

Screening Assessment

**Ethanamine, 2-[4-[(1*Z*)-1,2-diphenyl-1-butenyl]phenoxy]-*N,N*-dimethyl-
(Tamoxifen)**

**Chemical Abstracts Service Registry Number
10540-29-1**

**Environment Canada
Health Canada**

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment of the substance ethanamine, 2-[4-[(1*Z*)-1,2-diphenyl-1-butenyl]phenoxy]-*N,N*-dimethyl-, Chemical Abstracts Service Registry Number 10540-29-1, also known as tamoxifen. This substance was identified as a priority for assessment because it had been found to meet the categorization criteria for bioaccumulation and inherent toxicity to non-human organisms and is known to be in commerce in Canada. Tamoxifen was also 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.

Drugs containing tamoxifen 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.

Tamoxifen is an organic substance that does not occur naturally in the environment. It can be manufactured as chemical-grade tamoxifen (CAS RN 10540-29-1) or as pharmaceutical-grade tamoxifen citrate (CAS RN 54965-24-1). Tamoxifen with CAS RN 10540-29-1 is the only substance of the two that is listed on the *Domestic Substances List*. In Canada, tamoxifen citrate is primarily used as a human pharmaceutical, and chemical-grade tamoxifen can also be used as a laboratory research tool. Specifically, tamoxifen is used as a therapeutic substance to treat estrogen-responsive breast cancer; in research, its properties as a selective estrogen receptor modulator are used to examine mechanisms of endocrine function. The citrate moiety associated with tamoxifen is expected to have negligible ecotoxicological effects.

Tamoxifen is highly metabolized in the liver, and both the parent compound and its metabolites are excreted from the body when ingested. The hydroxylated metabolites of tamoxifen, 4-hydroxytamoxifen and endoxifen, which are structurally very similar to the parent compound, can be released to the environment together with unmetabolized tamoxifen and remain biologically active. Therefore, their properties are considered concurrently with the properties of tamoxifen in this screening assessment.

Commercially available data on pharmaceutical sales in Canada for 2011 and 2012 indicate that over 300 kg of tamoxifen citrate was purchased by hospitals and pharmacies for prescription in each of those years. Similar data were also available to estimate that 250 kg of the substance was purchased by hospitals and pharmacies for prescription across Canada in 2007. There are several pharmaceutical companies that are licensed to market tamoxifen in Canada for

human use. Chemical-grade tamoxifen can also be purchased from major chemical manufacturers.

Based on its physical and chemical properties, if released to the environment, tamoxifen is expected to reside in water, soil and sediment, depending on the compartment of release. Based on the modelled data and empirical evidence, tamoxifen, 4-hydroxytamoxifen and endoxifen are expected to persist in the water, soil and sediment. Tamoxifen is not expected to bioaccumulate in organisms due to its low water solubility, relatively large cross-sectional diameter (resulting in restricted uptake across the gill as a result of steric hindrance) and the high potential for fish to metabolize it. Modelled data also indicated that 4-hydroxytamoxifen and endoxifen have a limited bioaccumulation potential.

Tamoxifen is registered for pharmaceutical use in Canada. Tamoxifen can potentially make its way to surface waters through release from manufacturing or formulation sites. Tamoxifen and its metabolites, 4-hydroxytamoxifen and endoxifen, can be found in surface water as a result of releases of these substances in feces or urine from the therapeutic drug use of tamoxifen. Given these potential releases, the main source of ecological exposure to tamoxifen is through water. Because no information was available regarding actual releases of this substance in Canada, realistic conservative exposure scenarios, selected for a site-specific industrial operation and for down-the-drain releases through prescribed use of tamoxifen were developed to estimate discharges of the substance into the aquatic environment. Tamoxifen and its metabolites, 4-hydroxytamoxifen and endoxifen, are considered to be highly toxic to aquatic organisms, and have potential for endocrine disruption. The predicted environmental concentrations in water were below the predicted no-effect concentration calculated for aquatic organisms.

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 tamoxifen. It is concluded that tamoxifen does not meet the criteria under paragraph 64(a) or (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 to tamoxifen, the principal source of exposure is drinking water containing the drug. The exposure to tamoxifen present in drinking water is significantly smaller than the exposure to tamoxifen through its use as a pharmaceutical.

For this assessment, conservative assumptions were used when estimating the potential indirect exposure of the general population to tamoxifen. Limited data

on tamoxifen in Canadian waters are currently available. Very low concentrations of tamoxifen were measured in the influent, effluent, and biosolids samples collected from select Canadian waste water treatment plants, and leachate samples from Canadian landfills. Therefore, for the purposes of this assessment, modelled data in surface water in Canada and the reporting limits for the samples collected from Canadian wastewater effluent were used as conservative proxies for Canadian drinking water concentrations. Upper-bounding estimated intakes from environmental media were very low (< 0.1 ng/kg body weight per day). Based on these low exposures, risks from this substance are not expected. To further support this risk characterization, the upper-bounding estimated indirect exposures of the general population were compared with the lowest therapeutic dose. The margins of exposure ranged from $> 82\,000$ to $> 4\,000\,000$.

Based on the information presented in this screening assessment, it is concluded that tamoxifen 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 tamoxifen does not meet any of the criteria set out in section 64 of CEPA 1999.

Table of Contents

1. Introduction.....	1
2. Substance Identity.....	3
2.1.1 Metabolites.....	3
3. Physical and Chemical Properties	8
4. Sources and Uses.....	12
5. Releases to the Environment	14
6. Measured Environmental Concentrations.....	14
7. Environmental Fate	16
7.1 Environmental Persistence	18
7.1.1 Empirical Data.....	18
7.1.2 Modelling Results.....	20
7.2 Potential for Bioaccumulation.....	25
7.2.1 Metabolism.....	25
7.2.2 Estimating BCF and BAF	25
8. Potential to Cause Ecological Harm	29
8.1 Ecological Effects Assessment	29
8.1.1 Aquatic Toxicity of Tamoxifen	29
8.1.2 Aquatic Toxicity of Tamoxifen Metabolites	40
8.1.3 Derivation of the PNEC	42
8.2 Ecological Exposure Assessment	43
8.2.1 Industrial Release	43
8.2.2 Down-the-Drain Releases from Pharmaceutical Use	45
8.3 Characterization of Ecological Risk.....	47
8.4 Uncertainties in Evaluation of Ecological Risk	49
9. Potential to Cause Harm to Human Health.....	50
10. Uncertainties in Evaluation of Risk to Human Health.....	53
11. Conclusion	54
11.1.1 Considerations for Follow up.....	54
12. References	56

Table of Tables

Table 1a. Substance identity: tamoxifen and tamoxifen citrate	4
Table 1b. Substance identity for 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen.....	6
Table 1c. Substance identity: endoxifen, a metabolite of tamoxifen.....	7
Table 2a. Physical and chemical properties of neutral form of tamoxifena	8
Table 2b. Physical and chemical properties of 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen	10
Table 2c. Physical and chemical properties of endoxifen, a metabolite of tamoxifen.....	11
Table 3. Results of Level III fugacity modelling for tamoxifen (EQC 2003), indicating the percentage of tamoxifen partitioning into each compartment	17
Table 4a. Modelled data for degradation of tamoxifen	21
Table 4b. Modelled data for degradation of 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen	23
Table 4c. Modelled data for degradation of endoxifen, a metabolite of tamoxifen	24
Table 5a. Summary of modelled data for bioaccumulation of tamoxifen (neutral and ionized forms).....	26
Table 5b. Summary of modelled data for bioaccumulation of 4-HT, a metabolite of tamoxifen.....	27
Table 5c. Summary of modelled data for bioaccumulation of endoxifen, a metabolite of tamoxifen	27
Table 6a. Empirical data for aquatic toxicity of tamoxifen	36
Table 6b. In vitro empirical data for the toxicity of 4-HT, a metabolite of tamoxifen	41
Table 7a. Summary of input values used for estimating aquatic concentrations resulting from industrial releases of tamoxifen	44
Table 7b. Summary of input values used for estimating aquatic concentrations resulting from prescribed use of tamoxifen.....	46

1. Introduction

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

A screening assessment was undertaken on the substance ethanamine, 2-[4-[(1*Z*)-1,2-diphenyl-1-butenyl]phenoxy]-*N,N*-dimethyl-, Chemical Abstracts Service Registry Number (CAS RN) 10540-29-1. This substance will be referred to by its common name, tamoxifen. Tamoxifen was identified as a priority for assessment because it had been found to meet the categorization criteria for bioaccumulation and inherent toxicity to non-human organisms and is known to be in commerce in Canada. Tamoxifen was also 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.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999). Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution¹.

This screening assessment includes consideration of information on chemical properties, 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 critical studies and lines of evidence most pertinent to the conclusion.

¹ A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 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.

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

This 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 and comments from the 60-day public comment period following the publication of the draft screening assessment. 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. 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 ethanamine, 2-[4-[(1*Z*)-1,2-diphenyl-1-butenyl]phenoxy]-*N,N*-dimethyl- will be referred to by its common name, tamoxifen. Tamoxifen can be manufactured as chemical-grade tamoxifen (CAS RN 10540-29-1) or as pharmaceutical-grade tamoxifen citrate (CAS RN 54965-24-1). Tamoxifen with CAS RN 10540-29-1 is the only substance of the two that is listed on the Domestic Substances List (DSL). Tamoxifen citrate is available as a pharmaceutical for humans in products marketed under different names, such as Novaldex-D, Apo-Tamox and Mylan-Tamoxifen (DPD 2010)(see Table 1a).; however, both forms of tamoxifen (i.e., chemical and pharmaceutical grade), have been used in a variety of toxicity studies. For the purpose of the present screening assessment, both the pharmaceutical-grade tamoxifen citrate and the chemical-grade tamoxifen are treated equally and interchangeably. The presence of citrate salt is generally omitted from discussion, given that its function is predominantly pharmacokinetic and it is not expected to contribute to the toxicity of tamoxifen itself. Therefore, tamoxifen is the primary subject of this screening assessment.

2.1.1 Metabolites

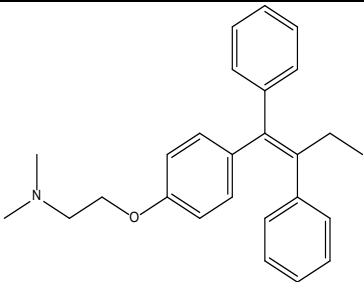
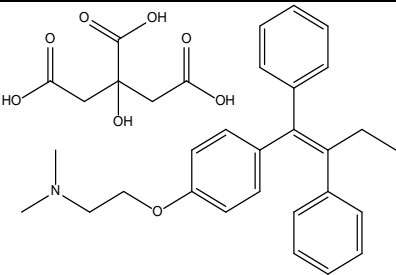
Tamoxifen is a pharmaceutical substance, known as a selective estrogen receptor modulator (SERM) substance (Williams et al. 2007). When ingested by humans, tamoxifen is extensively metabolized in the liver by cytochrome p450 enzymes, and both tamoxifen and its metabolites are known to be excreted from the body (Kisanga et al. 2005). Hydroxylated metabolites, 4-hydroxytamoxifen (4-HT) and endoxifen, are the major active metabolites of tamoxifen; they exhibit properties similar to those of the parent compound and are both very powerful antiestrogens (Williams et al. 2007; Lim et al. 2005; Kisanga et al. 2005). Moreover, tamoxifen has been referred to as a pro-drug, in that the effectiveness of the therapeutic treatment depends on its adequate conversion to the hydroxylated metabolites (Jaremko et al. 2010).

Metabolism of tamoxifen to 4-HT and endoxifen occurs in steps that include hydroxylation and demethylation. Consequently, tamoxifen and its hydroxylated metabolites are very similar structurally, differing mainly by the addition of the hydroxyl (OH) group to one of the phenyl rings in both metabolites and the replacement of the dimethylamino group by the methylamino group in one of the metabolites, endoxifen (Goetz and Loprinzi 2003; Kisanga et al. 2005). Therefore, since both hydroxylated metabolites of tamoxifen, 4-HT and endoxifen, remain biologically active, are excreted from the human body upon ingestion, are structurally very similar to the parent compound and can co-occur with unmetabolized tamoxifen in the environment, their toxicity properties are evaluated concurrently with those of tamoxifen in this screening assessment.

Substance identity information for tamoxifen, 4-HT and endoxifen is provided in Tables 1a, 1b and 1c, respectively.

Table 1a. Substance identity: tamoxifen and tamoxifen citrate

Substance name	Tamoxifen	Tamoxifen citrate
CAS RN	10540-29-1	54965-24-1
DSL name	Ethanamine, 2-[4-[(1Z)-1,2-diphenyl-1-butenyl]phenoxy]-N,N-dimethyl-	n/a
NCI names ¹	Ethanamine, 2-[4-[(1Z)-1,2-diphenyl-1-butenyl]phenoxy]-N,N-dimethyl- (ASIA-PAC, DSL, NZIoC); Ethanamine, 2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethyl-, (Z)- (AICS); Tamoxifen (English, German) (REACH, EINECS); Tamoxifene (French) (EINECS); Tamoxifeno (Spanish) (EINECS)	
Other names	(Z)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine; Ethanamine, 2-[4-[(1Z)-1,2-diphenyl-1-buten-1-yl]phenoxy]-N,N-dimethyl-; Ethylamine, 2-[p-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethyl-, (Z)-; ICI 47699; Mammaton; Novaldex; Tamoxifen and its salts; <i>trans</i> -Tamoxifen; Z-Tamoxifen	(Z)-(2-(4-(1,2-Diphenylbut-1-enyl)phenoxy)ethyl)dimethylamm onium dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate; Ethanamine, 2-(4-((1Z)-1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1); Ethanamine, 2-(4-(1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethyl, (Z)-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1) Novaldex-D, Apo-Tamox, Mylan-Tamoxifen ² Caditam; Apo-Tamox, Caditam, Farmifeno, Genox, Ginarsan, Emblon, Jenoxifen, Kessar, Ledertam, Nolgen, Noltam, Nourytan, Noxitem, Oncotam, Soltamox, Tafoxen, Tamofen,

		Tamoplex, Tamox-Puren, Tamoxasta, Taxus, Terimon, TMX, Zemide, Zitazonium, Zynoplex ³
Chemical group (DSL stream)	Discrete organics	Discrete organics
Major chemical class or use	Aliphatic amines	Aliphatic amines
Major chemical subclass	Phenols	Phenols
Chemical formula	C ₂₆ H ₂₉ NO	C ₂₆ H ₂₉ NO·C ₆ H ₈ O ₇
Chemical structure		
SMILES	<chem>C(=C(/c1ccccc1)CC)(\c1ccc(OCCN(C)C)cc1)c1ccccc1</chem>	<chem>C(=C(/c1ccccc1)CC)(\c1ccc(cc1)OCCN(C)C)c1ccccc1.C(CC(O)=O)(CC(O)=O)(C(O)=O)O</chem>
Molecular weight (g/mol)	371.52	563.62

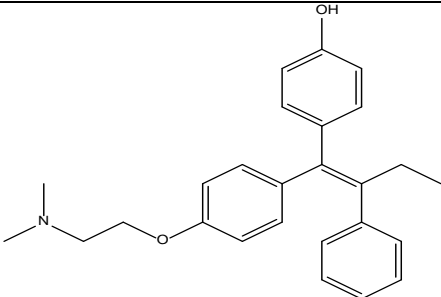
Abbreviations: n/a, not available; SMILES, Simplified Molecular-Input Line-Entry System

¹National Chemical Inventories (NCI) (2009): AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing Commercial Chemical Substances); ELINCS (European List of Notified Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); *NZIoC* (New Zealand Inventory of Chemicals); PICCS (Philippine Inventory of Chemicals and Chemical Substances); *REACH* (Registration, Evaluation, Authorisation and Restriction of Chemical substances); and TSCA (*Toxic Substances Control Act* Chemical Substance Inventory).

²DPD 2010

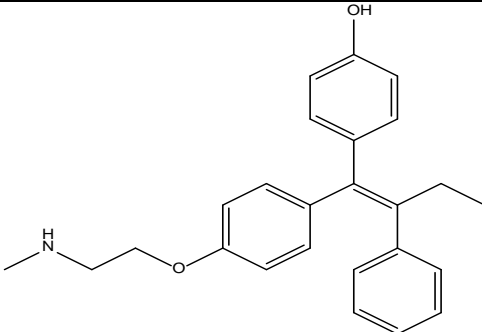
³ChemIDPlus 1993–

Table 1b. Substance identity for 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen

CAS RN	68047-06-3
Other registry numbers¹	65213-48-1, 72732-26-4, 76276-99-8
Systematic name¹	Phenol, 4-((1 <i>Z</i>)-1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)-; Phenol, 4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)-, (<i>Z</i>)-
Synonyms	(<i>Z</i>)-4-Hydroxytamoxifen (US NLM); 4-((1 <i>Z</i>)-1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)phenol (US NLM); BRN 4910749 (RTECS); Hydroxytamoxifen (RTECS); ICI 79,280 (RTECS); ICI 79280 (US NLM); <i>trans</i> -4-(1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenyl-1-(butenyl)phenol (RTECS); <i>trans</i> -4-Hydroxytamoxifen (US NLM)
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Aliphatic amines
Major chemical subclass	Phenols
Chemical formula	C ₂₆ H ₂₉ NO ₂
Chemical structure	
SMILES²	<chem>c1cccc(\C(CC)=C(\c2ccc(cc2)O)c2ccc(OCCN(C)C)cc2)c1</chem>
Molecular mass (g/mol)	387.52

¹ US National Library of Medicine (NLM), Medical Subject Headings File (MeSH) (Chem ID Plus 1998–)² Simplified Molecular Input Line Entry System

Table 1c. Substance identity: endoxifen, a metabolite of tamoxifen

Chemical Abstracts Service Registry Number (CAS RN)	110025-28-0
Systematic name¹	Phenol, 4-(1-(4-(2-(methylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)-
Synonyms¹	4-Hydroxy- <i>N</i> -demethyltamoxifen (MeSH); Endoxifen (MeSH, US NLM)
Other names	4-Hydroxy- <i>N</i> -desmethyltamoxifen (MeSH)
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Aliphatic amines
Major chemical sub-class	Phenols
Chemical formula	C ₂₅ H ₂₇ NO ₂
Chemical structure	
SMILES	<chem>c1(ccc(cc1)C(=C(/CC)c1ccccc1)\c1ccc(cc1)OCCNC)O</chem>
Molecular mass (g/mol)	373.49

¹ US National Library of Medicine (NLM), Medical Subject Headings File (MeSH) (Chem ID Plus 1998–)² Simplified Molecular Input Line Entry System

3. Physical and Chemical Properties

Table 2a contains the available experimental and modelled physical and chemical properties of tamoxifen that are relevant to its environmental fate. In addition, physical and chemical properties for the hydroxylated tamoxifen metabolites, 4-HT and endoxifen, were modelled and are presented in Tables 2b and 2c, respectively. Experimental physical and chemical properties of 4-HT and endoxifen were not found in the published literature.

Models based on quantitative structure–activity relationships (QSARs) were used to generate data for some of the physical and chemical properties of tamoxifen and its hydroxylated metabolites, 4-HT and endoxifen. These models (except WSKOWWIN) are mainly based on fragment addition methods, i.e., they rely on the structure of a chemical. Since these models accept only the neutral form of a chemical as input (in SMILES form), the modelled values shown in Tables 2a, 2b and 2c are for the neutral forms of tamoxifen, 4-HT and endoxifen. However, the relatively high acid dissociation constant (pK_a) of 8.69 for the basic functional group indicates that 50% of tamoxifen will be found in its ionized form at pH 8.69 (ACD/pKaDB 2005). In water bodies at environmentally relevant pH values (pH 6–9), about 33% (pH 9) to 100% (pH 6) of tamoxifen will be in its neutral form, indicating that aquatic exposure to tamoxifen can be to both the neutral and ionized forms. The proportion of dissociated tamoxifen [0.2% (pH 6) to 67% (pH 9)] (ACD/pKaDB 2005) indicates that partitioning behaviour predicted using the log D is appropriate for the ionized form of the substance. Therefore, the physical and chemical property predictions likely do not fully represent the properties and environmental behaviour of tamoxifen as an ionized compound. However, as the neutral form of tamoxifen is also present in the environment, the predicted physical and chemical properties remain relevant.

Table 2a. Physical and chemical properties of neutral form of tamoxifena

Property	Type	Value ^{a,b}	Temperature (°C)	Reference
Melting point (°C)	Modelled	180.85*		MPBPWIN 2008
Boiling point (°C)	Modelled	468.20		MPBPWIN 2008
Density (kg/m ³)	Experimental	N/A		
Vapour pressure (Pa)	Modelled	4.62×10^{-6} *	25	EPI Suite 2008

Property	Type	Value ^{a,b}	Temperature (°C)	Reference
Henry's Law constant (Pa·m ³ /mol)	Modelled	4.55×10 ^{-5*}	25	MPBPWIN 2008
Log K _{ow} (dimensionless)	Modelled	6.3		KOWWIN 2008
Log D (dimensionless)	Experimental ^c	Log D _{oct} = 4.51*		CEREP c2010a
Log D (dimensionless)	Modelled	Log D _{cyc} = 4.60		ACD/pKaDB 2005
Log K _{oc} (dimensionless)	Modelled	6.42		KOCWIN 2008
Water solubility (mg/L)	Experimental ^c	0.4829* (1.3 µM)		CEREP c2010b
Water solubility (mg/L)	Modelled (estimated from log K _{ow})	0.1936	25	WSKOWWIN 2008
Water solubility (mg/L)	Modelled (estimated from fragments)	0.0246	25	WSKOWWIN 2008
pK _a (dimensionless)	Experimental	9.24		Flexser et al. 1935
pK _a (dimensionless)	Modelled	8.69		ACD/pKaDB 2005

Abbreviations: cyc, cyclohexane; D, distribution coefficient taking into account the presence of the ionic species; represents a net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; N/A, not available; oct, *n*-octanol; pK_a, acid dissociation constant

^a An asterisk (*) indicates a value selected for modelling.

^b Value in parentheses represents the original one as reported by the authors.

^c Water solubility and log D values for tamoxifen were determined experimentally by a biotechnology company CEREP for the commercially available CEREP assays for determination of aqueous solubility and partition coefficient Log D (CEREP c2010a, b). *n*-Octanol and cyclohexane were used in the organic phase in the partition coefficient assays. Experimental details regarding determination of the water solubility and log D of tamoxifen were not available. Nonetheless, confidence in these experimental values is high, as tamoxifen is used as a reference compound and internal control along with eight other pharmaceutical substances in the CEREP commercial assays.

Table 2b. Physical and chemical properties of 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen

Property	Type	Value ^a	Temperature (°C)	Reference
Melting point (°C)	Modelled	211.01*		MPBPWIN 2008
Boiling point (°C)	Modelled	503.04		MPBPWIN 2008
Density (kg/m ³)	Experimental	N/A		
Vapour pressure (Pa)	Modelled	4.14×10 ⁻⁹ *	25	EPI Suite 2008
Henry's Law constant (Pa·m ³ /mol)	Modelled	4.73×10 ⁻⁹ *	25	MPBPWIN 2008
Log K _{ow} (dimensionless)	Modelled	5.82		KOWWIN 2008
Log K _{oc} (dimensionless)	Modelled	6.53		KOCWIN 2008
Water solubility (mg/L)	Modelled (estimated from log K _{ow})	1.51	25	WSKOWWIN 2008
Water solubility (mg/L)	Modelled (estimated from fragments)	0.73*	25	WSKOWWIN 2008
pK _a (dimensionless)	Modelled	10.35 (acid) 8.7 (base)		ACD/pK _a DB 2005
Log D (dimensionless)	Modelled	4.25*		ACD/pK _a DB 2005

Abbreviations: D, distribution coefficient taking into account the presence of the ionic species; represents a net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; N/A, not available; pK_a, acid dissociation constant

^a An asterisk (*) indicates a value selected for modelling.

Table 2c. Physical and chemical properties of endoxifen, a metabolite of tamoxifen

Property	Type	Value ^a	Temperature (°C)	Reference
Melting point (°C)	Modelled	212.24*		MPBPWIN 2008
Boiling point (°C)	Modelled	501.85		MPBPWIN 2008
Density (kg/m ³)	Experimental	N/A		
Vapour pressure (Pa)	Modelled	4.32×10 ⁻⁹ *	25	EPI Suite 2008
Henry's Law constant (Pa·m ³ /mol)	Modelled	2.16×10 ⁻⁹ *	25	MPBPWIN 2008
Log K _{ow} (dimensionless)	Modelled	5.61		KOWWIN 2008
Log K _{oc} (dimensionless)	Modelled	6.56		KOCWIN 2008
Water solubility (mg/L)	Modelled (estimated from log K _{ow})	2.79	25	WSKOWWIN 2008
Water solubility (mg/L)	Modelled (estimated from fragments)	2.19*	25	WSKOWWIN 2008
pK _a (dimensionless)	Modelled	10.36 (acid) 9.4 (base)		ACD/pK _a DB 2005
Log D (dimensionless)	Modelled	3.74*		ACD/pK _a DB 2005

Abbreviations: D, distribution coefficient taking into account the presence of the ionic species; represents a net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; N/A, not available; pK_a, acid dissociation constant

^a An asterisk (*) indicates a value selected for modelling.

4. Sources and Uses

The substance tamoxifen is not produced naturally in the environment. Tamoxifen is a pharmaceutical that has been used as an antiestrogen to treat estrogen-responsive human breast cancers.

Tamoxifen is known as a selective estrogen receptor modulator (SERM) substance, able to act as both an estrogen antagonist and an estrogen agonist in different types of tissues (Williams et al. 2007; Isidori et al. 2009; Marty et al. 2011). It has become a research tool in the field of fish endocrinology and also has applications in aquaculture and ecotoxicology (Williams et al. 2007). Moreover, to improve understanding of the potential impacts of selective estrogen receptor modulators in aquatic life, tamoxifen was recommended for use as a reference compound in fish chronic testing in a 2003 Society of Environmental Toxicology and Chemistry Pellston Workshop (Williams et al. 2007).

To date, a survey pursuant to section 71 of CEPA 1999 has not been issued for this substance. Entry characterization for tamoxifen in Canada consisted of searching for information on sources and uses of the substance in relevant databases to identify potential for exposure of the general population from all sources, including pharmaceutical use (Canada [1978]; HSDB 1983– ; Household Products Database 1993– ; LNHPD 2008; DPD 2010; EAFUS 2011; NHPID 2011). Based on notifications submitted under the Cosmetic Regulations to Health Canada, tamoxifen 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).

Tamoxifen is not an approved drug for use in livestock feed in Canada (personal communication, email from the Canadian Food Inspection Agency [CFIA] to Health Canada, dated October 29, 2010; unreferenced). It is not listed in the CFIA's Compendium of Medicating Ingredient Brochures, which lists drugs approved for administration through feed for specific purposes, with detailed claims and use levels (CFIA 2007). Tamoxifen also does not have any licensed Drug Identification Numbers (DINs) for use in veterinary applications in Canada (DPD 2010). However, there is still the possibility that veterinarians may be prescribing the drug for uses that have not been approved (i.e., off-label uses). Outside of Canada, applications of tamoxifen in the food industry have been identified, including its use in promoting growth in poultry (Park et al. 2004) and as a potential tool in aquaculture to mass produce sterile or monosex fish populations (Singh 2013). Information available for this substance indicates that in Canada, its use is limited to pharmaceuticals and research. Searches for this substance were conducted up to March 2013, and no information was found regarding alternative uses or releases of this substance in Canada.

For the years 2011 and 2012, data on pharmaceutical sales in Canada were obtained from IMS Health (IMS 2013). According to this source, in 2011, 54.9 kg of tamoxifen was sold to hospitals and 250.9 kg was sold to pharmacies, for a total of 305.8 kg. In 2012, the total amount of tamoxifen sold in Canada was higher than in 2011, at 320.2 kg, where 58.8 kg originated from hospital sales, and 261.4 kg from pharmacies (IMS 2013). Similar data were available to estimate that 250 kg of the substance was purchased by hospitals and pharmacies for prescription across Canada for the year 2007 (McLaughlin and Belknap 2008). There are several pharmaceutical companies that are licensed to market tamoxifen in Canada, including two innovator companies and five generic companies (DPD 2010). Pharmaceutical-grade tamoxifen (tamoxifen citrate) is sold in either 10 mg or 20 mg tablets (DPD 2010). The annual quantities of chemical-grade tamoxifen imported, manufactured or sold in Canada remain undetermined. Chemical-grade free base tamoxifen and tamoxifen citrate salt can be purchased from major chemical manufacturers; the typical format is 1 g or 5 g vials, as well as radiolabelled tamoxifen in 1 mL vials (Sigma-Aldrich c2010). For research applications (i.e., animal testing), tamoxifen is used in solution in concentrations ranging between micrograms and milligrams per kilogram body weight or in parts per million (ppm, equivalent to mg/L). Therefore, the total quantity stemming from laboratory research use in Canada is not expected to exceed, and is likely to be significantly less than that used for human therapeutic purposes.

Although there is the possibility that drug products containing the substance may be imported into Canada, no information is available on the quantity of such imports.

5. Releases to the Environment

The production and use of tamoxifen as a pharmaceutical may result in its release to the environment through various waste streams. In general, wastewater is a common point of entry of a substance into water through wastewater system² effluent. The potential for exposure to tamoxifen from indirect sources (i.e., down-the-drain releases from patients using the substance for cancer therapy) and direct sources (i.e., released during manufacture or formulation) was considered in this assessment. Given these potential releases, the main compartment of concern for exposure to this substance is water. Pharmaceutical substances used in human and veterinary medicine can enter the aquatic environment following manufacture, use or excretion following administration of the drug in the form of non-metabolized parent compounds and as metabolites (Ashton et al. 2004). An additional source of the pharmaceuticals in water is the incorrect disposal of unused drugs into household wastewater. No information was available regarding actual releases of this substance from manufacturing or formulation. There is also insufficient information available to estimate potential down-the-drain releases of tamoxifen from research facilities as a result of tamoxifen use in basic research. It is expected, based on concentrations typically used for research purposes, that this is not a significant source of tamoxifen in water.

6. Measured Environmental Concentrations

In Canada, data are available for concentrations of tamoxifen in samples collected from a variety of wastewater treatment plants representing typical Canadian treatment systems and geographic variations. Tamoxifen was detected in the range of 5.97×10^{-7} – 1.04×10^{-6} mg/L (0.597–1.04 ng/L) in influent samples, in the range of 1.30×10^{-6} – 1.73×10^{-6} mg/L (1.30–1.73 ng/L) in effluent samples, and in the range of 6.66×10^{-3} – 5.08×10^{-3} mg/kg (0.666–5.08 ng/g) in biosolids. Municipal landfill leachate discharged into the wastewater treatment system was also sampled, and tamoxifen was detected in the range of 1.3×10^{-3} – 4.54×10^{-3} mg/kg (1.33–4.54 ng/g) (Teslic and Smyth 2013). No data on Canadian environmental concentrations of the hydroxylated metabolites of tamoxifen were identified.

² In this assessment, the term wastewater system does not include sewer networks or collection systems.

Given the limited scope of information regarding the presence of tamoxifen in Canadian waters, environmental concentrations of the substance were estimated from available information, including estimated substance quantities, release rates and size of receiving water bodies, for the purpose of ecological risk assessment (see “Ecological Exposure Assessment” section).

Concentrations of tamoxifen in surface waters and wastewater have been reported in several locations in Europe. In addition, in a recent study, the presence of the tamoxifen metabolites endoxifen and hydroxy-tamoxifen (it is not clear whether it is 4-HT, therefore the metabolite name as it appears in the publication is used) was also identified in wastewater samples in Europe (Ferrando-Climent et al. 2013).

Ferrando-Climent et al. (2013) collected wastewater samples from hospitals in the towns of Coimbra (Portugal), Valencia and Girona (both in Spain), as well as from municipal water treatment plants located in Girona and Toulouse (Spain). The sizes of the hospitals included in the study ranged from 400 to over 1400 beds. The population sizes of the towns featured in the study fell between 145 000 (Girona) and nearly 800 000 (Valencia) inhabitants.

Tamoxifen was detected in all hospital effluent samples collected, at concentrations ranging from 2.6×10^{-5} to 1.33×10^{-4} mg/L (26.3 to 133.4 ng/L). The substance was also detected in all wastewater treatment plant influent samples, at concentrations ranging from 3.0×10^{-5} to 5.83×10^{-5} mg/L (30 to 58.3 ng/L). The tamoxifen metabolites endoxifen and hydroxy-tamoxifen were also identified in some of the hospital effluent samples using the information-dependent acquisition tool (IDA), a method based on searching IDA-generated chromatographs for the theoretical molecular ions of the target metabolites. Characteristics of the metabolite retention time were used to confirm the IDA results in the absence of metabolite standards. The presence of hydroxy-tamoxifen and endoxifen was identified in two out of four hospital influent samples, even though tamoxifen was detected in all samples.

Tamoxifen has been detected in surface water samples collected in the UK from some estuaries (sampling sites were located at the lower reaches of the rivers Tyne, Tees, Mersey, Thames and Belfast Lough) at concentrations ranging from less than 4×10^{-6} to 7.1×10^{-5} mg/L (< 4 to 71 ng/L) (Thomas and Hilton 2004). It was detected in 2 of 45 samples collected from sewage treatment plants (at Corby, Great Billing, East Hyde, Harpenden and Ryemeads, located in the southeastern UK) at concentrations of 2.0×10^{-5} to 4.0×10^{-5} mg/L (20 and 40 ng/L), but was not detected in samples of surface water collected upstream or downstream from the plants, at a detection limit of 1.0×10^{-5} mg/L (10 ng/L) (Ashton et al. 2004). Slightly higher, but comparable, environmental concentrations of tamoxifen in the wastewater effluent and surface waters of the lower river Tyne in the UK have been reported by Roberts and Thomas (2006). In this study,

tamoxifen concentrations ranged from 2.7×10^{-5} to 2.12×10^{-4} mg/L (27 to 212 ng/L) in the surface waters [with a median concentration of 5.3×10^{-5} mg/L (53 ng/L)] and from 1.46×10^{-4} mg/L to 3.69×10^{-4} mg/L (146 to 369 ng/L) in the final effluent from the wastewater treatment works at Howdon. Zhou et al. (2009) took samples from three wastewater treatment plants located near the river Ouse in the UK. The concentrations of tamoxifen measured in the effluents from these plants ranged from 2.0×10^{-7} to 7.0×10^{-7} mg/L (0.2 to 0.7 ng/L), although concentrations were below the detection limit in samples of river water taken both upstream and downstream of the site.

In France, discharge from a conventional wastewater treatment plant located in Alés, in the Languedoc Roussillon region in the south, into the Gardon River has been studied (Coetsier et al. 2009). Tamoxifen concentrations in the surface waters in the Gardon River ranged from less than 5.8×10^{-6} to 2.5×10^{-5} mg/L (< 5.8 to 25 ng/L), whereas measured concentrations in the effluent samples ranged from less than 5.8×10^{-6} to 1.02×10^{-4} mg/L (< 5.8 to 102 ng/L). It is noted that tamoxifen consumption in France for the year 2006 was estimated to be 335 kg, based on quantities of medicine paid for by the French social health care system and distributed throughout the French population of 62.9 million inhabitants (Coetsier et al. 2009).

Tauxe-Wuersch et al. (2006) measured the concentrations of tamoxifen in 37 samples of hospital and urban wastewaters in Lausanne and Morges, Switzerland. Tamoxifen was detected in almost all samples of raw sewage between the limit of detection and limit of quantification of the method used [1.0×10^{-6} and 4.0×10^{-6} mg/L (1 and 4 ng/L)], but was not detected in any samples of treated effluent. It was reported that 156 kg of tamoxifen is sold in Switzerland annually.

In Spain, Bueno et al. (2010) sampled 10 different rivers across the country for tamoxifen. With a detection limit of 1.1×10^{-5} mg/L (11 ng/L), tamoxifen was not detected in any samples.

7. Environmental Fate

Level III fugacity modelling (EQC 2003) simulates the distribution of a substance in a hypothetical environment according to chemical partitioning, reactivity and inter-media transport processes. The mass fraction values shown in Table 3 for tamoxifen represent the net effect of these processes under conditions of continuous release when a non-equilibrium “steady state” has been achieved. Given that the EQC model results for tamoxifen metabolites were very similar, to avoid duplication, only a brief summary is provided for these substances. Model inputs to EQC (2003) are summarized in Environment Canada (2014) and noted with an asterisk in Tables 2a, 2b and 2c.

Based on the physical and chemical properties of tamoxifen (Table 2a), the results of Level III fugacity modelling, presented in Table 3, suggest that the neutral form of tamoxifen is expected to reside in water, soil and sediment, depending on the compartment of release. However, the relatively high pK_a of 8.69 for the basic functional group indicates that 50% of tamoxifen will be found in its ionized form at pH 8.69 (ACD/pKaDB 2005). In water bodies at environmentally relevant pH values (pH 6–9), about 33% (pH 9) to 100% (pH 6) of tamoxifen will be in its neutral form, indicating that aquatic exposure to tamoxifen can be from both the neutral and ionized forms. The proportion of dissociated tamoxifen [0.2% (pH 6) to 67% (pH 9)] indicates that partitioning behaviour predicted using the log D is appropriate to the ionized form of the substance (ACD 2011). However, the level III EQC model cannot address the potential for tamoxifen to ionize in the aquatic environment as a salt or the likelihood that the salt form will be more soluble than the free acid form (i.e., non-salt form). Nor can the model address the potential for binding in soil from electrostatic interactions (cation exchange) or binding to clays which are negatively surface charged. Therefore, the model cannot fully simulate the fate distribution of tamoxifen in the environment.

Table 3. Results of Level III fugacity modelling for tamoxifen (EQC 2003), indicating the percentage of tamoxifen partitioning into each compartment

Substance released to:	Percentage partitioning into each compartment			
	Air	Water	Soil	Sediment
Air (100%)	2	2	94	2
Water (100%)	Negligible	50	Negligible	50
Soil (100%)	Negligible	Negligible	100	Negligible

If released to air, a very small amount of the substance is expected to reside in air (Table 3). Based on its negligible modelled vapour pressure of 4.62×10^{-6} Pa and Henry's Law constant of 4.55×10^{-5} Pa·m³/mol, tamoxifen is non-volatile. Therefore, if released solely to air, it will tend to be deposited to soil from wet and dry deposition (approximately 94%; see Table 3).

If released into water, tamoxifen is expected to strongly adsorb to suspended solids and sediment, based on the very high estimated log K_{oc} value of 6.42 for the neutral form. Volatilization from water surfaces is expected to be an unimportant fate process based upon this compound's estimated Henry's Law constant. Thus, if water is a receiving medium, tamoxifen is expected to reside in water and sediment in approximately equal proportions (see Table 3).

If released to soil, tamoxifen is expected to have high adsorptivity to soil (i.e., expected to be immobile) based on its estimated log K_{oc} for the neutral form. Volatilization from moist soil surfaces seems to be an unimportant fate process based on its estimated Henry's Law constant. This chemical is not expected to

volatilize from dry soil surfaces based on its vapour pressure. Therefore, if released to soil, tamoxifen will reside mainly in this environmental compartment, with less than 1% advected to water and sediments and negligible amounts partitioning to air, as illustrated by the results of the Level III fugacity modelling (see Table 3).

EQC model results for the hydroxylated metabolites of tamoxifen generated using their respective modelled physical and chemical properties indicated that the metabolites 4-HT and endoxifen would also likely be distributed in a manner similar to the parent compound, tamoxifen.

7.1 Environmental Persistence

In order to provide the best possible weight of evidence for determination of the persistence of tamoxifen, both empirical and modelled data were considered. Model estimates of the persistence of tamoxifen are strictly structure based and not expected to be influenced by chemical speciation. Chemical speciation, however, may affect bioavailability for biodegradation. This is not accounted for in the model estimates of biodegradation.

7.1.1 Empirical Data

Although pharmaceuticals can be degraded by biotic or abiotic processes, they may act as quasi-persistent compounds (also called pseudo-persistent) simply because of their continual release into surface waters via wastewater treatment plant (WWTP) effluents, which may result in multi-generational exposure for the resident organisms (Daughton and Ternes 1999; Ferrari et al. 2003). Therefore, the aquatic compartment is of key importance for evaluating the environmental persistence of pharmaceutical substances such as tamoxifen. Knowledge of physical, biological and chemical processes, such as adsorption, degradation, photolysis and hydrolysis, as well as the presence of possible transformation products in the environment (including metabolites), is needed to understand the fate, effects and risk associated with the presence of tamoxifen in the aquatic environment.

To date, few studies have addressed the degradation potential of tamoxifen in water. Degradation processes studied in water include photolysis (DellaGreca et al. 2007) and radiolytic oxidation (Legu  n   et al. 2001). In addition, studies of the degradation of tamoxifen by microbes have also been performed (El-Sharkawy and Abul-Hajj 1987; El-Sharkawy 1991).

Tamoxifen is photosensitive. It exhibits a strong ultraviolet (UV) absorption band at a wavelength of 277 nm and a tail at wavelengths > 310 nm (DellaGreca et al. 2007). Moreover, product monographs indicate that pharmaceutical products containing tamoxifen should be protected from light during storage (DPD 2010).

Irradiation of tamoxifen in water by sunlight, a solar simulator and a UV lamp under close to natural conditions was studied by DellaGreca et al. (2007). The effects of pH as well as of natural photosensitizers, including humic acid and nitrate, on the rate of degradation of tamoxifen were also investigated using irradiation by a solar stimulator (DellaGreca et al. 2007). Methylene blue-sensitized photo-oxygenation of tamoxifen was also carried out (Foote et al. 1995). In all experimental scenarios, the main products derived from photolysis of tamoxifen in water were identified following evaporation of water from the test samples and analysis of the residue composition by thin-layer chromatography and nuclear magnetic resonance spectroscopy.

Experiments showed that tamoxifen was recovered unchanged by keeping it in the dark in an aqueous solution for 30 days. Irradiation of tamoxifen by a solar simulator for 80 hours produced the following stable degradation products: trace amounts of *cis*-isomer of tamoxifen and two phenanthrenes at a yield of about 2% and 90% of the unchanged parent compound tamoxifen. Similar 80-hour experiments were carried out in the presence of nitrate and humic acid, at pH 4 and 9. Tamoxifen remained unchanged in the presence of nitrate and humic acid at pH 9. At pH 4, 70% of tamoxifen remained unchanged, *cis*-isomer was identified at about 4% and a mixture of two phenanthrenes was identified at about 8%. The increasing degradation rate at acidic pH was likely due to the greater solubility of the protonated drug in water compared with the neutral form. Dispersions of tamoxifen were exposed to solar light for 1 month at pH 4. Photoproducts of tamoxifen were identified as 50% of the parent compound tamoxifen, 10% of a complex polar fraction that contained benzoic acid, 4% *cis*-isomer of tamoxifen and trace amounts of two phenanthrenes and a ketone. Tamoxifen solutions at pH 2 saturated by either argon or oxygen were irradiated by a UV lamp for 7 hours. The residues obtained from irradiation under an oxygen atmosphere were 23% parent compound tamoxifen, two phenanthrenes at 23%, 9% ketone and *cis*-isomer of tamoxifen at 2%. Following irradiation under an argon atmosphere, photoproducts were 47% parent compound tamoxifen, 36% *cis*-isomer and trace amounts of two phenanthrenes (DellaGreca et al. 2007).

Solar simulator irradiation of tamoxifen aqueous dispersions carried out for 57 hours in the presence of methylene blue under an oxygen atmosphere produced 85% of the parent compound tamoxifen, 6% of a ketone and minor unidentified products. Methylene blue is known to be an efficient sensitizer for singlet oxygen, which adds to the C=C double bond, leading to dioxetanes, and, in the presence of allylic hydrogens, gives an ene-type reaction, leading to allylic hydroperoxides (Foote et al. 1995). The authors noted that this reaction proceeded very slowly (Foote et al. 1995).

Overall, the degradation products of tamoxifen formed by photo-induced reactions included the *cis*-isomer, phenanthrenes and ketones. Isomerization,

cyclization and, to some degree, photo-oxygenation were the main photo-induced reactions of tamoxifen. However, in general, these reactions proceeded very slowly when experimental settings closely mimicked natural conditions. At most, approximately 50% of tamoxifen was converted to photoproducts over the exposure period of up to 1 month. It should be noted that if water turbidity and water depth are considered, then photodegradation would be limited.

In another study, the antioxidant properties of tamoxifen were investigated *in vitro* by Legu  n   et al. (2001). Specifically, the ability of tamoxifen to scavenge OH and HO₂ free radicals that are produced by water radiolysis was investigated. Aqueous solutions of tamoxifen were gamma-irradiated in aerated acidic conditions. Tamoxifen reacted quantitatively with the OH free radicals, but not with the HO₂ free radicals, under the experimental conditions (Legu  n   et al. 2001). In addition, tamoxifen metabolites resulting from both fungal and microbial transformation were identified (El-Sharkawy and Abul-Hajj 1987; El-Sharkawy 1991). It was noted that some microbes display the full range of drug metabolism observed in mammals (El-Sharkawy and Abul-Hajj 1987). It was found that tamoxifen was generally resistant to microbial and fungal metabolism, and only a few species exhibited the ability to metabolize this substance. In the El-Sharkawy (1991) study, 48 microbial species representing 20 genera were screened. Tamoxifen was metabolized to desmethyltamoxifen and tamoxifen-*N*-oxide by eight species (*Cunninghamella blakesleeana*, *C. bainieri*, *C. echinulata*, *Caenorhabditis elegans*, *Mucor ramannianus*, *Beauveria bassiana*, *Curvularia lunata*, *Rhizopus stolonifer*), while only one species (*Streptomyces rimosus*) was able to biotransform tamoxifen to 4-HT. In the El-Sharkawy and Abul-Hajj (1987) study, 96 fungal species were screened, and only 1 species (*Gliocladium roseum*) was able to metabolize tamoxifen. The resulting biotransformation products obtained were *N*-oxide and *N*-desmethyl metabolites. The results of microbial and fungal transformation studies (El-Sharkawy and Abul-Hajj 1987; El-Sharkawy 1991) indicated that tamoxifen was very resistant to metabolic transformation by a multitude of fungi and microbes.

7.1.2 Modelling Results

Since few experimental data on the degradation of tamoxifen are available, a QSAR-based weight of evidence approach (Environment Canada 2007) was also applied using the degradation models shown in Table 4a. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that tamoxifen is expected to be released exclusively to this compartment, biodegradation in water was primarily examined. Tamoxifen does contain functional groups expected to undergo hydrolysis. Table 4a summarizes the results of available QSAR models for degradation in various environmental media.

A QSAR-based weight of evidence approach was also applied to the tamoxifen metabolites 4-HT and endoxifen. Results using the available degradation models are shown in Tables 4b and 4c for 4-HT and endoxifen, respectively.

Tamoxifen and its hydroxylated metabolites 4-HT and endoxifen exhibit short predicted atmospheric oxidation half-lives of 0.04 day and ozone reaction half-lives of 0.001 day. Therefore, with a half-life of < 2 days via reactions with hydroxyl radicals, photolysis and potentially with ozone, tamoxifen and its hydroxylated metabolites 4-HT are not considered persistent in air.

In water, hydrolysis half-lives could not be predicted for tamoxifen, 4-HT or endoxifen, as the model HYDROWIN (2008) does not estimate hydrolysis rate constants for these types of structures.

Ultimate biodegradation model results for tamoxifen collectively suggest a very slow biodegradation rate in water. In contrast, the primary biodegradation (BIOWIN Sub-model 4) result of 3.1 for tamoxifen falls just above the recommended conservative threshold of 3.0, adapted for indication of a faster rate of biodegradation (Aronson et al. 2006). However, based on the overall modelled evidence and the weight of inference given to results obtained from the ultimate biodegradation models (BIOWIN Sub-models 3, 5 and 6; TOPKAT and CATABOL), it is considered that the model evidence for biodegradation of tamoxifen indicates slow biodegradation rates in water. Based on the ultimate biodegradation model results, the half-life of tamoxifen is expected to be ≥ 182 days in this compartment (see Table 4a).

Similarly, for 4-HT and endoxifen, ultimate biodegradation model results also suggest that these substances biodegrade slowly in water, but the primary biodegradation results (from BIOWIN Sub-model 4) indicate a faster rate of biodegradation. Considering the consistency of biodegradation results from ultimate models suggesting a slow rate of biodegradation (especially TOPKAT, CATABOL and BIOWIN Sub-models 5 and 6) and the weight of evidence obtained from these results, it is considered that 4-HT and endoxifen biodegrade slowly in water. As a result, the predicted half-lives of 4-HT and endoxifen in water are expected to be ≥ 182 days (see Tables 4b and c).

Table 4a. Modelled data for degradation of tamoxifen

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008 ^a	$t_{1/2} = 0.04$ day	< 2

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Ozone reaction	AOPWIN 2008 ^a	$t_{1/2} = 0.001$ day	< 2
Hydrolysis	HYDROWIN 2008 ^a	n/a ^b	n/a
Primary biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 4: Expert Survey (qualitative results)	3.1 ^c “biodegrades fast”	< 182
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	-0.018 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 6: MITI non-linear probability	0.009 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 Probability	0 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	CATABOL c2004-2008 % BOD	% BOD = 0.07 “biodegrades very slowly”	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan);

$t_{1/2}$, 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.

Table 4b. Modelled data for degradation of 4-hydroxytamoxifen (4-HT), a metabolite of tamoxifen

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008 ^a	$t_{1/2} = 0.04$ day	< 2
Ozone reaction	AOPWIN 2008 ^a	$t_{1/2} = 0.001$ day	< 2
Hydrolysis	HYDROWIN 2008 ^a	n/a ^b	n/a
Primary biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 4: Expert Survey (qualitative results)	3.1 ^c “biodegrades fast”	< 182
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	-0.0098 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 6: MITI non-linear probability	0.008 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 Probability	0 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	CATABOL c2004-2008 % BOD	% BOD = 0.07 “biodegrades very slowly”	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan);

$t_{1/2}$, 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.

Table 4c. Modelled data for degradation of endoxifen, a metabolite of tamoxifen

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008 ^a	$t_{1/2} = 0.04$ day	< 2
Ozone reaction	AOPWIN 2008 ^a	$t_{1/2} = 0.001$ day	< 2
Hydrolysis	HYDROWIN 2008 ^a	n/a ^b	n/a
Primary biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 4: Expert Survey (qualitative results)	3.5 ^c “biodegrades fast”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 3: Expert Survey (qualitative results)	2.4 ^c “may biodegrade fast”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 5: MITI linear probability	0.15 ^d “biodegrades slowly”	≥ 182
Ultimate biodegradation (aerobic)	BIOWIN 2008 ^a Sub-model 6: MITI non-linear probability	0.021 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 Probability	0 ^d “biodegrades very slowly”	≥ 182
Ultimate biodegradation (aerobic)	CATABOL c2004-2008 % BOD	% BOD = 0.08 “biodegrades very slowly”	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan);

^t_{1/2}, 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.

Finally, 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 tamoxifen as well as 4-HT and endoxifen are expected to be persistent in soil and sediment.

Based on the empirical data, and in particular evidence for the slow photodegradation of tamoxifen in aqueous systems described by DellaGreca et al. (2007) as well as the modelled data for both tamoxifen and its hydroxylated metabolites, 4-HT and endoxifen, it is concluded that tamoxifen, 4-HT and endoxifen is persistent in water, soil and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days), but not in air (half-life in air ≤ 2 days).

7.2 Potential for Bioaccumulation

The high modelled log K_{ow} of 6.3 for tamoxifen suggests that the neutral form of tamoxifen may have potential to bioaccumulate in biota (see Table 2a). The experimental log D value of 4.51 for the ionized fraction of tamoxifen suggests a lower potential for bioaccumulation in biota. However, the partition coefficient in isolation is not considered sufficient evidence to determine bioaccumulation potential, as it cannot account for physiological parameters such as metabolism.

7.2.1 Metabolism

Tamoxifen is extensively metabolized by the cytochrome p450 enzyme system (Kisanga et al. 2005), a highly conserved system of enzymes among vertebrate and invertebrate organisms that catalyzes oxidation of organic substances. Small differences in p450 enzymes exist among species, and result in differences in drug metabolism, including substrate specificity and catalytic activity (Martignoni 2006). For example, uptake rates of tamoxifen from oral dosing were observed to be higher in fish than in rats, and 4-HT was noted to be the dominant metabolite of tamoxifen in fish, whereas in rats, the metabolite N-desmethyltamoxifen was prevalent (Mills et al. [date unknown]).

7.2.2 Estimating BCF and BAF

Since no experimental bioaccumulation factor (BAF) or bioconcentration factor (BCF) data for tamoxifen were available, a predictive approach was applied using available BAF and BCF models, as shown in Tables 5a, 5b and 5c.

Kinetic mass balance modelling is in principle considered to be the most reliable prediction method for determining 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 this reason, BAF are the preferred metric for assessing the bioaccumulation potential of substances.

Given that tamoxifen has the potential to ionize, both the log D (for the ionized form) and the log K_{ow} (for the neutral form) values were used as model inputs into the BCFBAF (2008) model. BCF and BAF estimates, corrected for potential biotransformation, were generated using the BCFBAF (2008) model (in EPI Suite 2008) and CPOPs (2008). Metabolic rate constants (k_M) were derived using structure–activity relationships described further in Arnot et al. (2008a; 2008b; 2009). Modelled BCF and BAF values corrected for metabolism are presented in Table 5a for tamoxifen. Modelled BCF and BAF values corrected for metabolism are presented in Tables 5b and 5c for the tamoxifen metabolites 4-HT and endoxifen, respectively.

Table 5a. Summary of modelled data for bioaccumulation of tamoxifen (neutral and ionized forms)

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	k_M (/day)	Reference
Fish	BCFBAF Sub-model 2 (mass balance) Log D = 4.51	BCF ^a	538	0.16	BCFBAF 2008
Fish	BCFBAF Sub-model 3 (Gobas mass balance) Log D = 4.51	BAF ^a	552	0.16	BCFBAF 2008
Fish	BCF _{max} with mitigating factors Log D = 4.51	BCF ^b	61	0.03	CPOPs 2008
Fish	BCFBAF Sub-model 2 (mass balance) Log K_{ow} = 6.3	BCF ^a	1 695	0.16	BCFBAF 2008
Fish	BCFBAF	BAF ^a	10 640	0.16	BCFBAF 2008

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	k_M (/day)	Reference
	Sub-model 3 (Gobas mass balance) Log K_{ow} = 6.3				
Fish	BCF _{max} with mitigating factors Log K_{ow} = 6.3	BCF ^b	587	0.03	CPOPs 2008

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

^a Results generated using weight, lipid and temperature for a middle trophic level fish.

^b Possible mitigating factors include ionization, molecular size, metabolism and water solubility.

Table 5b. Summary of modelled data for bioaccumulation of 4-HT, a metabolite of tamoxifen

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	k_M (/day)	Reference
Fish	BCFBAF Sub-model 2: mass balance	BCF	299	1.01	BCFBAF 2008
Fish	BCFBAF Sub-model 3: Gobas mass balance	BAF	302	1.01	BCFBAF 2008

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

Table 5c. Summary of modelled data for bioaccumulation of endoxifen, a metabolite of tamoxifen

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	k_M (/day)	Reference
Fish	BCFBAF Sub-model 2: mass balance	BCF	328	0.15	BCFBAF 2008

Test organism	Model and model basis	Endpoint	Value (L/kg wet weight)	k_M (/day)	Reference
Fish	BCFBAF Sub-model 3: Gobas mass balance	BAF	333	0.15	BCFBAF 2008

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

The modelled results indicate that the ionized form of tamoxifen is not expected to have high bioaccumulation potential. The predicted BCF and BAF values obtained from the BCFBAF (2008) and the CPOPs (2008) models and corrected for metabolism were less than 5000. For the neutral form of tamoxifen, the predicted BCF values indicate a low potential for bioconcentration, and the predicted BAF value from the BCFBAF (2008) model indicates a high potential for bioaccumulation. There is, however, high uncertainty in the predicted values for k_M for both the neutral and ionized forms, given the lack of chemical structures similar to pharmaceutical substances such as tamoxifen in the training sets of models such as BCFBAF (2008).

The modelled bioaccumulation results for 4-HT and endoxifen also indicate that these substances do not have a high potential to bioaccumulate in fish. The predicted BCF and BAF values, corrected for metabolism, were slightly lower than the BCF and BAF values predicted for tamoxifen by the same models (i.e., BCFBAF 2008 Sub-models 2 and 3) and well below 5000.

Information regarding molecular size and cross-sectional diameters is also useful to consider as weight of evidence for bioaccumulation potential. Analysis relating fish BCF data to molecular size parameters (Dimitrov et al. 2002, 2005; Sakuratani et al. 2008) suggests 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 maximum diameter is greater than approximately 1.5 nm and much more so for molecules having a maximum diameter of greater than 1.7 nm. It was observed that substances that do not have a very high bioconcentration potential often have a D_{max} of > 2.0 nm and an effective diameter (D_{eff}) > 1.1 nm. Based on three-dimensional analysis of conformers, calculated using the BCF_{max} Model with Mitigating Factors (Dimitrov et al. 2005), the maximum diameters (D_{max}) for the ionized form of tamoxifen range from 1.51 to 1.91 nm, and the D_{max} values for the neutral form of tamoxifen range from 1.62 to 1.91 nm. This suggests that both forms of tamoxifen may experience restricted uptake from steric effects at the gill surface.

The available weight of evidence indicates that tamoxifen as well as its metabolites 4-HT and endoxifen are not expected to significantly bioaccumulate in biota. Modelled results suggest that these substances, especially in ionized form, are characterized by low BCF and BAF values. In addition, these substances have low water solubilities, which may limit their availability for uptake from water. It was determined that tamoxifen has a relatively large cross-sectional diameter, and this can further restrict its uptake across the gills as a result of steric hindrance. It is also expected that tamoxifen has a high potential to be metabolized by fish. Therefore, based on the available data, it is concluded that tamoxifen and its hydroxylated metabolites 4-HT and endoxifen have low bioaccumulation potential.

8. Potential to Cause Ecological Harm

8.1 Ecological Effects Assessment

Tamoxifen as well as its hydroxylated metabolites are not expected to be released to any degree into environmental compartments other than water. However, based on their modelled physical and chemical properties (see Tables 2a, 2b and 2c) and the results of fugacity modelling (see Table 3), tamoxifen, 4-HT and endoxifen may partition to sediments. No ecological effects studies in sediment were found for these compounds.

In order to provide the best possible weight of evidence for assessing the ecological effects of tamoxifen and its metabolites, empirical data were considered. Since waterborne tamoxifen mainly originates from tamoxifen citrate pharmaceutical formulations as they are ingested and excreted into wastewater by humans, it was relevant to evaluate the effects of tamoxifen citrate in fish. It is expected, based on the toxicity profile of citric acid (OECD 2001), that the citrate moiety associated with tamoxifen would have negligible ecotoxicological effects on aquatic organisms.

It has been indicated that 15.2 mg of tamoxifen citrate in tablets is equivalent to 10 mg of tamoxifen (Drug Infonet c1996–2005). Therefore, to illustrate exposure concentrations and effects from the tamoxifen active ingredient, a factor of 0.66 is applied to exposure concentrations and ecological endpoints obtained when tamoxifen citrate was used [i.e., endpoints from the unpublished AstraZeneca studies reported in Williams et al. 2007; Knacker et al. 2010].

8.1.1 Aquatic Toxicity of Tamoxifen

Studies evaluating the toxicity of tamoxifen to aquatic invertebrates and vertebrates, including crustaceans, rotifer species, fish and frogs, as well as *in vitro* cytotoxic assays in fish cell lines are presented in Table 6a and discussed

below. Since tamoxifen is not readily soluble in water (solubility is < 0.5 mg/L; CEREP c2010b), carrier solvents such as acetone, triethylene glycol (trigol) or dimethyl sulfoxide (DMSO) are typically used to make experimental stock solutions. Appropriate solvent controls were incorporated into study designs.

Tamoxifen is an endocrine-active substance; hence effects observed in pelagic organisms are endocrine disrupting in nature, particularly in studies with vertebrates where the underlying estrogenic mode of action of tamoxifen becomes apparent. Of note are the shifts in sex ratios observed in tamoxifen-exposed fish populations (e.g., Van der Ven et al. 2007; Knacker et al. 2010; Liu et al. 2010; Singh 2013), and alteration in gonadotropin expression levels in female frogs (Urbatzka et al. 2006, 2007).

The toxicity of tamoxifen and its photochemical derivatives to aquatic invertebrates was investigated by DellaGreca et al. (2007). Products of tamoxifen derived in water from exposure to sunlight were identified, and their chronic and acute toxicities were evaluated using a rotifer species, *Brachionus calyciflorus*, and three crustaceans, *Thamnocephalus platyurus*, *Daphnia magna* and *Ceriodaphnia dubia*. Irradiation of tamoxifen by a solar simulator and sunlight produced five stable products: a *cis*-isomer (Product 1), two phenanthrenes (Products 2 and 3), a ketone (Product 4) and benzoic acid.

Acute 24-hour bioassays were performed on *B. calyciflorus* and *T. platyurus* to evaluate mortality (i.e., LC_{50}) and on *D. magna* to evaluate immobilization (i.e., EC_{50}) from exposure to tamoxifen and its photochemical derivatives. As a result of exposure to tamoxifen, the acute LC_{50} values for *B. calyciflorus* and *T. platyurus* were 0.97 and 0.40 mg/L, respectively, and the acute EC_{50} for *D. magna* was 1.53 mg/L. Exposure to photodegradates of tamoxifen (i.e., Products 1–4) resulted in LC_{50} values in the range of 0.95–1.31 mg/L for *B. calyciflorus* and 0.47–1.59 mg/L for *T. platyurus* and an EC_{50} in the range of 1.74–3.27 mg/L for Products 1–3 in *D. magna*, with no effects noted for Product 4 at exposure concentrations up to 5 mg/L.

Chronic tests to establish EC_{50} values for tamoxifen and its photodegradates were also carried out in *B. calyciflorus* and *C. dubia* for 48 hours and 7 days, respectively. EC_{50} values resulting from exposure to tamoxifen were 0.25 mg/L for *B. calyciflorus* and 8.1×10^{-4} mg/L for *C. dubia*. Moreover, EC_{50} values established from exposure to products 1–4 were in the range of 0.123–0.26 mg/L for *B. calyciflorus* and in the range of 4.1×10^{-4} – 9.6×10^{-3} mg/L for *C. dubia*, indicating an equivalent or higher toxicity of photodegradation products compared with that of the parent compound. Photodegradation products of tamoxifen investigated in the chronic tests revealed greatest effects on *C. dubia*, with a toxic potential 3 orders of magnitude higher than that established in acute tests for the related species *D. magna*. *C. dubia* was also the most sensitive to undegraded tamoxifen in chronic exposure tests. Overall, the findings by

DellaGreca et al. (2007) indicated that exposure to tamoxifen and its photodegradates in water posed a hazard to aquatic invertebrates, particularly in longer-term or chronic exposure scenarios.

The toxicity of tamoxifen was also investigated in a marine crustacean species, *Acartia tonsa*, during early developmental stages of the larvae known as naupliar stages (Andersen et al. 200; Hilton et al. 2003). *A. tonsa* larvae with juvenile morphology are named nauplii, whereas larvae possessing the adult morphology are referred to as copepodites. These differences in morphology make it feasible to test effects of chemicals on growth and development, with the test endpoint being the fraction of juveniles that develop into copepodites in a given time frame. The effect of tamoxifen was assessed in a semistatic test, covering the period of development from egg until approximately 50% of larvae in the control had reached the copepodite stage. It was observed that tamoxifen inhibited development of *A. tonsa* at low exposure concentrations. Reported EC₅₀ and EC₁₀ values following a chronic 5-day test were 0.049 and 0.0087 mg/L, respectively.

Reproductive effects of tamoxifen citrate in the fathead minnow (*Pimephales promelas*) in partial and full cycle studies were undertaken by AstraZeneca PLC to define potential adverse effects of chronic waterborne tamoxifen exposure using established regulatory endpoints, and to generate biomarker data to improve current understanding of tamoxifen influence on the endocrine homeostasis in fish (Williams et al. 2007). In the partial life cycle study, adverse effect endpoints were examined in fathead minnows during the F₀ (first breeding pair) reproduction phase and F₁ (first generation from first breeding pair) embryo-larval phase following exposure to tamoxifen at mean measured concentrations ranging between 7.3×10^{-5} and 0.012 mg/L (1.1×10^{-4} – 0.018 mg/L tamoxifen citrate) over 42 days (Williams et al. 2007). Endpoints studied were F₀ fecundity, fish weight and length, F₀ vitellogenin levels and, for the first generation, F₁ hatching success at 4 days, larval length and weight at 28 days and vitellogenin levels at 42 days. In the full life cycle study, similar adverse effect endpoints were examined for F₀ and F₁ fish generations following exposure to tamoxifen at mean measured concentrations ranging from 5×10^{-6} to 2.7×10^{-3} mg/L (7×10^{-6} – 4.1×10^{-3} mg/L tamoxifen citrate). For F₀, measurements were taken for survival during the spawning phase, fish lengths and weights at 112 and 211 days post-hatch (dph), vitellogenin levels at 211 dph and, for F₁, lengths and weights at 28 and 112 dph and vitellogenin levels at 112 dph in both males and females.

Data from partial and full life cycle studies were analyzed statistically to identify significant differences between control and treatment groups. The full life cycle study showed no statistically significant reduction in F₀ and F₁ hatching success after exposure to tamoxifen at concentrations up to the maximum mean measured concentration of 2.7×10^{-3} mg/L (4.1×10^{-3} mg/L tamoxifen citrate). Moreover, F₀ fecundity was not reduced significantly by exposure concentrations

up to the maximum mean measured concentration of 2.7×10^{-3} mg/L (4.1×10^{-3} mg/L tamoxifen citrate). However, in the partial life cycle study, a 42-day exposure to tamoxifen at 0.012 mg/L (0.018 mg/L tamoxifen citrate) caused a 70% reduction in spawning ($p < 0.01$). Survival was unaffected by tamoxifen at exposure concentrations up to the maximum mean measured concentration of 2.7×10^{-3} mg/L (4.1×10^{-3} mg/L tamoxifen citrate) over 211 days in the full life cycle study and at exposure concentrations of ≤ 0.012 mg/L after 42 days in the partial life cycle study. The no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) were calculated based on the chronic data from the partial and full life cycle studies based on adverse effect endpoints such as altered development, growth and reproduction, excluding the 28 dph larval growth data. The NOEC and LOEC values for the fathead minnow established in Williams et al. (2007) were 0.0034 mg/L (0.0051 mg/L tamoxifen citrate) and 0.0037 mg/L (0.0056 mg/L tamoxifen citrate), respectively. Acute (96-hour) LC_{50} values for other fish species previously determined in unpublished studies by AstraZeneca were also mentioned in Williams et al. (2007). The LC_{50} values were 0.15 mg/L (0.23 mg/L tamoxifen citrate) for the bluegill sunfish and 0.27 mg/L (0.41 mg/L tamoxifen citrate) for rainbow trout (Williams et al. 2007).

Williams et al. (2007) expressed the biomarker responses (i.e., vitellogenin levels) as ^{biomarker}NOEC and ^{biomarker}LOEC. However, the authors advised that biomarker responses alone should not be used for calculating PNECs. Measured vitellogenin levels appeared to be gender and life stage specific. Exposure to tamoxifen citrate had no effect on plasma vitellogenin levels in adult fish in a 42-day study. However, there was a 50% reduction ($p < 0.01$) in whole-body vitellogenin levels in F_1 fish larvae at exposure concentrations of ≤ 0.012 mg/L (≤ 0.018 mg/L tamoxifen citrate) after 42 days, and in the full life cycle study, there was a significant increase in plasma vitellogenin levels in F_0 males at 211 dph and also in F_1 females at 112 dph at an exposure concentration of 0.0034 mg/L (0.0051 mg/L tamoxifen citrate). Discrete values for ^{biomarker}NOEC and ^{biomarker}LOEC were not provided.

Toxicological effects of tamoxifen were also studied long term, life cycle studies using zebrafish (*Danio rerio*) (Knacker et al. 2010; Van der Ven et al. 2007).

Van der Ven et al. (2007) studied the effects of tamoxifen in a partial life cycle assay with zebrafish (*Danio rerio*), where parental zebrafish (P) and their progeny (F_1) were exposed to the substance during reproduction, sexual differentiation and development (Van der Ven et al. 2007). Reproductive parameters (fertility, fecundity), mortality and growth, as well as vitellogenin expression and histology, were evaluated. Knacker et al. 2011 evaluated effects of tamoxifen citrate in a two-generation study, encompassing the P generation, the filial F_1 generation (early life stages, juvenile growth and reproduction), and the early life stage phase of the F_2 generation. For P and F_1 generations, vitellogenin and sex steroid 11-keto testosterone were also measured.

In Van der Ven et al. (2007), general toxicity was observed at higher, range-finding exposure concentrations of 0.01–10 mg/L (27–27 000 nM) over a 10-day period. At an exposure concentration of 1 mg/L (2 700 nM), mortality was observed in fish larvae, whereas juvenile fish showed increased mortality at 0.1 mg/L, and no progeny were present at an exposure concentration of 0.3 mg/L. At exposure concentrations of 1 mg/L and higher, hemorrhage, disturbed locomotion and disturbed respiration in both juvenile and adult fish were observed. At lower tamoxifen exposure concentrations of 0.003, 0.01, 0.03, 0.1 and 0.3 mg/L (8.6, 27, 86, 270 and 860 nM), female zebrafish in all tamoxifen exposure groups had oviducts filled with degenerated eggs.

In Knacker et al. (2010), observations in the P generation included a reduced rate of egg fertilization rate at the highest tested concentration of 0.009 mg/L (0.014 mg/L tamoxifen citrate), significantly decreased vitellogenin levels in both male and female fish at the two highest tamoxifen concentration levels of 0.0026 mg/L and 0.009 mg/L (0.004 mg/L and 0.014 mg/L tamoxifen citrate, respectively), and a reduced concentration of 11-keto testosterone in male fish at exposure concentration of 0.009 mg/L (0.014 mg/L tamoxifen citrate). For F1 generation, both the hatching rate and growth were significantly reduced at tamoxifen concentration of 0.007 mg/L (0.011 mg/L tamoxifen citrate). There were no effects on the hatching success and post-hatch survival in the F2 generation, but a slight increase in length was observed at the highest concentration tested of 0.0015 mg/L (0.0023 mg/L tamoxifen citrate).

Significant alternations in the male to female population sex ratio in the F1 generation were observed by both Van der Ven et al. (2007) and Knacker et al. (2010). In Van der Ven et al. (2007), an exposure concentration of 0.03 mg/L significantly increased proportion of males to females, and also caused a higher proportion of individuals with undifferentiated gonads. Knacker et al (2010) observed changes in the population sex ratio at a much lower tamoxifen exposure concentration of 0.00051 mg/L (0.00077 mg/L). A complete sex-reversal where no females were found was observed at the highest concentration tested of 0.007 mg/L (0.011 mg/L tamoxifen citrate).

Tamoxifen was also shown to affect the population sex ratio in other fish species (Singh 2013; Liu et al. 2010). In a study using carp (*Cyprinus carpio*), exposure to tamoxifen through diet significantly affected sex differentiation and gonadal maturity (Singh 2013). Carp fingerlings were exposed to tamoxifen mixed in feed at concentrations of 100 and 200 mg/kg feed for 60 days and fed twice daily. Exposure to the higher tamoxifen concentration of 200 mg/kg feed brought about 82.5% masculinization of the fish population (Singh 2013). Elsewhere, it was also reported that treatment with tamoxifen is effective in inducing female to male sex reversal in the southern catfish (*Silurus meridionalis*) (Liu et al. 2010).

Other relevant information identified, comprising several ecotoxicological endpoints from unpublished studies by AstraZeneca and including LOEC, NOEC and LC₅₀ values for algal species, *D. magna* and fish species, was summarized in the Swedish Drug Database (2011). These values, determined by exposing organisms to tamoxifen citrate, are presented in Table 6a. For the algae *Selenastrum capricornutum* and the blue-green algae *Microcystis aeruginosa*, LOEC and NOEC values for growth rate were determined in chronic tests. Tamoxifen was highly toxic to both species: for *S. capricornutum*, the LOEC and NOEC were 0.008 mg/L (0.012 mg/L tamoxifen citrate) and 0.003 mg/L (0.0049 mg/L tamoxifen citrate), respectively, and for *M. aeruginosa*, the LOEC and NOEC were 0.13 mg/L (0.2 mg/L tamoxifen citrate) and 0.065 mg/L (0.098 mg/L tamoxifen citrate), respectively. Tamoxifen was also highly toxic to *D. magna* in chronic tests addressing reproduction and growth rate; the LOEC for reproduction was determined to be 0.09 mg/L (0.14 mg/L tamoxifen citrate), whereas NOEC values for reproduction and length were 0.05 mg/L (0.078 mg/L tamoxifen citrate) and 0.03 mg/L (0.043 mg/L tamoxifen citrate), respectively. Lastly, in addition to the LC₅₀ values for rainbow trout and bluegill sunfish reported by Williams et al. (2007), endpoints from another unpublished rainbow trout study were also summarized in the Swedish Drug Database (2011). For *Salmo gairdneri*, the LC₅₀ and NOEC in an acute 96-hour study were determined to be 0.21 mg/L (0.32 mg/L tamoxifen citrate) and 0.18 mg/L (0.27 mg/L tamoxifen citrate), respectively.

Endocrine disrupting effects of tamoxifen were studied in adult frogs, *Xenopus laevis*, at an exposure concentration of 0.0037 mg/L (10^{-8} M) over a period of 4 weeks (Urbatzka et al. 2006, 2007). The chosen exposure concentration was regarded to be within the physiological range of circulating sex steroids in an adult *X. laevis*, but higher than environmental concentrations, measured in the range of 1×10^{-6} – 2×10^{-5} mg/L (1–20 ng/L). In general, reproduction in vertebrates is under endocrine control of the hypothalamus–pituitary–gonad axis, which governs circulating sex steroids that exert effects in several peripheral target organs, including the liver (Urbatzka et al. 2006). Therefore, biomarker genes were selected for the detection of endocrine disrupting activity, including three transport proteins in the plasma: retinol binding protein (RBP), involved in the transport of the vitamin A precursor, retinol; transferrin (TF), an iron transporter; and transthyretin (TTR), involved in transporting thyroid hormones (Urbatzka et al. 2007). The messenger ribonucleic acid (mRNA) expression of the three transporter genes in the liver of male and female *X. laevis* was compared to that of vitellogenin at the mRNA and plasma protein level. mRNA expression of vitellogenin in the liver was decreased in female frogs, but plasma vitellogenin levels were not changed following exposure to tamoxifen. TF mRNA expression was increased in the female frogs; however, the RBP basal expression level as well as the TTR mRNA expression level were not altered following tamoxifen treatment. Hence, the TF and vitellogenin mRNA expression pattern in the liver indicated an anti-estrogenic response that may impact

processes involved in protein homeostasis. In addition, measured plasma concentrations of testosterone were not changed in either male or female frogs, and plasma concentrations of 17β -estradiol were increased in the females following exposure to tamoxifen, reflecting modification in a negative feedback mechanism on the hypothalamus–pituitary–gonad axis.

As well, brain (including the pituitary gland) mRNA expression patterns of hypophyseal gonadotropins—follicle stimulating hormone (FSH) and luteinizing hormone (LH), key reproductive hormones involved in gonadal development—and gonadotropin-releasing hormone (GnRH) were investigated to determine potential disturbance of reproductive processes in response to exposure to tamoxifen (Urbatzka et al. 2006). Effects were noted in female frogs only, in which LH and FSH mRNA levels were increased by approximately 1.5- and 2.5-fold, respectively. Levels of GnRH mRNA remained unchanged in both male and female frogs. Overall, these results indicated that expression of gonadotropins in amphibians can be significantly altered in a gender-specific pattern by endocrine disrupting compounds, including tamoxifen.

Tamoxifen was also tested *in vitro* in several cytotoxic assays, such as the thiazolyl blue tetrazolium bromide (MTT), neutral red (NR), lactate dehydrogenase (LDH), alamarin-blue (AB) and 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) assays (see footnote below Table 6a for brief assay descriptions), in fish cell lines (Caminada et al. 2006; Bopp and Lettieri 2008). These quantitative colorimetric or fluorometric assays employ different cellular modes of action to indicate cytotoxicity of the test compound, such as disruption of membrane integrity and detection of enzyme or metabolic activity (Caminada et al. 2006; Bopp and Lettieri 2008). Cytotoxicity assays in fish cell lines can be a valuable tool in the risk assessment to estimate and rank the acute toxicity of compounds in order to minimize *in vivo* acute toxicity testing.

In the Caminada et al. (2006) study, it was noted that both the NR and MTT assays produced equal cytotoxicity results; that is, experimental EC_{50} values from both tests were in the same order of magnitude in the two fish cell lines tested, PLHC-1 (*Poeciliopsis lucida* hepatoma cell line) and RTG-2 (rainbow trout gonadal cell line). The reported EC_{50} values resulting from exposure to tamoxifen using the MTT assay were 7.4 and 7.09 mg/L in the PLHC-1 and RTG-2 cell lines, respectively. The EC_{50} value from the NR assay using the RTG-2 cell line was 7.2 mg/L. In addition, there was a clear correlation ($p < 0.0001$) between the EC_{50} values and the tamoxifen log D value (at pH 7), indicating that cytotoxicity is due to non-specific toxicity or narcosis. Log D considers the partitioning of a compound at a specific pH; therefore, it tends to reflect the situation occurring in the cytotoxicity assay, such that compounds that are more lipophilic generally tend to be more toxic.

Four cytotoxic assays were performed in a zebrafish (*Danio rerio*) liver cell line, ZFL (Bopp and Lettieri 2008). Two assays were colorimetric, the MTT assay and the LDH assay, and two assays were fluorometric, the AB assay and the CFDA-AM assay. There were no significant differences in the EC₁₀ or EC₅₀ values established from the four assays; however, the authors indicated that, overall, the fluorometric assays were more precise, more robust and consequently better suited for cytotoxicity assessment.

EC₁₀ and EC₅₀ values were established for tamoxifen and its metabolite 4-HT in the ZFL cell line (see Table 6b for 4-HT results). For tamoxifen, EC₁₀ values ranged between 0.23 and 0.94 mg/L, whereas EC₅₀ values ranged between 0.70 and 1.28 mg/L (see Table 6a). It should be noted that the MTT assay was performed in both the Caminada et al. (2006) and Bopp and Lettieri (2008) studies, and the EC₅₀ values for tamoxifen were approximately 10 times less in the ZFL cell line than those established by Caminada et al. (2006) in the RTG-2 and PLHC-1 fish cell lines, indicating that there may be cell line-specific differences in sensitivity.

Table 6a. Empirical data for aquatic toxicity of tamoxifen

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Blue-green algae (<i>Microcystis aeruginosa</i>)	Chronic (21 days)	LOEC (growth rate)	0.13 (0.2 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a
Blue-green algae (<i>Microcystis aeruginosa</i>)	Chronic (21 days)	NOEC (growth rate)	0.065 (0.098 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a
Green algae (<i>Selenastrum capricornutum</i>)	Chronic (14 days)	LOEC (growth rate)	0.008 (0.012 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a
Green algae (<i>Selenastrum capricornutum</i>)	Chronic (14 days)	NOEC (growth rate)	0.003 (0.0049 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
<i>Thamnocephalus platyurus</i>	Acute (24 h)	LC ₅₀	0.40	DellaGreca et al. 2007 ^b
Water flea (<i>Daphnia magna</i>)	Acute (24 h)	LC ₅₀	1.53	DellaGreca et al. 2007 ^b
<i>Ceriodaphnia dubia</i>	Chronic (7 days)	EC ₅₀ (population growth inhibition)	0.00081	DellaGreca et al. 2007 ^b
Water flea (<i>Daphnia magna</i>)	Chronic (21 days)	LOEC (reproduction)	0.09 (0.14 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011
Water flea (<i>Daphnia magna</i>)	Chronic (21 days)	NOEC (length)	0.03 (0.043 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011
Water flea (<i>Daphnia magna</i>)	Chronic (21 days)	NOEC (reproduction)	0.05 (0.078 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011
Marine copepod (<i>Acartia tonsa</i>)	Chronic (5 days)	EC ₅₀ (inhibition of napular development)	0.049	Andersen et al. 2001
Marine copepod (<i>Acartia tonsa</i>)	Chronic (5 days)	EC ₁₀ (inhibition of napular development)	0.0087	Andersen et al. 2001
<i>Brachionus calyciflorus</i>	Chronic (48 h)	EC ₅₀ (population growth inhibition)	0.25	DellaGreca et al. 2007
<i>Brachionus calyciflorus</i>	Acute (24 h)	LC ₅₀	0.97	DellaGreca et al. 2007

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Zebrafish (<i>Danio rerio</i>)	Chronic or full life cycle (filial generation)	LOEC _(sex reversal)	0.00051 (0.00077 mg/L tamoxifen citrate)	Knacker et al. 2010 ^a
Fathead minnow (<i>Pimephales promelas</i>)	Chronic or full life cycle (284 days)	NOEC	0.0034 (0.0051 mg/L tamoxifen citrate)	Williams et al. 2007 ^a
Fathead minnow (<i>Pimephales promelas</i>)	Chronic or full life cycle (284 days)	LOEC	0.0037 (0.0056 mg/L tamoxifen citrate)	Williams et al. 2007 ^a
Fathead minnow (<i>Pimephales promelas</i>)	Partial life cycle (42 days)	EC ₇₀ (spawning)	0.012 (0.018 mg/L tamoxifen citrate)	Williams et al. 2007 ^a
Bluegill sunfish (<i>Lepomis macrochirus</i>)	Acute (96 h)	LC ₅₀	0.15 (0.23 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Williams et al. 2007 and Swedish Drug Database 2011 ^a
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Acute (96 h)	LC ₅₀	0.27 (0.41 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Williams et al. 2007 and Swedish Drug Database 2011 ^a

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Rainbow trout (<i>Salmo gairdneri</i>)	Acute (96 h)	LC ₅₀	0.21 (0.32 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a
Rainbow trout (<i>Salmo gairdneri</i>)	Acute (96 h)	NOEC	0.18 (0.27 mg/L tamoxifen citrate)	Unpublished AstraZeneca study in Swedish Drug Database 2011 ^a
<i>Poeciliopsis lucida</i> (desert topminnow) hepatoma fish cell line PLHC-1	MTT assay ^c	EC ₅₀ (damage to cell membranes)	7.4 (0.02 mM)	Caminada et al. 2006 ^d
<i>Poeciliopsis lucida</i> (desert topminnow) hepatoma fish cell line PLHC-1	NR assay ^e	EC ₅₀ (damage to cell membranes)	7.2 (0.0194 mM)	Caminada et al. 2006 ^d
Rainbow trout gonadal cell line RTG-2	MTT assay ^c	EC ₅₀ (damage to cell membranes)	7.09 (0.0191 mM)	Caminada et al. 2006 ^d
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	LDH assay ^f	EC ₁₀	0.23 (0.61 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	LDH assay ^f	EC ₅₀	0.70 (1.88 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	MTT assay ^c	EC ₁₀	0.52 (1.39 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	MTT assay ^c	EC ₅₀	1.28 (3.46 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	AB assay ^g	EC ₁₀	0.51 (1.37 µM)	Bopp and Lettieri 2008

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	AB assay ^g	EC ₅₀	1.12 (3.12 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	CFDA-AM assay ^h	EC ₁₀	0.94 (2.52 µM)	Bopp and Lettieri 2008
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	CFDA-AM assay ^h	EC ₅₀	1.24 (3.35 µM)	Bopp and Lettieri 2008

Abbreviations: AB, alamarin-blue; CFDA-AM, 5-carboxyfluorescein diacetate acetoxymethyl ester; EC₁₀, the concentration of a substance that is estimated to cause some effect on 10% of the test organisms; EC₅₀, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC₅₀, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LDH, lactate dehydrogenase; LOEC, lowest-observed-effect concentration, the lowest concentration in a toxicity test that caused a statistically significant effect in comparison with the controls; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or thiazolyl blue tetrazolium bromide; NOEC, no-observed-effect concentration, the highest concentration in a toxicity test not causing a statistically significant effect in comparison with the controls; NR, neutral red

^a Tamoxifen citrate (CAS RN 54965-24-1, molecular weight 563.62 g/mol) was used in the studies (Williams et al. 2007; unpublished AstraZeneca studies cited in Williams et al. 2007 and Swedish Drug Database 2011; Knacker et al. 2010). Ecotoxicological endpoint values for tamoxifen citrate are reported in parentheses below the values calculated for tamoxifen using a 0.66 conversion factor.

^b Analytical standard-grade tamoxifen (purity 90%) supplied by Aldrich was used in the study (DellaGreca et al. 2007).

^c The MTT assay is based on the uptake of MTT and its reduction to MTT formazan in the mitochondria of living cells, whereas in dead cells this cleavage reaction typically does not take place.

^d The molecular weight of tamoxifen is 371.515 g/mol; it was used to convert the reported EC₅₀ units from mM to mg/L.

^e The NR assay is based on uptake and accumulation of NR in the lysosomes of living cells, whereas damaged cells have altered uptake rates and dead cells do not retain the dye.

^f The LDH assay is based on the detection of LDH, which is released from the cytosol of damaged or lysed cells; cytotoxicity is reflected in plasma membrane integrity.

^g The AB assay measures cellular metabolic activity and is based on the conversion of the non-fluorescent dye resazurin to a fluorescent dye resorufin by mitochondrial and other enzymes.

^h The CFDA-AM assay is based on conversion of a non-toxic esterase substrate, CFDA-AM, to a fluorescent dye, carboxyfluorescein; cytotoxicity is reflected in plasma membrane integrity, as only living cells support esterase activity.

8.1.2 Aquatic Toxicity of Tamoxifen Metabolites

In vivo studies addressing the aquatic toxicity of the hydroxylated tamoxifen metabolites, 4-HT and endoxifen, were not identified in the published literature. Similarly to tamoxifen, 4-HT was tested *in vitro* in several cytotoxic assays performed in fish cell lines (Caminada et al. 2006; Bopp and Lettieri 2008). EC₁₀ and EC₅₀ values from these studies are presented in Table 6b. Similar studies were not identified for endoxifen.

EC₅₀ values for 4-HT established from the MTT assays were 5.3 and 5.6 mg/L in the PLHC-1 and RTG-2 cell lines, respectively, and an EC₅₀ of 1.8 mg/L was

established from the NR assay in the PLHC-1 cell line (Caminada et al. 2006). In the ZFL cell line, EC₅₀ values ranged from 0.45 to 0.69 mg/L, and EC₁₀ values ranged from 0.28 to 0.58 mg/L, as established in four cytotoxicity assays (Bopp and Lettieri 2008). It should be noted that the EC₅₀ results from the MTT assay were approximately 10 times higher in the PLHC-1 cell line than in the ZFL cell line, at 5.3 mg/L versus 0.69 mg/L, indicating that there may be cell line-specific differences in sensitivity (Table 6b). An analogous trend was observed when the parent compound tamoxifen was tested using the MTT assay in these cell lines (see Table 6a).

Table 6b. In vitro empirical data for the toxicity of 4-HT, a metabolite of tamoxifen

Test cell line	Type of test	Endpoint	Value (mg/L)	Reference
<i>Poeciliopsis lucida</i> (desert topminnow) hepatoma fish cell line PLHC-1	MTT assay ^a	EC ₅₀ (damage to cell membranes)	5.3 (0.0138 mM)	Caminada et al. 2006 ^b
<i>Poeciliopsis lucida</i> (desert topminnow) hepatoma fish cell line PLHC-1	NR assay ^c	EC ₅₀ (damage to cell membranes)	1.8 (0.004 64 mM)	Caminada et al. 2006 ^b
Rainbow trout gonadal cell line RTG-2	MTT assay ^a	EC ₅₀ (damage to cell membranes)	5.6 (0.0145 mM)	Caminada et al. 2006 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	LDH assay ^d	EC ₁₀	0.28 (0.73 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	LDH assay ^d	EC ₅₀	0.63 (1.62 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	MTT assay ^a	EC ₁₀	0.58 (1.49 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	MTT assay ^a	EC ₅₀	0.69 (1.78 µM)	Bopp and Lettieri 2008 ^b

Test cell line	Type of test	Endpoint	Value (mg/L)	Reference
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	AB assay ^e	EC ₁₀	0.31 (0.79 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	AB assay ^e	EC ₅₀	0.45 (1.17 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	CFDA-AM assay ^f	EC ₁₀	0.42 (1.08 µM)	Bopp and Lettieri 2008 ^b
Zebrafish (<i>Danio rerio</i>) liver cell line ZFL	CFDA-AM assay ^f	EC ₅₀	0.67 (1.73 µM)	Bopp and Lettieri 2008 ^b

Abbreviations: AB, alamarBlue; CFDA-AM, 5-carboxyfluorescein diacetate acetoxymethyl ester; EC₁₀, the concentration of a substance that is estimated to cause some effect on 10% of the test organisms; EC₅₀, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LDH, lactate dehydrogenase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or thiazolyl blue tetrazolium bromide; NR, neutral red

^a The MTT assay is based on the uptake of MTT and its reduction to MTT formazin in the mitochondria of living cells, whereas in dead cells this cleavage reaction typically does not take place.

^b The molecular weight of 4-HT is 387.52; it was used to convert the reported EC_{10/50} units from mM to mg/L.

^c The NR assay is based on uptake and accumulation of NR in the lysosomes of living cells, whereas damaged cells have altered uptake rates and dead cells do not retain the dye.

^d The LDH assay is based on the detection of LDH, which is released from the cytosol of damaged or lysed cells; cytotoxicity is reflected in plasma membrane integrity.

^e The AB assay measures cellular metabolic activity and is based on the conversion of the non-fluorescent dye resazurin to a fluorescent dye resorufin by mitochondrial and other enzymes.

^f The CFDA-AM assay is based on conversion of a non-toxic esterase substrate, CFDA-AM, to a fluorescent dye, carboxyfluorescein; cytotoxicity is reflected in plasma membrane integrity, as only living cells support esterase activity.

8.1.3 Derivation of the PNEC

A conservative predicted no effect concentration (PNEC) for tamoxifen in the aquatic environment was derived from the critical toxicity value (CTV) of 0.00051 mg/L for zebrafish (*Danio rerio*) (see Table 6a; the CTV is indicated in bold). This CTV is considered to be the most sensitive, population-relevant endpoint, and it is also several orders of magnitude lower than the cytotoxicity values for tamoxifen metabolite 4-HT (see Tables 6a and 6b). Based on a robust study summary (RSS), the study from which the value originated (Supplement A in Knacker et al. 2010) was found to be reliable with satisfactory confidence (Environment Canada 2014).

The CTV of 0.00051 mg/L, a LOEC for the sex reversal effect on the filial generation of fish, was divided by an assessment factor of 10 (to consider inter-species and intra-species variability in sensitivity, and to account for potential higher toxicity, including the more potent anti-estrogenic properties of the tamoxifen metabolites, 4-HT and potentially endoxifen) to derive a PNEC value of 5.1×10^{-5} mg/L. The value of the assessment factor also reflects the fact that the critical study is based on chronic exposure (a two-generation study), that there is a relatively large set of toxicity data, and that the CTV is approximately 10-fold lower than most other measured or calculated chronic toxicity data. The aquatic PNEC for tamoxifen of 5.1×10^{-5} mg/L is also considered applicable to both 4-HT and endoxifen.

8.2 Ecological Exposure Assessment

Limited data on concentrations of tamoxifen in water in Canada have been identified. Therefore, environmental concentrations have been estimated from available information, including estimated substance quantities, estimated release rates and characteristics of the receiving environment. Environmental concentrations have been estimated for an industrial release scenario and a down-the-drain release scenario.

8.2.1 Industrial Release

It is currently unknown whether tamoxifen is manufactured in Canada. However, releases from potential manufacturing activities into water are estimated below, based on the total quantity of the substance sold in Canada in the year 2012. Aquatic exposure to tamoxifen is expected if the substance is released during its manufacture at a pharmaceutical production facility to a wastewater treatment plant and the treatment plant discharges its effluent to a receiving water body. The concentration of the substance in the receiving water near the discharge point of the wastewater treatment plant is used as the predicted environmental concentration (PEC) in evaluating the aquatic risk of the substance. It is calculated using the equation:

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

where:

PEC _{aq} :	Aquatic concentration resulting from industrial releases (mg/L)
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).

If produced in Canada, some tamoxifen would be expected to be released to water during production, and a conservative industrial release scenario is used to estimate the aquatic concentration of the substance. The scenario is made conservative by assuming that the total quantity of the substance used as a pharmaceutical in Canada is produced at a single production facility. The facility is assumed to be located in Mississauga (Ontario), a typical Canadian manufacturing site for pharmaceuticals. The loss of the substance (L), resulting from the cleaning of process equipment, to the local WWTP (i.e., located in Mississauga), is estimated to be low, at 0.5% (L) of the total quantity produced,. The WWTP is a secondary system, has an effluent flow (F) of 160 244 m³/day and discharges to Lake Ontario. The scenario also assumes that the release occurs 21 days/year (N), the WWTP removes 83.5% (R) of the substance, as predicted by a model (ASTreat 2006), and the receiving water (Lake Ontario) dilution factor is 10 (D). Based on the above assumptions, the substance at a total industrial production quantity (Q) of approximately 320 kg/year yields an aquatic concentration of 7.9×10^{-6} mg/L in the aquatic receiving water near the discharge point of the wastewater treatment plant (Environment Canada 2013a).

Table 7a. Summary of input values used for estimating aquatic concentrations resulting from industrial releases of tamoxifen

Input	Value	Justification and reference
Quantity (kg/year)	320	Estimated quantity as prescribed at hospitals and pharmacies across Canada for the year 2012, as the most conservative quantity in comparison with estimates for years 2007 and 2011 (McLaughlin and Belknap 2008; IMS 2013)
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)]

Input	Value	Justification and reference
Wastewater system removal efficiency (%)	83.5	Based on estimation from ASTreat (2006)
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)	160 244	Effluent flow of a large wastewater treatment plant (that uses 2 ^{ry} treatment) located in Mississauga, Ontario (a typical Canadian pharmaceuticals manufacturing site, assumed to be located in Mississauga)
Receiving water dilution factor (dimensionless)	10	Environment Canada's default assumption for large lakes, the WWTP in the scenario discharges to Lake Ontario

8.2.2 Down-the-Drain Releases from Pharmaceutical Use

As tamoxifen can be released into water as a result of its prescribed use (i.e., patients ingesting and subsequently excreting the pharmaceutical substance), an aquatic exposure scenario resulting from down-the-drain releases was developed. It has been shown that in humans approximately 65% of the administered tamoxifen is excreted in feces (24.7%), bile (11.5%) and urine (26.7%) (Kisanga et al. 2005). Releases of tamoxifen metabolites, 4-HT and endoxifen, were also considered. Some quantities of tamoxifen can also be released down the drain as a result of tamoxifen use in laboratory research. Few data are available to confirm the exact quantities of tamoxifen used by research facilities. However, it is expected, based on the methodologies and concentrations described in research papers, that these use quantities are low, considerably lower than the quantities of tamoxifen in commerce for human consumption. Based on these considerations, the down-the-drain release scenario is limited to exposure from tamoxifen and its metabolites stemming from human pharmaceutical use.

A down-the-drain release from pharmaceutical use scenario was employed to estimate the tamoxifen concentration in multiple water bodies receiving wastewater treatment system effluents to which the substance may have been released (Environment Canada 2009). This calculation also includes consideration of the tamoxifen metabolites 4-HT and endoxifen.

The loss to wastewater resulting from the prescribed use of tamoxifen was assumed to be 100%. This assumption included losses due to the metabolized tamoxifen, i.e., its metabolites 4-HT and endoxifen, and unmetabolized tamoxifen. It is noted that model input parameters that affect model calculations, such as the wastewater removal efficiencies, were very similar for tamoxifen, 4-HT and endoxifen [i.e., 84% for tamoxifen, and more than 85% for both 4-HT and endoxifen (ASTreat 2006)]. Therefore, it was considered that capturing the percent loss of the unmetabolized tamoxifen and tamoxifen metabolites as 100% was a simplified and appropriate approach.

The realistic assumptions include:

- loss to sewer at 100% (i.e., includes unmetabolized tamoxifen and tamoxifen metabolites, 4-HT and endoxifen);
- WWTP removal rate estimated at 0.0% in case of no treatment, 55% for primary treatment and 84% for primary–secondary combined treatment;
- number of annual release days at 365 days/year;
- receiving water dilution factor in the range of 1–10.

The number of annual release days was assumed to be 365 to account for the variable use of the drug throughout the year as well as the variability between locations (i.e., hospitals where the drug is administered). As distribution of use across Canada is unknown, a variability factor of 2 was applied on every location to account for uneven distribution.

Given the above assumptions, the maximum PEC of tamoxifen in receiving water bodies was estimated to be 3.2×10^{-5} mg/L. The estimate is based on a total of 320 kg/year for the quantity of the substance used (estimated amount of tamoxifen purchased by hospitals and pharmacies for prescription pharmaceutical needs for the year 2012). The equation and inputs used to calculate the PEC are also described in Environment Canada (2013b).

Table 7b. Summary of input values used for estimating aquatic concentrations resulting from prescribed use of tamoxifen

Input	Value(s)	Justification and reference
Quantity (kg/year)	320	Estimated quantity as prescribed at hospitals and pharmacies across Canada for the year 2012, as the most conservative quantity in comparison with estimates for years 2007 and 2011 (McLaughlin and Belknap 2008; IMS 2013)

Input	Value(s)	Justification and reference
Loss to wastewater (%)	100% (includes tamoxifen, 4-HT and endoxifen)	It was determined that model parameters for tamoxifen, 4-HT- and endoxifen were very similar, therefore it was considered appropriate to capture % loss with one value.
Variability factor ^a	2	Default
Wastewater system removal efficiency (%)	84	Based on estimation from ASTreat (2006)
Number of annual release days (days/year)	365	Assumes that the drug is taken daily
Receiving water dilution factor (dimensionless)	1–10	Environment Canada Existing Substances default assumption

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

8.3 Characterization of Ecological Risk

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

Tamoxifen is a pharmaceutical approved for the market in Canada, and it also has applications as an investigative tool in research laboratories. Based on its uses, it has potential for dispersive releases into the Canadian environment. Once released into the environment, it will be found mainly in water in both the ionized and neutral forms. Upon ingestion, tamoxifen is metabolized to active metabolites, most notably 4-HT and endoxifen. Tamoxifen metabolites are expected to be excreted into water systems along with the parent compound. From the water compartment, tamoxifen and its metabolites may also partition to the sediments. Tamoxifen as well as its hydroxylated metabolites are expected to be persistent in water, soil and sediment. Tamoxifen, 4-HT and endoxifen have potential to harm aquatic organisms at low concentrations. They also have anti-estrogenic properties, and the hydroxylated metabolites are known to have a much greater affinity for estrogen receptor α than the parent compound tamoxifen.

A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The conservative industrial release scenario presented above yielded a PEC of 7.9×10^{-6} mg/L. A PNEC of 5.1×10^{-5} mg/L was derived based on the most sensitive, chronic experimental toxicity value, and dividing this value by an assessment factor of 10 that accounts for inter- and intra-species variability in sensitivity and the potential higher toxicity associated with the more potent anti-estrogenic properties of the tamoxifen metabolites. This resulted in a risk quotient (PEC/PNEC) of 0.15 for releases from industry. Therefore, harm to aquatic organisms is unlikely for industrial releases, even under conservative (protective) assumptions.

For exposure resulting from down-the-drain releases from pharmaceutical use, the PEC (the maximum PEC was determined to be 3.2×10^{-5} mg/L) did not exceed the PNEC (5.1×10^{-5} mg/L) at any site across Canada (Environment Canada 2010b). Therefore, based on the estimated number of receiving water bodies that will not be negatively affected by the use of tamoxifen, coupled with the magnitude of the risk quotient and the more realistic scenario run, it is proposed that tamoxifen is unlikely to cause harm to aquatic organisms from down-the-drain releases.

When tamoxifen is released into a water body, it may partition into suspended particulate matter and to bottom sediments, where sediment-dwelling organisms would be exposed to the substance. However, no environmental monitoring data or toxicity data specific to sediment-dwelling organisms are available for this substance. For this substance, a risk quotient based on exposure in sediment pore water may be calculated based on the aquatic compartment PEC and PNEC values presented above and used for sediment risk characterization. In the calculation, bottom sediment and its pore water are assumed to be in equilibrium with the overlying water, and benthic and pelagic organisms are assumed to have similar sensitivities to the substance. Therefore, the PEC and PNEC for sediment pore water are considered to be the same as for the aquatic compartment. This equilibrium approach would result in a risk quotient (PEC/PNEC) for the sediment compartment that is the same as for the aquatic compartment. Therefore, harm to sediment-dwelling organisms from tamoxifen and its metabolites, 4-HT and endoxifen, in Canada is unlikely.

Together, the information available suggests that there is low risk of harm to organisms or the broader integrity of the environment from these substances. It is therefore concluded that tamoxifen does not meet the criteria set out in 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 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 regarding tamoxifen use quantities in research laboratories and associated releases into the environment. The available information, including tamoxifen concentrations used for research purposes and quantity formats available for sale from chemical companies, is very limited and insufficient to derive a quantitative estimate that would help determine the importance of this source. For estimation of the releases stemming from potential manufacturing of tamoxifen in Canada, the proportion of the substance manufactured and released from each individual industrial facility is unknown. Therefore, it was conservatively assumed that all tamoxifen used in Canada was manufactured at a single location. Similarly, as the distribution of use across Canada is unknown, a variability factor of 2 was applied on every location in Mega Flush to account for uneven distribution. Due to the limited information regarding the environmental presence of tamoxifen metabolites and the difficulty in discerning their overall hazard contribution based on tamoxifen metabolism, characterization of exposure from tamoxifen metabolites was based on assumptions of no metabolism of tamoxifen and incorporated a PNEC value that accounted for the known increased endocrine potency of these substances.

The partitioning and physical and chemical property models cannot address the potential for tamoxifen to ionize in the aquatic environment or the potential for binding in soil from electrostatic interactions (cation exchange) or binding to clays which are negatively surface charged. Therefore, these model predictions likely do not represent the properties and environmental behaviour of tamoxifen both as an ionized and neutral compound.

The bioaccumulation assessment is limited by the absence of empirical bioaccumulation data. Modelled bioaccumulation and bioconcentration factors were derived and all predictions using models have some degree of error. There is some uncertainty, as tamoxifen may not be in their training sets; many of the structural classes of pharmaceuticals are not amenable to model prediction because they are considered “out of the model domain of applicability” (e.g., structural and water solubility domains). In addition, there is concern that the lack of metabolic transformation data for tamoxifen may provide results that could be interpreted as a false positive.

Also, regarding ecotoxicity, based on the predicted partitioning behaviour of this chemical, the significance of sediment as an important medium of exposure is not well addressed by the available effects data. 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

Tamoxifen has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC 1996, 2012) and as a known human carcinogen by the National Toxicology Program in the United States (NTP 2011).

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

Releases of tamoxifen 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 7.9×10^{-6} mg/L (7.9 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 drug 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 the 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 these pharmaceuticals through drinking water.

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

For this assessment, conservative assumptions were used when estimating the potential indirect exposure of humans to tamoxifen. 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 100% of the pharmaceutical that was purchased by hospitals and pharmacies was prescribed and administered to patients and excreted into wastewater after administration (i.e., no absorption or metabolism of the drug). It was also assumed that removal of tamoxifen during wastewater treatment was dependent on the treatment process applied (see above section on down-the-drain releases).

This scenario estimates concentrations in approximately 1000 waterways across Canada. The highest values estimated by this model are typically in small waterways with low dilution capacity, which are unlikely sources of drinking water. As a result, this scenario would be expected to highly overestimate actual concentrations in drinking water. The maximum PEC was 3.2×10^{-5} mg/L (32 ng/L).

Limited measured concentration data for tamoxifen were identified in Canada and other countries of the world and are summarized in the “Measured Environmental Concentrations” section above. Concentrations measured in wastewater effluent, surface water, groundwater and drinking water were examined when available. Overall, the studies indicated that the amount of pharmaceutical measured decreases significantly as the substance moves from the wastewater treatment plant and is released into surface water. As there is variability in the use of pharmaceuticals in different countries (due to different population levels, prescription preferences, drug registrations, etc.), the measured concentrations in other countries are not necessarily representative of concentrations in Canadian waters. They do, however, account for releases from all potential sources and for potential reductions in drug concentrations resulting from metabolism, environmental degradation, removal via wastewater, drinking water treatment, etc. For these reasons, measured concentrations are preferable to modelled concentrations for characterizing human exposure, even if measurements were taken in other countries.

Limited measured concentration data for tamoxifen were identified. In a study of tamoxifen in samples collected from a variety of wastewater treatment plants representing typical Canadian treatment systems and geographic variations, tamoxifen was detected in the range of 1.30×10^{-6} – 1.73×10^{-6} mg/L (1.30–1.73 ng/L) in effluent samples (Teslic and Smyth 2013). 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 tamoxifen 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 of tamoxifen, based on the maximum value detected in samples of wastewater effluent of 1.73×10^{-6} mg/L (1.7 ng/L), is 0.18 ng/kg bw per day. Based on the modelled concentration of 32 ng/L in surface water, the estimated intake would be 3.4 ng/kg bw per day.

Given the low levels of estimated exposure, the potential risk of indirect exposure to tamoxifen is expected to be low.

To further characterize potential risks associated with the intake of tamoxifen via drinking water, the lowest therapeutic dose (LTD) for tamoxifen 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 tamoxifen products currently registered for use in Canada by humans are all tablets for oral ingestion (DPD 2010). Dosage information for these products indicates a recommended dose of 20–40 mg/day (Pharmascience Inc. 2003; Pharmel Inc. 2003; Apotex Inc. 2004; Teva Canada Limited 2011; AstraZeneca Canada Inc. 2012; Mylan Pharmaceuticals ULC 2012). Using an adult body weight of 70.9 kg (Health Canada 1998) for conversion, an LTD of 20 mg/day is equivalent to a dose of 0.28 mg/kg bw per day.

MOEs were derived using the equation below:

$$\text{MOE} = \text{LTD}/\text{Intake}$$

where:

- MOE: Margin of exposure (dimensionless)
- LTD: Lowest therapeutic dose (mg/kg bw per day)
- Intake: Maximum estimated intake for drinking water derived from modelled or measured concentrations (mg/kg bw per day)

For tamoxifen, this results in an MOE >1 000 000, based on an intake calculated using the maximum value measures in effluent samples taken from wastewater effluent in Canada. The MOE calculated using the maximum modelled PEC would be >82 000. 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 tamoxifen are low.

10. Uncertainties in Evaluation of Risk to Human Health

There is uncertainty regarding the estimation of exposure due to the lack of representative measured concentrations of tamoxifen 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 to tamoxifen in Canadian drinking water would be lower than the exposures estimated using both the model and the maximum concentrations measured in surface water outside of Canada. This is supported by 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 concentration data from Canadian surface water and/or drinking water for this substance.

Potential exposures to tamoxifen 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.

Tamoxifen 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. These potential releases may be accounted for in the measured concentrations if they are occurring in the area of study.

It is recognized that the LTD represents an exposure level at which a desired pharmacological response is achieved and further that at this exposure level,

adverse effects, in addition to intended effects, may occur in some patients. For certain indications and certain classes of drugs, the nature of these unintended effects may be significant. However, the LTD is developed for patients who require treatment for a particular illness and therefore are likely to be more susceptible to potential effects than a healthy individual. Although the use of the LTD provides a tier 1 type of assessment that does not utilize all the toxicity data that may be available for the substance, the highly conservative exposure defaults that have been used still lead to significant MOEs 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.

11. Conclusion

Considering all 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 tamoxifen 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 information presented in this screening assessment, it is concluded that tamoxifen 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 tamoxifen does not meet any of the criteria set out in section 64 of CEPA 1999.

11.1.1 Considerations for Follow up

Monitoring of future use quantities of tamoxifen is considered important given its ecotoxicological and hazard properties. Estimates of use quantities between 2007 and 2012 indicate an increase in demand of approximately 20%. Although no ecological risk in the Canadian environment due to exposure to tamoxifen was determined, a rising demand may lead to increases in its use quantities and consequently to increases in environmental releases and exposure, potentially leading to ecological harm. It is noted that tamoxifen (CAS RN 10540-29-1) is listed on the DSL, while tamoxifen citrate (CAS RN 54965-24-1) is neither on the DSL nor Non-Domestic Substance List (NDSL). Tamoxifen citrate is the prevalent form available as a prescribed pharmaceutical; tamoxifen is the active

ingredient in the medicinal products. Options on how best to monitor changes in the use profile of this substance such as monitoring of international activities or surveillance of the Canadian marketplace will be investigated. Tamoxifen may be considered for inclusion in the Domestic Substances List inventory update initiative.

12. References

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