)CC(C)C)=O)C(C)C)=O)C)CC(C)C)=O)C)=O)C)=O
Molecular mass	1188.61 g/mol

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; NCI, National Chemical Inventories; SMILES, simplified molecular input line entry system

[1] NCI (2007)

2.1 Analogues

Structural analogues having relevant empirical data may be used to help assess those substances that lack empirical data. Structural analogues are chemicals that are structurally similar to one another and therefore are expected to have

similar physical and chemical properties, to behave similarly in the environment and to demonstrate similar toxicities in non-human organisms (as a function of bioavailability and chemical reactivity).

In the case of this assessment, analogues for cyclosporin A were identified through literature review; however, no corresponding persistence, bioaccumulation or ecotoxicity data were available for read-across purposes.

Based on expert judgement, cyclosporin E is considered to be structurally and chemically similar to cyclosporin A (i.e., molecular weight, amino acid, cyclic peptide and protein), such that any differences (i.e., the absence of an additional methyl group for cyclosporin E) would not impact the functionality or toxicity of the substance. In addition, the modelled physical and chemical properties for cyclosporin E are comparable to those of cyclosporin A. Given the paucity of empirical data for cyclosporin E and the potential error associated with model predictions, selected empirical physical and chemical properties [i.e., melting point, octanol–water partition coefficient (log K_{ow}), solubility], bioaccumulation data and toxicity data for cyclosporin A were used directly (as read-across data) to support the weight of evidence and conclusions for cyclosporin E in this screening assessment.

3. Physical and Chemical Properties

Table 3-1 contains experimental and modelled physical and chemical properties of cyclosporin A that are relevant to its environmental fate. Table 3-2 contains the modelled physical and chemical properties of cyclosporin E, as no experimental physical and chemical properties were found for cyclosporin E.

As described in the preceding section on analogues, cyclosporin E is considered to be structurally and chemically similar to cyclosporin A, such that any differences would not impact the functionality or toxicity of the substance. In addition, the modelled physical and chemical properties for cyclosporin E are comparable to those for cyclosporin A. Therefore, the experimental physical and chemical properties for cyclosporin A are used directly (as read-across data) for cyclosporin E.

As shown in Table 3-1, experimental water solubilities for cyclosporin A range from 7.3 to 200 mg/L, depending on the temperature. The water solubility of cyclosporin A is inversely proportional to the temperature; that is, at higher temperatures, there is a decrease in solubility (see Table 3-1; Ismailos et al. 1991). The physical state of cyclosporin A is a crystalline white powder. Cyclosporin A and cyclosporin E are considered to be non-ionizing substances.

Table 3-1. Physical and chemical properties of the neutral form of cyclosporin A

Property	**Type**	**Value[a]**	**Temperature (°C)**	**Reference**
Melting point (°C)	Experimental	148*–151	NA	Budavari 1989
Boiling point (°C)	Modelled	1730	NA	MPBPWIN 2008
Bulk density (kg/m^3)	Experimental	200–450	NA	Novartis 2006
Vapour pressure(Pa)	Modelled	10.2* (0.0764 mmHg)[b]	25	MPBPWIN 2008
Henry's Law constant (Pa·m^3/mol)	Modelled	Bond estimate (incomplete) Group estimate (incomplete)	25	HENRYWIN 2008
Log K_{ow} (dimensionless)	Experimental	2.92* (pH 7.4)	NA	El Tayar et al. 1993
Log K_{ow} (dimensionless)	Modelled	0.99	NA	KOWWIN 2008
Log K_{oc} (dimensionless)	Modelled	1×10^{10} (estimate from MCI) 53.6 (estimate from log K_{ow} of 2.92)	25	KOCWIN 2008
Water solubility (mg/L)	Unknown	200	20	Novartis 2006
Water solubility (mg/L)	Experimental	27.7*	25	Ran et al. 2001
Water solubility (mg/L)	Experimental	32.9 (26.2–39.6)	20	Ismailos et al. 1991
Water solubility (mg/L)	Experimental	101.5 (63.8–139.2)	5	Ismailos et al. 1991
Water solubility (mg/L)	Experimental	38.8 and 48.5	10	Ismailos et al. 1991
Water solubility (mg/L)	Experimental	12.2 (11.6–12.8)	30	Ismailos et al. 1991
Water solubility (mg/L)	Experimental	7.3	37	Ismailos et al. 1991

Property	Type	Value[a]	Temperature (°C)	Reference
		(6–8.6)		
Water solubility (mg/L)	Modelled	0.000 04	25	WSKOWWIN 2008
Water solubility (mg/L)	Unknown	40	NA	Apotex Inc. 2011

Abbreviations: K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; MCI, molecular connectivity index; NA, not available

[a] Values marked with an asterisk (*) are values selected for modelling purposes. Values in parentheses represent ranges.

[b] Value in parentheses is the original one as estimated by the model.

Table 3-2. Physical and chemical properties of the neutral form of cyclosporin E

Property	Type	Value	Temperature (°C)	Reference
Melting point (°C)	Modelled	349	NA	MPBPWIN 2008
Boiling point (°C)	Modelled	1751	NA	MPBPWIN 2008
Vapour pressure (Pa)	Modelled	0	25	MPBPWIN 2008
Henry's Law constant ($Pa \cdot m^3/mol$)	Modelled	Bond estimate (incomplete) Group estimate (incomplete)	25	HENRYWIN 2008
Log K_{ow} (dimensionless)	Modelled	0.78	NA	KOWWIN 2008
Log K_{oc} (dimensionless)	Modelled	13.8 (estimate from MCI) 0.57 (estimate from log K_{ow} of 0.78)	25	KOCWIN 2008
Water solubility (mg/L)	Modelled	0.003	25	WSKOWWIN 2008

Abbreviations: K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; MIC, molecular connectivity index; NA, not available

4. Sources and Uses

The substances cyclosporin A and cyclosporin E are naturally produced in the environment. Cyclosporins are produced by certain species of fungi, including *Tolypocladium inflatum* Gams [also formally defined as *Beauveria nivea* (Dong et al. 2011)], *Neocosmospora vasinfecta,* and *Verticillium* spp. (Nakajima et al. 1888; Issac et al. 1990; Bonnet et al. 2003). Cyclosporins may be released by the fungi as toxins or to impair immune responses within the infected organisms (e.g., insects), thereby facilitating fungal development (Podsiadlowski et al. 1998; Vilcinskas et al. 1999; Jegorov et al. 2000).

Cyclosporin can also be produced synthetically from *N*-methyl-*C*-9-amino acid, with subsequent additions of appropriate peptides, followed by cyclization (IARC 1990).

Cyclosporin A is a therapeutic and immunosuppressant agent that is commonly used in humans to prevent the rejection of allograft/organ transplants and to treat rheumatoid arthritis and psoriasis (Apotex Inc. 2011). There are several pharmaceutical companies licensed to market cyclosporin A in Canada for human or veterinary use (e.g., for dogs) (DPD 2010). Pharmaceutical-grade cyclosporin A may be sold in 10, 25, 50 and 100 mg tablets or as an oral solution at 50 and 100 mg/L (DPD 2010). Chemical-grade cyclosporin A can be purchased from major chemical manufacturers (Sigma-Aldrich 2010).

To date, a survey pursuant to section 71 of CEPA 1999 has not been issued for cyclosporin A or cyclosporin E. Therefore, entry characterization for cyclosporin A and cyclosporin E in Canada consisted of searching for information on sources and uses of the substances in relevant databases to identify potential for exposure of the general population from all sources, including pharmaceutical use (Canada [1978]; HSDB 1983– ; Household Products Database 1993– ; LNHPD 2008; DPD 2010; EAFUS 2011; NHPID 2011). Based on notifications submitted under the Cosmetic Regulations to Health Canada, neither cyclosporine A nor cyclosporine E are used in cosmetic products in Canada (2012 email from the Consumer Product Safety Directorate, Health Canada, to the Existing Substance Risk Assessment Bureau, Health Canada; unreferenced). Information available for cyclosporin A indicates that its uses are limited to pharmaceuticals and research. Searches for these substances were conducted up to March 2013, and no information was found regarding alternative uses of these substances in Canada. Data were available to estimate that 622 kg of cyclosporin A was purchased by hospitals and pharmacies for prescription across Canada for the year 2007 (McLaughlin and Belknap 2008). Data were also available to estimate that 548 kg and 544 kg of cyclosporin A were sold to hospitals and pharmacies across Canada for the years 2011 and 2012, respectively (IMS 2013). Cyclosporin A may also be used for additional off-label or veterinary uses that are not considered in this assessment. The quantity of the substance being used for these purposes is unknown.

Additionally, there is the possibility that pharmaceutical products containing cyclosporin A may be imported into Canada, but no information is available on the quantity of such imports.

Cyclosporin E is currently not registered for pharmaceutical use in Canada (DPD 2010), and no information was identified regarding its use in Canada.

5. Releases to the Environment

Pharmaceuticals can make their way into surface waters through release from manufacturing or formulation sites and/or release of the unmetabolized substances or their metabolites in feces or urine from consumers directly using these substances.

The production and use of pharmaceutical products containing cyclosporin A may result in the release of cyclosporin A to the environment through various waste streams. However, specific information regarding actual releases of cyclosporin A from its manufacture or formulation in Canada was not available. Cyclosporin A as well as its metabolites can also be released to the environment from indirect sources, i.e., down-the-drain releases from patients using the drug. In humans, cyclosporin A is primarily metabolized by multiple forms of the hepatic mono-oxygenase cytochrome P450 3A enzyme system in the liver, gastrointestinal tract and kidney (Apotext Inc. 2011). At least 15–25 metabolites of cyclosporin A have been identified from human bile, feces, blood and urine. Nine of these metabolites have been isolated and identified, all of which have the intact cyclic oligopeptide structure of the parent compound. Structural modifications during metabolism include mono- and dihydroxylation as well as *N*-demethylation, mainly at the *N*-methyl leucines. Metabolites and the unchanged form of cyclosporin are excreted into the bile, with only 6% of the oral dose excreted in the urine; only 0.1% is excreted in the urine as the unchanged cyclosporin. More than 44% of cyclosporin A appears in the bile as metabolites. However, the biological activity of the metabolites and their contributions to toxicity are known to be less than those of the parent compound (Bowers 1990; Copeland et al. 1990; Dai et al. 2004; Novartis 2006; Apotex Inc 2011). Metabolites of cyclosporin A are, therefore, not assessed further in this screening assessment.

No information regarding the use of cyclosporin E in Canada was identified. Information on the metabolism and metabolites of cyclosporin E was also not found in the literature. As such, there are no known or expected releases of the substance and its metabolites to the Canadian environment.

6. Measured Environmental Concentrations

No data on concentrations of cyclosporin A or cyclosporin E in the environment have been identified in Canada or elsewhere. Therefore, environmental concentrations of cyclosporin A were estimated from available information,

including estimated substance quantities, release rates and size of receiving water bodies (see “Ecological Exposure Assessment” section).

7. Environmental Fate

Level III fugacity modelling (EQC 2003) simulates the distribution of a substance in a hypothetical, evaluative environment known as the “unit world.” The EQC model simulates the environmental distribution of a chemical at a regional scale and outputs the fraction of the total mass in each compartment from an emission into the unit world and the resulting concentration in each compartment. Environment Canada uses only the mass fraction distribution results for general information on the environmental fate of a substance and generally does not use the compartmental concentration results for the predicted environmental concentration (PEC) in a substance assessment. Some exceptions to this may occur, such as when a wide dispersive release of a substance suggests that regional-scale concentrations are appropriate for the PEC(s).

These results represent the partitioning of the substance in a hypothetical evaluative environment resulting from inter-media partitioning and loss by both advective transport (out of the modelled environment) and degradation/transformation processes. The partitioning values shown in Table 7-1 represent the expected net effect of these processes under conditions of continuous release when a non-equilibrium “steady state” has been achieved.

Table 7-1. Summary of the Level III fugacity modelling (EQC 2003) for cyclosporin, showing percent partitioning into each medium for three release scenarios.

Substance released to:	Air (%)	Water (%)	Soil (%)	Sediment (%)
Air (100%)	98.5	0.7	0.8	0.02
Water (100%)	14.1	83.8	0.1	2
Soil (100%)	13.1	0.8	86.1	0.01

If released to air, given their moderate volatility (vapour pressure of 10.2 Pa), cyclosporin A and cyclosporin E are expected to exist in the ambient atmosphere. However, it is not likely that cyclosporin A or cyclosporin E would be released to air through human activity.

If released into water, cyclosporin A and cyclosporin E are expected to reside in water as non-polar or very weakly polar substances, given their chemical structures, water solubilities and low log K_{ow}. Thus, if water is a receiving medium, cyclosporin A and cyclosporin E are expected to reside mainly in water and, to some extent, to partition to air (see Table 7-1).

If released to soil, cyclosporin A and cyclosporin E are expected to adsorb to the soil. Volatilization from moist soil surfaces also seems to be a likely fate process,

given their moderate volatility. Therefore, if released to soil, cyclosporin A and cyclosporin E will reside mainly in soil and, to some extent, will partition to air.

Based on their physical and chemical properties (Tables 3-1 and 3-2) and the results of Level III fugacity modelling (Table 3-1), cyclosporin A and cyclosporin E are expected to reside predominantly in the compartment of release (i.e., water or soil). However, as cyclosporin A is expected to occur in surface waters through its release from manufacturing or formulation sites and/or the release of the unmetabolized substance or its metabolites in feces or urine from consumers directly using the substance, this assessment examined water as the main source of exposure in the ecological environment. The application of biosolids containing cyclosporin A or cyclosporin E to agricultural land is a possibility, but it cannot be quantified in the absence of toxicity data and data on concentrations of cyclosporin A and cyclosporin E in soil/biosolids in Canada.

7.1 Environmental Persistence

In order to provide the best possible weight of evidence for the persistence of cyclosporin A and cyclosporin E in the environment, both empirical and modelled data were considered.

Table 8-1 presents the empirical biodegradation data (Novartis 2006) for cyclosporin A. The Organisation for Economic Co-operation and Development (OECD) Test Guideline 301B (1981) modified Sturm test showed 84% biodegradation (at an initial concentration of 11 mg/L) over 28 days. This test indicates that the half-life in water is likely to be shorter than 182 days (6 months) and that the substance is likely not persistent in water. Nevertheless, given the structural features of cyclosporin A, it is expected that the substance is subject to some primary biodegradation (release of carbon dioxide due to small portions of the molecule being metabolized by bacteria); ultimately, however, complete mineralization is unlikely.

Table 8-1: Empirical data for degradation of cyclosporin A

Medium	Fate process	Degradation value	Degradation endpoint / units	Reference
Water	Biodegradation	84	Biodegradation / %	Novartis 2006

Since few experimental data on the degradation of cyclosporin A are available and no data are available for cyclosporin E, a quantitative structure–activity relationship (QSAR)-based weight of evidence approach (Environment Canada 2007) was applied using the degradation models shown in Table 8-2. Given the ecological importance of the water compartment and given that cyclosporin A is expected to be released to this compartment, biodegradation in water was primarily examined. Table 8-2 summarizes the results of available QSAR models for degradation for cyclosporin A.

Table 8-2: Modelled data for degradation of cyclosporin A

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008[1a]	$t_{½}$ = 22.2 min	< 2
Ozone reaction	AOPWIN 2008[a]	1.75 h	< 2
Hydrolysis	HYDROWIN 2008[a]	n/a[b]	n/a
Primary biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 4: Expert Survey (qualitative results)	4.77[c] "biodegrades fast"	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 3: Expert Survey (qualitative results)	0.4[c] "biodegrades very slowly"	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 5: MITI linear probability	−1.25[d] "biodegrades very slowly"	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 6: MITI non-linear probability	0[d] "biodegrades very slowly"	≥ 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 Probability	n/a[e]	n/a
Ultimate biodegradation (aerobic)	CATABOL c2004-2008 % BOD	% BOD = 0.05[f] "biodegrades very slowly"	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); n/a, not applicable; $t_{½}$, half-life

[a] EPI Suite (2008).

[b] Hydrolyzable function detected: amides. With the exception of a few halogenated acetamides, most amides hydrolyze to acids extremely slowly at 25°C and pH 7, with half-lives measured in the centuries. Electronegative groups on carbon or nitrogen greatly accelerate base-catalyzed hydrolysis, but alkyl groups on nitrogen retard both acid- and base-catalyzed processes. No neutral hydrolysis is evident.

[c] Output is a numerical score from 0 to 5.

[d] Output is a probability score.

[e] Model cannot provide an estimate for this type of structure.
[f] Out of the parameter domain, but mostly within the structural domain.

The TOPKAT 2004 model could not reliably provide an estimate for cyclosporin A, as chemicals with comparable structures are not contained in the training sets. For CATABOL (c2004-2008), the model predictions for cyclosporin A were out of the parameter domain (molecular weight and log K_{ow}), but mostly within the structural domain, and were thus judged acceptable.

In air, a predicted atmospheric oxidation half-life of approximately 22.2 minutes (Table 8-2) demonstrates that cyclosporin A is likely to be rapidly oxidized. The substance is not expected to react appreciably with other photo-oxidative species in the atmosphere, such as ozone, nor is it likely to degrade via direct photolysis. Therefore, it is expected that reactions with hydroxyl radicals will be the most important fate process in the atmosphere for cyclosporin A and cyclosporin E Cyclosporin A and cyclosporin E are not persistent in air.

Cyclosporin A and cyclosporin E do not contain functional groups expected to undergo hydrolysis in water. The HYDROWIN (2008) model indicated that the only hydrolyzable function detected was amides. However, with the exception of a few halogenated acetamides, most amides hydrolyze to acids extremely slowly, with half-lives measured in centuries at 25°C and at an environmentally relevant pH of 7. Electronegative groups on carbon or nitrogen greatly accelerate base-catalyzed hydrolysis, but alkyl groups on nitrogen retard both acid- and base-catalyzed processes. Therefore, no neutral hydrolysis is evident (HYDROWIN 2008).

The result of the BIOWIN Sub-model 4 (primary survey model) suggests that the substance has a primary half-life of < 182 days. However, all the ultimate biodegradation models suggest that biodegradation is very slow and that the half-life in water would be ≥ 182 days. In addition, the results from BIOWIN Sub-models 3, 5 and 6 exceed the suggested thresholds for “slow biodegradation,” indicating that the substance is likely to remain stable in the environment for long periods of time. Therefore, considering all model results and structural features, the weight of evidence suggests that the biodegradation half-life of cyclosporin A and cyclosporin E is ≥ 182 days in water.

Using an extrapolation ratio of 1:1:4 for water:soil:sediment biodegradation half-lives (Boethling et al. 1995), the half-life in soil is also ≥ 182 days, and the half-life in sediment is ≥ 365 days. This indicates that cyclosporin A and cyclosporin E are expected to be persistent in soil and sediment.

Based on the modelled and empirical data (see Tables 8-1 and 8-2), cyclosporin A and cyclosporin E are expected to remain in the environment for long periods of time.

7.2 Potential for Bioaccumulation

No experimental bioaccumulation factor (BAF) or bioconcentration factor (BCF) data for cyclosporin A or cyclosporin E were available. Experimental and modelled log K_{ow} values (2.92 and 0.99) for cyclosporin A and a modelled log K_{ow} value (0.78) for cyclosporin E suggest a low potential for bioaccumulation in biota (see Tables 2-1 and 2-2).

In order to provide the best possible weight of evidence analysis of the bioaccumulation potential of cyclosporin A and cyclosporin E, the physical and chemical properties (i.e., log K_{ow}, solubility), empirical metabolism/excretion data and modelled data were considered.

7.2.1 Estimating BCF and BAF

Environment Canada estimated the BCF and BAF of cyclosporin A using both structure-based models and a three trophic level kinetic mass balance model (Table 8-3). All estimates of BCF and BAF, except those estimated using sub-model 1 of the BCFBAF (2008) model in EPIWIN version 4.0 (EPI Suite 2008), were corrected for metabolism, because it represents a fundamental elimination pathway for many chemicals. This correction was performed by deriving metabolic rate constants (k_M) using available empirical BCF or BMF study information or using a structure-based QSAR method. The empirical method is preferred when data are available. BAF values were also adjusted for dietary assimilation efficiency when this information was available.

Metabolic rate constants (k_M) were derived using structure–activity relationships described further in Arnot et al. (2008a, b, 2009). The middle trophic level fish was used to represent overall model output, as suggested by the model developer, and is most representative of the fish weight likely to be consumed by an avian or terrestrial piscivore. After statistical normalization routines, the median k_M is > 25.0/day.

Table 8-3: Modelled bioaccumulation data for cyclosporin A

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	Reference
Fish	BCFBAF Sub-model 1: linear regression	BCF	39.2	BCFBAF 2008
Fish	BCFBAF Sub-model 2: mass balance	BCF	5.4	BCFBAF 2008
Fish	BCFBAF	BAF	5.4	BCFBAF 2008

	Sub-model 3: Gobas mass balance			

The BCFBAF model flagged that the predicted biotransformation rate constant (i.e., 100/day for a 10 g fish) exceeds the theoretical whole-body maximum value, suggesting that cyclosporin A may readily metabolize in fish. In addition, at a log K_{ow} of 2.92, the BAF value suggests insignificant dietary uptake. Metabolism is also less important, as the main loss process is gill exchange (i.e., BAF = BCF). The model predictions for bioaccumulation were considered acceptable as an indication of fast metabolism, although there is some uncertainty (i.e., error would suggest that the k_M will not be slow, with potential for a false negative).

A metabolism/toxicity study examined the effects of ingesting cyclosporin-infected mosquito larvae by adult brown bullheads (*Ameiurus nebulosus*), a benthic freshwater species (Jegorov et al. 2000). To mimic this ingestion pathway, a dosing volume of 0.5–2.0 m of cyclosporin A and 100 mg/mL of cyclosporin A was applied by gastric probe (to simulate intestinal absorption via the foodborne route of exposure) to adult brown bullheads weighing 300–480 g. The brown bullheads were then covered with a wet towel and maintained for 30 minutes in a wet box rinsed with oxygen, as the fish may eliminate cyclosporin A if immersed in water immediately after treatment. After 30 minutes, the brown bullheads were released into an aquarium, and blood samples were taken at 0.5, 1, 2, 3, 5, 8, 12 and 24 hours to monitor the absorption of cyclosporin A. The study found that brown bullheads had some side effects (lethargy, problems with balance, fungal infection) at the highest dose applied (500 mg/kg bw), but these effects were reversible, and the fish recovered after 2–3 days. High concentrations of cyclosporin A (up to 80 mg/L) and its metabolites (also up to 80 mg/L) were reached in the blood at 24 hours. The study found that brown bullheads metabolized cyclosporin A to hydroxyl and demethylated derivatives, which were then excreted into the surrounding water (i.e., phase I biotransformation). However, it was noted that there were considerable fish-to-fish differences, reflecting the different metabolic activity of each individual fish.

The available evidence indicates that cyclosporin A and cyclosporin E are expected to have low bioaccumulation potential due to their physical and chemical properties (i.e., high molecular weight, low log K_{ow}, high potential for fish to metabolize cyclosporin A) and likely a very fast rate of biotransformation. Metabolism-corrected BCF and BAF values are low, with a sufficient margin for the consideration of uncertainty regarding the rate of metabolism. The available empirical biotransformation data and kinetic-based modelled values corrected for metabolism are consistent and agree with the intended design of these substances as biologically active pharmaceuticals.

8. Potential to Cause Ecological Harm

8.1 Ecological Effects Assessment

8.1.1 Mode of Action

In some aquatic organisms, cyclosporin A adversely impacts the multi-xenobiotic resistance (MXR) mechanism. MXR represents a general biological defence mechanism for the protection of organisms against both endogenous and environmental toxicants (Faria et al. 2011). MXR is mediated by transmembrane transport proteins that recognize a wide variety of potential environmental toxicants and then pump them out of the cell. Environmental toxicants may interfere with the MXR transporter activity, thereby increasing the intracellular concentrations of those environmental toxicants and enhancing their deleterious effects in the organism (Faria et al. 2011). The protective role of the MXR mechanism and the presence of the P-glycoprotein family (a membrane-associated transporter protein) have been demonstrated in more than 40 aquatic species, such as the killifish (*Fundulus heteroclitus*), rainbow trout (*Oncorhynchus mykiss*), flounder (*Pleuronectes americanus*), turbot (*Scophthalmus maximus*), zebrafish (*Danio rerio*) and marine and freshwater bivalves (Bard. 2000; Zaja et al. 2008).

Cyclosporin A adversely impacts the MXR mechanism by inhibiting the function of the P-glycoprotein, thereby preventing the excretion of environmental toxicants (Bard and Gadbois 2007). Podsiadlowski et al. (1998) suggested that cyclosporin A may inhibit P-glycoprotein-mediated adenosine triphosphate (ATP) consumption and thus promote the poisoning of the infected insect by chemicals normally removed by the P-glycoprotein pump. Podsiadlowski et al. (1998) also found that *Chironomus riparius* larvae are more sensitive to the combined P-glycoprotein inhibitory effect of cyclosporin A (3 µM, or 3.6 mg/L) and ivermectin (an insecticide), with a 24-hour LC_{50} of 2.12 ng/mL, compared with an LC_{50} of 5.96 ng/mL for ivermectin alone. Exposure to 3 µM (3.6 mg/L) cyclosporin A alone did not result in mortality. The impact of cyclosporin A is of most concern in organs that have an excretion (liver, kidneys), absorption (intestine) or blood–brain barrier function. It is expected that cyclosporin E would have a similar effect.

8.1.2 Empirical Aquatic Toxicity Studies

Suitable studies on the ecological effects of cyclosporin A on aquatic and terrestrial organisms were found. Empirical ecotoxicity studies for cyclosporin E were not found.

Modelled toxicity predictions were not considered to be acceptable, as the structural properties of cyclosporin A and cyclosporin E were outside of the domain of applicability of the models. The structural class of peptides to which

cyclosporin A and cyclosporin E belong is “difficult to model” using toxicity QSARs. The physical and chemical properties of many of the structural classes of peptides are not amenable to modelling toxicity because they are considered “out of the model domain of applicability” (e.g., structural domains). Therefore, models could not be used due to the reactive nature of the substances and the lack of structural coverage. Analogues were found using ChemIDplus (1993–), but none had any ecotoxicological read-across data.

Table 8-4 shows a range of aquatic toxicity values obtained from various experimental toxicity studies for cyclosporin A. Several of these values were considered unreliable toxicity estimates for cyclosporin A, as the results indicated that acute effects would be expected at concentrations above its water solubility (i.e., 27.7 mg/L at 25°C; Table 2a). However, given that concentrations for both toxicity and water solubility are often uncertain, toxicity values that exceeded solubility estimates by up to a factor of 10 were considered to be acceptable. The experimental endpoints for cyclosporin A using short-term (acute) water-only exposure at high exposure concentrations from Sanderson and Thomsen 2009 are considerably above the water solubility (27.7 mg/L), and, given the chemical structure and non-polar or very weakly polar nature of cyclosporin A, it is likely that exposure to and uptake of cyclosporin A are through food (e.g., ingestion of contaminated insects) and water. Thus, the results from Sanderson and Thomsen (2009), were not considered acceptable for use in this assessment.

There is experimental evidence that cyclosporin A causes direct harm to insects at low concentrations (see Table 6). Weiser and Matha (1987) exposed L4 larvae of the common mosquito (*Culex pipiens autogenicus*) to nominal cyclosporin A concentrations of 1, 2, 5, 10 and 15 mg/L. Mortalities appeared after 24 hours, with a 48-hour median lethal concentration (LC_{50}) of 0.6 mg/L. The authors suggested that cyclosporins are likely filtered by the mosquito larvae from suspensions of microparticles and digested out from the hyphae when they are deposited in the midgut. Bonnet et al. (2003) used an *in vitro* cellular model with the freshwater ciliated protozoan, *Tetrahymena pyriformis*. The ciliated protozoan has a short generation time of 3 hours, allowing for effects to be seen through several generations over a short period of time. The effect of cyclosporin A on the population growth of the ciliated protozoan was evaluated. The final test concentrations were 5, 10, 20, 30, 40, 50, 60 and 70 mg/L. The results showed that cyclosporin A exerted a concentration-dependent inhibitory effect on the growth of *T. pyriformis* populations, with an average median inhibitory concentration (IC_{50}) of 50.55 ± 5.57 mg/L over a period corresponding to three generations.

In the Jegorov et al. 2000 study examining the effects of the ingestion of cyclosporin-infected mosquito larvae by adult brown bullheads (*Ameiurus nebulosus*)), brown bullheads had some side effects (lethargy, problems with balance, fungal infection) at the highest dose applied (500 mg/kg bw), but these effects were reversible, and the brown bullheads recovered after 2–3 days. High

concentrations of cyclosporin A (up to 80 mg/L) as well its metabolites (also up to 80 mg/L) were reached in the blood at 24 hours.

Faria et al. (2011) found that cyclosporin A was marginally toxic to zebra mussel (*Dreissena polymorpha*) embryonic development, with a median effective concentration (EC_{50}) <20 µM (<24.5 mg/L).

Table 8-4: Empirical toxicity data for cyclosporin A

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Mosquito L4 larvae (*Culex pipiens autogenicus*)	Acute (72 h)	LC_{50}	0.6	Weiser and Matha 1987
Zebrafish (*Danio rerio*)	Acute	LC_{50}	< 2	Belyaeva et al. 2008
Zebrafish (*Danio rerio*)	Not available	Teratogenicity, nephrotoxicity, hepatotoxicity, cardiotoxicity	69	Belyaeva et al. 2008
Ciliated protozoan (*Tetrahymena pyriformis*)	Chronic (cell proliferation rate)	IC_{50}	42.03 µM (50.6 mg/L)	Bonnet et al. 2003
Daphnia	Acute (48 h)	EC_{50} (endpoint not described)	20	Sanderson and Thomsen 2009
Fish (*Oncorhynchus mykiss*)	Acute (96 h)	LC_{50} (endpoint not described)	100	Sanderson and Thomsen 2009
Brown bullhead (*Ameiurus nebulosus*)	Acute (24 h)	LD_{50}	Mortality not reached at highest dose of 500 mg/kg bw	Jegorov et al. 2000
Unknown bacterial mixture	Activated sludge respiration inhibition test	EC_{50}	> 100	Sanderson and Thomsen 2009
Chironomus riparius larvae	Acute (24 h)	LC_{50}	Mortality not reached at	Podsiadlowski et al. 1998

			highest concentration of 3.6 mg/L	
Zebra mussel (*Dreissena polymorpha*)	Acute (48 h)	EC_{50}	<20 µM (<24.5 mg/L)	Faria et al. 2011

Abbreviations: EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; IC_{50}, the concentration of a substance that is estimated to cause some inhibitory effect on 50% of the test organisms; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LD_{50}, the dose of a substance that is estimated to be lethal to 50% of the test organisms

8.1.3 Other Ecological Effects - Immunotoxicity

Cyclosporin A suppressed the humoral immune response of greater wax moth (*Galleria mellonella*) larvae (Fiolka 2008). In general, *G. mellonella* immune reactions involve the synthesis and release into the hemolymph of antibacterial immune proteins (i.e., lysozyme and antimicrobial peptides). In this study, larvae were injected at the last abdominal proleg with 39 and 78 ng lipopolysaccharide from *Pseudomonas aeruginosa*, a highly virulent entomopathogenic bacterium. Cyclosporin A was then injected into the hemocele of the larvae. The injection of cyclosporin A (15 µg/g) alone induced the release of lysozyme into the hemolymph of the larvae. The decrease of lysozyme activity and total lack of antibacterial peptides in larvae injected with cyclosporin A and *P. aeruginosa* resulted in *P. aeruginosa* multiplying in the celomic cavity of the larvae. These larvae died with symptoms of septicemia commonly attributed to the effects of *P. aeruginosa*. In effect, cyclosporin A–treated larvae had 30% reduced immunity.

In another study, Vilcinskas et al. (1999) infected the last instar larvae of *G. mellonella* with the fungus, *Beauveria bassiana* strain M227, either by external contamination with conidia or by injection of blastospores that were propagated within submerged cultures. Cyclosporin A was injected in amounts of 10 µg or 30 µg per larva. Direct toxicity was not induced (injection of solubilized or particle-bound cyclosporin A did not cause mortality or other pathological alterations); however, the humoral immune reactions were activated and resulted in the release of lysozymes and cecropin-like molecules.

8.1.4 Derivation of the Predicted No-Effect Concentration (PNEC)

The empirical data for cyclosporin A suggest that both cyclosporin A and cyclosporin E are expected to cause acute harm to aquatic organisms at low concentrations (acute LC_{50}s ≤ 1.0 mg/L). A conservative predicted no-effect concentration (PNEC) was derived from an *in vivo* acute LC_{50} for mosquito larvae of 0.6 mg/L (Weiser and Matha 1987). A robust study summary analysis found the Weiser and Matha (1987) study to be reliable, with satisfactory confidence. This value was chosen as the critical toxicity value (CTV), as it is the most sensitive and environmentally relevant endpoint. The CTV was divided by an

assessment factor of 200 (10 to account for interspecies and intraspecies variability in sensitivity, 10 to estimate a long-term no-effects concentration from a short-term LC_{50} and 2 for the concern over the adverse effect of cyclosporin A on the MXR mechanism and the nominal concentrations provided in the study by Weiser and Matha [1987]), to give a value of 0.003 mg/L.

8.2 Ecological Exposure Assessment

No data concerning concentrations of cyclosporin A or cyclosporin E in Canadian waters or elsewhere have been identified. As cyclosporin E is not registered for pharmaceutical use in Canada (DPD 2010), an ecological exposure assessment was not conducted for cyclosporin E. Environmental concentrations for cyclosporin A are estimated from available information, including estimated substance quantities, release rates and size of receiving water bodies. Since the biological activity of Cyclosporin A metabolites and their contributions to toxicity are less than those of the parent compound (Bowers 1990; Copeland et al. 1990; Dai et al. 2004; Novartis 2006; Apotex Inc 2011), they are not assessed in this screening assessment.

Predicted environmental concentrations (PECs) for Cyclosporin A have been estimated from manufacturing/formulation of the pharmaceutical in an industrial release scenario, and from patients using the drug in a down-the-drain release scenario, as described in the following sections.

8.2.1 Industrial Release

Aquatic exposure to cyclosporin A is expected from the release during its manufacture at a pharmaceutical production facility to a wastewater treatment plant and the subsequent discharge of effluent from the treatment plant 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 PEC in evaluating the aquatic risk of the substance. It can be calculated using the equation:

$$PEC_{aq} = (1000 \times Q \times L) \times (1 - R) / (N \times F \times D)$$

where:

PEC_{aq}:	Aquatic concentration resulting from industrial releases (mg/L)
Q:	Total substance quantity produced annually at an industrial site (kg/year)
L:	Loss to wastewater (fraction)

R: Wastewater treatment plant removal rate (fraction)

N: Number of annual release days (days/year)

F: Wastewater treatment plant effluent flow (m^3/day)

D: Receiving water dilution factor (dimensionless)

A conservative industrial release scenario is used to estimate the aquatic concentration of the substance. The scenario is made conservative by assuming that the total quantity of the substance manufactured in Canada is produced at a single production facility. The facility is further assumed to be located in Mississauga, Ontario, a typical Canadian pharmaceuticals manufacturing site. Based on the assumptions indicated in Table 8-5, the substance at a total industrial production quantity of approximately 622 kg/year yields a concentration of 0.000 044 mg/L in the receiving water near the discharge point of the wastewater treatment plant (Environment Canada 2010a).

Table 8-5. Summary of input values used for estimating aquatic concentrations resulting from industrial releases of cyclosporin A

Input	Value	Justification and reference
Q: Quantity (kg/year)	622	McLaughlin and Belknap 2008; IMS 2013 Estimated quantity as prescribed at hospitals and pharmacies across Canada for the year 2007 as the most conservative quantity in comparison with the years 2011 and 2012
L: Loss to wastewater (%)	0.5	Health Canada (pers. comm.)*
R: Wastewater treatment plant removal efficiency (%)	1.9	EPI Suite 2008
N: Number of annual release days (days/year)	21	Assumed to be manufactured or processed in small batches over 1 month, due to the assumption of the low substance quantity manufactured or processed per industrial site
F: Wastewater treatment plant effluent flow (m^3/day)	332 624	Effluent flow of a large wastewater treatment plant located in Mississauga (a typical Canadian pharmaceuticals manufacturing site, assumed to be located in Mississauga)
D: Receiving water dilution factor (dimensionless)	10	Environment Canada's default assumption for large lakes, the WWTP in the scenario discharges to Lake Ontario

[*]Technical Support Document for Pharmaceutical Spreadsheets, 2007. Personal communication to Exposure Unit, Existing Substances, Environment Canada from Environmental Assessment Unit, New Substances, Health Canada

8.2.2 Down-the Drain Releases from Pharmaceutical Use

As cyclosporin A is used in pharmaceutical products and can be released to water as a result of its prescribed uses, an aquatic exposure scenario resulting from down-the-drain releases from pharmaceutical uses was developed. The scenario estimates the concentration of cyclosporin A in multiple water bodies receiving wastewater treatment system effluents where pharmaceutical products that contain cyclosporin A may have been released (Environment Canada 2009). This scenario provides estimates for approximately 1000 release sites across Canada.

The conservative/protective assumptions include:

- loss to sewer at 100% (no uptake or metabolism of the substance within humans);
- wastewater treatment plant removal rate estimated to be 0% in case of no treatment, 0% for primary-only treatment and 1.9% for primary–secondary combined treatment;
- number of annual release days of 365 days/year;
- receiving water dilution factor in the range of 1–10.

Input values used to estimate aquatic exposure resulting from down-the-drain releases from pharmaceutical use are summarized in Table 8-6.

Table 8-6. Summary of input values used for estimating aquatic concentrations resulting from prescribed use of cyclosporin A

Input	Value(s)	Justification and reference
Quantity (kg)	622	McLaughlin and Belknap 2008, IMS 2013 Estimated quantity sold to hospitals and pharmacies across Canada for the year 2007 as the most conservative quantity in comparison to the years 2011 and 2012
Loss to wastewater (%)	1) 0.1 - 6% 2) 100% (assumes no metabolism)	1. Assumes some uptake or metabolism of the substance within human body (Rowney et al. 2009.and Mahnik et al. 2007) 2. Assumes no metabolism in light of the uncertainty relating to the environmental stability of the metabolites of cyclosporin A
Variability factor [a]	2	Default
Wastewater treatment plant removal efficiency (%)	1.9	EPIsuite 2008
Number of annual release days (days)	365	number of annual release days
Dilution factor (–)	1- 10	Environment Canada Existing Substances default assumption

[a] The variability factor is used to define the level of variability of the use of a pharmaceutical in the country. When multiple pharmaceuticals are on the same market, one may be used at a different average rate by inhabitants in one region compared with those in another region. By default, a value of 2 is used as a realistic worst-case scenario applied to all sites.

Metabolites and the unchanged form of cyclosporin A are excreted into the bile, with only 6% of the oral dose excreted in the urine; only 0.1% is excreted in the urine as unchanged drug. The half-life of cyclosporin A in humans is approximately 18 hours (range 7.7–26.9 hours). More than 44% of a cyclosporin A dose appears in the bile as metabolites. However, in light of the uncertainty relating to the environmental stability of the metabolites of cyclosporin A, a

conservative environmental concentration value was obtained by not considering metabolism in the derivation of the PEC. The number of annual release days was assumed to be 365 to account for the variable use of the drug by consumers throughout a year as well as the variability between locations (e.g., hospitals where the drug is administered).

Given the above assumptions, the maximum PEC of cyclosporin A in the receiving water bodies was estimated to be 0.000 93 mg/L. The equation and inputs used to calculate the PEC are described in Environment Canada (2010b).

8.3 Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine various 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 conservative risk quotient calculation as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substances.

Cyclosporin A and cyclosporin E are expected to be persistent in water, soil and sediment and are also expected to have a low bioaccumulation potential. Once released into the environment, cyclosporin A and cyclosporin E will be found mainly in water, as pharmaceutical products containing cyclosporin A or cyclosporin E are expected to occur in surface waters through the release from manufacturing/formulation sites and/or through the release of the unmetabolized substances or their metabolites in feces or urine from consumers directly using these substances. Given these potential releases, this assessment examined water as the main source of exposure in the ecological environment. The application of biosolids containing cyclosporin A or cyclosporin E to agricultural land is a possibility, but it cannot be quantified in the absence of toxicity data and data on concentrations of cyclosporin A and cyclosporin E in soil/biosolids in Canada.

The information on the use of cyclosporin A in Canada indicates a potential for dispersive release into the Canadian environment. Cyclosporin E is not registered for use in Canada, and therefore no releases are expected into Canadian waters or soil/biosolids.

A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. Cyclosporin A and cyclosporin E have been demonstrated to have high potential for toxicity to aquatic organisms. The conservative industrial scenario presented above yielded a PEC of 0.000 044 mg/L. A PNEC was derived from the acute toxicity value of 0.6 mg/L (as the most sensitive valid experimental value) for mosquito (*Culex pipiens autogenicus*) L4 larvae, to give a value of 0.003 mg/L. The resulting risk quotient

(PEC/PNEC) is 0.015. Therefore, harm to aquatic organisms is unlikely from the industrial use of cyclosporin A or cyclosporin E.

The PEC (0.000 93 mg/L) will not exceed the PNEC (0.003 mg/L) at any site across Canada for exposures resulting from down-the-drain releases through the consumption of pharmaceutical products that contain cyclosporin A (Environment Canada 2010b). Based on the estimated number of receiving water bodies that will not be negatively affected by the use of the substances, coupled with the magnitude of the risk quotient and the more realistic scenario run, it is concluded that cyclosporin A and cyclosporin E are unlikely to cause harm to aquatic organisms from down-the-drain releases. This information suggests that cyclosporin A and cyclosporin E do not have the potential to cause ecological harm in Canada from prescribed uses.

Together, the information available suggests that there is low risk of harm to organisms or the broader integrity of the environment from these substances. It is therefore concluded that cyclosporin A and cyclosporin E do not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as they are not 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 or that constitute or may constitute a danger to the environment on which life depends.

8.4 Uncertainities in Evaluation of Ecological Risk

There is a lack of information on the sources of environmental concentrations and manufacture, import and use quantities of cyclosporin A and cyclosporin E in Canada. The proportion of cyclosporin A manufactured and released from each individual industrial facility is also unknown. Therefore, it was conservatively assumed that all cyclosporin A used in Canada was manufactured at a single location. Uncertainties are also associated with the fraction of the substance that is released during use, as there is no information available in Canada. These uncertainties were addressed by making conservative assumptions using best model estimates. Additionally, the locations of the release sites are unknown. As such, the quantitative results provide only a general indication of the magnitude of the potential risk to aquatic organisms. Uncertainties are also associated with the fractions of the substances that are released during use and with the fraction that is removed in wastewater treatment plants.

Based on the predicted partitioning behaviour of cyclosporin A and cyclosporin E, the significance of soil and sediment as media of exposure is not well addressed by the available effects data. The application of biosolids containing cyclosporin A or cyclosporin E to agricultural land is a possibility, but it cannot be quantified in the absence of toxicity data and information on concentrations of cyclosporin A or cyclosporin E in soil/biosolids in Canada.

The bioaccumulation assessment is limited by the few empirical bioaccumulation data; this necessitated the use of models to predict the bioaccumulation and biotransformation potential of the substances. Although all predictions using models have some degree of error, the metabolism-corrected model outputs provide support for the expectation that cyclosporin A and cyclosporin E have a low bioaccumulation potential given their structural characteristics, low experimental log K_{ow} and the high potential for fish to metabolize cyclosporin A.

9. Potential to Cause Harm to Human Health

Cyclosporin A has been classified as a known human carcinogen by the National Toxicology Program in the United States (NTP 2011). It is also classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC 1990, 2012). Cyclosporin E has no such classifications, but it is a close analogue of cyclosporin A, and there were no toxicity data identified to suggest that cyclosporin E would not have the same toxicological effects as cyclosporin A.

No sources of exposure have been identified for cyclosporin E.

Drugs containing cyclosporin A as an ingredient are assessed under the F&DA with respect to their safety, effectiveness and quality. This assessment focused on uses and exposures that were not covered as part of the F&DA assessment, specifically the risks posed by the residues resulting from manufacture, formulation and disposal after use.

Releases of cyclosporin A could occur during its manufacture from a pharmaceutical production facility to a wastewater treatment plant and the subsequent discharge of effluent from the treatment plant to a receiving water body. A conservative industrial release scenario is used to estimate the aquatic concentration of the substance and yields a concentration of 0.000 044 mg/L (44 ng/L) in the receiving water near the discharge point of the wastewater treatment plant (see section 8.2.1).

When patients use pharmaceuticals, some of the drugs may not be absorbed or metabolized, and even drugs that are metabolized may have active metabolites or may revert to the parent form in environmental media. This may lead to excretion of active drug residues into the wastewater system and the release of the wastewater effluent containing these residues into surface water (i.e., lakes, rivers), and this surface water has the potential to be used as drinking water. Additionally, the drug may be released to wastewater during the manufacturing process or via incorrect disposal of the excess pharmaceutical. Therefore, a focus of this assessment is on the potential for indirect exposure of humans to these pharmaceuticals through drinking water.

Only a portion of the cyclosporin A used in Canada would be released into the wastewater system. Metabolism results in a smaller portion of the pharmaceutical being excreted by the patient in the urine and/or feces. This amount can be further reduced as a result of wastewater treatment, environmental biodegradation and/or drinking water treatment prior to consumption. The concentration in the water source is also significantly reduced via dilution as the waste is released into waterways.

For this assessment, conservative assumptions were used when estimating the potential indirect exposure of humans to cyclosporin A. Releases to surface water were modelled using a down-the-drain release from pharmaceutical use scenario, as described above. For the purposes of modelling, it was assumed that 100% of the pharmaceutical that was purchased by hospitals and pharmacies was prescribed and administered to patients and excreted into wastewater after administration (i.e., no absorption or metabolism of the drug). It was also assumed that a maximum of 1.9% of the cyclosporin A was removed during wastewater treatment.

This scenario estimates concentrations in approximately 1000 waterways across Canada. The highest values estimated by this scenario are typically in small waterways with low dilution capacity, which are unlikely to be sources of drinking water. As a result, this scenario would be expected to highly overestimate actual concentrations in drinking water. The maximum PEC was 0.000 93 mg/L (as derived above).

No measured data were identified for cyclosporin A in the environment in Canada or elsewhere.

The estimated intakes of cyclosporin A in drinking water by humans can be represented by formula-fed infants 0–6 months of age, which is considered to be the most highly exposed age class, on a body weight basis, of those examined. The equation for deriving the estimated intake is given below:

$$\text{Intake} = (\text{PEC} \times \text{IR}) / \text{bw}$$

where:

Intake: Estimated intake of the substance from drinking water (mg/kg bw per day)

PEC: Predicted environmental concentration in receiving water from modelled or measured data (mg/L)

IR: Ingestion rate of drinking water for formula-fed infants: 0.8 L/day (Health Canada 1998)

bw: Default body weight for infants 0–6 months of age: 7.5 kg (Health Canada 1998)

The maximum estimated intake for cyclosporin A, based on a modelled concentration of 0.000 93 mg/L, would be 0.000 099 mg/kg bw per day, or 99 ng/kg bw per day. It is expected that these estimates provide conservative upper-bounding estimates of possible exposure and that actual exposures would be significantly lower. Given the low levels of estimated exposure, potential risk from exposure to this substance is expected to be low.

To further characterize potential risks associated with the intake of cyclosporin A via drinking water, the lowest therapeutic dose (LTD) for cyclosporin A was identified, and a margin of exposure (MOE) was calculated to determine the ratio between the upper-bounding estimate of intake by the general population and the dose that would be expected to produce a pharmacological effect. This approach is consistent with methodology described elsewhere (Webb et al. 2003; Schwab et al. 2005; Watts et al. 2007; Bull et al. 2011; WHO 2011). The LTD is the lowest concentration that evokes a desired therapeutic effect among target populations and is equivalent to the lowest dose prescribed or recommended, taking into account the number of doses per day (WHO 2011). These values are derived from an assessment of the balance between safety and efficacy.

Dosage information for the oral form of cyclosporin A indicates a recommended dose of 2 mg/kg bw per day (Sandoz Canada Inc. 2008; Apotex Inc. 2011). Conservative MOEs were derived using the equation below:

MOE = LTD/Intake

where:

MOE: Margin of exposure (dimensionless)

LTD: Lowest therapeutic dose (mg/kg bw per day)

Intake: Maximum estimated intake for drinking water derived from modelled or measured concentrations (mg/kg bw per day)

For cyclosporin A, this results in an MOE greater than 20 000. Given the very conservative nature of the exposure inputs and the use of human data to derive a point of departure for risk characterization, this MOE supports the determination that risk from indirect exposure to cyclosporin A is low.

Since cyclosporin E is not identified to be in commerce in Canada, exposure and hence risk are not expected.

9.1 Uncertainties in Evaluation of Risk to Human Health

There is uncertainty regarding the estimation of exposure due to the lack of representative measured concentrations of cyclosporin A in Canadian drinking water and the use of models for estimating risk to human health. However, confidence is high that actual exposures to cyclosporin A in Canadian drinking water would be lower than the ones used from both models. This is supported by the highly conservative default assumptions used. The uncertainty in the human risk estimates could be reduced significantly by the use of measured concentration data for cyclosporin A in Canadian surface water and/or drinking water.

Potential exposures to cyclosporin A could occur via other sources, such as ingestion of fish or swimming in waters where the pharmaceutical is present, but these exposures are expected to be much lower than exposure through drinking water and so are not considered in this assessment.
Cyclosporin A may also be used for additional off-label or veterinary uses that are not considered in this assessment. The quantity of the substance being used for these purposes is unknown, and so estimation of releases is not possible at this time.

It is recognized that the LTD represents an exposure level at which a desired pharmacological response is achieved and further that at this exposure level, adverse effects, in addition to intended effects, may occur in some patients. For certain indications and certain classes of drugs, the nature of these unintended effects may be significant. However, the LTD is developed for patients who require treatment for a particular illness and therefore are likely to be more susceptible to potential effects than a healthy individual. Although the use of the LTD provides a tier 1 type of assessment that does not utilize all the toxicity data that may be available for each substance, the highly conservative exposure defaults that have been used lead to significant MOEs between the LTD and the estimated intakes.

10. Conclusion

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from cyclosporin A and cyclosporin E It is concluded that both substances do not meet the criteria under paragraphs 64(a) or (b) of CEPA 1999 as they are not 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 or that constitute or may constitute a danger to the environment on which life depends

Based on the information presented in this screening assessment, it is concluded that cyclosporin A and cyclosporin E do not meet the criteria set out in paragraph 64(*c*) of CEPA 1999, as they are 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 concluded that these substances do not meet any of the criteria set out in section 64 of CEPA 1999.

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