Screening Assessment

Furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[[4,6-O-(1R)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, (5R,5aR,8aR,9S)-

(Etoposide)

Chemical Abstracts Service Registry Number

33419-42-0

Environment Canada

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Synopsis

Pursuant to section 68 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment of the substance furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5a*H*)-one, 9-[[4,6-*O*-(1*R*)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, (5*R*,5a*R*,8a*R*,9*S*)-, Chemical Abstracts Service Registry Number 33419-42-0. This substance will be referred to by its common name, etoposide. Etoposide 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.

Drugs containing etoposide 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.

Etoposide is an organic substance, derived from the laboratory transformation of the root of the mayapple tree (*Podophyllum peltatum*), that is registered for use in Canada as a chemotherapeutic agent for the treatment of small lung tumours and testicular cancer. A total of 23 kg of etoposide was sold to hospitals and pharmacies across Canada in 2012.

Based on etoposide’s chemotherapeutic use in humans, a small amount of this substance may be released to wastewater systems after passing through the human gastrointestinal tract or renal system. Etoposide has moderate solubility in water, minimal volatility and no tendency to partition to lipids of organisms. Due to these physical and chemical properties, etoposide is expected to reside predominantly in water and soil, depending on the compartment of release. Empirical and modelled data suggest that etoposide has the potential to persist in water, soil and sediment.

Etoposide has low bioaccumulation potential based on a qualitative assessment of its physical and chemical properties (i.e., high molecular weight, low octanol–water partition coefficient [log K_{ow}]) and the high potential for fish to metabolize and readily excrete etoposide.

Based on empirical and modelled effects data, etoposide is expected to be moderately toxic to organisms in the aquatic environment. There are indications that etoposide may induce genotoxicity and affect endocrine function in mammals and aquatic organisms. To account for these sublethal effects, which would not be detected by standard acute toxicity tests, a high assessment factor was selected to determine the predicted no-effect concentration (PNEC), given that these effects often have an impact at the population level rather than at the organism level.

For the ecological assessment, realistic, conservative exposure scenarios were selected for the aquatic environment based on expected releases for a site-specific industrial operation and for down-the-drain releases of the substance. The predicted environmental concentrations in water were below the PNEC calculated for aquatic organisms.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms or to the broader integrity of the environment from etoposide. It is therefore concluded that etoposide does not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as it is 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 containing the pharmaceutical. The exposure to etoposide present in drinking water is significantly smaller than the exposure to etoposide through its use as a pharmaceutical.

For this assessment, conservative assumptions were used when estimating the potential indirect exposure of the general population to etoposide. Etoposide was not detected in samples taken from wastewater treatment plant influent and effluent at six plants across Canada. Upper-bounding estimated intakes of environmental residues based on the detection limit from that study and on modelled surface water concentrations were very low (< 1.5 ng/kg body weight per day). Based on low exposures, risks from these substances 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. The margins of exposure ranged from > 2 000 000 to 3 000 000.

Based on the adequacy of the margins of exposure, it is concluded that etoposide does not meet the criteria under 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.

Conclusion

It is concluded that etoposide does not meet any of the criteria set out in section 64 of CEPA 1999.

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Tables and Figures

1. Introduction

Pursuant to section 68 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 substance furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[[4,6-O-(1R)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, (5R,5aR,8aR,9S)-, Chemical Abstracts Service Registry Number (CAS RN) 33419-42-0. This substance will be referred to by its common name, etoposide. Etoposide was identified as a priority for assessment because it had been identified as posing a potential high hazard to human health based on classifications by other national or international agencies for carcinogenicity. This substance did not meet the ecological categorization criteria for persistence or bioaccumulation potential, but it was categorized as being inherently toxic to aquatic organisms.

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 using precaution.[1]

This screening assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposure. Relevant data were identified up to March 2013. Key studies were critically evaluated, along with modelled results, to reach conclusions. When available and relevant, information presented in risk and hazard assessments from other jurisdictions was considered. The 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.

Drugs containing etoposide as an ingredient are assessed under the F*ood 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

[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 on the substances in the Chemicals Management Plan (CMP) is not relevant to, nor does it preclude, an assessment against the hazard criteria for the *Workplace Hazardous Materials Information System* (WHMIS) that are specified in the *Controlled Products Regulations* 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.

assessment, specifically the risks posed by the residues resulting from manufacture, use and disposal.

The 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 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 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

For the purposes of this document, the substance furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[[4,6-O-(1R)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, (5R,5aR,8aR,9S)-, will be referred to as etoposide, its common name.

Etoposide can be manufactured as a pure substance (CAS RN 33419-42-0) or as a more soluble pharmaceutical product, etoposide phosphate (CAS RN 117091-64-2). Etoposide and etoposide phosphate are both available commercially as pharmaceutical products for human consumption. Although the pharmaceutical products contain 1% ethanol for solubilization purposes, studies using the chemical grade as well as studies using the drug product are presented in the text.

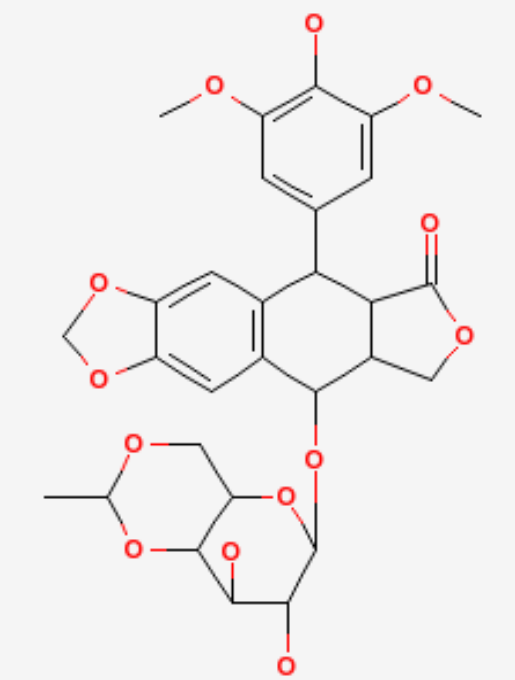
For the purpose of this screening assessment, both forms of etoposide are treated equally. Etoposide phosphate is not expected to be found in the environment, as the substance is rapidly transformed in the human body after the drug is injected. Therefore, the presence of the phosphate is generally omitted from the discussion, given that its function is predominantly pharmacokinetic and it is not expected to contribute to the toxicity or to the exposure pathway of etoposide.

The substance identity information on etoposide is presented in Table 2-1.

Table 2-1: Substance identity: etoposide

CAS RN	**33419-42-0**
DSL name	Furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[[4,6-O-(1R)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-

	3,5-dimethoxyphenyl)-, (5R,5aR,8aR,9S)-
NCI names	Etoposide (EINECS, REACH); Furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[(4,6-O-ethylidene-β-D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, [5R-[5a,5ab,8aa,9b(R*)]]- (AICS); Furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[[4,6-O-(1R)-ethylidene-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, (5R,5aR,8aR,9S)- (ASIA-PAC, NZIoC)
Other names	(−)-Etoposide; 4′-Demethyl-1-O-[4,6-O-(ethylidene)- β-D-glucopyranosyl]epipodophyllotoxin; 4′-Demethylepipodophyllotoxin 9-(4,6-O-ethylidene-β-D-glucopyranoside); 4′-Demethylepipodophyllotoxin ethylidene-β-D-glucoside; Celltop; EPE; Epipodophyllotoxin VP 16213; Epipodophyllotoxin, 4′-demethyl-, 4,6-O-ethylidene-b-D-glucopyranoside; Eposin; Eto-Gry; Etosid; Furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 9-[(4,6-O-ethylidene-β-D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-, [5R-[5a,5ab,8aa,9b(R*)]]-; Fytosid; Lastet; NSC 141540; Toposar; trans-Etoposide; VePesid; Vepesid J; VP 16; VP 16 (pharmaceutical); VP 16-123; VP 16-213; Zuyeyidal
Chemical group (DSL s**tream)**	Organic
Major chemical class or use	Pharmaceuticals
Chemical formula	$C_{29}H_{32}O_{13}$

Chemical structure	
SMILES	O1C2COC(C)OC2C(O)C(O)C1OC3C4COC(=O)C4C(c5cc(OC)c(O)c(OC)c5)c6cc7OCOc7cc36
Molecular mass	**588.56 g/mol**

Abbreviations: AICS, Australian Inventory of Chemical Substances; ASIA-PAC (Asia-Pacific Substances Lists; CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; EINECS, European Inventory of Existing Commercial Chemical Substances; NCI, National Chemical Inventories; *NZIoC*, New Zealand Inventory of Chemicals; *REACH*, Registration, Evaluation, Authorisation and Restriction of Chemical Substances; SMILES, simplified molecular input line entry system
Source: NCI (2009)

3. Physical and Chemical Properties

A summary of experimental and modelled physical and chemical properties of etoposide that are relevant to its environmental fate and ecotoxicity is presented in Table 3-1. Key studies from which experimental data were reported for some of these properties were critically reviewed for validity. Results from these reviews (robust study summaries [RSS]) are found in Appendix A.

Models based on quantitative structure–activity relationships (QSARs) were used to generate data for some of the physical and chemical properties of etoposide. These models are based mainly on fragment addition methods; that is, they sum the contributions of sub-structural fragments of a molecule to make predictions for a property or endpoint. Most of these models rely on the neutral form of a chemical as input.

Table 3-1: A summary of the physical and chemical properties of etoposide

Property	Type	Value[a]	Temperature (°C)	Reference
Physical form	NA	White crystalline powder	NA	Gennaro 1995
Melting point (ºC)	Experimental	236–251°C (mean value used for modelling purposes: 244°C*)	NA	Keller-Juslén et al. 1971
Melting point (ºC)	Modelled	263	NA	ACD/Percepta ©1997–2012
Melting point (ºC)	Modelled	334	NA	MPBPWIN 2008
Boiling point (ºC)	Modelled	759	NA	MPBPWIN 2008
Density (kg/m^3)	Modelled	1.55×10^3	NA	ACD/Percepta ©1997–2012
Vapour pressure (Pa)	Modelled	7.20×10^{-21} (5.40×10^{-23} mmHg)	25	ChemIDplus 1993–
Vapour pressure (Pa)	Modelled	9.77×10^{-20}* (7.32×10^{-22} mmHg)	25	MPBPWIN 2008
Henry's Law constant (Pa·m^3/mol)	Estimated	1.77×10^{-25} (1.75×10^{-30} atm·m^3/mol)	25	Meylan and Howard 1991
Henry's Law constant (Pa·m^3/mol)	Modelled	1.12×10^{-19} (6.04×10^{-24} atm·m^3/mol)	25	HENRYWIN 2008
Log K_{ow} (dimensionless)	Estimated	0.6*	NA	Hansch et al. 1995
Log K_{ow}	Experimental	1.0[b]	25	Shah et al. 1989

Property	Type	Value[a]	Temperature (°C)	Reference
(dimensionless)				
Log K_{ow} (dimensionless)	Modelled	0.04	NA	KOWWIN 2008
Log K_{ow} (dimensionless)	Modelled	0.28	NA	ACD/Percepta ©1997–2012
Log K_{ow} (dimensionless)	Modelled	1.03	NA	FASS 2011
Log K_{oc} (dimensionless)	Modelled (from MCI)	2.29	NA	KOCWIN 2009
Log K_{oc} (dimensionless)	Modelled (from K_{ow})	0.28	NA	KOCWIN 2009
Log K_{oc} (dimensionless)	Estimated from K_{ow} and a regression-derived equation described in Lyman et al. (1990)	1.71	NA	HSDB 1983–
Log K_{oc} (dimensionless)	Modelled	1.53	NA	ACD/Percepta ©1997–2012
Water solubility (mg/L)	Experimental	93.8*	Room temperature	Shah et al. 1995
Water solubility (mg/L)	Experimental	150	37	Du and Vasavada 1993
Water solubility (mg/L)	Estimated	58.7	25	Meylan et al. 1996
Water solubility	Modelled	105.7	NA	WSKOWWIN 2008

Property	Type	Value[a]	Temperature (°C)	Reference
(mg/L)				
Water solubility (mg/L)	Modelled	~30	NA	Gennaro 1995
Log K_{oa} (dimensionless)	Modelled	13.25	NA	KOAWIN 2008
pK_a (dimensionless)	Experimental	9.8	NA	O'Neil 2001
pK_a (dimensionless)	Modelled	9.9	NA	ACD/Percepta ©1997–2012

Abbreviations: K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; MCI, molecular connectivity index; NA, not applicable; pK_a, acid dissociation constant.

[a] Values in parentheses represent the original ones as reported by the authors or as estimated by the models. Values marked with an asterisk (*) are values selected for modelling purposes.

[b] This experimental study was rejected because it was conducted at a concentration above the water solubility (see details in Appendix A).

4. Sources and Uses

Etoposide is a semi-synthetic podophyllotoxin transformed in laboratory from the root of the mayapple tree (*Podophyllum peltatum*) (Zounková 2010) and is not reported to occur naturally in the environment.

Entry characterization consisted of searching for information on sources and releases of the substance in relevant databases (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, etoposide is not 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 this substance indicates that its uses are limited to pharmaceuticals and positive controls in research. Literature searches were conducted up to March 2013, and no information was found regarding alternative uses or releases of this substance in Canada. To date, a survey pursuant to section 71 of CEPA 1999 has not been issued for this substance. Data were available to estimate that 22 kg and 23 kg of the substance were sold to hospitals and pharmacies across Canada during the years 2011 and 2012, respectively (IMS 2013).

In Canada, etoposide is registered in Health Canada's Drug Product Database as an active ingredient in licensed pharmaceuticals (DPD 2010). This prescription drug is an intravenous and oral chemotherapeutic agent used in the treatment of lung and

testicular cancer (Hospira Healthcare Corporation 2007; Bristol-Myers Squibb Company 2008).

Etoposide 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.

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 substance in feces or urine from consumers directly using these substances. For this assessment, potential releases of etoposide from indirect sources (i.e., down-the-drain releases from patients using the substance for cancer therapy) and direct sources (i.e., releases during manufacture, formulation or packaging) were assessed. In both cases, releases are expected to end up mainly in wastewater. No information was available regarding actual releases of this substance from the manufacture or formulation of pharmaceuticals containing it. Data were available to estimate the amount (23 kg) of the substance sold to hospitals and pharmacies across Canada for the year 2012 (IMS 2013).

Anthropogenic releases to the environment depend upon various losses that occur during the manufacture, industrial use, prescribed use and disposal of a substance. In order to estimate potential releases to the environment occurring at different stages of the life cycle of a substance, Environment Canada compiles information on the sectors and product lines relevant to the substance. In addition to providing an overview of stages where releases are possible, an effort is made to quantify the percent release going to wastewater, land and air at different stages of the life cycle.[2] Relevant factors are considered, uncertainties are recognized and assumptions may be made during each stage, depending on information available.

The information is compiled to give an overview of the potential losses occurring at different stages of the life cycle and the receiving media involved, as well as identifying stages of the life cycle that are likely larger contributors to the overall environmental concentration. Recycling activities and transfer to waste disposal sites (landfill,

[2] The percent releases are presented as a range. Assumptions and input parameters used in making the release estimates are based on information obtained from a variety of sources, including responses to regulatory surveys, Statistics Canada, manufacturers' websites and technical databases and documents. Of particular relevance are emission factors, which are generally expressed as the fraction of a substance released to the environment, particularly during its manufacture, processing and use associated with industrial processes. Sources of such information include emission scenario documents, often developed under the auspices of the Organisation for Economic Co-operation and Development (OECD), and default assumptions used by different international chemical regulatory agencies. It is noted that the level of uncertainty in the mass of a substance and quantity released to the environment generally increases towards the end of the life cycle.

incineration) are also considered. However, unless specific information on the rate of or potential for release of the substance from landfills and incinerators is available, releases to the environment from disposal are not quantitatively accounted for.

This information is used to further develop exposure characterization scenarios to estimate resulting environmental concentrations. Presented in Table 5-1 is a summary of the expected releases for etoposide over its life cycle.

Table 5-1: Summary of the estimated percent release to compartments resulting from different life cycle stages for etoposide[a].

Compartment of release	Industrial use (%)	Prescribed use (%)
Wastewater[b]	0.5	45–74
Metabolized	NA	15
Land[c]	0	0
Air	0	0

Abbreviation: NA, not applicable

[a] Information from the following documents was used to estimate releases to the environment and the distribution of the substance as summarized in this table: Hande 1998; Hospira Healthcare Corporation 2007; Bristol-Myers Squibb Company 2008). Values presented for release to environmental media do not account for possible mitigation measures that may be in place at some locations.

[b] The loss to wastewater refers to raw wastewater prior to any treatment.

[c] The loss to land does not include transfers subsequent to a substance's use and service life (e.g., land application of biosolids). These will be discussed in the "*Ecological Exposure Assessment*" section.

The above loss estimates indicate that etoposide has a potential for release to the environment. Most etoposide is released to wastewater subsequently to the administration of the drug, in feces and urine (i.e., little absorption or metabolism of the drug).

In general, wastewater is a common point of entry of a substance into water through wastewater system[3] effluent and a potential point of entry into soil through the subsequent waste management of biosolids. When a substance is transferred to land (i.e., the general public may discard un-used or expired pharmaceutical products containing active medicinal ingredients in household garbage which will likely end up in landfills), it may be washed into the sewer or surface water or transferred by wind or rain to nearby soil. Finally, landfills have the potential to leach substances into groundwater (potentially reaching surface water). In many landfill sites in Canada, leachate is collected and treated either on-site or off-site prior to release to receiving water.

Unchanged etoposide has been found to be excreted in both urine and bile (Hospira Healthcare Corporation 2007; Teva Parenteral Medicines 2007). The fraction of

[3] In this assessment, the term wastewater system does not include sewer networks or collection systems.

etoposide released to the wastewater system varies according to the mode of administration of the drug. When the drug was administered intravenously, the proportion of unchanged etoposide recovered in urine represented 29% of the dose (Allen and Creaven 1975). With this same mode of administration, it is estimated that an additional 1.5–16% of the etoposide is recovered unchanged in feces (Hospira Healthcare Corporation 2007; Teva Parenteral Medicines 2007). The maximum value of 16% is conservatively used for this assessment. Therefore, it is estimated that 45% of the intravenous dose of etoposide will be released to the wastewater system from prescribed use. This is the lower value in the range of releases to wastewater from prescribed use.

The bioavailability of etoposide in the human body is reduced by half when the drug is taken orally (Bristol-Myers Squibb Company 2008), due to the drug remaining in the lumen. It is assumed that the total unabsorbed oral dose (52%) will be found unchanged in feces (Bristol-Myers Squibb Company 2008). Up to 16% of the absorbed 48% may return to the lumen to be excreted, for a total of 8% of the original dose. To the unabsorbed fraction (52%) is therefore added the maximal amount of absorbed etoposide returning to the lumen (8%), to reach a maximum of 60% of the oral dose being excreted in feces.

Similarly, a total of 14% of the etoposide oral dose unchanged in urine is obtained by the product of 29%, the fraction of etoposide intravenously administered found in urine (Allen and Creaven 1975), and 48%, the fraction absorbed from an oral dose.

Therefore, 60% and 14% of the administered dose are expected to be found unchanged in feces and urine, respectively, for a total maximum release of etoposide of 74% from oral dosing. This accounts for the higher value in the range of releases to wastewater from prescribed use.

A negligible amount of etoposide is estimated to be lost through waste disposal and recycling. Etoposide phosphate administered intravenously and etoposide are expected to have similar pharmacokinetics (Hande 1998) and therefore proportionate releases to wastewater.

6. Measured Environmental Concentrations

In Canada, samples collected at six municipal wastewater treatment plants selected to represent typical Canadian treatment systems and geographic variations were analyzed for etoposide (Smyth and Teslic 2013). These data were collected through an existing national wastewater monitoring program that was initiated under the Chemicals Management Plan in 2008. The wastewater monitoring program for 2012–2013 incorporates a multi-media approach by including municipal landfill leachate that is discharged into the wastewater treatment system, in order to better understand the influence of leachate inputs to wastewater treatment plants and the impact, if any, on the receiving water.

Etoposide was not detected in any of the influent or effluent samples or in any of the biosolid samples, with detection limits for the samples ranging from 6.32 to 47.8 ng/L. The results presented in Smyth and Teslic (2013) do not include winter sampling results, which tend to show poorer removals of compounds. However, since etoposide was not detected in the wastewater treatment plant influent in summer, seasonal variations are expected to be negligible.

In other countries, data on concentrations of etoposide in municipal wastewater effluent, hospital wastewater and receiving water bodies in other countries have been identified.

The concentrations of a few antineoplastic drugs, including etoposide, were measured in the effluents of two hospitals in France (Catastini et al. 2008). One of the hospitals specialized in the treatment of cancers. Samples were collected in quintuplicate at the exit of the sewage pipe of both hospitals and in the municipal wastewater treatment plant influent and effluent. Concentrations of etoposide in the hospital effluents were between the detection limit (0.11 µg/L) and 5.0 µg/L. At the municipal wastewater treatment plant, etoposide was not detected in the influent or effluent.

The effluents of 21 hospitals in China prior to wastewater treatment were analyzed for nine cytostatic compounds, including etoposide (Yin et al. 2010). Sixty-five effluent samples were analyzed for etoposide on different days. Etoposide was detected in 15 of the 65 samples with concentrations up to 380 ng/L and a median concentration of 42 ng/L, but was under the detection limit of 5 ng/L in the remaining 50 samples. The results varied significantly from day to day for the same hospital’s wastewater effluents, which is likely due to the occurrence of a patient being treated in the hospital during a designated sampling day.

In Spain, Martín et al. (2011) studied methods to improve the analytical detection performance of various pharmaceutical compounds, including etoposide. Using high-performance liquid chromatography (HPLC) coupled with mass spectroscopy to analyze etoposide concentrations, the authors reached a recovery efficiency ranging from 91% to 105% in a hospital effluent, before and after the hospital’s wastewater system, and in the receiving river. Mean etoposide concentrations (n = 3) were 15 ng/L in the wastewater system influent and 3.4 ng/L in the wastewater system effluent; etoposide was not detected in the river, at a detection limit of 2.2 ng/L.

Ferrando-Climent et al. (2013) similarly worked on the development of analytical methods for cytostatic drugs in another region of Spain. A detection limit of 24 ng/L was obtained by this group. To test the proposed method, wastewater samples were collected at the effluent of four hospitals and at the influent of three municipal wastewater treatment plants. Etoposide was below the detection limit in two of the hospital effluents, while it reached concentrations of 98 ng/L and 406 ng/L in the other two hospital effluents. Two of the three municipal wastewater treatment plant influents showed no traces of etoposide, whereas the etoposide concentration was 83 ng/L in the third influent.

7. Environmental Fate

7.1 Metabolites

A fraction of administered etoposide is excreted via urine conjugated to sulfate, or as a glucuronide metabolite (Williams et al. 2002). Etoposide glucuronide, 6-[4-[5-[(2,8-dihydroxy-7-methyl-4,4a,6,7,8,8a-hexahydropyrano[3,2-d][1,3]dioxin-6-yl)oxy]-8-oxo-5a,6,8a,9-tetrahydro-5H-[2]benzofuro[5,6-f][1,3]benzodioxol-9-yl]-2,6-dimethoxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid, CAS RN 100007-55-4 is the major metabolite found in human urine, at between 8% and 29% of the injected dose (IARC 2000). Etoposide glucuronide is not listed on the Domestic Substances List (DSL). *In vitro*, etoposide glucuronide was shown to be less cytotoxic than etoposide (Schmidt and Monneret 2003). There are no indications that the addition of the glucose ring on etoposide (forming etoposide glucoronide) could impact the toxicity of the substance. Furthermore, etoposide glucuronide is expected to be less bioavailable than etoposide, due to its larger diameter. Therefore, the metabolite is not evaluated concurrently with etoposide in this screening assessment.

The hydroxy acid metabolite of etoposide is formed by opening of the lactone ring (the D-ring). It has been detected in human urine at concentrations of 0.2–2.2% of the administered dose (IARC 2000). The catechol metabolite of etoposide, 1,2-benzenediol, has also been detected in urine, but in very small quantities (< 2% of the administered dose) (Stremetzne et al. 1997). Catechol (CAS RN 120-80-9) has previously been found to meet the paragraph 64(*c*) criterion under CEPA 1999 during the Challenge initiative, for its use as a photographic developer (Environment Canada and Health Canada 2008). Its volume released as a metabolite of etoposide is very low compared with the estimated releases from the use of catechol in photography. Due to the low percentages of the administered dose of the drug being excreted in these forms, these metabolites are not evaluated in this screening assessment.

7.2 Modelling Results

In the environment, CATALOGIC (2012) predicts that a degradation product, very similar to etoposide, may be formed following biodegradation: 1-((7,8-dihydroxy-2-methylhexahydropyrano[3,2-d][1,3]dioxin-6-yl)oxy)-6,7-dihydroxy-4-(3,4,5-trihydroxyphenyl)-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid. No information was located for this substance. Due to its structural similarity to etoposide, QSAR model predictions, predicting the fate in the environment and the effects, are very similar for this metabolite and for etoposide.

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 (i.e., 100 000 km^2) 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).

Etoposide is ionizable, with an acid dissociation constant (pKa) of 9.8. Under environmentally relevant pH conditions (between pH 6 and pH 9), etoposide would essentially be in its neutral form. Therefore, the EQC model can provide reliable estimates. Model inputs to EQC (2003) are provided in Appendix B.

The mass fraction distribution of etoposide is given in Table 7-1 using individual steady-state emissions to air, water and soil. The level III EQC model assumes non-equilibrium conditions between environmental compartments, but equilibrium within compartments. The results in Table 7-1 represent the net effect of chemical partitioning, inter-media transport and loss by both advection (out of the modelled region) and degradation/transformation processes.

The results of Level III fugacity modelling (Table 7-1) suggest that etoposide is expected to reside predominantly in water or soil, depending on the compartment of release.

Table 7-1: Summary of the Level III fugacity modelling (EQC 2003) indicating the percentage of etoposide partitioning into each compartment

Substance released to:	Percentage partitioning to air	Percentage partitioning to water	Percentage partitioning to soil	Percentage partitioning to sediment
Air (100%)	0.001	1.8	98.1	0.04
Water (100%)	0	97.8	0	2.2
Soil (100%)	0	0.04	100	0.001

When released to water, etoposide is expected to remain in that medium. Volatilization from water surfaces should not occur based on this compound's estimated Henry's Law constant of 1.77×10^{-25} Pa·m^3/mol. Nevertheless, if water is the receiving medium, a small mass fraction of etoposide is expected to reside in sediment (Table 7-1).

If released to soil (e.g., biosolids application), etoposide is expected to adsorb slightly onto solid particles based on its estimated organic carbon–water partition coefficient (K_{oc}) values of 1.9 to 199 (the maximum log K_{oc} is estimated to be ~2.3), which indicates that etoposide is expected to be fairly mobile in soil. Volatilization from moist soil surfaces is not expected to occur based on its low Henry's Law constant; therefore, releases to soil result in most of the mass fraction remaining in soil or groundwater and slow losses to reaction (degradation). However, releases to the soil compartment are expected to be minimal (see Table 5-1 above). For example, little of the substance is expected to associate with biosolids, given its low soil sorption coefficient.

A negligible amount of the substance is expected to reside in air (see Table 5-1 above). Based on the low vapour pressure of 9.77×10^{-20} Pa and low Henry's Law constant of 1.77×10^{-25} Pa·m^3/mol, etoposide is not volatile. Therefore, if released solely to air, it will deposit predominately onto soil (98.1%; Table 7-1).

Due to the very low proportion of etoposide expected to partition to air (Table 7-1) and the short half-life of the substance in air (0.84 hour; see Table 7-2 below), it is considered unlikely that etoposide would be transported through the atmosphere. Its long-range atmospheric transport potential is considered negligible.

From its use as a prescription drug, etoposide is expected to be released to wastewater from excretion and drug manufacture (Table 5-1). It is not expected to be retained in large proportions in wastewater systems and would be found in surface water, where it would remain in its neutral form, according to its physical and chemical properties. Therefore, the fate and effects of etoposide in environmental media other than surface water, such as soil and air, will not be assessed further, since exposure of non-aquatic organisms is negligible.

7.3 Environmental Persistence

As presented in Table 5-1, no significant releases are expected in any media other than wastewater. Once wastewater reaches the wastewater treatment system, only 1.51% of the etoposide is expected to be retained in biosolids. However, 98.14% of the etoposide would be found in the wastewater treatment system effluent, as modelled by the wastewater treatment plant (WWTP) fugacity model (EQC 2003). When etoposide is released to water, etoposide will reside predominately in surface water, with a negligible proportion of the substance settling to sediment (Table 7-1).

In order to provide the best possible weight of evidence for the persistence of etoposide, empirical and modelled data for the substance were considered.

7.3.1 Empirical data

Lu et al. (2000) reported that ultraviolet light (248 nm) ionized etoposide at room temperature. The effects of other wavelengths were not evaluated by the authors. The photolysis rate of etoposide exposed to a 248 nm sunbeam is dependent on the substance's concentration and has been reported to be 2.8×10^9 L/mol per second (Lu et al. 2000). Photolysis may occur in water in the first ~30 cm below the surface.

Shah et al. (1989) prepared etoposide solutions of 100 mg/L in water buffered at various pH values in amber-coloured bottles to avoid photolysis. Stability tests were conducted, and the solutions were analyzed by HPLC until the remaining etoposide level was negligible. The results were plotted versus time to determine half-lives. Only half-lives from environmentally relevant pH values are shown in Table 5a. The degradation rates were higher at higher pH.

No biodegradation studies were found for etoposide. The Millipore Material Safety Data Sheet notes that etoposide failed the closed bottle test and Zahn-Wellens tests for ready and inherent biodegradability (Millipore Corporation 2011). Details on the methodology were not mentioned. Although the reliability could not be verified, the result of this study—not readily biodegradable—is consistent with modelling results and structural interpretation.

Table 7-2: Empirical data for degradation of etoposide

Medium	Fate process	Degradation value	Degradation endpoint / units	Reference	RSS reliability category [a]
Water	Photolysis	2.8×10^9 (248 nm)	Photolysis rate / $L \cdot mol^{-1} \cdot s^{-1}$	Lu et al. 2000	NR
Water	Hydrolysis	63.00	Half-life (pH 5.00) / days	Shah et al. 1989	Low
Water	Hydrolysis	49.50	Half-life (pH 6.15) / days	Shah et al. 1989	Low
Water	Hydrolysis	27.72	Half-life (pH 7.30) / days	Shah et al. 1989	Low

Abbreviations: NR, not reviewed; RSS, robust study summaries
[a] Robust study summaries were used to determine the quality of the studies and are available in Appendix A.

7.3.2 Modelling results

Since limited experimental data on the degradation of etoposide are available, a QSAR-based weight of evidence approach (Environment Canada 2007) was applied using the degradation models shown in Table 7-3. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that etoposide is expected to be released to this compartment, biodegradation in water was primarily examined.

Table 7-3 summarizes the results of available QSAR models for degradation in various environmental media.

Table 7-3: Modeled data for degradation of etoposide

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008[a]	$t_{½}$ = 0.035 day	< 2
Ozone reaction	AOPWIN 2008[a]	NA[b]	NA
Hydrolysis	HYDROWIN 2008[a]	NA	NA
Primary biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 4: Expert	3.72[c] “biodegrades fast”	< 182

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
	Survey (qualitative results)		
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 3: Expert Survey (qualitative results)	2.1[c] “biodegrades slowly”	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 5: MITI linear probability	0.65[d] “biodegrades fast”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 6: MITI non-linear probability	0.02[d] “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	CATALOGIC 2009	% BOD = 13.9 “biodegrades slowly”	130

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

[a] EPI Suite (2008).

[b] Model does not provide an estimate for this type of structure.

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

[d] Output is a probability score.

Etoposide’s biodegradation model predictions should be viewed with some caution. Biodegradation processes involve biological activity that may be altered or improved by pharmaceuticals. As etoposide’s mode of action decreases cell viability, it is possible that the substance has antibiotic activity in the environment as well. Therefore, biodegradation rates would be underestimated. Although cell viability is not an intrinsic structural factor to estimate persistence, bacterial cell viability is directly proportional to the biodegradation rates that influence the stability of the substance in the environment.

The estimates proposed by DS TOPKAT (©2005–2009) were not considered reliable to describe etoposide’s biodegradation rate, as etoposide was considered outside of the model domain for DS TOPKAT. It is believed that chemicals with comparable structures are not contained in DS TOPKAT’s training set. Therefore, DS TOPKAT estimates are not shown in Table 7-3.

The predictions for CATALOGIC (2009) were 46.7% in the structural domain and were within the parameter domain for octanol–water partition coefficient (K_{ow}). CATALOGIC (2009) is able to predict metabolic pathways and transformation products from abiotic reactions and microbial transformations. For etoposide, the model estimates that one compound would be formed predominantly. The resulting structure is similar to etoposide, but with the two five-carbon rings open at the oxygen location. Several transformation steps are required to obtain this metabolite: ester hydrolysis, primary hydroxyl group oxidation, aldehyde oxidation, decarboxylation and oxidative O-dealkylation at three locations on the structure. Each reaction has an occurrence probability of between 73% and 100% in the environment. Summing the uncertainties of occurrence of every reaction, the probability of obtaining the metabolite is 47% (CATALOGIC 2009).

The primary biodegradation model BIOWIN Sub-model 4 estimates that degradation of etoposide has a primary half-life of < 182 days. The ultimate biodegradation models suggest that biodegradation is slow and that the half-life in water would be ≥ 182 days, whereas the result of the BIOWIN Sub-model 5 would suggest that the substance has a half-life of < 182 days. The results from BIOWIN Sub-models 3 and 6 and the CATALOGIC model suggest a slow to very slow rate of biodegradation. Some of etoposide's structural features may not be biodegradable, as the structure includes a large number of rings and branches, whereas other of its structural features are easily biodegradable, such as esters and benzene rings with various substitutions (easily biodegradable for compounds with K_{ow} < 2.18) and cyclic chemicals consisting only of C, O, N and H. Therefore, considering all model results, empirical data and structural features, there is more reliable evidence to suggest that the biodegradation half-life of etoposide is ≥ 182 days in water.

In air, a predicted atmospheric oxidation half-life of 0.035 day (see Table 7-3) demonstrates that this substance 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; however, etoposide is susceptible to direct photolysis in ultraviolet light (Lu et al. 1999). Therefore, it is expected that reactions with hydroxyl radicals will be the most important fate process in the atmosphere for etoposide. The substance is not expected to be found in air, but could be degraded by sunlight in the first centimetres below the surface of receiving water bodies.

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 sediments is ≥ 365 days. This indicates that, overall, etoposide is expected to be persistent in water, soil and sediment.

7.3.3 Conclusion on persistence

Photolysis and primary biodegradation half-lives of etoposide in water suggest that etoposide is readily biodegradable. However, results of CATALOGIC (2009) and BIOWIN (2008) Sub-models 3 and 6 indicate that ultimate biodegradation in the

environment is slow. CATALOGIC (2009) predicts a major metabolite resulting from etoposide degradation that is very similar to etoposide, meaning that primary biodegradation may be fast, but the skeleton of the parent structure could be minimally transformed. Accordingly, etoposide is considered to be persistent in the environment.

7.4 Potential for Bioaccumulation

Experimental and modelled log K_{ow} values of 0.04–1.0 for etoposide (value used for modelling: 0.6) suggest that the substance has a low potential to bioaccumulate in biota (see Table 3-1). In order to provide the best possible weight of evidence analysis of the bioaccumulation potential of etoposide, modelled data, empirical pharmacokinetic studies and other physiological parameters, such as metabolism and elimination, were considered in order to arrive at an overall conclusion.

7.4.1 Metabolism and elimination

A pharmacokinetic study indicates that 34–66% of the administered dose is recovered in human urine after 72 hours (Allen and Creaven 1975). In a similar analysis, Joel et al. (1995) found that 44% of the dose was recovered in urine. This suggests that etoposide is rapidly eliminated from humans and would not result in significant body burdens over time. In the human body, a fraction of the administered etoposide is metabolized by lactone hydrolysis to generate the hydroxy acid form of the substance; this metabolite appears to be pharmacologically inactive (McEvoy 2004). The drug can also be transformed by the action of the cytochrome enzyme CYP3A4 and by sulfate and glucoronate conjugation (Williams et al. 2002). Generally, the metabolism of etoposide in the human body follows two pathways: a phase I process that essentially involves CYP3A4 to produce a hydroxy acid compound and a phase II pathway that would lead to the excretion of glucuronide and sulfate metabolites. The biotransformation in hepatic cells is a CYP3A4 (cytochrome P450–mediated demethylation) reaction (IARC 2000). A homologue of this enzyme is present in mammals, fish and most other species, and it is deemed that detoxification will occur in a similar fashion in most aquatic species.

The major metabolite found in human urine is the etoposide glucuronide (IARC 2000), at 8–29% of the administered dose. In patients with normal renal and hepatic efficiencies, the elimination half-life of etoposide was estimated to be 5.6 ± 0.4 hours (D’Incaici et al. 1986). The low elimination half-life suggests that both etoposide and its metabolite will be rapidly excreted.

For aquatic organisms, the metabolic competency of an organism can be related to body weight and temperature. The lipid content of fish differs from the lipid content of humans, and the temperature of Canadian waters is on average lower than normal room temperature. Although phase I and II metabolic activity in fish may be significantly reduced compared with human metabolic activity, it is likely that elimination processes in humans are rapid enough to suggest that the bioaccumulation potential in aquatic species would be low.

7.4.2 Estimating BCF and BAF

Given its use as an antineoplastic drug, etoposide is expected to show high metabolic activity. Therefore, the modelling methods for estimating bioaccumulation based on comparison of empirical bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) of similar molecules or fragments are not suitable for this substance. However, a mass balance model such as the Arnot-Gobas model may provide reliable estimates because it includes the metabolic rate in its calculations.

Since no experimental BAF or BCF data were available for etoposide, a predictive approach was applied using available BAF and BCF models, as shown in Table 7-4.

An estimated BCF of 3 was reported for etoposide (Millipore Corporation 2011) using a log K_{ow} of 0.60 and a regression-derived equation. This BCF suggests that the potential for bioconcentration in aquatic organisms is very low.

Measures of BAF are the preferred metric for assessing bioaccumulation potential of substances. This is because BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log K_{ow} > ~4.0 (Arnot and Gobas 2003). Kinetic mass balance modelling is in principle considered to provide the most reliable prediction method for determining the bioaccumulation potential because it allows for metabolism correction as long as the log K_{ow} of the substance is within the log K_{ow} domain of the model. For etoposide, BAF estimates are deemed to be similar to BCF values because of the negligible dietary uptake for a substance with a low K_{ow}.

BCF and BAF estimates, corrected for potential biotransformation, were generated using the BCFBAF (2008) model. Metabolic rate constants (k_M) were derived using structure–activity relationships, described further in Arnot et al. (2008a, b). The middle trophic level fish was used to represent overall model output and is most representative of the fish weight likely to be consumed by an avian or terrestrial piscivore. The metabolic rate constant (k_M) is < 125.0/day, depending on the fish weight. The results of the BCF modelling are given in Table 7-4.

Table 7-4: Summary of modelled data for bioaccumulation of etoposide

k_M (/day)	Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	Reference
125[a]	Fish	BCFBAF Sub-model 1 (linear regression)	BCF	0.97	BCFBAF 2008
70.3	Fish	BCFBAF Sub-model 2 (mass balance)	BCF[b]	1.02	BCFBAF 2008
0.17	Fish	BCF_{max} without	BCF[c]	11.95[d]	CPOPs 2008

		mitigating factors[c]			
39.5	Fish	BCFBAF Sub-model 3 (Gobas mass balance)	BAF[b]	1.03	BCFBAF 2008

Abbreviations: BAF, bioaccumulation factor; BCF, bioconcentration factor; k_M, metabolic rate constant

[a] Predicted value exceeds the theoretical whole-body maximum value, so the whole body maximum values are provided and recommended by the model to replace the original model predictions.

[b] Results generated using weight, lipid and temperature for a middle trophic level fish.

[c] Possible mitigating factors include ionization, molecular size, metabolism and water solubility.

[d] Number of “unknown fragments” is 57.14%, which is too high to be acceptable.

The BCFBAF (2008) model flagged that the predicted metabolic rate constant (i.e., 100/day for a 10 g fish) exceeds the theoretical whole-body maximum value, suggesting that etoposide may be readily metabolized in fish.

Based on three-dimensional analysis of conformers calculated using the BCF_{max} Model with Mitigating Factors (CPOPs 2008), the maximum (D_{max}) and effective (D_{eff}) diameters of etoposide range from 1.43 to 2.06 nm. This suggests that etoposide may also experience restricted uptake from steric effects at the gill surface. Information regarding molecular size and cross-sectional diameters is useful to consider and is commonly used by international jurisdictions such as the European Union (ECHA 2008) as weight of evidence for bioaccumulation potential. Recent investigations relating fish BCF data to molecular size parameters (Dimitrov et al. 2002, 2005) suggest that the probability of a molecule crossing cell membranes as a result of passive diffusion declines significantly with increasing D_{max}. The probability of passive diffusion decreases appreciably when the D_{max} is > ~1.5 nm and more so for molecules having a D_{max} of > 1.7 nm. Sakuratani et al. (2008) also investigated the effect of cross-sectional diameter on passive diffusion in a BCF test set of about 1200 new and existing chemicals. They observed that substances that do not have a very high bioconcentration potential (BCF < 5000) often have a D_{max} of > 2.0 nm and a D_{eff} of > 1.1 nm. However, as Arnot et al. (2010) noted, there are uncertainties associated with the thresholds proposed by Dimitrov et al. (2002, 2005) and Sakuratani et al. (2008), since the BCF studies used to derive them were not critically evaluated. Arnot et al. (2010) pointed out that molecular size influences solubility and diffusivity in water and organic phases (membranes), and larger molecules may have slower uptake rates. However, these same kinetic constraints apply to diffusive routes of chemical elimination (i.e., slow in = slow out). Thus, significant bioaccumulation potential may remain for substances that are subject to slow absorption processes, if they are slowly biotransformed or slowly eliminated by other processes. However, if the rate of gill uptake is sufficiently mitigated by steric hindrance to the point where the rate of elimination exceeds uptake, bioconcentration will be lowered.

The available evidence indicates that etoposide is expected to have low bioaccumulation potential due to its physical and chemical properties (i.e., high molecular weight, low log K_{ow}), relatively large cross-sectional diameter, resulting in

restricted uptake from steric effects at the gill surface, and high metabolic activity in many species, which can accelerate the excretion of etoposide from the cells. Metabolism-corrected BCF and BAF values are also < 5000.

7.4.3 Conclusion on bioaccumulation potential

Because of its low log K_{ow}, rapid total rate of elimination, rapid gill exchange and increased metabolic activity, etoposide is not expected to bioaccumulate, bioconcentrate or biomagnify in aquatic biota.

8. Potential to Cause Ecological Harm

8.1 Ecological Effects Characterization

In order to provide the best possible weight of evidence for assessing the ecological effects of etoposide, empirical and modelled data were considered in the assessment. The QSAR models are based on similarities with a large number of compounds, which include a limited number of biologically active compounds such as drugs. Etoposide is considered within the limits of domain applicability for most models, but the level of variability between models is very high.

8.1.1 Mode of action

In mammals, etoposide is an inhibitor of topoisomerase II, an enzyme essential for deoxyribonucleic acid (DNA) replication, transcription, recombination and chromosomal segregation. Etoposide forms a ternary complex with DNA and the topoisomerase II enzyme, preventing religation of the DNA strands. The biotransformation in hepatic cells involves cytochrome P450–mediated demethylation (CYP3A4) (IARC 2000). A version of this enzyme is present in most species, so it is suggested that detoxification will occur using the same enzymatic system in aquatic species.

8.1.2 Empirical aquatic toxicity data

Empirical aquatic toxicity data for etoposide are presented in Table 8-1. Relatively few data are available on gross or whole body level effects (e.g., abnormal development). However, a number of biomarker test results are available and are presented in the next subsections. Due to the potential impact of etoposide on endocrine function and carcinogenicity, much of the research on etoposide has focused on these areas. They are largely *in vitro* studies and therefore cannot be readily used for developing a predicted no-effect concentration (PNEC) for risk characterization because of the difficulty in extrapolating from adverse biochemical effects to the ecological population.

Table 8-1: Empirical aquatic toxicity data for etoposide

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Bacteria	Acute (16 h)	NOEC	200	Zounková et al.

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Pseudomonas putida				2007
Bacteria *Pseudomonas putida*	Acute (16 h)	LOEC	250	Zounková et al. 2007
Bacteria *Pseudomonas putida*	Acute (16 h)	EC_{50}	630	Zounková et al. 2007
Green alga *Pseudokirchneriella subcapitata*	Acute (96 h)	NOEC	< 10	Zounková et al. 2007
Green alga *Pseudokirchneriella subcapitata*	Acute (96 h)	LOEC	10	Zounková et al. 2007
Green alga *Pseudokirchneriella subcapitata*	Acute (96 h)	EC_{50}	250	Zounková et al. 2007
Crustacean *Daphnia magna*	Acute (48 h)	NOEC	10	Zounková et al. 2007
Crustacean *Daphnia magna*	Acute (48 h)	LOEC	30[a]	Zounková et al. 2007
Crustacean *Daphnia magna*	Acute (48 h)	EC_{50}	30[a]	Zounková et al. 2007

Abbreviations: EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; NOEC, the no-observed-effect concentration, the highest concentration in a toxicity test not causing a statistically significant effect in comparison with the controls; LOEC, the lowest-observed-effect concentration, the lowest concentration in a toxicity test that caused a statistically significant effect in comparison with the controls;
[a] Critical value for inherent toxicity to non-human organisms.

The effects of etoposide on species from three major trophic levels of the aquatic compartment were tested: producers, consumers and decomposers (Zounková et al. 2007). The growth inhibition of the unicellular green alga *Pseudokirchneriella subcapitata*, a producer, in cell cultures was measured over 96 hours for the amount of chlorophyll using the response to green light absorbance (680 nm) at five etoposide concentrations. The assay was repeated in three or four replicates used to generate dose–response curves. Statistical tests were used to determinate no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC) and median effective concentration (EC_{50}), presented in Table 8-1. The effects of etoposide on newly hatched daphnid, *Daphnia magna*, a consumer, were assessed at six concentrations. After 48 hours of exposure, the immobilized organisms were counted, and the results were expressed as the percentage of control. Statistical tests were performed to calculate NOEC, LOEC and EC_{50}, shown in Table 8-1. The growth inhibition of cell cultures of the bacteria *Pseudomonas putida*, a decomposer, was measured from the absorption at 590 nm. The test was performed at six etoposide concentrations to generate dose–response curves. The article met quality standards, and a robust study summary was used to determine the quality of the studies and is appended in Appendix A. Standardized statistical toxicity test results are presented in Table 8-1.

8.1.3 Mechanisms of toxicity

Four-week studies of toxicity were conducted in monkeys treated intravenously at 0.4–3.6 mg/kg body weight (bw) per day. The main effects observed were myelosuppression with anemia, leucopenia, thrombocytopenia and some hepatotoxicity (IARC 2000).

The induction of p53 protein and apoptosis rate were investigated in fish desert topminnow (*Poeciliopsis lucida*) hepatocytes (Rau Embry et al. 2006). In mammals, the p53 protein protects normal cells from aberrant growth by its ability to modulate the genes involved in cell growth, notably caspase-3. In desert topminnow, p53 protein level was not affected by etoposide, but a significantly higher level of apoptosis, as shown by dose-dependent induction of caspase-3, was observed at concentrations of 5.9 and 14.7 mg/L. This suggests that the p53 protein was activated by an alternative mechanism in fish, compared with mammals. Therefore, at the cellular level, the mechanism of toxicity of etoposide may not be comparable in fish and mammals.

In order to validate the activity of endosulfan in reducing the apoptosis rate in spleen cells from Nile tilapia (*Oreochromis niloticus*), Tellez-Bañuelos et al. (2011) used etoposide for the positive control. Using flow cytometry, the authors counted apoptotic cells in a culture exposed to endosulfan alone, to etoposide alone or to both substances and in a control culture not exposed to xenobiotics. The cell density of splenocytes exposed to etoposide and subject to early apoptosis decreased by 22%, 14% and 13% relative to control cells after 24, 48 and 72 hours, respectively.

8.1.4 Potential for genotoxicity

The genotoxicity potential of etoposide was assessed using a pair-wise matching technique by Jackson et al. (1996) to generate a genetic activity profile. While the data clearly show a potential for genotoxicity at low doses of 0.01–50 mg/kg bw in humans and other mammals, this effect is not observed in most prokaryotes or in lower eukaryotes, for which almost no effects are observed following exposure to etoposide concentrations between 74 and 740 mg/L.

DNA degradation in mussel hematocytes and gills resulting from exposure to etoposide was assessed by Mičić et al. (2002). The separation of DNA strands in mussels following exposure to etoposide was examined. As expected, DNA strands were broken by the action of etoposide, but response to the substance resulted from non-random separation of DNA fragments. The results were not provided, but the authors suggested that etoposide’s mechanism of genotoxicity in mussels is due to its action on specific DNA sequence targets in hematocytes and gills.

In order to examine the genotoxicity potential of etoposide, growing cultures of *Escherichia coli* and *Salmonella choleraesius subsp. choleraesius* were exposed to various concentrations of etoposide by Zounková et al. (2007). In *E. coli*, the induction of SOS-chromotest[4] response resulting from an alteration of the genetic information was measured indirectly using the ratio of β-galactosidase and alkaline phosphatase relatively to the negative control. To simulate the influence of metabolic activity, the experiment was repeated with the addition of rat liver homogenate. This addition inhibited etoposide's minimum genotoxic concentration by ~3 times after 2 hours (Table 8-2), indicating that metabolism may be rapid. In *Salmonella choleraesius subsp. choleraesius*, the effects of etoposide were measured by the strain's growth inhibition, rather than its metabolic activity. The *Salmonella* strain is less sensitive than *E. coli* to etoposide, as shown by its higher minimum genotoxic concentration.

Table 8-2: Empirical genotoxicity data for etoposide

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Bacterium *Escherichia coli*	Genotoxicity – 2 hours	MGC	2.4 (without metabolic activation), 6.4 (with metabolic activation)	Zounková et al. 2007
Yeast *Saccharomyces cerevisiae*	Genotoxicity – 16 hours	MGC	150 (140–168)	Zounková et al. 2007

Abbreviations: MGC, minimum genotoxic concentration; RSS, robust study summary

Some evidence of the genotoxicity potential of etoposide in mice, rats and humans is presented in IARC (2012), which concludes that etoposide is carcinogenic to humans (Group 1). Although it is known that etoposide has genotoxic activity in mussels and prokaryotes, it is not possible to conclude with certainty that genotoxicity would be observed in other aquatic organisms.

8.1.5 Other ecological effects

Milan et al. (2003) assessed life-threatening arrhythmia resulting from exposure of zebrafish larvae to etoposide. The two arrhythmia symptoms that indicate heart rate disorder are QT interval prolongation on the electrocardiogram and *torsades de pointes*.

[4] **SOS-chromotest:** a bacterial genotoxicity test with the genetically modified bacterial tester strain *Escherichia coli* PQ 37. β-Galactosidase activity was measured (reporter enzyme for genotoxicity induced along with DNA repair system) using a chromogenic substrate *ortho*-nitrophenyl-β-D-galactopyranoside. At the same time, activity of alkaline phosphatase (marker of viability/cytotoxicity) was assessed using *p*-nitrophenyl phosphate chromogenic substrate. The concentrations causing more than 50% inhibition were excluded from genotoxicity evaluations. The SOS induction factor was then calculated for each tested concentration, and the minimum genotoxic concentration at which the induction factor, 1.5, was determined. Values > 1.5 indicate significant genotoxicity.

These symptoms were monitored using a camera directed towards the fish heart and analyzed using software measuring pixel density, plotted against time. After 24 hours of exposure to etoposide at 1, 10 or 100 mg/L, no significant effect on heart rate or arrhythmia was observed.

The multi-xenobiotic resistance of sea urchin embryos to etoposide alone or mixed with known resistance inhibitors was assessed by Smital et al. (2004). Embryos tested with only etoposide at 2.94 mg/L did not show any significant increase in cell death. However, when the drugs verapamil and reversin 205 were added to the mixture at low concentrations, the ratio of apoptotic cells to normal cells was up to 10-fold higher than it was following exposure to etoposide alone. The authors proposed that cell death is generated in embryos by high alterations of the genetic material. These co-exposure experiments indicate that the apoptotic resistance system of sea urchin embryos is affected by drug mixtures that could be present in effluents from large drug point sources, such as hospitals.

Etoposide has been shown to be teratogenic and embryocidal in mice and rats at doses of 1–3% of the recommended human dose based on body surface area (McEvoy 2004). Etoposide induced thymic atrophy in gestating female rats at 10 mg/kg bw. As the thymus is a gland of the endocrine and immune system, etoposide is suspected of affecting endocrine function. However, there is not enough information to conclude this with high certainty.

8.1.6 Modelled aquatic toxicity results

As the empirical data are limited, QSAR models were used to read across similar structures in order to verify the consistency between this approach and the experimental studies presented in Tables 8-1 and 8-2. Confidence in modelled results is low because of the lack of complete structural coverage for etoposide for most of the models tested (e.g., OASIS, ECOSAR, DS TOPKAT, CPOPs). Consequently, only the AIEPS (©2010–2012) model was selected. In addition, only the effect values of the top 10 structures that had over 60% similarity with etoposide were averaged and are presented in Table 8-3. For the green alga *Pseudokirchneriella subcapitata*, only the values from the top five most similar structures were averaged, as only these had over 60% similarity with etoposide (Table 8-3). The results achieved by this read-across approach are consistent with the empirical results of Tables 8-1 and 8-2.

Table 8-3: Modelled acute aquatic toxicity data for etoposide

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish (f*athead minnow Pimephales promelas*)	Acute (96 h)	LC_{50}	47.6	AIEPS ©2010–2012
Crustacean *(Daphnia magna)*	Acute (48 h)	LC_{50}	74.9	AIEPS ©2010–2012
Alga (*Pseudokirchneriella subcapitata*)	Acute (72 h)	EC_{50}	10.2	AIEPS ©2010–2012

Abbreviations: EC_{50}, the concentration of a substance that is estimated to cause some 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

The predicted critical effect concentration of etoposide in fish was calculated by Fick et al. (2010), using the fish plasma model proposed by Huggett et al. (2003). This model generates a concentration ratio based on the compound's K_{ow} between human therapeutic plasma concentration and the fish steady-state plasma concentration. Based on this, the model predicts the concentration of concern in fish from the pharmaceutical response in humans. The theoretical plasma bioconcentration ratio was estimated to be low, and the critical effect concentration of etoposide in fish was 7.1 mg/L.

8.1.7 Derivation of the PNEC

The acute toxicity tests for etoposide with algae, crustaceans and bacteria indicate a potential for effects on a variety of aquatic organisms. Green algae are the most sensitive class of aquatic organisms tested at low concentration, with low acute effect concentrations of 10 mg/L. However, an effect level for microscopic algae may be overly conservative for other aquatic organisms. At higher concentrations, a larger proportion of the population of *D. magna* is affected by etoposide. The EC_{50} of *D. magna* exposed to etoposide is as low as its LOEC; the lowest concentration having significantly different effect value from control is identified as the EC_{50}. This is likely owing to large variability between replicates or poor design of the range-finding study to determine exposure concentrations, raising the uncertainty for all tested concentrations. The critical toxicity value (CTV) was selected to be 30 mg/L, the LOEC and EC_{50} for *D. magna*.

A conservative PNEC was derived by dividing the CTV identified (30 mg/L) by an uncertainty factor of 500 to account for uncertainties and possible long-term subchronic effects resulting from exposure to etoposide, as follows: a factor of 100 was applied to account for uncertainty related to interspecies and intraspecies variability in sensitivity, extrapolation from acute to chronic effects and extrapolation from laboratory conditions to the field. A supplementary factor of 5 was applied to account for the possible effects related to genotoxicity and endocrine function. These subchronic effects would not be seen in standard short-term laboratory tests because they are not designed to observe cellular or gene-level interactions. Possible carcinogenic, mutagenic or hormonal effects from reactive substances may not be observed over the lifetime of the organism, but "molecular initiating events" may commence quite rapidly upon permeation of the cell by a reactive compound (in this case a cancer treatment drug), disrupting cellular processes. Therefore, additional precaution is warranted to account for this non-quantifiable source of uncertainty, which could result in non-predictable excess toxicity in a species. It has been demonstrated that etoposide has synergistic effects with other drugs likely to be released from the same source (Smital et al. 2004).

This calculation results in a PNEC of 0.06 mg/L.

8.2 Ecological Exposure Assessment

No data concerning the concentrations of etoposide in any media in Canada have been identified. PECs have been estimated from available information, including estimated substance quantities, estimated release rates and characteristics of the receiving aquatic environment. PECs have been estimated for an industrial release scenario and a down-the-drain release scenario, as described in the following subsections. Since the biological activity of etoposide metabolites and their contributions to toxicity are deemed less than those of the parent compound, they are not assessed in this screening assessment.

8.2.1 Industrial release

An aquatic exposure to etoposide is expected if the industrial substance is released during its manufacture and processing to a wastewater system that discharges its effluent to a receiving surface water body. The concentration of the substance in the receiving water near the discharge point of the wastewater system is used as the PEC in evaluating the aquatic risk of the substance. It is calculated using the following 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)

1000: Conversion factor (g/kg)

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)

Table 8-4 presents the inputs used to estimate aquatic concentrations close to the industrial point of discharge. Three companies were identified as having industrial activities related to etoposide, but the proportion of the drug manufactured or imported by each of the individual facilities is unknown. Therefore, it was conservatively assumed that all the etoposide mass on the Canadian market is manufactured by one facility. Based on these assumptions, this scenario yields a PEC of 1.61×10^{-6} mg/L (Environment Canada 2013a). This PEC value represents the level of exposure in the receiving water away from the point of discharge from the wastewater system at the site.

Table 8-4: Summary of input values used for estimating aquatic concentrations resulting from industrial releases of etoposide from the pharmaceutical industry

Input	Value	Justification and reference
Q: Quantity (kg/year)	23	Estimated quantity as prescribed at hospitals and pharmacies across Canada for the year 2012 (IMS 2013)
L: Loss to wastewater (%)	0.5	Personal communication, Technical Support Document for Pharmaceutical Spreadsheets, from Environmental Assessment Unit, New Substances [Health Canada], to Exposure Unit, Existing Substances [Environment Canada], dated 2007; unreferenced
R: Wastewater system removal efficiency (%)	2	WWTP fugacity model from EPI Suite (2008): total removal from a wastewater system
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
Wastewater system effluent flow (m^3/day)	332 624	Effluent flow of a large wastewater treatment plant located in Mississauga (a typical Canadian pharmaceutical manufacturing site, assumed to be located in Mississauga)
Receiving water dilution factor (dimensionless)	10	Environment Canada default assumption for large lakes, the WWTP in the scenario discharges to Lake Ontario

Abbreviation: WWTP, wastewater treatment plant

8.2.2 Down-the-Drain Releases from Pharmaceutical Use

As etoposide is used in pharmaceutical products and can be released to water, an aquatic exposure scenario resulting from down-the-drain releases from pharmaceutical use was developed. The scenario estimates the concentration of etoposide in multiple water bodies receiving wastewater treatment system effluents where pharmaceutical products that contain etoposide may have been released (Environment Canada 2009). This scenario provides estimates for approximately 1000 release sites across Canada (Environment Canada 2013b).

The total mass of etoposide used in Canada was assumed to be evenly distributed within the country. The releases are from the unabsorbed and unchanged fraction of the drug excreted by patients in feces and urine. The number of annual release days was estimated to be 365, based on use of the drug across patients. Some variability between sites is expected because of the location of hospitals where the drug is administered. Table 8-5 presents a summary of the inputs used to estimate aquatic concentrations resulting from the use of etoposide. The approach and equations used to calculate the PECs are described in Environment Canada (2009).

Table 8-5: Summary of the input values used for estimating aquatic concentrations resulting from use of pharmaceuticals containing etoposide

Input	Value(s)	Justification and reference
Quantity (kg/year)	23	Estimated quantity as sold to hospitals and pharmacies across Canada for the year 2012 (IMS 2013)
Loss to wastewater (%)	99	Assumes some uptake or metabolism of the substance within human body (Hande 1998; Hospira HealthCare Corporation 2007; Bristol-Myers Squibb Company 2008); 74% is the higher value in the range of the calculated fraction released to water (see Table 5-1) Assumes no metabolism in light of the uncertainty relating to the environmental stability of the metabolites of etoposide, the etoposide glucuronide
Variability factor[a]	2	Default realistic worst-case value
Wastewater system removal efficiency (%)	2	WWTP fugacity model from EPI Suite (2008): total removal from a wastewater system
Number of annual release days (days/year)	365	This product is expected to be administered year-long
Receiving water dilution factor (dimensionless)	Maximum 10	Environment Canada Existing Substances default assumption

Abbreviation: WWTP, wastewater treatment plant

[a] The variability factor is used to define the level of variability of the use of a product in the country. When multiple products 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.

The PECs of etoposide in the receiving water bodies were estimated to be in the range of 3.8×10^{-8} to 1.6×10^{-5} mg/L.

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, inherent or ecological toxicity, sources and fate of the substance, and presence and distribution in the environment.

Quantities of etoposide sold in Canada are low, primarily for use as a pharmaceutical product in medical oncology. As etoposide is released to wastewater from both industrial

and prescribed use, it will be treated by the wastewater treatment system. It is not expected to sorb significantly to sludge or to be removed efficiently from the wastewater system. Therefore, once released into the environment, it will be found mainly in water.

Etoposide is expected to be persistent in water, soil and sediment, but it is not expected to be found significantly in media other than water. Based on its low K_{ow}, its high molecular weight and its high metabolic activity, etoposide is expected to be minimally absorbed by gills and easily excreted. Therefore, etoposide is expected to have a low bioaccumulation potential.

Etoposide has also been demonstrated to have moderate potential for toxicity to aquatic organisms. Some of the evidence of harm for etoposide relates to endpoints such as developmental and reproductive toxicity, genotoxicity and endocrine function. These effects are part of the weight of evidence indicating that etoposide has the potential to be hazardous to organisms. It is acknowledged that cancer generally occurs infrequently in wild animals and that it is difficult to assess the potential for the manifestation of cancer endpoints in individual organisms and to estimate the overall impact on individuals or local populations of organisms. When there is evidence, as in the case for etoposide, that a substance causes cancer in laboratory animals (particularly through a genotoxic mechanism), such information could be considered to contribute to the weight of evidence suggesting potential to cause ecological harm under CEPA 1999. However, this would not necessarily be sufficient as a sole or primary basis for concluding that a substance meets the criteria under paragraph 64(*a*) of CEPA 1999.

A risk quotient analysis, integrating realistic worst-case estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The site-specific industrial scenario (considering the actual receiving water body) presented above yielded a PEC of 1.61×10^{-6} mg/L (Environment Canada 2013a).

This PEC value is then used to calculate a risk quotient, as shown in the following equation:

$$RQ = PEC/PNEC$$

where:

RQ: Risk quotient (dimensionless)

PEC: Predicted environmental concentration in receiving water (mg/L)

PNEC: Predicted no-effect concentration (mg/L)

The PNEC for aquatic organisms was evaluated to be of 0.06 mg/L. An assessment factor of 500, which may be considered excessive to protect the environment, is deemed to be conservative enough to account for subtle long-term effects such as genotoxicity and endocrine disrupting effects. The current scientific knowledge on the potential disruption of the ecosystem from these long-term effects is low, and precaution is needed. The resulting risk quotient (PEC/PNEC) is 2.68×10^{-5}. Therefore, harm to aquatic organisms is unlikely at this site.

The risk quotients are less than one for all sites across Canada for exposures resulting from down-the-drain releases through the consumption of pharmaceutical products that contain etoposide. The maximum risk quotient at one location is 0.0003. Based on the estimated number of receiving water bodies that will not be negatively affected by the use of the substance, coupled with the magnitude of the risk quotient and the realism of the scenario run, etoposide is not expected to cause harm to aquatic organisms from down-the-drain releases.

Together, the information available suggests that there is low risk of harm to organisms or the broader integrity of the environment from this substance. It is therefore concluded that etoposide does not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as it is 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 Uncertainties in Evaluation of Ecological Risk

There are uncertainties due to the lack of information on environmental concentrations in Canada, the lack of information on manufacturing and the quantity of etoposide imported into Canada. Although no information was requested from industry, data were available to estimate the amount of the substance prescribed at hospitals and pharmacies across Canada for the years 2011 and 2012 (IMS 2013).

The proportion of etoposide manufactured and released from each individual industrial facility is unknown. Therefore, it was conservatively assumed that all etoposide used in Canada was manufactured at a single location. Similarly, as the distribution of the use across Canada is unknown, a variability factor of 2 was applied to every location in Mega Flush to account for uneven distribution.

The confidence in modelled results for a biologically active substance like etoposide is low. The model estimates often considered only fragments of etoposide to compare with other substances, and a large fraction was out of the domain of applicability. The models to estimate effects cannot address specific modes of action, such as DNA binding, that are characteristic of a drug like etoposide, nor could models estimate correctly genotoxicity or endocrine function effects. Therefore, empirical data and other lines of evidence contributed to the weight of evidence.

The bioaccumulation assessment is limited by the absence of empirical bioaccumulation data and the difficulties in relying on bioaccumulation models. Therefore, a qualitative assessment based on K_{ow}, metabolic activity and a mass balance model was used to predict the bioaccumulation potential of etoposide. The mass balance model cannot address non-passive diffusion routes of uptake and partitioning of the substance to non-lipid phases in the organisms. There is, however, a low potential that model results would be interpreted as false negative, given the substance's low K_{ow}.

Regarding ecotoxicity, based on the predicted partitioning behaviour of this substance, the significance of soil and sediment as important media of exposure is not well addressed by the effects data available. Indeed, the only effects data identified apply primarily to pelagic aquatic exposures, although the water column may not be the medium of primary concern, based on partitioning estimates.

9. Potential to Cause Harm to Human Health

Etoposide, by itself and in combination with cisplatin and bleomycin, has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC 2012).

Drugs containing etoposide 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 etoposide 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 1.61×10^{-6} mg/L (1.6 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 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 etoposide through drinking water.

Only a portion of the pharmaceutical used in Canada would be released into the wastewater system. The metabolism of the substance 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 the general population to etoposide. Releases to surface water were modelled using the down-the-drain releases from pharmaceutical use scenario, as described above. For the purposes of modelling, it was assumed that 74% of the pharmaceutical that was prescribed was excreted and released into wastewater. It was also assumed that only 2% of etoposide 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 estimated was $1.21 \times 10^{-}$ mg/L (12.1 ng/L).

Limited measured concentration data for etoposide were identified. The concentrations measured in hospital effluent were not deemed relevant for this assessment, given the various reductions in concentration that can occur between the release of effluent from the hospital and consumption by humans. Smyth and Teslic (2013) attempted to measure etoposide in wastewater from six wastewater treatment plants in Canada. Etoposide was not detected in the influent or effluent, with detection limits ranging from 6.32 to 47.8 ng/L. As this substance was not detected even at the lowest detection limit, this value (6.32 ng/L) is considered to be a conservative proxy to actual concentrations. It is recognized that this concentration would not be expected to be found in drinking water, as it would be further reduced via dilution after the effluent was released to surface water and possibly reduced during the drinking water treatment process prior to consumption. However, this value can be used as a conservative estimate of exposure of Canadians.

The estimated intakes of etoposide by humans can be represented by formula-fed infants 0–6 months of age, which is estimated 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 etoposide, based on the detection limit of 6.32 ng/L in wastewater treatment plant effluent in Canada in which etoposide was not detected, is 0.674 ng/kg bw per day. Based on the consumer release scenario's modelled concentration of 12.1 ng/L, the estimated intake would be 1.3 ng/kg bw per day.

Given the low levels of estimated exposure, the potential risk of indirect exposure to etoposide is expected to be low. This determination is further supported by consideration of two additional lines of evidence for evaluation of potential harm to human health.

A comparison was made between the estimated intake value for etoposide and the threshold of toxicological concern (TTC) value of 2.5 ng/kg bw per day originally proposed by Kroes et al. (2004). The estimated intake is below the TTC. The TTC provides a reference point against which the range of estimated intakes can be compared. TTC values, which are derived using probabilistic approaches, establish generic human exposure threshold values below which it is expected that the probability of adverse effects is low. A TTC value of 0.15 µg/day (equivalent to 2.5 ng/kg bw per day) has been established for potentially carcinogenic substances with structural alerts for genotoxicity. Additional higher TTC values have been established for substances not containing similar structural alerts by examining available toxicity data for large groups of substances and are indicative of a very low probability of risk to human health (Munro et al. 1996a, b; Kroes et al. 2004; EFSA 2012; Dewhurst and Renwick 2013).

A second comparison was also made to evaluate potential risk. The lowest therapeutic dose (LTD) for etoposide 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.

The products currently registered for use in Canada can be administered intravenously or orally (DPD 2010); however, as the exposure route for the general population is through oral ingestion of drinking water, an oral dose is the most relevant for characterizing the potential risks. Dosage information for the oral form indicates a recommended dose of 100–200 mg/m^2 per day (Bristol-Myers Squibb Company 2008). Using an adult body weight of 70.9 kg (Health Canada 1998) and a body surface area of 1.82 m^2 for an adult (Health Canada 1995), the LTD of 100 mg/m^2 is equivalent to a dose of 2.6 mg/kg bw per day.

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)

Using the intake based on the detection limit of samples of wastewater influent and effluent in which etoposide was not detected results in an MOE of > 3 000 000. The MOE using the maximum modelled PEC would be > 2 000 000. Given the highly conservative nature of the exposure inputs and the use of human data to derive a point of departure for risk characterization, these MOEs support the determination that risks from indirect exposure to etoposide are likely to be negligible.

It is therefore concluded that etoposide does not meet the criteria set out 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.

9.1 Uncertainties in Evaluation of Risk to Human Health

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

Potential exposures to etoposide 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 less than the exposure through drinking water and so are not considered in this assessment.

Etoposide 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 severe. 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 this substance, the highly conservative exposure defaults that have been used lead to significant margins between the LTD and the estimated intakes. The LTD also allows for derivation of an MOE based on a human dose as the point of departure, which is preferable to using a point of departure developed using experimental animals.

10. Conclusion

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms or the broader integrity of the environment from this substance. It is therefore concluded that etoposide does not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as it is 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 etoposide does not meet the criteria set out 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 concluded that etoposide does not meet any of the criteria under section 64 of CEPA 1999.

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Appendix A: Robust Study Summaries

Description of the Reliability Evaluation

To evaluate the reliability of studies for key ecological endpoints (i.e., inherent toxicity to aquatic organisms, bioaccumulation potential, persistence), a strategy generally analogous to the Klimisch approach (Klimisch et al. 1997) has been developed. It involves the use of a standardized Robust Study Summary (RSS) form and a scoring system to quantitatively evaluate the studies. The RSS form is an adaptation of the OECD RSS templates (OECD 2002). It consists of a checklist of criteria reflecting information on the test substance, method, test organism, test design/conditions, ecological relevance and results (column 1). Most items are weighted according to their criticality to the quality of the study (column 2). For each item, the evaluator must indicate whether the item has been addressed in the study by answering “Yes” (Y), “No” (N) or “Not applicable” (n/a) (column 3). The most important or critical items (which describe parameters/factors that have the most direct influence on the quality of the study) have been given a higher weight (3 points), while the less critical items have been given a lower score (1 or 2 points). The weighting is based on expert judgement.

Once all the questions have been answered, an overall RSS score for the study is calculated as:

$$\text{Overall study score (\%)} = \frac{\sum W_{Yes}}{\sum W_{Yes+No}} \times 100\%$$

where:

W_{Yes} = weight of applicable “Yes” answers;

W_{Yes+No} = weight of applicable “Yes” and “No” answers.

The overall score’s corresponding reliability code and category are determined using the four categories inspired from the Klimisch approach and based on the score ranges as described in Table A.1.

Table A-1: Scoring grid for overall study reliability

Reliability code	Reliability category	Overall study score range
1	High confidence	≥ 80%

2	Satisfactory confidence	60–79%
3	Low confidence	40–59%
4	Not acceptable	< 40%

The RSS for log Kow was performed on the study by Shah et al. 1989 (Table A.2). The RSS score was 65%, and the reliability code was 2. Overall, the reliability of this study was found to be satisfactory. Although it is predicted that the level of confidence is satisfactory, the octanol–water partition coefficient value should not be considered as the most relevant, because the test was performed with the pharmaceutical formulation of etoposide instead of the pure compound. Also, the quantity of etoposide added to the solution was higher than its water solubility.

Table A.2: Robust study summary for log Kow (Shah et al. 1989)

Item	Weight	Response	Mark
Could you repeat the experiment with available information?	5	Not easily, but basic information is presented	3
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	0
Are the results presented in detail, clearly and understandably?	3	No	0
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	No. 10 mg of etoposide was added to 5 mL of water and 5 mL of octanol. 10 mg/10 mL is one order of magnitude over etoposide’s water solubility	0
Were particulates absent?	2	Not mentioned, but HPLC was used, therefore the solution might have been filtered	1
Was a reference chemical of known constant tested?	3	Not mentioned	0

Were other fate processes considered?	5	Yes; degradation products	5
Was a control (blank) run?	3	Not mentioned	0
Was temperature kept constant?	5	Yes	5
Was the experiment done near room temperature (15–30°C)?	3	Yes: 25°C	3
Is the purity of the test chemical reported (> 98%)?	3	No, but it comes from a drug company and is deemed to be pure enough in the formulation	2
Was the chemical's identity proven?	3	No	0
Is the source of the chemical reported?	1	Yes; Bristol-Myers	1

The RSS for water solubility was performed on the study by Shah et al. 1995 (Table A.3). The RSS score was 89%, and the reliability code was 1. Overall, the reliability of this study has high confidence; however, the test was performed with the pharmaceutical formulation of etoposide instead of the pure compound.

Table A.3: Robust study summary for water solubility (Shah et al. 1995)

Item	Weight	Response	Mark
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	Yes, distilled	2
Are the results presented in detail, clearly and understandably?	3	Tables are missing	1
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	Yes	5
Were particulates absent?	2	Yes, filtered through a 0.45 µm membrane filter	2
Was a reference chemical of known constant tested?	3	Not mentioned	0
Were other fate processes	5	Yes, degradation	5

Item	Weight	Response	Mark
considered?		and photosensitivity	
Was a control (blank) run?	3	Not mentioned	0
Was temperature kept constant?	5	Yes, approximately: room temperature	3
Was the experiment done near room temperature (15–30°C)?	3	Yes: Room temperature	3
Is the purity of the test chemical reported (> 98%)?	3	No, but it comes from a drug company and is deemed to be pure enough in the formulation	2
Was the chemical's identity proven?	3	No	0
Is the source of the chemical reported?	1	Yes; Bristol-Myers	1

The RSS for persistence in water was performed on the study by Shah et al. 1995 (Table A.4). The RSS score was 57.9%, and the reliability code was 3. Overall, the reliability of this study is low.

Table A.4: Robust study summary for persistence in water (Shah et al. 1995)

Item	Weight	Yes/No	Specify
Substance identity: chemical name(s)	n/a		Etoposide
Chemical composition of the substance	2	Y	Product was used as received from the drug company, product monograph is available with detailed information
Chemical purity	1	N	n/a
Reference	1	N	n/a

OECD, EU, national or other standard method?	3	N	n/a
Justification of the method/protocol if a standard method was not used	2	Y	Explanations are provided for details of experiment
GLP	3	n/a	Not applicable: the study was completed in 1989, and GLP was not implemented
Test type (i.e., hydrolysis, biodegradation, etc.)	n/a	Y	Hydrolysis
Test conditions type (aerobic or anaerobic)	n/a	N	Anaerobic
Test medium (water, sediment or soil)	n/a	Y	Water
Test duration	n/a	Y	Until remaining etoposide level was negligible
Negative or positive controls?	1	Y	Negative
Number of replicates (including controls)	1	Y	Three replicates
Measured concentrations reported?	3	N	n/a
Analytical method / instrument	1	Y	HPLC
Type of biodegradation (ready or inherent) reported?	2	n/a	n/a
When type of biodegradation (ready or inherent) is not reported, if there is indirect information allowing identification of biodegradation type?	1	n/a	n/a
Inoculum source	1	n/a	n/a
Inoculum concentration or number of microorganisms	1	n/a	n/a
Were inoculum pre-conditioning and pre-adaptation reported?	1	n/a	n/a
Were inoculum pre-conditioning and pre-adaptation appropriate for the method used?	n/a	n/a	n/a
Temperature	1	n/a	n/a

Has percentage degradation of the reference compound reached the pass levels by day 14?	n/a	n/a	n/a
Soil: soil moisture reported?	1	n/a	n/a
Soil and sediments: background soil organic matter content reported?	1	n/a	n/a
Soil and sediments: clay content reported?	1	n/a	n/a
Soil and sediments: cation exchange capacity reported?	1	n/a	n/a
pH values reported?	1	Y	1.3; 2.03; 3.05; 5.00; 6.15; 7.30; 8.00; 10.00
Temperature	1	Y	25°C
Were appropriate concentrations of the substance used?	1	Y	Top range of water solubility limit
If solvent was used, was it done appropriately?	1	Y	The solvent (buffer) has no interaction with the tested molecule, but controls pH
Temperature	1	n/a	n/a
Light source	1	n/a	n/a
Light spectrum (nm)	1	n/a	n/a
Relative intensity based on sunlight intensity	1	n/a	n/a
Spectrum of a substance	1	n/a	n/a
Indirect photolysis: sensitizer (type)	1	n/a	n/a
Indirect photolysis: concentration of sensitizer	1	n/a	n/a
Endpoint and value	n/a	n/a	Many. See text.

Breakdown products	n/a	n/a	Four breakdown products are observed but not identified

Abbreviations: EU, European Union; GLP, good laboratory practice; HPLC, high-performance liquid chromatography; N, no; n/a, not applicable; OECD, Organisation for Economic Co-operation and Development; Y, yes

The RSS for aquatic toxicity was performed on the study by Zounková et al. 2007 (Table A.5). The RSS score was 76.7%, and the reliability code was 2. Overall, the reliability of this study is satisfactory.

Table A.5: Robust study summary for aquatic toxicity (Zounková et al. 2007)

Item	Weight	Yes/No	Specify
Substance identity: chemical name(s)	n/a	Y	Etoposide
Chemical composition of the substance	2	Y	The drug was supplied by a hospital, the composition is described in the product monograph
Chemical purity	1	N	n/a
Persistence/stability of test substance in aquatic solution reported?	1	N	n/a
Reference	1	Y	European standards
OECD, EU, national or other standard method?	3	Y	The Czech standard (identical to the European standard EN ISO 6341:1996)
Justification of the method/protocol if a standard method was not used	2	n/a	n/a
GLP	3	Y	Follows standards. Duplicates, statistical analysis and test duration were adequate.
Organism identity: name	n/a	n/a	*Pseudomonas putida,*

Item	Weight	Yes/No	Specify
			Pseudokirchneriella subcapitata, Daphnia magna
Latin or both Latin & common names reported?	1	Y	n/a
Life cycle age / stage of test organism	1	Y	Yes for daphnia, not applicable for the other organisms
Length and/or weight	1	n/a	n/a
Sex	1	n/a	n/a
Number of organisms per replicate	1	Y	20 daphnia per vial. Not applicable for other organisms
Organism loading rate	1	Y	5 animals/10 ml for daphnia, not applicable for the other organisms
Food type and feeding periods during the acclimation period	1	n/a	n/a. Acute studies
Test type (acute or chronic	n/a	Y	Acute
Experiment type (laboratory or field)	n/a	Y	Laboratory
Exposure pathways (food, water, both)	n/a	Y	Direct contact via water
Exposure duration	n/a	Y	16 hours bacteria, 96 hours algae and 48 hours daphnid
Negative or positive controls (specify)	1	Y	Negative. Control organisms were in buffer.
Number of replicates (including controls)	1	Y	Triplicate
Nominal concentrations reported?	1	N	n/a
Measured concentrations reported?	3	N	n/a
Food type and feeding periods during the long-term tests	1	n/a	n/a
Were concentrations measured periodically (especially in the chronic test)?	1	Y	Not mentioned for bacteria. For algae and daphnia, every 24 hours (daphnia Zounková 2010)
Were the exposure media conditions	3	N	Water properties

Item	Weight	Yes/No	Specify
relevant to the particular chemical reported? (e.g., for the metal toxicity – pH, DOC/TOC, water hardness, temperature)			are not mentioned
Photoperiod and light intensity	1	Y	16 hours:8 hours light:dark cycle (Zounková 2010)
Stock and test solution preparation	1	Y	Stock buffered saline solution and further diluted with water
Was solubilizer/emulsifier used, if the chemical was poorly soluble or unstable?	1	Y	1% v/v ethanol
If solubilizer/emulsifier was used, was its concentration reported?	1	Y	1% v/v ethanol
If solubilizer/emulsifier was used, was its ecotoxicity reported?	1	N	
Analytical monitoring intervals	1	Y	For algae and daphnia (Zounková 2010)
Statistical methods used	1	Y	Analysis of variance and Dunnett's test
Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g., when mortality in the control > 10%) or physical effects (e.g., "shading effect")?	n/a	n/a	n/a
Was the test organism relevant to the Canadian environment?	3	Y	*Daphnia magna* found in Canada
Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	EU standards used
Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	EU standards used
Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	EU standards used
Was temperature of the test water within	1	Y	Mentioned in

Item	Weight	Yes/No	Specify
the range typical for the Canadian environment (5 to 27°C)?			Zounková 2010 (18–23°C)
Was toxicity value below the chemical's water solubility?	3	Y	Generally yes. For the values over the water solubility, ethanol in the product is expected to solubilize etoposide
Toxicity values (specify endpoint and value)	n/a	n/a	Many. See text
Other endpoints reported – e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	Y	LOEC/NOEC
Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	Y	Genotoxicity

Abbreviations: BAF, bioaccumulation factor; BCF, bioconcentration factor; DO, dissolved oxygen; DOC, dissolved organic carbon; EU, European Union; GLP, good laboratory practice; HPLC, high-performance liquid chromatography; LOEC, lowest-observed-effect concentration; N, no; n/a, not applicable; NOEC, no-observed-effect concentration; OECD, Organisation for Economic Co-operation and Development; TOC, total organic carbon; Y, yes

Appendix B: PBT[5] Model Input Summary Tables

Table B.1: PBT model input summary table for physical-chemical models

Model input parameters	EPI Suite (all models, including AOPWIN, KOCWIN, BCFBAF, BIOWIN and ECOSAR)
SMILES code	X
Molecular weight (g/mol)	NA
Melting point (°C)	X
Boiling point (°C)	X
Data temperature (°C)	NA
Density (kg/m^3)	NA
Vapour pressure (Pa)	X
Henry's Law constant (Pa·m^3/mol)	X
Log K_{aw} (dimensionless)	NA
Log K_{ow} (dimensionless)	X
K_{ow} (dimensionless)	NA
Log K_{oc} (L/kg)	NA
Water solubility (mg/L)	X
Log K_{oa} (dimensionless)	NA

Abbreviations: K_{aw}, air–water partition coefficient; K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; SMILES, simplified molecular input line entry system; NA, not applicable

Table B.2: PBT model input summary table for fate modelling

Model input parameters	STP (1), ASTreat (2), SimpleTreat (3), (required inputs are different, depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	Arnot-, Gobas BCF/BAF Model
SMILES code	NA	NA	X
Molecular weight (g/mol)	X (1, 2, 3)	X (I, II)	NA
Melting point (°C)	NA	X (I)	NA

[5] Persistence, bioaccumulation, toxicity.

Model input parameters	**STP (1), ASTreat (2), SimpleTreat (3), (required inputs are different, depending on model)**	**EQC (required inputs are different if Type I vs. Type II chemical)**	**Arnot-, Gobas BCF/BAF Model**
Boiling point (°C)	NA		NA
Data temperature (°C)	NA	X (I, II)	NA
Density (kg/m^3)	X (2)		NA
Vapour pressure (Pa)	X (1, 3)	X (I)	NA
Henry's Law constant (Pa·m^3/mol)	X (3)		X
Log K_{aw} (dimensionless)	X (2)	X (II)	NA
Log K_{ow} (dimensionless)	X (1)	X (I)	X
K_{ow} (dimensionless)	X (2, 3)	NA	NA
Log K_{oc} (L/kg)	NA	NA	NA
Water solubility (mg/L)	X (1, 3)	X (I)	X
Log K_{oa} (dimensionless)			NA
Soil–water partition coefficient (L/kg)[a]	NA	X (II)	NA
Sediment–water partition coefficient (L/kg)[a]	NA	X (II)	NA
Suspended particles–water partition coefficient (L/kg)[a]	X (2)	X (II)	NA
Fish–water partition coefficient (L/kg)[b]	NA	X (II)	NA
Aerosol–water partition coefficient (dimensionless)[c]	NA	X (II)	NA
Vegetation–water partition coefficient (dimensionless)[a]	NA	NA	NA
Enthalpy (K_{ow})	NA	NA	NA
Enthalpy (K_{aw})	NA	NA	NA
Half-life in air (days)	NA	X (I, II)	NA
Half-life in water (days)	NA	X (I, II)	NA
Half-life in sediment (days)	NA	X (I, II)	NA
Half-life in soil (days)	NA	X (I, II)	NA
Half-life in vegetation (days)[d]	NA		NA
Metabolic rate constant (1/day)	NA	NA	*

Model input parameters	STP (1), ASTreat (2), SimpleTreat (3), (required inputs are different, depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	Arnot-, Gobas BCF/BAF Model
Biodegradation rate constant (1/day or 1/h) – specify	X (3, 1/h) (2, 1/day)	NA	NA
Biodegradation half-life in primary clarifier ($t_{½\text{-}p}$) (h)	X (1)	NA	NA
Biodegradation half-life in aeration vessel ($t_{½\text{-}s}$) (h)	X (1)	NA	NA
Biodegradation half-life in settling tank ($t_{½\text{-}s}$) (h)	X (1)	NA	NA

Abbreviations: BCF, bioconcentration factor; K_{aw}, air–water partition coefficient; K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; SMILES, simplified molecular input line entry system; NA, not applicable

[a] Derived from log K_{oc}.

[b] Derived from BCF data.

[c] Default value.

[d] Derived from half-life in water.

Table B.3: PBT model input summary table for PBT profiling and ecotoxicity

Model input parameters	CPOPs (including CATALOGIC, BCF Mitigating Factors Model, OASIS Toxicity Model)	AIES / DS TOPKAT/ ASTER
SMILES code	X	X
Molecular weight (g/mol)	NA	NA
Melting point (°C)	NA	NA
Boiling point (°C)	NA	NA
Data temperature (°C)	NA	NA
Density (kg/m^3)	NA	NA
Vapour pressure (Pa)	NA	NA
Henry's Law constant ($Pa \cdot m^3/mol$)	NA	NA
Log K_{aw} (dimensionless)	NA	NA

Model input parameters	**CPOPs (including CATALOGIC, BCF Mitigating Factors Model, OASIS Toxicity Model)**	**AIES / DS TOPKAT/ ASTER**
Log K_{ow} (dimensionless)	X	X
K_{ow} (dimensionless)	NA	NA
Log K_{oc} (L/kg)	NA	NA
Water solubility (mg/L)	X	X
Log K_{oa} (dimensionless)	NA	NA

Abbreviations: AIES, Artificial Intelligence Expert System; K_{aw}, air–water partition coefficient; K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; SMILES, simplified molecular input line entry system; NA, not applicable