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Screening Assessment for the Challenge

Benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]- (Mitotane)

Chemical Abstracts Service Registry Number 53-19-0

Environment and Climate Change Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment on benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-, hereinafter referred to as mitotane. The Chemical Abstracts Service Registry Number (CAS RN[1]) for mitotane is 53-19-0. Mitotane was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA.

Mitotane is a substance that may be formed upon the degradation of dichlorodiphenyltrichloroethane (DDT) and may be present as residual in DDT and dicofol, two active ingredients formerly found in pest control products that are no longer registered for use in Canada. Residues of both DDT and dicofol may still be found in soil and sediment. Additionally, mitotane is registered for use in Canada as a chemotherapeutic agent for the treatment of adrenal cancer. The substance is not naturally occurring and is not manufactured in Canada. Between 100 and 1000 kg of mitotane were imported into Canada in 2005. Mitotane was not reported to be imported in 2006 above the reporting threshold of 100 kg nor used above the reporting threshold of 1000 kg. The pharmaceutical industry provided information that indicates that quantities imported and used in Canada is in the range of 100 to 1000 kg per year. Its use as a chemotherapeutic agent suggests that it could be released into the Canadian environment.

It is expected that most of the total quantity of mitotane currently present in the environment is the result of historical use of DDT and dicofol. Based on mitotane's chemotherapeutic use, a small amount of this substance may be released to wastewater treatment systems. Mitotane has low solubility in water, minimal volatility and a tendency to partition to particles and lipids of organisms because of its hydrophobic nature. For these reasons, mitotane may still be predominantly found in soil and, to a lesser extent, in sediment. It is expected to be present in small proportions in water and air.

Because of its physical and chemical properties, mitotane is not expected to degrade rapidly in the environment. It is expected to be persistent in air, water, soil and sediments. Mitotane also has the potential to bioaccumulate in aquatic organisms and may biomagnify in freshwater piscivorous food chains. Such accumulation is unlikely in birds or mammals, however, because of their

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enhanced metabolic capacities. The substance has been determined to meet both the persistence and bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, analogue and modelled aquatic toxicity data indicate that the substance is highly hazardous to aquatic organisms.

There are long-term risks associated with persistent and bioaccumulative substances that cannot, at present, be reliably predicted. Since accumulations of persistent and bioaccumulative substances may be widespread and are difficult to reverse, a conservative response to uncertainty is justified. However, it is acknowledged that the level of concern for a persistent and bioaccumulative substance is dependant on the rate and nature of releases to the environment.

While limited quantities of mitotane are consumed as a pharmaceutical in Canada, a certain proportion of this amount may be released through excretion at a limited small number of wastewater treatment sites. Comparison of estimated levels of mitotane in lakes and rivers near wastewater treatment system effluent discharge points, with a level expected to cause harm to sensitive aquatic organisms, indicates potential for ecological harm.

Given the empirical health effects data for mitotane, the adrenal glands are expected to be the target tissue for humans. Margins between conservative upper-bounding estimates of exposure to mitotane from environmental media and the lowest-observed-effect level in laboratory studies are considered adequate to address uncertainties in the health effects and exposure databases.

On the basis of the information available, it is concluded that mitotane meets the criteria under paragraph 64(a) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. However, mitotane does not meet the criteria under paragraph 64(b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends. It is also concluded that mitotane does not meet the criteria in paragraph 64(c) of CEPA 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.

On the basis of the information available, it is concluded that mitotane meets one or more of the criteria set out in section 64 of CEPA. Mitotane has been determined to meet the persistence and bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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1. Introduction

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

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

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

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

The substance, benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-, was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA (ECCC, HC [modified 2007]). The “Challenge” for this substance was published in the *Canada Gazette* on December 26, 2009 (Canada 2009a, 2009b). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. No submissions of information were received in response to the Challenge.

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

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

This screening assessment includes consideration of information on chemical properties, hazards, uses and exposure. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents and stakeholder research reports and from literature searches up to September 2010 for the ecological and health sections of the document. In February 2017, a rapid search of the literature did not identify any significant new information that could influence the outcome of this assessment. Key studies were critically evaluatedand modelling results may have been used to reach conclusions.

When available and relevant, information presented in hazard assessments from other jurisdictions was considered.

This screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment and Climate Change Canada and incorporates input from other programs within these departments. The ecological portions of this assessment have undergone external written peer review/consultation. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. No external comments were received on the draft screening assessment. The final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

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

[2] A determination of whether one or more of the criteria of section 64 of CEPA are met is based on 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 products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in *the Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarily, a conclusion based on the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

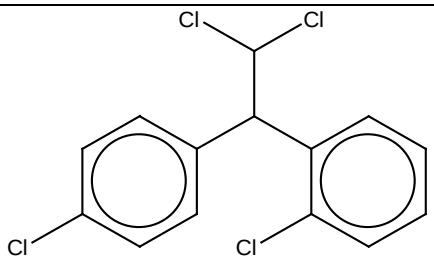
2. Substance identity

For the purposes of this document, this substance will be referred to as mitotane, its common name. Mitotane, also known as *o,p'*-DDD, can be both a residue in, and a degradation product of a component of the insecticide, dichlorodiphenyltrichloroethane (DDT). The components and their proportions in the DDT product are listed in Table 4-1. DDT has not been registered for use in Canada since 1985. DDT derivatives may also be present at low levels as residues in dicofol (a miticide) products.

The *p,p'*-DDD isomer (CAS RN 72-54-8; see Figure 3-1) is structurally similar to *o,p'*-DDD but is the substance more commonly measured in the environment and more commonly referred to in the literature, as the name DDD generally refers to the *p,p'*-DDD isomer.

Table 2-1. Substance identity for mitotane

Chemical Abstracts Service Registry Number (CAS RN)	**53-19-0**
DSL name[a]	**Benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-**
National Chemical Inventories (NCI) names[b]	*Benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-* (AICS, ASIA-PAC, NZIoC) *mitotane* (EINECS)
Other names	*o,p'-DDD; o,p'-TDE; Lysodren®; Mitotan; 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane; 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene; 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane; 2,2-bis(2-chlorophenyl-4-chlorophenyl)-1,1-dichloroethane; 2,4'-dichlorodiphenyl dichloroethane; Benzene, 1-chloro-2-(2,2-dichloro-1-(4-chlorophenyl)ethyl); CB 313; Chloditan; Chlodithane; Ethane, 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)-; NCI-C04933; NSC-38721; o,p'-Dichlorodiphenyldichloroethane*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Organohalides
Major chemical sub-class	Chlorophenyls
Chemical formula	$C_{14}H_{10}Cl_4$

Chemical structure	
SMILES[c]	ClC(Cl)C(c1ccc(Cl)cc1)c2ccccc2Cl
Molecular mass	320.05 g/mol

[a] DSL (Domestic Substances List)

[b] National Chemical Inventories (NCI). 2009: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); EINECS (European Inventory of Existing Commercial Chemical Substances); and NZIoC (New Zealand Inventory of Chemicals).

[c] Simplified Molecular Input Line Entry System.

3. Physical and chemical properties

Mitotane has two optically active forms called enantiomers, labelled as dextro (+) or laevo (-) (Konwick et al. 2006). This property of a substance is called chirality. Although the gross chemical properties of a chiral molecule are the same for both enantiomers, biological activity can be enantiomer-specific. This issue is discussed further in the bioaccumulation and ecological effects sections of this report.

Table 3-1 contains experimental and modelled physical and chemical properties of mitotane that are relevant to its environmental fate. Key studies from which experimental data were reported (i.e., water solubility and K_{ow}) were critically reviewed for validity.

Models based on quantitative structure-activity relationships (QSAR) were used to generate data for some of the physical and chemical properties of mitotane. These models (except for WSKOWWIN 2008) are mainly based on fragment addition methods, i.e., they rely on the structure of a chemical.

Because of the lack of empirical data for K_{OW}, bioaccumulation and ecotoxicity for mitotane, the isomer *p,p'*-DDD (CAS RN 72-54-8) (see Figure 3-1) was used as an analogue. The substance, *p,p'*-DDD, has been historically used as an insecticide but has not been registered for use in Canada since 1978. The only difference between the chemical structure of *p,p'*-DDD and mitotane is the location of one of the chlorine atoms on the benzene rings. Thus, because of their similar structures, EPI Suite (2008) models predict that both mitotane and *p,p'*-DDD have the same physical and chemical properties, persistence, bioaccumulation in fish, and aquatic toxicity, provided there is no additional input of experimental physical and chemical property data. Therefore, the slight difference in chemical structure should not affect the gross physical and chemical

properties such as those listed in Table 3-1. Evidence of this may be seen, for example, in the similarity of the experimental water solubilities for mitotane and *p,p′*-DDD, which are 0.1 mg/L and 0.09 mg/L, respectively (Biggar and Riggs 1974). The location of the chlorine atoms may, however, cause differences in bioaccumulation and toxicity in certain types of organisms, such as birds and mammals, as there is evidence that the two substances accumulate and metabolize differently in such organisms. The suitability of the analogue *p,p′*-DDD for toxicity and bioaccumulation is discussed, as appropriate, in the respective sections.

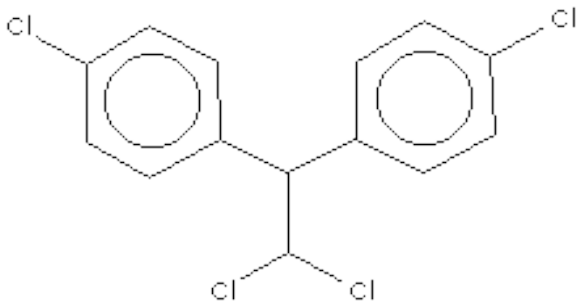


Figure 3-1. Structure of *p,p′*-DDD

Table 3-1. Physical and chemical properties for mitotane

Property	Type	Value[a]	Temperature (°C)	Reference
Melting point (ºC)	Experimental	77*	-	PhysProp 2006
Melting point (ºC)	Modelled	114.3	-	MPBPWIN 2008
Boiling point (ºC)	Modelled	366.75	-	MPBPWIN 2008
Density (kg/m^3)	No information available	No information available	No information available	No information available
Vapour pressure (Pa)	Modelled	2.253E-3 (1.69E-5 mm Hg)	25	MPBPWIN 2008
Vapour pressure (Pa)	Experimental	2.586E-4 (1.94E-6 mm Hg)	30	Suntio et al. 1988
Vapour pressure (Pa)	Experimental	2.51E-3*	25	Zhang et al. 2009
Henry's law constant ($Pa \cdot m^3/mol$)	Calculated[b]	8.035*	25	-
Henry's law constant ($atm \cdot m^3/mol$)	Calculated[b]	7.93E-5	25	-
Henry's law constant ($Pa \cdot m^3/mol$)	Modelled	4.40	25	HENRYWIN 2008
Henry's law constant ($Pa \cdot m^3/mol$)	Modelled	4.341E-5	25	HENRYWIN 2008
log K_{ow} (octanol-water partition	Modelled	5.87	-	KOWWIN 2008

Property	Type	Value[a]	Temperature (°C)	Reference
coefficient) (dimensionless)				
log K_{ow} (dimensionless)	Modelled	6.55	-	Karickhoff et al. 1991 (cited in Meador et al. 1997)
log K_{ow} (dimensionless)	Experimental (value is for mitotane's analogue, *p,p'*-DDD)	6.02	-	Sangster 1994 (cited in Physprop 2006)
log K_{ow} (dimensionless)	Experimental (value is for mitotane's analogue, *p,p'*-DDD)	6.22*	25	DeBruijn et al. 1989
log K_{ow} (dimensionless)	N/A	5.69	-	Hansch and Leo 1979 (cited in Gossett et al. 1983)
log K_{oc} (organic carbon-water partition coefficient) (dimensionless)	Modelled	5.19	-	ATSDR 2002
log K_{oc} (dimensionless)	Modelled (from K_{ow})	5.40*	-	KOCWIN 2008
log K_{oc} (dimensionles	Modelled (from MCI)	5.08	-	KOCWIN 2008

Property	Type	Value[a]	Temperature (°C)	Reference
s)				
Water solubility (mg/L)	Experimental	0.1*	25	Biggar and Riggs 1974
Water solubility (mg/L)	Modelled	0.1192	Not specified	WSKOWWIN 2008
Other solubilities (g/L)	Experimental (alcohol, isooctane, hexane and carbon tetrachloride)	"Soluble"	Not specified	USP 2008; O'Neil 2006
pK_a (acid dissociation constant) (dimensionless)	The substance is not ionizable under environmental conditions	Not applicable	Not applicable	Not applicable

[a] Values in parentheses represent the original ones as reported by the authors or as estimated by the models.
[b] Henry's law constant = vapour pressure/water solubility, both vapour pressure and water solubility are experimental values.
* indicates selected value for modelling.
'-' Data not available.

4. Sources

Mitotane is not reported to occur naturally in the environment.

Information was collected through a CEPA section 71 notice conducted for the 2005 calendar year (Canada 2006). The results indicated that mitotane was not manufactured in Canada. However, two companies imported mitotane into Canada in the 100 to 1000 kg/year range.

For the 2006 calendar year, information gathered through the CEPA section 71 notice indicated that mitotane was not manufactured, imported or used in Canada in a quantity above the reporting thresholds. One Canadian company did, however, identify itself as having a stakeholder interest in the substance (Canada 2009a).

In Canada, mitotane is a substance in products regulated under the *Food and Drugs Act* (Canada 1985b). *Domestic Substances List* (DSL) registration information—which is based on activity between January 1, 1984, and December 31, 1986—indicates that products were in commerce under this use. Mitotane was originally added to the DSL without accompanying information, such as quantity in commerce and number of notifiers (Environment Canada 1988). Mitotane is registered in Health Canada's Drug Product Database as an ingredient in a licensed pharmaceutical drug (DPD 2010). Details provided in the public comment period following publication of the draft assessment indicates that imported quantities are in the range of 100 to 1000 kg.

Historical use of the pesticides DDT and dicofol is another environmental source of mitotane. Mitotane may be present as a residue (0.1%) in the commercial pesticide product DDT (Table 4-1) (GdCh 1998). Mitotane is also a degradation product or metabolite of an isomer of DDT (*o,p'*-DDT) which is typically present in the range of 15% to 21% in the insecticide DDT (ATSDR 2002). The isomer of DDT, *o,p'*-DDT, is present at detectable levels in dicofol (HSDB 2010). DDT was once widely used as a broad-spectrum insecticide. In 1970, Canada restricted the use of the insecticide DDT, and registration was suspended in 1985 (CCME 1999, Canada 2002). In 1972, the United States excluded the use of DDT as the active ingredient of registered pesticide products. However, it is still in use in some countries (ATSDR 2002). DDT was found to be a harmful persistent organic pollutant (POP) by international institutions such as the United Nations Environment Programme (UNEP). The worldwide production of DDT has largely been reduced and is subject to international control under the Stockholm Convention on Persistent Organic Pollutants (UNEP 2001).

Table 4-1. Components of commercial DDT formulations, and their degradation products

DDT component	DDT formulation (%) (GdCh 1998)	Degradation products (IPCS 1989)
p,p′-DDT	77.1	*p,p′*-DDE (aerobic); *p,p′*-DDD (anaerobic)
o,p′-DDT	14.9	*o,p′*-DDE (aerobic); mitotane (anaerobic)
p,p′-DDE	4	*p,p′*-DDMU; 4-chlorobenzaldehyde
o,p′-DDE	0.1	Not available
p,p′-DDD	0.3	*p,p′*-DDMU
Mitotane (*o,p′*-DDD)	0.1	*o,p′*-DDA and hydroxylates

DDT and its derivatives can also be present in dicofol formulations as impurities (ATSDR 2002). The content of *o,p′*-DDT is generally < 0.1% of the total volume of the dicofol product (HSDB 2010), while mitotane (*o,p′*-DDD) was not detectable in dicofol formulations (Turgut et al. 2009; Qiu et al. 2005). Sales of dicofol were voluntarily discontinued in Canada in December 2008, and all uses of dicofol products expired on December 31, 2011. Dicofol products can no longer be legally sold or used in Canada (PMRA 2010).

Low levels of mitotane resulting from historical DDT and dicofol applications have the potential to still be present in the environment or to enter the Canadian environment through long-range transport (e.g., in air) from other countries (see Section 8.2).

5. Uses

In Canada, mitotane is registered in Health Canada's Drug Product Database (DPD) as an ingredient in a licensed pharmaceutical drug (DPD 2010). The drug containing this substance as an ingredient was previously assessed under the *Food and Drugs Act* (FDA) with respect to its safety, effectiveness and quality. The assessmentAssessment related to the pharmaceutical use of mitotane in this report focused on environmental exposures that were not covered as part of the FDA assessment.

This prescription drug is an oral chemotherapeutic agent used in the treatment of cancer of the adrenal gland (ATSDR 2002; Canadian Cancer Society 2010; University of Michigan 2010). Specifically, mitotane is indicated in the treatment of inoperable, metastatic and recurrent adrenocortical cancers (Attivi, 2010; Bristol-Myers Squibb Company 2010). Most clinicians consider mitotane the drug of choice for the treatment of adrenocortical cancers (AHFS 2010). An estimated 93 kg, 100 kg and 60 kg were used in 2007, 2011 and 2012, respectively, in Canada (McLaughlin and Belknap 2008, IMS 2013). Information from the

pharmaceutical industry indicates that actual use in Canada varies from year to year, but is generally in the range of 100 to 1000 kg per year. An average dose of at least 8 to 10 grams per day is recommended by the distributor, but the dose can range from 2 to 16 grams per day (Bristol-Myers Squibb Company 2010).

Mitotane is listed in the Natural Health Products Ingredients Database with a non-natural health product role because “listed in the Prescription Drug List as follows: Mitotane” (NHPID 2017). It is not listed in the Licensed Natural Health Products Database as being present in currently licensed natural health products (LNHPD 2017). Therefore, no current licensed natural health products contain this substance as a medicinal ingredient or as a non-medicinal ingredient (LNHPD 2010). Mitotane is not listed in the lists of permitted food additives as an approved food additive under the *Food and Drugs Act* (Canada 1978) and associated marketing authorizations (Health Canada 2013). It has not been identified as used and/or present in formulations of incidental additives or food packaging materials (personal communication from Food Directorate, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada, 2010; unreferenced).

In other countries, mitotane is reported to be used to treat Cushing’s syndrome (hyperadrenocorticism) in humans and dogs (ATSDR 2002; HSDB 2010).

6. Releases to the environment

No specific information on industrial releases of mitotane has been identified. No information was received from industrial importers/users of the substance in response to the CEPA section 71 survey for 2006 (Canada 2009a), and the substance is not reportable to the National Pollutant Release Inventory (NPRI 2006).

Mitotane does not occur naturally. Presence in the environment occurs as a result of either past application of the insecticide formulations DDT and dicofol (PMRA 2007) or its current use as a prescription drug.

Mitotane is a degradation product of *o,p'*-DDT, which is present at up to 21% in formulations of DDT. In dicofol, mitotane may be released through the degradation of *o,p'*-DDT, which is found in dicofol formulations at <1%. In Canada, these pesticides were used in agricultural areas and forests. The resulting releases of mitotane were predominantly to soils and, through spray drift, to air. When used as a prescription drug, mitotane is released to wastewater, and it is therefore expected to be present in sediments, surface water and biosolids.

Hence, as a degradation product of a component of both DDT and dicofol, mitotane from historical use is believed to be widely dispersed in the environment. A significantly larger proportion of mitotane present in the

environment is expected to be from the degradation of *o,p'*-DDT. However, concentrations near current points of discharge to water can be elevated as a result of recent drug use.

Mitotane is registered in Canada as a chemotherapeutic agent and is ingested in tablets. According to the product monograph, retrieved from the Drug Product Database (2010), mitotane is absorbed in the body or converted to a water-soluble metabolite. Consumer use of the drug product containing mitotane accounts for all mitotane releases of the drug product.

Unchanged mitotane has not been found in urine or bile (see information on metabolism of mitotane in health effects assessment section) (FDA 2009). An average dose of 8 to 10 grams per day is recommended by the distributor (Bristol-Myers Squibb Company 2010). The capacity of the intestine to dissolve that quantity of mitotane, as required for uptake, is limited. Since the information available on the fate and possible transformation of the unabsorbed product is limited and uncertain, it is conservatively assumed that 60% is excreted unchanged in the feces (Moy 1961).

Patients' response to mitotane is highly variable (Attivi 2010). Until a stable regimen is found (i.e., the maximum tolerable dose is reached), the patient remains in the hospital (Bristol-Myers Squibb Company 2010). The recommended mitotane plasma concentration (14 to 20 mg/L) is generally achieved after a period of 2 to 3 weeks (Moy 1961). The patient then returns home for the remainder of the treatment period, which ranges from 4 to 48 months (Hutter and Kayhoe 1966; Baudin et al. 2001; Terzolo, Angeli et al. 2007; Attivi 2010; Brunton et al. 2005, ASHP 2010).

After the excreted mitotane reaches the wastewater treatment systems, it will preferentially partition to sludge, which may be transformed to biosolids. Releases from biosolids that are applied to agricultural fields is a potential source of entry of mitotane to soils in Canada. However, this pathway results in disperse release and is expected to be negligible compared to the quantities of mitotane formed from ongoing *in situ o,p'*-DDT degradation.

7. Environmental fate

Because of its physical and chemical properties (Table 3-1) and on the basis of the results of Level III fugacity modelling (Table 7-1), mitotane is expected to predominantly reside in soil and sediment, depending on the compartment of release. Currently, the only release of mitotane to water is through wastewater, following its use as a prescription drug.

Table 7-1. Results of the Level III fugacity modelling (EQC 2003)

Substance released to:	Percentage of substance	Percentage of substance	Percentage of substance	Percentage of substance

	partitioning into air	partitioning into water	partitioning into soil	partitioning into sediment
Air (100%)	11.0	2.20	59.9	26.8
Water (100%)	0.16	7.50	0.87	91.5
Soil (100%)	0.00	0.01	99.9	0.11

These fugacity modelling results represent the partitioning of the substance in a hypothetical evaluative environment resulting from intermedia partitioning, and loss by both advective transport (i.e., out of the modelled region) and degradation/transformation processes. The partitioning values shown in Table 7-1 represent the net effect of these processes under conditions of continuous release when a non-equilibrium "steady-state" has been achieved.

If released to water, mitotane is expected to strongly adsorb to suspended solids and sediment because of its high estimated log K_{oc} value of ~5.09. Given this compound's estimated Henry's law constant, volatilization from water surfaces may occur. Nevertheless, if water is a receiving medium, mitotane is expected to partition mainly to sediment (Table 7-1).

Mitotane released to soil is expected to have high adsorptivity to soil (i.e., expected to be relatively immobile) because of its estimated log K_{oc}. Considering its Henry's law constant, volatilization from moist soil surfaces may occur. Its vapour pressure is such that it may also volatilize slightly from dry soil surfaces. However, wind erosion can contribute to mitotane loss from soil and transfer to air in an agricultural context (Natural Resources Canada 2010).

A small amount of the substance is expected to reside in air (see Table 7-1 above). Given its low experimental vapour pressure of 2.51×10^{-3} Pa and moderate Henry's law constant of 8.035 $Pa \cdot m^3/mol$, mitotane is considered to be minimally volatile. Therefore, if released solely to air, it will partition largely to soil and sediment (59.9% and 26.8%, respectively; Table 7-1), but the proportion that remains in the air is not negligible.

7.1 Environmental persistence

Table 7-2 presents empirical hydrolysis and biodegradation data for mitotane.

Table 7-2. Empirical data for degradation of mitotane

Medium	Fate process	Endpoint	Degradation endpoint / days	Reference
Water	Hydrolysis of DDD[a]	Half-life, pH 9 and 27°C	570 [b]	Wolfe et al. 1977
Water	Hydrolysis of DDD[a]	Half-life, pH 5 and 27°C	190 [c]	Wolfe et al. 1977

Medium	Fate process	Endpoint	Degradation endpoint / days	Reference
Sediments	Primary biodegradation, anaerobic	Half-life, concentration > 19.2 mg/kg dry weight (dw)	< 100	Huang et al. 2001
Sediments	Primary biodegradation, anaerobic	Half-life, concentrations 9.6–19.2 mg/kg dw	(persistent) > 250 days	Huang et al. 2001

[a] Includes both *o,p′*-DDD (mitotane) and *p,p′*-DDD.
[b] Estimate based on Cristol et al. (1952).
[c] Estimate based on Bensley and Kohnstam (1957).

Huang et al. (2001) studied anaerobic biodegradation of DDT and its metabolites in slurries of sediments and overlying site water (10% solids w/v) collected from the Keelung River, Taiwan. The presence of *p*,*p′*-DDT and its metabolite *p*,*p′*-DDD had been previously reported in sediment from this river, presumably as a result of deposition before the ban in the 1980s in China (Huang et al. 2001). An initial mitotane concentration of 39.5 µM—equivalent to 126.4 mg/kg dw of sediment—was reduced to 6 µM—equivalent to 19.2 mg/kg dw sediment—in 165 days after a lag phase of 25 days (Huang et al. 2001). On subsequent addition of mitotane (134.4 mg/kg dw), the removal rate increased. However, mitotane remained persistent after it had fallen to 19.2 mg/kg dw. There were no observable changes of mitotane in sterile controls during the sampling period. The authors determined the initial removal rate of mitotane to be 1.33 mg/kg dw/day. The degradation products of mitotane were not reported in this study.

Huang et al. (2001) also found a pH-dependence for the rates of *p*,*p′*-DDT dechlorination (see Table 4-1) and the rates of formation and further transformation of *p*,*p′*-DDD (i.e., analogue for mitotane in this assessment). Although the pH-dependence of mitotane transformation was not discussed, the degradation pathway of the *p,p′*-isomers is similar to that of the *o,p′*-isomers, and pH-dependence may therefore be comparable. The highest dechlorination activity for *p,p′*-DDD was observed at pH value of 6.7, whereas little or no transformation occurred at pH values of 5.9 and 9.0.

Huang et al. (2001) found that at higher concentrations (i.e., > 19.2 mg/kg dw), the primary transformation half-lives of the *o*,*p′*- and *p*,*p′*- isomers of DDT, DDD and DDE were less than 100 days, but there was a lag phase for the transformation of mitotane (*o,p′*-DDD) that was not observed for any of the other DDT residues studied. Huang et al. (2001) also found that all of the isomers of DDD and DDE were persistent at concentrations below 9.6 to 19.2 mg/kg dw. This observation may help to explain why environmental levels of DDT and its metabolites have generally declined since the 1970s but still remain ubiquitous at

concentrations ranging from 1 to 100 µg/kg in soil and sediment throughout the United States and around the world (Table 8-7, 8-10).

Kurt-Karakus et al. (2006) and Meijer et al. (2003) measured concentrations of DDT residues, including mitotane, in agricultural soil at the University of Guelph Muck Crops Research Station, Holland Marsh, Ontario, although it is not known whether the soil samples in the two studies were collected from the same field. In the two studies, mitotane concentrations in soil were 400 and 1200 µg/kg dw, respectively, and *o,p'*-DDT concentrations were 3200 and 3000 µg/kg dw, respectively. The date of the last DDT application at that location is not known, but was probably close to the time that DDT use in Canada was phased out in the mid-1970s (Kurt-Karakus et al. 2006). This demonstrates the potential for persistence of mitotane and/or *o,p'*-DDT in soil, as they were present in relatively high concentrations approximately 30 years after the last DDT application. Huang et al. (2001) determined degradation rates of *o,p'*-DDT and mitotane in anoxic slurries of sediment from the Keelung River in Taiwan. Mitotane was degraded 8.3 times slower than *p,p'*-DDT and 5.5 times slower than *o,p'*-DDT in anoxic sediments.

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

Table 7-3. Modelled data for degradation of mitotane

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008[a]	$t_{1/2}$ = 2.5 days	>= 2
Ozone reaction	AOPWIN 2008[a]	N/A[b]	N/A
Hydrolysis	HYDROWIN 2008[a]	N/A[b]	N/A
Primary biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 4: Expert Survey (qualitative results)	2.8[c] “weeks”	<= 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 3: Expert Survey (qualitative results)	1.7[c] “biodegrades very slowly”	>= 182
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 5: MITI linear probability	-0.22[d] “biodegrades very slowly”	>= 182

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Ultimate biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 6: MITI non-linear probability	0.0007[d] "biodegrades very slowly"	>= 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 Probability	0.003[d] "biodegrades very slowly"	>= 182
Ultimate biodegradation (aerobic)	CATABOL c2004-2008 % BOD (biological oxygen demand)	% BOD = 0.003 "biodegrades very slowly"	>= 182

[a] EPI Suite (2008)
[b] N/A (not applicable) – 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.

The BIOWIN (2008) model results are considered to be reliable, as the relevant structural features of mitotane are included in the structural fragment library of this model (i.e., aliphatic and aromatic chloride, alkyl substituent on aromatic ring). As well, mitotane is within the model structural domain of CATABOL (c2004-2008) (structural match = 94%) and within the optimum prediction space of TOPKAT (2004), with all structural fragments covered.

In air, a predicted atmospheric oxidation half-life value of 2.5 days (Table 7-3) demonstrates that this substance is likely to be slowly oxidized, and it is therefore considered to be persistent in air (i.e., half-life > 2 days). Mitotane is not expected to degrade via direct photolysis.

In water, empirical hydrolysis half-life values of 190 to 570 days, depending upon the pH (Table 7-2), demonstrate that this substance is likely to be slowly hydrolyzed. However, other fate processes in water, such as biodegradation, need to be considered to determine overall persistence in this medium.

All of the ultimate biodegradation models (i.e., for which degradation leads to complete mineralization where final products are CO_2, H_2O and, generally, elements of the periodic table, Table 7-3) are in agreement that mitotane biodegrades very slowly and is therefore considered to be persistent in water, even if primary biodegradation may occur more rapidly.

Mitotane also contains structural features associated with chemicals that are not easily biodegraded (e.g., aliphatic Cl, double-ringed aromatic structure with Cl, and log K_{ow} > 2.2) (Arnot et al. 2009). Therefore, considering all model and

empirical results and structural features, there is reliable evidence to suggest that the ultimate biodegradation half-life of mitotane is > 182 days in water.

Using an extrapolation ratio of 1:1:4 for water:soil:sediment biodegradation half-life (Boethling et al. 1995), the ultimate degradation half-life in soil is also > 182 days and the half-life in sediments is > 365 days. This indicates that mitotane is expected to be persistent in aerobic soil and sediment.

There is some evidence that mitotane may undergo long-range atmospheric transport (LRAT) to remote regions. Concentrations of mitotane (*o,p'*-DDD) have been measured in Arctic air in Canada and Norway. At Svalbard, Norway, mitotane concentrations in air were 0.15 and 0.02 pg/m^3 in the summers of 2004 and 2005, respectively (Becker et al. 2009). At the Alert research station in Nunavut, Canada, air concentrations of mitotane ranged from 0.09 to 1.15 pg/m^3 in 2006–2007 (Hung et al. 2010; personal communication, emails from Air Quality Research, Environment Canada, to Ecological Assessment Division, Environment Canada, dated August 27, 2010; unreferenced). Mitotane has also been found in Arctic fish species (Berg et al. 1997; Table 8-11). However, it is likely that the long-range transport of *o,p'*-DDT released from soil (by wind erosion) has contributed to the presence of mitotane in the Arctic, since a portion of the *o,p'*-DDT in air that reaches the Arctic will eventually degrade, and mitotane will be formed as a degradation product under anaerobic conditions.

The Transport and Persistence Level III Model (TaPL3) (TaPL3 2000) was used to estimate the characteristic travel distance (CTD), defined as the maximum distance traveled in air by 63% of the substance. Beyer et al. (2000) have proposed CTDs of > 2000 km as representing high long-range atmospheric transport potential (LRATP), 700 to 2000 km as moderate LRATP, and < 700 km as low LRATP. On the basis of the CTD estimate of 1156 km, the long-range atmospheric transport potential of mitotane is considered to be moderate. This means that mitotane may be transported through the atmosphere to areas moderately far from its emission sources.

The OECD POP screening model can also be used to help identify chemicals with high persistence and long-range transport potential (Scheringer et al. 2006). The OECD model is a global model that compartmentalizes the earth into air, water and soil. This model is “transport-oriented” rather than “target-oriented” as it simply identifies the CTD without indicating specifically where a substance may be transported (Fenner et al. 2005). Klasmeier et al. (2006) have suggested that a threshold of 5098 km, derived on the basis of the model’s CTD estimate for PCB-180, can be used to identify substances with high long-range transport potential. PCB-180 is empirically known to be found in remote regions. The CTD calculated for mitotane using the OECD model is 1047 km, indicating that mitotane does not have a high potential for transport in air, as this is below the boundary suggested for global pollutants by Klasmeier et al. (2006). The OECD POP screening model also calculates the transfer efficiency (TE), which is the

percentage of emission flux to air that is deposited to the surface (water and soil) in a remote region (TE % = D/E x 100, where E is the emission flux to air and D = the deposition flux to surface media in a target region). The TE for mitotane was calculated to be 0.000084%, which is below the boundary of 0.00065% (PCB-28) established on the basis of the model's reference substances empirically known to be deposited from air to soil or water. The low TE means that although mitotane has the potential to travel some distance in the atmosphere, it is unlikely to be deposited to Earth's surface in any remote region, according to the model.

Mitotane has been measured in Arctic air and wildlife as previously discussed. However, it is likely that much of the mitotane measured in the Arctic air is the result of the transformation of *o,p'*-DDT, since *o,p'*-DDT is much more abundant than mitotane in the atmosphere and *o,p'*-DDT has been shown to undergo LRAT to the Arctic (Wania 2006; Environment Canada 2006b). Furthermore, the LRATP models only rate mitotane as having moderate LRATP. Bidleman and Leone (2004) calculated *o,p'*-DDT fugacity in air. The fraction of *o,p'*-DDT in air from pesticidal uses of DDT was predicted to be 0.42, the mean value in air over soils was 0.49, and the mean value in in regional air was 0.43 to 0.45. It is reasonable to assume that long-range transport and deposition of *o,p'*-DDT followed by *in situ* degradation to mitotane is the only source of mitotane in ecosystems impacted solely by atmospheric deposition.

On the basis of the empirical and modelled data (Tables 7-2 and 7-3), it is concluded that mitotane is persistent in all media: air, water, soil and sediment.

7.2 Potential for bioaccumulation

7.2.1 Empirical measures of bioaccumulation

As noted previously, because of their similar structures, *o,p'*-DDD (mitotane) and *p,p'*-DDD are expected to generally have similar physical and chemical properties. However, a slight difference in their chemical structures is expected to result in different bioaccumulation potentials in some organisms. The cytochrome P450 enzymes that are responsible for the metabolism of many different classes of xenobiotic compounds in higher organisms (CYP2B) may not be found in most aquatic organisms (Norstrom 1997). In the case of non-fused ring aromatic compounds, the major requirement for ease of metabolism by such enzymes is the presence of unsubstituted *meta-para* positions on a ring structure (Norstrom 1997). This structural feature is present in one ring of *o,p'*-DDD, but not in *p,p'*-DDD. Thus, terrestrial mammals and birds are expected to readily metabolize absorbed *o,p'*-DDD, reducing its bioaccumulation potential in such organisms relative to that of *p,p'*-DDD. However, since CYP2B enzymes appear to be absent in fish and invertebrates, *o,p'*-DDD and *p,p'*-DDD are expected to have similar bioaccumulation potentials in aquatic organisms.

Table 7-4 presents empirical bioaccumulation values for the analogue substance *p,p'*-DDD in fish and aquatic invertebrates.

Table 7-4. Empirical BAF and BCF data for a mitotane analogue (*p,p'*-DDD)

Test organism, location	Endpoint	Value (L/kg wet weight)	Reference
Fish (composite sample), Lake Ontario	BAF (*p,p'*-DDD)	892 473	Oliver and Niimi 1988
Hyallela azteca (amphipod)	BCF (*p,p'*-DDD)	16 720	Lotufo et al. 2000
Diporeia spp. (amphipod)	BCF (*p,p'*-DDD)	436 000	Lotufo et al. 2000

BAF = bioaccumulation factor.
BCF = bioconcentration factor.

Lotufo et al. (2000) conducted BCF studies with freshwater amphipods and *p,p'*-DDD. *Hyallela azteca* was exposed to 0.178 µg/L *p,p'*-DDD for 10 days, and *Diporeia* spp. were exposed to 0.174 µg/L for 28 days. For *H. azteca*, the authors determined by visual inspection of the uptake curves and on the basis of the fact that the exposure time corresponded to at least three elimination half-lives by this organism, that the organisms were either close to attaining or had attained steady state during this exposure time. On the other hand, *Diporeia* spp. was not close to attaining steady state during the study, as the authors calculated that this would take approximately 623 days. The BCF presented in Table 7-4 for *Diporeia* spp. was modified to take this into account and is, therefore, the calculated steady-state BCF.

Lotufo et al. (2000) attributed some of the differences in the bioaccumulation of *p,p'*-DDD between *H. azteca* and *Diporeia* spp. to factors such as the temperature at which the experiments were carried out (room temperature for *H. azteca* and 4°C for *Diporeia* spp.), the organism size, and the lipid content of the two organisms.

Lotufo et al. (2000) determined that *p,p'*-DDD was not biotransformed during a 28-day exposure period with *Diporeia* spp. or a 24-hour exposure period with *H. azteca*, since after these time periods, the fraction of ^{14}C activity in the organism tissues corresponding to this compound was greater than 98%.

As noted previously, mitotane has two optically active forms called enantiomers, labelled as dextro (+) or laevo (-). Although chemical properties are expected to be the same for both enantiomers, biological activity can be enantiomer-specific. For example, variable results were found among individual miniature pigs for enantiomer-selective metabolism of mitotane, suggesting that polymorphic factors affect the chiral aspects of metabolism (Cantillana et al. 2009). On the other hand, Konwick et al. (2006) did not observe any evidence of enantiomer-specific biotransformation of mitotane in rainbow trout; their observation was based on consistent enantiomeric fractions in the fish and its half-life falling on a

log K_{OW} to log $t_{½}$ relationship established for recalcitrant contaminants in fish (Fisk et al. 1998). Although data are limited, enantiomer specific metabolic activity for mitotane is thus not expected for aquatic organisms.

The high BAF of 892 473 L/kg (Table 7-4) and biomagnification factor (BMF) of 2.8 (Table 7-5) reported for mitotane by Oliver and Niimi (1988) and Konwick et al. (2006), respectively, should be considered as maximum values as the fish were also exposed to DDT. Uptake and metabolism of DDT probably contributed to the concentration of DDD in the fish.

Table 7-5. Other empirical bioaccumulation data for mitotane and *p,p′*-DDD

Test organism, location	Endpoint	Value[a]	Reference
Rainbow trout (*Oncorhynchus mykiss*), lab study	BMF (from food only; mitotane)	2.8	Konwick et al. 2006
Oligochaetes exposed to 3-400 µg *p,p′*-DDD/gram organic carbon	BSAF(*p,p′*-DDD)	0.17–3.8	Ingersoll et al. 2005
Amphipod *Rhepoxynius abronius*	BSAF(mitotane)	0.31 ± 0.10	Meador et al. 1997
Polychaete *Armandia brevis*	BSAF (mitotane)	2.4 ± 0.9	Meador et al. 1997
Bivalves (razor shell, carpet shell, mussel and oyster)	BSAF(mitotane)	13.9–41.0	Thompson et al. 1999

[a] Normalized to [lipid]/[organic carbon].
BMF = biomagnification factor
BSAF = biota-sediment accumulation factor

Ingersoll et al. (2005) conducted a 42-day invertebrate colonization study in a pond containing *p,p′*-DDD-spiked sediment trays with a range of *p,p′*-DDD concentrations (3 to 400 µg *p,p′*-DDD/g organic carbon (oc)). The authors measured the bioaccumulation of *p,p′*-DDD by oligochaetes colonizing the pond. Only the two highest concentrations (120 and 400 µg *p,p′*-DDD/g_{oc}) produced BSAFs greater than 1. Bioaccumulation of *p,p′*-DDD by the oligochaetes increased with increasing concentration of *p,p′*-DDD in the sediments. However, most of the concentrations tested are higher than what is found in most environmental samples. For example, the highest concentration of mitotane found in sediments in the Atlantic provinces was 0.083 µg/kg dry wt (Table 8-10).

Meador et al. (1997) conducted a 10-day BSAF study with the saltwater species amphipod *Rhepoxynius abronius* and the polychaete *Armandia brevis*, collected off the coast of Washington state and exposed to sediments taken from 7 different sites selected over a large geographical area of the Hudson-Raritan estuary in New York. The sediments from these sites contained varying concentrations of mitotane, ranging from approximately 0.6 to 80 µg/kg dry wt.

The BSAF values in Table 7-5 are mean values, as the values obtained at each site are not given. The 10-day uptake period was considered to have been sufficient for *A. brevis* to reach steady state; however, *R. abronius* was probably far from steady state (Meador et al. 1997), indicating that the BSAF value for this species may be even higher at steady state.

Thompson et al. (1999) compared concentrations of *o,p'*-DDT residues and *p,p'*-DDT residues in four species of bivalves and sediment from an Atlantic Ocean bay on the coast of France. Interestingly, the sediment dwellers (razor shell, *Solen marginatus,* and carpet shell, *Ruditapes philippinarum*) accumulated primarily mitotane (50% to 60% of total residues), even though *p,p'*-DDD and *p,p'*-DDT dominated in sediment, which may suggest that *p,p'*-DDD metabolism was rapid in these species. On the other hand, the fraction of mitotane to total DDD in mussels and oysters was similar to that in sediment, suggesting non-selective accumulation (or metabolism if it occurs) of DDE and DDD isomers. BSAFs reported in this study were 13.9 for sediment-living oysters, 26.5 for razor shells, 28.5 for mussels, 30.7 for carpet shells and 41.0 for water-living oysters.

7.2.2 Modelling of BCF and BAF

Estimates of the BCF and BAF for mitotane were generated using quantitative structure-activity relationship (QSAR) and kinetic mass balance models. The results of this modelling are presented in Table 7-6 below. All models, except for the BCF linear regression model, corrected for metabolism rate (rate constant k_M). Both the default no metabolism and metabolism-corrected values are listed. The CPOPs model calculates the rate of metabolism on the basis of the probability of Phase I and Phase II biotransformation reactions occurring according to the structure of mitotane and according to the transformation pathways library contained in the model (Dimitrov et al. 2005). The k_M estimated using this method is ~0.01 d^{-1}.

Mass-balance predictions of BCF and BAF were generated using BCFBAF model contained in EPIWIN v4.0, which is based on a three-trophic-level version of the mass-balance kinetic model developed by Arnot and Gobas (2003). Metabolic rate constants can be generated in the EPIWIN BCFBAF model using either a QSAR-based approach (Arnot et al. 2009) or using empirical k_M data contained in the training or validation data sets of the model (Arnot et al. 2008a; Arnot et al. 2008b). The k_M used in the BCFBAF model was generated using the Konwick et al. 2006 BMF study for *o,p'*-DDD. The recommended k_M for a 10-g fish at 15^oC according to this study is ~0.015 d^{-1} (depuration half-life of ~45 days). Predictions of BCF and BAF using the BCFBAF model are then normalized to the body weight (184 g) and temperature (10^oC) of a middle trophic level fish, which represents median exposure conditions in the Canadian environment. These are given in Table 7-6. The normalization routine is described in Arnot et al. (2008b). Finally, the default dietary assimilation efficiency of 49% was not corrected according to the alpha value of 42% ±3% reported in the Konwick et al. (2006)

BMF study as this would have little effect on the dietary uptake rate of mitotane and thus little impact on the calculated BAF.In all cases, mitotane is considered in all domains of all the models in Table 7-6. This includes the global parameter (log K_{ow} and molecular weight), metabolism (k_M), mechanistic (passive diffusion) and structural[3] (chemical structure) domains.

Table 7-5. Modelled data for bioaccumulation of mitotane (log K_{ow} =5.9)

Test organism	Model and model basis	Endpoint	Value wet weight (L/kg)	Reference
Fish	BCFBAF (linear regression)	BCF, no metabolism	3467	BCFBAF 2008
Fish	BCFBAF (middle trophic)	BCF, metabolism-corrected	11 376	BCFBAF 2008
Fish	BCFBAF (middle trophic)	BAF, metabolism-corrected	148 936	BCFBAF 2008
Fish	BCF (mitigating factors)	BCF metabolism-corrected	7413	CPOPs 2008

At a log K_{ow} of ~5.9, the predicted bioavailable fraction of mitotane in the water column according to the Arnot-Gobas mass-balance fish model is ~85%, which suggests that uptake from water via the gills is a very relevant exposure for mitotane. If the log K_{oc} of 5.4 is used, the predicted bioavailable fraction is even greater (~95%). These data suggest that BCF may be the most relevant metric for establishing the bioaccumulation potential of mitotane and that dietary routes of exposure will contribute less significantly to the overall body burden of mitotane in pelagic organisms. In summary, the majority of modelled BCF and BAF values (Table 7-6), as well as the empirical BAF and BMF values (Tables 7-4 and 7-5), indicate that mitotane is highly bioaccumulative.

The predicted BCF values suggest that mitotane is highly bioconcentrated via the gills from the bioavailable fraction in water. These results are consistent with BCF values observed in aquatic invertebrates. The above estimated values for BAF agree relatively well with the field measured BAFs for *p,p'*-DDD for Lake Ontario

[3] All structural features are represented in the structural fragment library (i.e., aliphatic chloride, aromatic chloride, alkyl substituent on an aromatic ring, aromatic CH, aromatic H, CH (linear), benzene).

(Oliver and Niimi 1988), suggesting that the BAF for mitotane is in the order of 1 x 10^5 when tissue concentrations are compared to water concentrations.

Arnot and Gobas (2006) critically evaluated available bioaccumulation data (BCF and BAF) for fish and other organisms. Part of their work was prompted by the Government of Canada's categorization effort in early 2000 and led to an empirical database of quality BCF and BAF values that Canada has used for categorization and for the Challenge (Arnot and Gobas 2003). In Arnot and Gobas (2006), at a log K_{ow} of 5.9 for mitotane and a metabolic transformation rate of ~0.01-0.02, the empirical distribution of "acceptable" fish BCF data shows that there are several chemicals with fish BCFs meeting the Canadian criteria of BAF or BCF ≥5000. Many of these are also halogenated organic compounds.

7.2.3 Conclusion

There is very good consistency between various lines of evidence to suggest that mitotane will bioaccumulate from both water and the dietary exposures in aquatic organisms and will also biomagnify in foodwebs. This is consistent with many halogenated organic compounds with low rates of biotransformation. The log K_{ow} and log K_{oc} indicate that there is potentially a significant bioavailable fraction of mitotane in natural waters and that uptake via the gills (which BCF tests measure) is therefore sufficient to expect that BCF is a relevant metric for assessing the bioaccumulation potential. The log K_{ow}, rate of metabolism and laboratory BCF of mitotane are comparable to those of other chemicals that have been empirically observed to bioconcentrate from water to factors exceeding 5000. In addition, the calculated and observed "slow" rates of biotransformation in fish suggest that this pathway of elimination will not be significant in fish and other aquatic organisms. Coupled with a relatively high rate of dietary assimilation efficiency, mitotane is expected to significantly accumulate in the tissues of aquatic organisms from dietary transfer from the gastrointestinal tract.

Therefore, considering the consistency of the above evidence and high measured and predicted BAFs and BCFs, it is concluded that mitotane has a high potential for bioaccumulation and meets the Canadian criteria of BAF or BCF ≥5000.

8. Potential to cause ecological harm

8.1 Ecological effects assessment

8.1.1 Water

Empirical and modelled aquatic toxicity data for mitotane itself are presented in Tables 8-1 and 8-2, respectively. Relatively few experimental data are available on gross or whole-body-level effects (e.g., abnormal development). However, a number of biochemical results are presented. Due to its adrenocortical drug use

in humans and dogs, most research on mitotane is focused on this area. These biochemical studies are not directly applicable on the organism scale, and the absence of quantitative data eliminates them from consideration for the development of a critical toxicity value for risk quotient analysis. Modelling results in Table 8-2 present the predicted effect of mitotane on growth and survival of aquatic organisms. Empirical whole-organism-level aquatic toxicity data are also available for *p,p'*-DDD, an analogue for mitotane (Table 8-3).

p,p'-DDD is considered an appropriate analogue for toxicity to aquatic organisms since, as noted in the bioaccumulation section, most aquatic organisms are expected to have limited capacities to metabolize both *p,p'*-DDD and o,*p'*-DDD. However, owing to o*,p'*-DDD's enhanced susceptibility to metabolic transformation in mammals and birds compared to that of *p,p'*-DDD, *p,p'*-DDD is not considered a suitable analogue for toxicity to birds or mammals.

Together, these results indicate that mitotane has high toxicity to aquatic organisms following short-term (acute) and long-term (chronic) exposure at relatively low concentrations (e.g., < 1 mg/L for acute toxicity).

Table 8-1. Empirical aquatic toxicity data for mitotane

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Mollusc: Purple sea urchin embryos *Strongylocentrotus purpuratus*	Abnormal development[a] , (96 hours)	EC_{50}[b]	0.0676	Roepke et al. 2005
Mollusc: Purple sea urchin embryos *Strongylocentrotus purpuratus*	Abnormal development[a] (51 hours)	LOEC[c]	0.005	Roepke et al. 2005
Fish: Rainbow trout *Oncorhynchus mykiss*	Cortisol stress response (14 days)	LOEC[c]	5 mg/kg-bw (injection)[d]	Benguira et al. 2002
Fish: Rainbow trout *Oncorhynchus mykiss* interrenal cells	Cell viability assay (1 hour)	EC_{50}[b]	123.37	Leblond and Hontela 1999
Fish: Rainbow trout *Oncorhynchus mykiss* interrenal cells	Cortisol secretion (1 hour)	EC_{50}[b]	41.60	Leblond and Hontela 1999

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish: Tilapia *Sarotherodon aureus* superfused interrenal tissue	Cortisol secretion (5 hours)	LOEC[c]	0.1	Ilan and Yaron 1980
Fish: Arctic char (*Salvelinus alpinus)*	Primary stress response (23 hours)	No statistical difference with control	75 mg (oral)	Jorgenson et al. 2001
Insect: Mosquito larvae	Mortality (48 hours)	LC_{50}[e]	0.015	Deonier et al. 1946

[a] Abnormal development included delayed, abnormal, elongated and hatched larvae.
[b] EC_{50}: The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
[c] LOEC: The lowest-observed-effect concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.
[d] bw: Body weight.

[e] LC_{50}: The concentration of a substance that is estimated to be lethal to 50% of the test organisms.

Table 8-2. Modelled toxicity data for mitotane

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 hours)	LC_{50}[a]	0.096	ECOSAR 2008
Fathead minnow	Acute (96 hours)	LC_{50}[a]	0.023	TOPKAT 2004
Fathead minnow	Acute (96 hours)	LC_{50}[a]	0.087[b]	CPOPs 2008
Fish	Chronic (30 days)	-	0.013	ECOSAR 2008
Daphnia	Acute (48 hours)	LC_{50}[a]	0.096	ECOSAR 2008
Daphnia	Acute (48 hours)	LC_{50}[a]	0.063	CPOPs 2008
Daphnia	Chronic[c]	- [e]	0.021	ECOSAR 2008
Green algae	Acute (96 hours)	EC_{50}[d]	0.246[b]	ECOSAR 2008
Green algae	Chronic	-	0.179[b]	ECOSAR 2008

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Earthworm	Chronic (14 days)	LC_{50}[a]	220.7[b]	ECOSAR 2008

[a] $LC_{50:}$ The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[b] Given that concentrations for both the toxicity and water solubility are often uncertain, only toxicity values that exceeded solubility estimates by up to a factor of 10 were considered to be acceptable.
[c] Time period not specified.
[d] $EC_{50:}$ The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
'-' Data not available.

Table 8-3. Empirical aquatic toxicity data for *p,p'*-DDD, an analogue for mitotane

Analogue	Test organism	Test type	Endpoint	Value (mg/L)	Reference
DDD[a]	Crustacean: *Daphnia magna*	Acute (26 hours)	LC_{50}[b]	0.0046	Frear and Boyd 1967
DDD[a]	Crustacean: *Hyalella azteca (in porewater)*	Chronic (10 days)	LC_{50}	0.00077	Lotufo et al. 2000
DDD[a]	Crustaceans: *Diporeia* spp.	Chronic (10 days)	LC_{50}	0.01168	Lotufo et al. 2000
DDD[a]	Crustaceans: *Diporeia* spp.	Chronic (10 days)	EC_{50}[c]	0.00287	Lotufo et al. 2000
DDD[a]	Crustaceans: *Diporeia* spp.	Chronic (28 days)	LC_{50}	0.00196	Lotufo et al. 2000
DDD[a]	Crustaceans: *Diporeia* spp.	Chronic (28 days)	EC_{50}	< 0.00090	Lotufo et al. 2000
p,p'-DDD	Crustacean: *Hyalella azteca*	Chronic (10 days)	LC_{50}	0.00019	Phipps et al. 1995
p,p'-DDD	Crustacean: *Hyalella azteca (in porewater)*	Chronic (10 days)	LC_{50}	0.00108	Hoke et al. 1994
p,p'-DDD	Crustacean: *Hyalella azteca*	Chronic (10 days)	LC_{50}	0.00019	Hoke et al. 1994
p,p'-DDD	Insect: Common malaria mosquito larvae *Anopheles quadrimaculatus*	Acute (48 hours)	LC_{95}[d]	0.0025	Deonier and Jones 1946
p,p'-DDD	Insect: Common malaria mosquito larvae *Anopheles quadrimaculatus*	Acute (48 hours)	LC_{100}[e]	0.005	Deonier and Jones 1946
p,p'-DDD	Insect: Midge *Chironomus*	Chronic (10 days)	LC_{50}	0.00018	Phipps et al. 1995

Analogue	Test organism	Test type	Endpoint	Value (mg/L)	Reference
	tentans				
p,p'-DDD	Insect: Flatworm *Phagocata gracilis*	Chronic (10 days)	LC_{50}	0.6	Bonner and Wells 1987
p,p'-DDD	Insect: Midge *Chironomus tentans*	Chronic (10 days)	LC_{50}	0.00034	Hoke et al. 1997
DDD[a]	Fish: Rainbow trout	Acute (96 hours)	LC_{50}	0.07	Mayer and Ellersieck 1986
DDD[a]	Fish: Fathead minnow	Acute (96 hours)	LC_{50}	4.4	Mayer and Ellersieck 1986
DDD[a]	Fish: Channel catfish	Acute (96 hours)	LC_{50}	1.5	Mayer and Ellersieck 1986
DDD[a]	Fish: Bluegill	Acute (96 hours)	LC_{50}	0.042	Mayer and Ellersieck 1986
DDD[a]	Fish: Largemouth bass	Acute (96 hours)	LC_{50}	0.042	Mayer and Ellersieck 1986
DDD[a]	Fish: Walleye	Acute (96 hours)	LC_{50}	0.014	Mayer and Ellersieck 1986

[a] Includes both mitotane and *p,p'*-DDD.
[b] LC_{50}: The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[c] EC_{50}: The concentration of a substance that is estimated to cause some effect (immobilization) on 50% of the test organisms.
[d] LC_{95}: The concentration of a substance that is estimated to be lethal to 95% of the test organisms.
[e] LC_{100}: The concentration of a substance that is estimated to be lethal to 100% of the test organisms.

While there is evidence that mitotane can affect steroid genesis and cortical secretion in fish, birds and mammals (Ilan and Yaron 1980; Brandt et al. 1992; Jönsson et al. 1993; Jönsson et al. 1994; Lund 1994; Leblond and Hontela 1999; Benguira et al. 2002; Lacroix and Hontela 2003), the most sensitive empirical endpoint for mitotane itself was the effect on abnormal growth in the purple sea urchin as described below.

The effects of mitotane on echinoderm physiology, reproduction and development were tested to determine if they could impede the normal development of two species of sea urchin larvae (*Strongylocentrotus purpuratus* and *Lyyechinus anamesus*) (Roepke et al. 2005). Mitotane was dissolved in dimethyl sulphoxide (DMSO) and added to the test vials, resulting in concentrations of 0.005, 0.05, and 0.1 mg/L. Following a 96-hour test, larvae were separated into five categories (normal, delayed, abnormal, elongated and

hatched), and were compared (using a microscope) to the standard morphology of the normal pluteus larvae in the seawater controls. Successful development to the pluteus larval stage, compared to control organisms, was used to define concentration-response relationships. The EC_{50} for mitotane was estimated to be 0.0676 mg/L in *S. purpuratus*; similar results were observed with *L. anamesus* (data not shown). Higher levels of mitotane resulted in increasing percentages of delayed and morphologically abnormal larvae, and at 0.16 mg/L, unhatched embryos constituted 30% of all embryos counted (Roepke et al. 2005).

With respect to the biochemical test results, the capacity to mount a neuroendocrine stress response is a fundamental characteristic of a healthy organism as it allows the organism to cope with stressful situations threatening homeostasis. Acute stress conditions activate the teleost hypothalamo-pituitary-adrenal (HPA) axis to release cortisol (Benguira et al. 2002). Resulting effects may include impaired growth and renal function as well as gonadosomatic effects. Impairment of the normal capacity to elevate plasma cortisol in response to acute stress has been observed in fish chronically exposed to some contaminants, including mitotane (Benguira et al. 2002).

Cortisol stress response was investigated in rainbow trout (*Oncorhynchus mykiss*) exposed to mitotane by inducing confinement stress in organisms (Leblond and Hontela 1999; Lacroix and Hontela 2003; Benguira et al. 2002). A dose-dependent decrease in plasma cortisol was evident on day 14 post injection, indicating that, unlike controls, fish treated with mitotane at 5 mg/kg had a reduced capacity to elevate plasma cortisol levels (Benguira et al. 2002). The effects of mitotane on cell viability and cortisol secretion in rainbow trout (*Oncorhynchus mykiss*) interrenal cells have also been examined using a 60-minute *in vitro* assay (Leblond and Hontela 1999). The LC_{50} and EC_{50} for mitotane were calculated to be 123.37 and 41.60 mg/L, respectively (Leblond and Hontela 1999).

Similar effects were observed by another group in the interrenal tissues of *Sarotherodon aureus* following both *in vivo* and *in vitro* exposures of mitotane. In this study, the inhibition of cortisol secretion by mitotane was observed *in vitro* in superfused interrenal cells at concentrations as low as 0.1 mg/L (Ilan and Yaron 1980). However, in another study that administered a 75-mg/kg oral dose of mitotane to Arctic char (*Salvelinus alpinus*), no effect on the physiological stress response (measured by post-stress plasma ACTH and cortisol levels) was observed 28 days after administration (Jorgensen et al. 2001).

The modelling data shown in Table 8-2 indicate that mitotane is predicted to have high acute and chronic toxicity to fish, *Daphnia* and algae. In ECOSAR (2008), mitotane was modelled as a neutral organic. Fish were indicated to be the most sensitive with a chronic effect value of 0.013 mg/L predicted for fish exposed for 30 days. The K_{ow} used for modelling was within the model's domain of applicability ($\log K_{ow} < 8.00$). In CPOPs (2008), mitotane was modelled as a

“base surface narcotic” and it was 89% within the structural domain of the model for the *Daphnia* prediction, and 94% in the structural domain for the fish prediction.

The effects of mitotane and its positional isomer, *p,p'*-DDD, on early fourth-instar mosquito (*Anopheles quadrimaculatus*) larvae were tested in acetone-water suspensions. Percent mortality was measured after 24 and 48 hours for concentrations between 0.0025 and 0.005 mg/L of mitotane (Deonier and Jones 1946). After 24 hours, exposure to 0.005, 0.0033 and 0.0025 mg/L mitotane resulted in 88%, 68% and 58% mortality in mosquito larvae, respectively. Similar toxicity effects are observed after 48 hours; 0.005, 0.0033 and 0.0025 mg/L mitotane resulted in 100%, 93% and 95% mortality of mosquito larvae, respectively. It is believed that the observed effects of *p,p'*-DDD on mosquito larvae may not be representative of mitotane toxicity. Therefore, although values are presented, they are not included in the quantitative assessment.

Aquatic toxicity of *p,p'*-DDD to *Chironomus tentans* was assessed in another water-only 10-day toxicity test (Phipps et al. 1995). The LC_{50} was estimated to be 0.00018 mg/L after adjusting for control mortality. Hoke et al. (1997) conducted a similar study with the same exposure period and aquatic organism as Phipps et al. (1995). Hoke et al. (1997) obtained a LC_{50} of 0.00034 mg/L. Bonner and Wells (1987) assessed the chronic aquatic toxicity of *p,p'*-DDD and other *p,p'*-DDT derivatives on flat worms (*Phagocata gracilis*). The 10-day LC_{50} value was 0.6 mg/L for *p,p'*-DDD. Interestingly, the toxicity value was lower than the same endpoint measured for *p,p'*-DDE (2.63 mg/L) and *p,p'*-DDT (3.98 mg/L).

The survival in *Daphnia magna* following exposure to DDD was tested at a 26-hour exposure. The median lethal concentration (LC_{50}) for DDD was measured to be 0.0046 mg/L (Frear and Boyd 1967). Aquatic toxicity of *p,p'*-DDD to *H. azteca* was assessed in another water-only 10-day toxicity test (Hoke et al. 1994). The LC_{50} was estimated to be 0.00019 mg/L after adjusting for control mortality. Phipps et al. (1995) achieved the same result in a similar toxicity test. Hoke et al. (1994) also measured the aquatic toxicity of *p,p'*-DDD to *Hyallela azteca* in a porewater 10-day toxicity test. The LC_{50} value obtained was 0.00108 mg/L. The chronic aquatic toxicity of DDD was tested for two species of amphipods, *Hyallela azteca* and *Diporeia* spp., at 10 days and 28 days for *Diporeia* and 10 days only for *Hyallela azteca* (Lotufo et al. 2000). The effect observed in amphipods was immobilization; death was assumed when organisms were completely immobile. The 10-day EC_{50} and LC_{50} values in *Diporeia* spp. were 0.00287 mg/L and 0.01168 mg/L, respectively. The *Hyallela azteca* 10-day test of Lutufo et al. (2000) in porewater resulted in a LC_{50} value of 0.00077 mg/L, slightly lower than the results obtained by Hoke et al. (1994) in a similar study. The 28-day EC_{50} was not conclusive, but the LC_{50} compared to a similar experiment conducted for 10 days clearly shows that exposure for additional time increases the effect on organisms. This study supports the assumption that mitotane may have significant long-term effects.

Mayer and Ellersieck (1986) conducted a series of acute toxicity measurements of DDD on six fish species (rainbow trout, fathead minnow, channel catfish, bluegill, largemouth bass and walleye). The 96-h LC_{50}s varied between 0.014 and 4.4 mg/L.

The chirality of the mitotane molecule, as discussed previously, indicated a potential for enantiomer-selective metabolism. Given that enantiomer-specific activity was not detected in rainbow trout (Konwick et al. 2006) and found only in mammals (Cantillana et al. 2009), Konwick et al. (2006) also found that depuration rates of mitotane and *p,p'*-DDD were also very similar. This finding suggest that the analogues have similar metabolic activity in fishes. A comparable metabolism may be an indicator of similar toxicity behaviour in aquatic organisms.

In summary, experimental and modelled data for mitotane and its analogue indicate that this substance is expected to cause acute and chronic harm to several species of aquatic organisms at low concentrations.

8.1.2 Other environmental compartments

No empirical data on the toxicity of mitotane in sediment to sediment-dwelling organisms were identified. However, empirical data for the analogue *p,p'*-DDD were available and are summarized below (Table 8-4).

8.1.2.1 Sediment toxicity

Table 8-4. Empirical data for sediment toxicity of the mitotane analogue DDD

Analogue	Test organism	Type of test	Endpoint	Value (µg/goc[c])	Reference
p,p'-DDD	Nematodes	Chronic (12 weeks)	IC_{25}[a]	260	Ingersoll et al. 2005
p,p'-DDD	Nematodes	Chronic (12 weeks)	IC_{50}[b]	400	Ingersoll et al. 2005
p,p'-DDD	Oligochaetes	Chronic (12 weeks)	IC_{25}	> 400	Ingersoll et al. 2005
p,p'-DDD	Oligochaetes	Chronic (12 weeks)	IC_{50}	> 400	Ingersoll et al. 2005
p,p'-DDD	Chironomids	Chronic (12 weeks)	IC_{25}	47	Ingersoll et al. 2005
p,p'-DDD	Chironomids	Chronic (12 weeks)	IC_{50}	85	Ingersoll et al. 2005

[a] IC_{25}: Concentration across DDD-spiked sediments inducing 25% inhibition in colonizing trays abundance.
[b] IC_{50}: Concentration across DDD-spiked sediments inducing 50% inhibition in colonizing trays abundance.
[c] goc: grams of organic carbon

Ingersoll et al. 2005 evaluated the abundance of several taxa of invertebrates in a 12-week colonization study. Sediment-spiked concentrations of *p,p'*-DDD (dissolved in acetone) ranged from 3.0 to 400 µg *p,p'*-DDD/goc (grams of organic carbon). Negative and solvent controls were included. No significant differences in the abundance of invertebrates were observed between the two controls. The abundance of nematodes was significantly reduced at 400 µg *p,p'*-DDD/goc, and chironomid numbers were significantly reduced at 120 and 400 µg *p,p'*-DDD/goc. The IC_{25} values ranged from 47 to > 400 µg *p,p'*-DDD/goc, and IC_{50} values ranged from 85 to > 400 µg *p,p'*-DDD/goc (Ingersoll et al. 2005). This study indicates that a close analogue of mitotane can affect the abundance of several aquatic invertebrate taxa exposed to contaminated sediments. Therefore, mitotane is expected to cause similar effects when present at similar concentrations in sediment (Table 8-4).

8.1.2.1.1 Terrestrial algal toxicity

In soil, empirical data for mitotane and the analogue *p,p'*-DDD were available (Table 8-5). Microalgae are used as surrogates for screening chemicals in aqueous toxicity assays because they are generally more sensitive than vascular plants (Chung et al. 2007). Algae live in humid soils as well as in the water environment (Wehr and Sheath 2002), and they make up an important part of the soil ecosystem (Chung et al. 2007). A 4-day solid-phase microalgal bioassay was developed to screen for the phytotoxic effects of DDD in the terrestrial ecosystem using three species of microalgae (*Selenastrum capricornutum*, *Chlorococcum hypnosporum* and soil microalgae *Chlorococcum meneghini*). The test medium was washed quartz sand. The effects of DDD on fluorescence values, algal cell density, and chlorophyll *a* concentrations were observed and the EC_{50} and EC_{10} values for each species were derived from dose-response relationships. *C. meneghini* was the algae species most sensitive to the effects of DDD on cell density (EC_{50} = 179 mg/kg), and chlorophyll *a* content (EC_{50} = 150 mg/kg). Cell density is directly related to the growth and reproduction of the algae. The cell density and the fluorescence results indicated that the efficiency of energy transfer from light harvesting protein to photosystem II was reduced under the influence of DDD. The results of these assays were compared to the US EPA standard seed germination/root elongation tests in *Lolium perenne.* The EC_{50} values with DDD for seed germination and root length were greater than 1000 mg/kg (Chung et al. 1997). Therefore, it was found that those microalgae assays were more sensitive.

Table 8-5. Empirical data for toxicity of mitotane and its analogue DDD to terrestrial organisms

Substance	Test organism	Type of test	Endpoint	Value (mg/kg)	Reference
DDD[a]	*Selenastrum capricornutum*	Chronic (4 days)	EC_{50}[b]	246–> 500	Chung et al. 2007
DDD[a]	*Selenastrum*	Chronic	EC_{10}[c]	0.77–> 6.5	Chung et al.

Substance	Test organism	Type of test	Endpoint	Value (mg/kg)	Reference
	capricornutum	(4 days)			2007
DDD[a]	*Chlorococcum hypnosporum*	Chronic (4 days)	EC_{50}	457– > 1000	Chung et al. 2007
DDD[a]	*Chlorococcum hypnosporum*	Chronic (4 days)	EC_{10}	57–> 1000	Chung et al. 2007
DDD[a]	*Chlorococcum meneghini*	Chronic (4 days)	EC_{50}	150–> 700	Chung et al. 2007
DDD[a]	*Chlorococcum meneghini*	Chronic (4 days)	EC_{10}	13–> 35	Chung et al. 2007
DDD[a]	*Lolium perenne*	Chronic (5 days)	EC_{50}	> 1000	Chung et al. 2007
DDD[a]	*Lolium perenne*	Chronic (5 days)	EC_{10}	114– > 1000	Chung et al. 2007

[a] Includes both *o,p'*-DDD and *p,p'*-DDD.
[b] EC50 – The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
[c] EC10 – The concentration of a substance that is estimated to cause some effect on 10% of the test organisms.

8.1.3 Toxicity conclusions

The acute and chronic toxicity tests for mitotane and its analogue, *p,p'*-DDD, with fish, molluscs, insects and crustaceans indicate a potential for effects to a variety of aquatic organisms at low concentrations. Insect larvae were the most sensitive class of aquatic organisms tested, with chronic effect concentrations as low as 0.00018 mg/L for midge larvae. This effect value of *p,p'*-DDD on insect larvae, although the most sensitive, was not considered to be suitable for risk quotient analysis, as there are indications that mitotane effect may differ from its analogue for insects. Crustaceans were a very sensitive organism class, with chronic effect concentration of 0.00019 mg/L for *Hyalella azteca*. Biochemical tests performed on fish indicate that adrenal cells and liver cells may be affected by mitotane at moderate concentrations.

8.2 Ecological exposure assessment

Data concerning concentrations of mitotane in the Canadian environment as well as in some other parts of the world have been identified (Tables 8-6 and 8-11). Given that many concentration data are available for mitotane, only results of more recent studies are presented, to represent current distributions in the environment.Most of the measured mitotane concentrations in sediment, air and soil are likely from historical pesticide use rather than the current prescription drug use.

Table 8-6. Concentrations of mitotane in air

Location	Year	Detection limit (pg/m^3)	Number of samples (% of samples over detection limit)	Concentration range (pg/m^3)	Median (pg/m^3)	Reference
Alert, Canada	1992–1995	0.3	154 (6.9%)	0.024–1.707	0.051	NatChem 2002
Alert, Canada	2002–2007	0.05-0.10	285 (14%)	d.l. – 1.599	0.061	Hung et al. 2010; Hayley Hung pers. comm., unreferenced
Burnt Island, Egbert and Point Petre, Canada	2003	N/A	Unknown (N/A)	d.l. – 1.354	0.248	NatChem 2002
Lethbridge, Lundbreck, Lacombe and Vegreville, Canada	1999–2000	50	68 (0%)	<d.l.	N/A	Kumar 2001
7 sites near Great Lakes, Canada	1996–2003	N/A	Unknown (N/A)	0.31–8.8 (gas phase)	(average) 2.08	Sun et al. 2006
Point Pelee, Canada	2004-2005	0.3-0.7	18 (N/A)	5 cm above soil: 25-360	(mean) 150	Kurt-Karakus et al. 2006
Point Pelee, Canada	2004-2005	0.3-0.7	18 (N/A)	20 cm above soil: 20-230	(mean) 120	Kurt-Karakus et al. 2006
Point Pelee, Canada	2004-2005	0.3-0.7	18 (N/A)	72 cm above soil: 10-110	(mean) 70	Kurt-Karakus et al. 2006

Location	Year	Detection limit (pg/m^3)	Number of samples (% of samples over detection limit)	Concentration range (pg/m^3)	Median (pg/m^3)	Reference
Point Pelee, Canada	2004-2005	0.3-0.7	18 (N/A)	200 cm above soil: 7-90	(mean) 40	Kurt-Karakus et al. 2006

N/A=not available

Table 8-7. Concentrations of mitotane in soil

Location	Year	Detection limit (µg/kg)	Number of samples	% of samples over detection limit	Concentration range (µg/kg)	Median (µg/kg)	Reference
Okanagan Valley and Fraser Valley, Canada	2000–2001	1	11	73	d.l.–250	36	Bidleman et al. 2006
Bradford/ Holland Marsh, Canada	2004–2005	1.1	Unknown	N/A	N/A	(mean) 400) (mean) 1200	Kurt-Karakus et al. 2006 Meijer et al. (2003
Point Pelee, Canada	1997–2006	0.58	275	N/A	d.l.–11400[a]	Geometric mean: 40[a]	Crowe and Smith 2007

[a] sum of *o,p'*-DDD and *p,p'*-DDD
N/A=not available

Table 8-8. Concentrations of mitotane in surface water

Location	Year	Detection limit	Number of samples	% of samples over detection limit	Concentration range (ng/L)	Median	Reference
Okanagan Valley and Fraser Valley, Canada	2003 – 2005	0.001 ng/L	27	37	d.l.– 0.2	N/A	Environment Canada 2009a
Johnson creek basin, Oregon, United States	1998 – 2002	0.001 µg/L	32	0	<d.l.	N/A	Tanner and Lee 2004

N/A=not available; dw=dry weight; d.l.=detection limit

Table 8-9. Concentrations of mitotane in precipitation

Location; year	Year	Detection limit	Number of samples	% of samples over detection limit	Concentration range	Median	Reference
Atlantic provinces, Canada	1995 – 2006	N/A	26	N/A	d.l.– 25.5 ng/L	8 ng/L	Environment Canada 2010

N/A=not available

Table 8-10. Concentrations of mitotane in sediment

Location	Year	Detection limit	Number of samples	% of samples over detection limit	Concentration range (µg/kg)	Median µg/kg)	Reference
Atlantic provinces, Canada	1995 – 2006	N/A	55	N/A	d.l.–0.08 dw	0.003	Environment Canada 2010
20 states sampled, United States	1992 – 1995	1 µg/kg dw	350	11%	d.l.–150 dw	N/A	Wong et al. 2000

dw=dry weight; d.l.=detection limit; N/A=not available

o,p'-DDT was not measured in sediments of the St. Lawrence River (Pham et al. 1993) but mitotane was detected. *o,p'*-DDT was present as a significant proportion of the DDT derivatives in the St. Lawrence River suspended particulate phase (Pham et al. 1993). It is expected that when *o,p'*-DDT settles on sediment, it is quickly degraded into mitotane. Garrison et al. (2000) demonstrated that *o,p'*-DDD is the major metabolite produced by degradation of *o,p'*-DDT in seawater sediments, while *o,p'*-DDE, the degradation product of *o,p'*-DDT in aerobic conditions (see Table 4-1), is present in a larger proportion in freshwater sediments (Wandiga et al. 2003).

In fact, for a large proportion of samples in all environmental compartments, mitotane is not detected. This could be due to high detection limits, especially in air. In addition, although there are an increasing number of countries restricting the use of DDT, there were no observed changes in atmospheric concentrations of mitotane between 1992–1995 (NatChem 2002) and 2002–2007 (Hung et al. 2010). Mitotane was detected in precipitation deposited in the Canadian Atlantic provinces where levels of mitotane were higher than those in surface water (see Tables 8-8 and 8-9). The atmosphere would seem to be an important vector of transportation for contamination in this area.

Relatively high mitotane concentrations were detected in soil in the intensive agricultural areas of the Okanagan Valley (British Columbia, Canada), Point Pelee (Ontario, Canada) and the Fraser Valley (British Columbia, Canada).

Data were found on mitotane and DDD isomer concentrations in aquatic organisms in Canada, the United States, and Greenland (Table 8-7). Concentrations of mitotane measured in organisms varied between 0.07 and 736 µg/kg wet weight (ww). The highest concentrations were generally found in the lipids of organisms.

Table 8-11. Concentrations of mitotane in biota

Organism	Location	Year	Tissue sampled	Number of samples	Concentration range (ww) µg/kg	Median (µg/kg)	Reference
Blue whale	St-Lawrence River, Canada	1992–1995	Whole (lipids)	Unknown	3.7–736[a]	130[a]	Trent University 2002
Common carp (*Cyprinus*	Las Vegas Bay, United States	1999–2000	Whole	129	5–122	7.9	USGS 2000

Organism	Location	Year	Tissue sampled	Number of samples	Concentration range (ww) µg/kg	Median (µg/kg)	Reference
carpio)							
Large mouth bass and Florida gar	Hillsboro Canal, South Florida, United States	1995	Whole	Unknown	5–34	N/A	USGS 2002
Fish (8 species)	Lake Michigan, United States	1990	Whole composite fishes	23	0.13–81.70	5.83	Giesy et al. 2004
Fish	20 states sampled, United States	1992–1995	Whole	231	5–360	N/A	Wong et al. 2000
Bivalve	20 states sampled, United States;	1992–1995	Whole	118	5–20	N/A	Wong et al. 2000
Salmon	Alaska, United States	2001–2002	Whole	Unknown	0.07–0.91	N/A	Alaska Department of Environmental Conservation 2010
Fish; Greenland halibut, smalleyed rabbit-fish, redfish, jelly wolf-fish, roughhead grenadier, black dogfish, blue hake, tusk	Davis Strait, West Greenland, Denmark	1992	Whole	Unknown	0.8-28	(mean) 7.5	Berg et al. 1997
Lake trout	Ontario	1999-	Fish	74	d.l.–	4.51	Personal

Organism	Location	Year	Tissue sampled	Number of samples	Concentration range (ww) µg/kg	Median (µg/kg)	Reference
	Lake, Michigan Lake, Huron Lake, Superior Lake, ON, Canada and United States	2000	composite		47.4		communication between the Ecological Assessment Division, Environment Canada, U.S. Environmental Protection Agency, Great Lakes National Program Office and Environment Canada, Canada Centre for Inland Waters, unreferenced[b]
Walleye	Erie Lake, ON, Canada and United States	1999-2000	Fish composite	13	d.l.–3.28	1.64	Personal communication between the Ecological Assessment Division, Environm

Organism	Location	Year	Tissue sampled	Number of samples	Concentration range (ww) µg/kg	Median (µg/kg)	Reference
							ent Canada, U.S. Environmental Protection Agency, Great Lakes National Program Office and Environment Canada, Canada Centre for Inland Waters, unreferenced[b]
Fish (13 species)	St-Lawrence River, QC, Canada	1995-1997	Whole	156	<1–26[a]	1.5[a]	Laliberté 2003

[a] sum of *o,p'*-DDD and *p,p'*-DDD.
[b] with the permission of the US Environmental Protection Agency, Great Lakes National Program Office, Chicago, Illinois, United States and Environment Canada, Canada Centre for Inland Waters, Burlington, Canada
d.l.=detection limit

8.2.1 Releases from use of mitotane as a prescription drug

Mitotane is present in a prescription drug and can be released to wastewater following human excretion of un-metabolizedunmetabolized mitotane. Mitotane is only partially removed from wastewater during treatment and, as such, it can be released to surface water as part of wastewater effluent. Concentrations of mitotane in surface water near discharge points of wastewater treatment systems were predicted to evaluate the risk of this substance to aquatic organisms. These predicted environmental concentrations (PECs) were calculated using the following equation:

$$PEC = (1000 \times Q \times L) \times (1 - R) / (F \times D)$$

where:

PEC: Predicted concentration of mitotane in surface water close to a point of discharge of a wastewater treatment system (mg/L)

1000: Conversion factor (mg/g)

Q: Mass of mitotane consumed daily by one patient (g/day)

L: Loss to wastewater (fraction)

R: Wastewater treatment system removal rate (fraction)

F: Wastewater treatment system effluent flow (L/day)

D: Receiving surface water dilution factor (dimensionless)

As the drug is prescribed to a limited number of patients in Canada, mitotane is expected to be released to only a few sites at any point in time. Given that the locations of the wastewater treatment systems receiving inputs of mitotane are unknown and since the number of patients using this drug across the country is low, the daily drug dose for a single patient (minus the metabolized and/or absorbed fraction) was used as the mass released to a given wastewater treatment system to identify the proportion of discharge points that would potentially show a risk if there was use and excretion of mitotane by one resident in that area. Predicted environmental concentrations for approximately 1000 discharge points for wastewater treatment system across Canada were calculated. The following inputs and assumptions were used:

- an average daily dose taken by patients of 9 grams (Bristol-Myers Squibb Company 2010);
- release to sewer of 60% of the daily dose (not absorbed and unmetabolized) recommended for a patient;
- wastewater treatment system removal rate estimated at 87% for aerated and facultative lagoons (estimated by STP-EX (2008)), 54.6% for primary only treatment (estimated by ASTreat 1.0 (2006)) and 68.1 % for primary-secondary combined treatment (estimated by SimpleTreat 3.0 (1997));
- receiving surface water dilution factor in the range of 1 to 10.

The treatment period ranges from 4 to 48 months (Hutter and Kayhoe 1966; Baudin et al. 2001; Terzolo,Angeli et al. 2007; Attivi 2010; Brunton et al. 2005; ASHP 2010). Although the prescription drug is administered for a variable period, it is estimated that the average treatment may last over the whole year. Between

100 and 1000 kg of mitotane is used in Canada in a year, and the number of patients using the drug annually is estimated to be between 30 and 304.

The predicted environmental concentrations (PECs) of mitotane in water bodies close to wastewater system discharge points were estimated to be in the range of 8.8E-5 to 0.25 mg/L. Additional details on how PECs were calculated are described in Environment Canada (2013).

8.3 Characterization of ecological risk

The approach taken in this ecological screening assessment was to examine relevant scientific and technical information and to develop conclusions using a weight-of-evidence approach and precaution as required under CEPA. Lines of evidence considered include information on sources and quantities of releases, persistence, bioaccumulation, ecotoxicity and results of risk quotient calculations.

8.3.1 Potential for releases

Up until the 1970s, DDT was applied in large quantities to land and sprayed in air in Canada. Under anaerobic conditions in soil, water and sediment, *o,p'*-DDT degrades to *o,p'*-DDD (mitotane). Results from fugacity modelling (Table 7-1) show that mitotane released to air and on land will ultimately reside in soil or sediment. Historical use of DDT and dicofol likely resulted in widespread, low concentrations in all environmental compartments. Ambient monitoring data from Tables 8-6 to 8-10 are deemed to result essentially from past historical use rather than current drug use. Mitotane was detected in biota for various species, suggesting environmental exposure to mitotane or one of its DDT parent compounds.

The current use of mitotane is as a chemotherapeutic agent. Given this use, mitotane may be released down-the-drain to water via wastewater treatment systems. Because of mitotane's low solubility, relatively low volatility and high log K_{oc}, mitotane released to the environment from usage as a prescription drug is expected to be found in water, in biosolids from wastewater treatment system sludge, and in sediments in proximity to local point source discharges.

Concentration of mitotane in the ambient environment (i.e., not close to wastewater treatment system discharge points) are expected to be largely the result of historical pesticide use rather than the ongoing pharmaceutical use of the substance.

8.3.2 Persistence

In sediments, the primary anaerobic degradation half-life of mitotane was estimated by Huang et al. (2001) to be > 250 days, after an initial period of relatively rapid removal. This is consistent with modelling results (Table 7-2). All

degradation models suggest that mitotane biodegrades very slowly in the aquatic compartment. Extrapolation to soils and sediment indicates that degradation is also very slow under aerobic conditions in those compartments. The available data thus support the conclusion that mitotane meets the criteria for persistence in air, water, sediment and soil as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Recent measurements of mitotane concentrations in different media that are likely associated with its historical use as a pesticide confirm its presence and persistence in the environment. There is also the possibility of long-range atmospheric transport as suggested by both modelling and empirical evidence of the presence of mitotane in the Canadian Arctic. However, this may also be due to transport of *o,p'*-DDT followed by subsequent degradation.

8.3.3 Bioaccumulation

Empirical bioconcentration and bioaccumulation factors were found for the mitotane analogue, *p,p'*-DDD. These data and modelled mitotane estimates indicate that mitotane is bioaccumulative in aquatic organisms. A biomagnification factor of nearly threefold in rainbow trout was calculated for mitotane showing that mitotane taken through food uptake is likely to be amplified through the piscivorous aquatic food chain, but likely not in birds and mammals. Furthermore, concentrations measured in higher-tier aquatic predators were generally higher than what was found in lower trophic level organisms (Table 8-7). BSAF values calculated for sediment-dwelling organisms were generally greater than 1, confirming that mitotane may bioaccumulate in sediment-dwelling organisms. Empirical BCF and BAF data for *p,p'*-DDD ranged from 16 720 to 892 473 L/kg, and modelled BCFs and BAFs ranged from 3467 to 148 936 L/kg. Therefore, mitotane meets the criteria for bioaccumulation potential (BCF or BAF $\geq$ 5000), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

8.3.4 Qualitative evaluation

There are special concerns with substances that are highly persistent, bioaccumulative and inherently toxic (i.e., PBiT). This is because the degree of exposure to substances such as mitotane, or *o,p*-DDD, cannot be easily quantified and remains uncertain. Persistent substances remain in the environment for long periods of time, increasing the probability and duration of exposure. This is of particular concern because releases of small amounts of persistent and bioaccumulative substances may lead to relatively high concentrations in organisms over time, which may result in effects not currently measured for mitotane (e.g., developmental toxicity in vertebrates). Some bioaccumulative and persistent substances may also biomagnify through the food chain, resulting in internal exposures for top predators. Since they are widespread, several different persistent and bioaccumulative substances may be

present simultaneously in the tissues of organisms, increasing the likelihood and potential severity of harm.

8.3.5 Risk quotient analysis

A risk quotient analysis integrating estimates of exposure with toxicity information was performed for surface water near sources of release.

The pharmaceutical use scenario yielded PECs ranging from 8.8E-5 to 0.25 mg/L (Environment Canada 2013). A predicted no-effect concentration (PNEC) was derived from the chronic toxicity value of 0.00019 mg/L as the most sensitive experimental value for crustaceans for the analogue *p,p'*-DDD for *Hyallela azteca*. A PNEC of 0.000019 mg/L is obtained by dividing the chronic toxicity value by an assessment factor of 10 to account for extrapolation from a laboratory LC_{50} to a predicted no-effects concentration for sensitive species in the field. Because of its persistent and bioaccumulative characteristics, the risk quotient calculations for mitotane may underestimate actual long-term risks associated with food-chain transfer.

On the basis of data for approximately 1000 wastewater treatment systems, it is predicted that the PECs for mitotane may exceed the PNEC near the point of wastewater discharge in about 81% of the receiving water bodies across Canada resulting from a single patient using mitotane (Environment Canada 2009b). However, it is recognized that there is a limited number of patients using mitotane in Canada at any time. Statistically, 81% of the areas where the estimated 30 to 304 patients reside may be at risk of potential ecological harm for aquatic organisms. Therefore, it is estimated that harm to aquatic organisms near the point of wastewater discharge could occur at 25 to 250 receiving water bodies annually through the use of mitotane as a prescription drug.

No environmental monitoring data specific to sediment-dwelling organisms and close to point sources of discharge are available for this substance. For mitotane, a risk quotient based on exposure in sediment porewater could not be calculated. Current evidence from spiked sediment tests for the analogue of mitotane suggests that sediment macroinvertebrates may be much less sensitive than pelagic or bentho-pelagic organisms, such as epibenthic crustaceans.

8.3.6 Summary

The overall presence of mitotane in the environment is largely due to historical pesticide use. However, given its reported use as a prescription drug, there is current release of this substance into the Canadian environment. Between 100 and 1000 kg of mitotane is used per year in Canada, 60% of which is estimated to be released to sewers. While these releases likely have a negligible influence on the overall environmental load of mitotane, PEC calculations indicate that levels in surface water receiving effluent from wastewater treatment systems

where patients release mitotane frequently exceed the predicted lowest effect levels for aquatic organisms. Furthermore, mitotane is highly persistent in soil, sediment, air and water. Once released in the environment, mitotane may be transported over moderate distances in air and has been detected in remote areas such as the Arctic, largely from transport of the parent compound, DDT, on suspended particles. While measured levels of mitotane in the environment from historical loading of DDT and dicofol are generally low, mitotane has a long residence time in the environment. Since the onset of effects in aquatic organisms occurs at very low concentrations, continued exposure to this substance remains of concern given its ability to readily accumulate in the tissues of organisms and be distributed over a wide geographical area. Continued exposure and accumulation of mitotane may result in chronic sublethal effects not currently measured in toxicity tests.

Comparison of no effect thresholds to predicted surface water concentrations close to discharge points of wastewater treatment systems suggests current use of mitotane as a pharmaceutical is expected to result in ecological harm. Historical use as a pesticide also presents an unquantifiable long-term exposure requiring consideration of additional precaution to account for the potential of unknown and potentially irreversible effects in organisms. Mitotane, therefore, has the potential to cause ecological harm at a wide range of spatial and temporal scales from different sources of release to the Canadian environment.

8.4 Uncertainties in evaluation of ecological risk

There is a lack of information on the sources of environmental concentrations of mitotane in Canada. The proportion of mitotane concentrations in water, sediment and biota in the ambient environment (Tables 8-6 and 8-7) resulting from its use as a prescription drug—as opposed to resulting from historical application of the pesticide DDT—is expected to be low. Given that use of the drug is localized, compared to wide-spread contamination from former use of DDT, the resulting concentrations near sources of wastewater discharge are expected to be higher as a result of current uses.

The bioaccumulation assessment is limited by the number of available empirical bioaccumulation data. This necessitated that an analogue and DDD (unknown proportion of mitotane and *p,p'*-DDD) be used. The suitability of *p,p'*-DDD as an analogue of mitotane for bioaccumulation was confirmed for invertebrates, fish and cetaceans, but was deemed unsuitable for terrestrial mammals and birds because of critical differences in the metabolization of the substances. Therefore, bioaccumulation of *p,p'*-DDD for mammals and birds was not considered in this assessment. Modelled bioaccumulation and bioconcentration factors were also derived and, although all predictions using models have some degree of error, the metabolism-corrected model outputs confirmed that mitotane, given its structural characteristics, can be expected to have a high bioaccumulation

potential. Empirical results for the analogue were in agreement with modelled data. This further confirms the validity of the modelled values for mitotane.

Regarding ecotoxicity, some of the evidence of harm relates to biochemical endpoints such as cell viability and endocrine effects. These effects add to the weight of evidence indicating that this substance has the potential to be highly hazardous. There are uncertainties regarding the representativeness of *p,p'*-DDD effects on organisms other than invertebrates and fish for mitotane. Mitotane has endocrine effects, while *p,p'*-DDD does not. Mitotane is metabolized differently by mammals and birds than the analogue. Consequently, data on the ecotoxicity of the analogue was not used for terrestrial organisms.

Uncertainties are also associated with the fraction of the substance that is released during use. Moy (1961) found that 60% of the drug was found unchanged in human feces. Although no study was found that disputed this finding, the method used by Moy (1961) to detect mitotane in feces is not highly specific to mitotane. These uncertainties were addressed by making conservative assumptions using the best information available (i.e., 60%). There is also high variability in the daily dose of mitotane used to treat adrenocortical carcinoma. The dose is dependent on the patient's response to the drug, the objective being to reach a plasma level of between 14 and 20 mg/mL (i.e., the maximum tolerable dose). The product sheet for the prescription drug (Bristol-Myers Squibb Company 2010) indicates that the dose averages between 8 and 10 grams per day. Many studies describe methods to improve absorption by the human body in order to decrease the dose required and the side effects (Attivi 2010). However, none of these methods seem to be representative of the current use of mitotane or to have passed the clinical stage. Therefore, a typical value, estimated on the basis of the average dose, was used in the quantitative risk characterization.

The confidentiality of the information provided by the pharmaceutical industry during the public comment period excludes the possibility of using discrete quantities for the quantitative assessment. Therefore, the higher end of the range of the number of patients using the drug in Canada may be overestimated.

The locations of the release sites vary, depending on the patients' place of residence. Therefore, a statistical approach was taken to estimate the number of sites that would be expected to present a risk if a patient in the area was served by the wastewater treatment system. In high population areas, it is likely that more than one patient is served by the same wastewater treatment system. If so, the maximum risk quotient calculated in this assessment may be underestimated, although the number of sites that present risk to aquatic organisms would proportionately decrease. The quantitative results thus provide only a general indication of the magnitude of the potential risk to aquatic organisms.

9. Potential to cause harm to human health

9.1 Exposure assessment

9.1.1 Environmental media

Upper-bounding estimates of total daily intake of mitotane by the Canadian general population are presented in Appendix B.

The general population may be exposed to mitotane indirectly through environmental media. Empirical data on concentrations of mitotane in environmental media in Canada and elsewhere were identified and are presented in Tables 8-6 to 8-10 of the Ecological Exposure Assessment section. Most measured mitotane concentrations presented in Table 8-6 to 8-10 are likely to be from historical pesticide uses.

Mitotane was detected in ambient air at various sites sampled around the Great Lakes between 1996 and 2003 (Sun et al. 2006). Concentrations ranged from 0.31 to 8.8 pg/m^3 in the gas phase (Sun et al. 2006). It was also detected in air samples collected from various heights above agricultural soil in southern Ontario (Kurt-Karakus et al. 2006). Maximum concentrations reported at 5, 20, 72 and 200 cm above soil were 360, 230, 110 and 90 pg/m^3, respectively (Kurt-Karakus et al. 2006). However, it was not detected in ambient air at four sites in an agricultural area of Alberta (limit of detection [LOD] 0.05 ng/m^3; Kumar 2001).

Mitotane was detected in surface water in the Okanagan Valley and Fraser Valley, British Columbia (Environment Canada 2009a). Concentrations ranged from < 0.001 to 0.2 ng/L (Environment Canada 2009a). Similarly, Pham et al. (1996) reported mitotane in surface water sampled from the St. Lawrence River and its tributaries up to 0.232 ng/L. However, it was not detected in surface water at multiple sites in the Johnson Creek Basin, Oregon, between 1988 and 2002 (LOD 0.001 µg/L; Tanner and Lee 2004). Mitotane was also detected at concentrations of up to 25.2 ng/L in precipitation samples collected in the Atlantic provinces of Canada between 1995 and 2006 (Environment Canada 2010).

Mitotane was detected in agricultural and orchard soils in British Columbia at concentrations of < 1 to 250 µg/kg (Bidleman et al. 2006). It was also detected in agricultural soils in Southern Ontario at a concentration of 400 ng/g (Kurt-Karakus et al. 2006). There were also other reports of DDD in soil, but they did not differentiate between *o,p'*-DDD (mitotane) and *p,p'*-DDD (an insecticide which has not been registered for use in Canada since 1978; personal communication from Health Canada Pest Management Regulatory Agency, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada, 2010; unreferenced). For example, Crowe and Smith (2007) reported a range of < 1 to 11.4 mg/kg of DDD in soil sampled from the same region.

There were no reports of mitotane in foods other than fish in Canada. However, it has been reported in foods elsewhere. Mitotane has been detected (3 to 78 µg/kg) in cheese in Spain (Bentabol and Jodral 1995). In a total diet study carried out in 1990–1991 in Spain, DDD concentrations were very low (< 1 to 7 µg/kg) in samples of 13 food groups (Urieta et al. 1996). It is important to note that the authors of that study did not differentiate between *o,p'*-DDD (mitotane) and *p,p'*-DDD. In another total diet study, also from Spain, concentrations of total DDT (which include *o,p'*-DDD) were a maximum of 9.9 µg/kg wet weight in fish (Lazara et al. 1996).

Mitotane has been detected in whales and fish at various sites throughout the United States, in Greenland (Denmark) and in Canada (see Ecological Exposure Assessment, Table 8-11). Additionally, mitotane has been reported in 11 consumer fish species from South China at a mean concentration of 4.4 µg/kg wet weight (Meng et al. 2009). The mitotane concentrations in fish reported by Giesy et al. (1994) were considered the most relevant to Canada given the location of sampling. In that 1990 study, mitotane was detected at a range of 0.13 to 81.70 µg/kg in fish (Giesy et al. 1994). It should be noted that the three rivers from which fish were collected in Michigan are Great Lakes influenced (Giesy et al. 1994).

Upper-bounding estimates of intakes of mitotane from ambient air, indoor air, drinking water, food and soil were derived on the basis of the environmental concentrations reported in the literature. They are summarized in Appendix B. The total upper-bounding estimates range from 0.0016 µg/kg-bw/day (kilograms-body weight per day) for infants (0 to 6 months) to 0.022 µg/kg-bw/day for children aged 0.5 to 4 years (see Appendix B).

9.1.2 Products available to consumers

There were no reports under section 71 for the use of mitotane in consumer products in Canada (Canada 2009a), and exposure to the general population from use of consumer products is therefore not expected. Mitotane is registered for use in Canada as an oral chemotherapeutic agent used in the treatment of certain cancers of the adrenal gland (DPD 2010). Direct exposure from use of mitotane as a therapeutic product is addressed under the *Food and Drug Regulations* (Canada 1978).

9.2 Health effects assessment

Appendix C contains a summary of the available health effects information for mitotane.

No classifications or assessments of the health effects of mitotane by national or international regulatory agencies were identified.

Mitotane did not induce mutation in *Salmonella typhimurium* (Mortelmans et al. 1986; Buselmaier et al. 1972). An unspecified isomer of mitotane did not induce unscheduled DNA synthesis in primary cultures of rat, mouse or Syrian hamster hepatocytes (Maslansky and Williams 1981). Mixed results were obtained for induction of chromosomal aberrations in cultured rodent cells; however, mitotane did not induce transformation in mouse embryo cells or sister chromatid exchange in Chinese hamster ovary cells (Galloway et al. 1987; Langenbach and Gingell 1975; Palmer et al. 1972). *In vivo*, mitotane induced selective increase in lung DNA synthesis, but showed negative results in the liver (Lund et al. 1990). According to the authors, this effect may be linked to the formation of a reactive metabolite in the mouse lung. The overall genotoxicity results indicate that mitotane is not mutagenic and not likely to be genotoxic.

Effects of mitotane on rats and dogs via oral administration have been investigated in studies with exposure periods varying from 4 days to 2 years. In a subchronic study in rats exposed orally to mitotane, Leydig cell tumours (LCTs) were reported in the testis of male rats after one year of exposure at a dose of 0.6 mg/kg-bw/day (Lacassagne 1971). These results were reported to be inconsistent with earlier studies, which may indicate involvement of a contaminant or strain variation in the response. No further details of this study were reported (HBPTO 2001). Also, there are several lines of evidence that suggest that human Leydig cells are quantitatively less sensitive than rats cells to chemically induced LCTs (Shenker et al. 1993; Quigley et al. 1995; Clegg et al. 1997).

The lowest oral lowest-observed-adverse-effect-level (LOAEL) was determined to be 4 mg/kg-bw per day on the basis of gross atrophy of the adrenal glands and degeneration of the cells of its inner cortex in dogs orally exposed to mitotane for 4 days (Cueto and Brown 1958). Long-term exposures to 50 mg/kg-bw per day of mitotane resulted in adrenocortical necrosis in dogs (Kirk and Jensen 1975; Lehman 1951). Damage to the zona fasciculata and zona reticularis was also observed in dogs exposed to mitotane for 10 days (138 mg/kg-bw per day) (Kirk et al. 1974). Decrease contractile force of the heart and plasma volume was observed in dogs exposed to 50 mg/kg-bw per day to mitotane for 14 days (Cueto 1970). In rats, atrophy of both the adrenal cortex and the thymolymphatic organs was observed following exposure to 121 mg/kg-bw per day of mitotane for 16 days (Hamid et al. 1974). Tissue damage of the adrenal glands (5 mg/kg-bw per day) and liver enlargement (20 mg/kg-bw per day) was also observed in rats fed mitotane for up to 2 years (Lehman 1952).

Dermal exposure to 200 mg/kg-bw per day to mitotane for 90 days caused severe effects (no further details provided) and was lethal to rabbits after 6 days of exposure to 400 mg/kg-bw per day (Lehman 1951).

Developmental toxicity of mitotane was investigated in rats administered mitotane in the diet during gestation. Slight, but significant delay (2 days) in vaginal

opening of pups was observed when Sprague-Dawley dams had received 28 mg/kg-bw per day of mitotane by gavage during gestation days 15 to 19. Pups derived from the previous experiment and exposed subcutaneously to 1 mg/kg-bw of mitotane on their second, third and fourth day of life showed persistent vaginal estrus and absence of corpora lutea during adult life (Gellert and Heinrichs 1975).

The results from acute toxicity studies indicated that the oral LD_{50} values in rats, mice and guinea pigs for mitotane were all greater than 4000 mg/kg-bw and that the dermal LD_{50} value in rabbits was greater than 1200 mg/kg-bw following a single exposure (Gaines 1969; Lehman 1951; RTECS 2009). No acute inhalation studies were identified.

The metabolism of mitotane has been studied in humans and a variety of other mammalian species. Absorption following ingestion of mitotane is evident in humans from both measurements of serum and adipose tissue concentrations of this chemical (ATSDR 2002). Forty percent of the administered dose of mitotane is absorbed from the gastrointestinal tract regardless of the dosage form (FDA 2009). A portion of the absorbed fraction is expected to be transformed into a water-soluble metabolite in the liver, while another fraction is stocked in fat tissues (Moy 1961). Following a single oral dose of 30 mg/kg-bw in Göttingen minipigs, mitotane reached a median maximum concentration in 8 hours before rapidly declining due to elimination and distribution to body tissues, with adipose tissue being the primary storage site. A high fat/plasma ratio on day 30 reveals the high lipophilicity of this substance (Hermansson et al. 2008). Mitotane inhibits the mitochondrial conversion of cholesterol to pregnenolone and the conversion of 11-deoxycortisol to cortisol and produces selective adrenocortical necrosis in the adrenal tumour and metastases in humans (De León et al. 2002) (see use as therapeutic drug below). Competitive binding assays have showed that mitotane also binds to the human estrogen receptor (but with much weaker affinity than estradiol) (ATSDR 2002). Mitotane is metabolized by the adrenal mitochondria via cytochrome P450 to reactive products that covalently bind to the mitochondria macromolecules. The adrenal metabolism and covalent binding activities of animal species was shown to correlate with the known sensitivity of these species to the adrenocorticolytic effect of mitotane (ATSDR 2002). For example, mitochondria in dogs had significantly greater metabolism of mitotane and covalent binding activities compared with mitochondria from rats, rabbits or guinea pigs (Martz and Straw 1980).

In humans, mitotane has been used to treat adrenocortical carcinoma and Cushing's disease[4] for at least four decades (Bergenstal et al. 1960; Wooten and

[4] Hyperadrenocorticism, which is secondary to excessive pituitary secretion of the adrenocorticotropic hormone (Dorland's Medical Dictionary 2000).

King 1993). The drug is administered as 500 mg tablets in which mitotane is incorporated in solid form. An average dose of at least 8 to 10 grams per day is recommended by the distributor, but the dose can range from 2 to 16 grams per day (DPD 2010). The therapeutic action is based on the induction of selective necrosis of the zona fasciculata and the zona fascicularis of the adrenal cortex and inhibition of cortisol synthesis (Hart and Straw 1971; Hart et al. 1971; Hart et al. 1973). The most significant effects associated with mitotane treatment at doses as high as 12 000 mg per day for up to 34 months were fatigue, nausea, anorexia, vomiting and diarrhea in patients with Cushing's syndrome and adrenocortical carcinoma. The compound did not produce any observable effects to the liver, kidney or bone marrow, and adverse effects cleared when dosing was discontinued (Bergenstal et al. 1960; Hoffman and Mattox 1972; Luton et al. 1979; Perevodcikova et al. 1972; Wallace et al. 1961).

The confidence in the health effects database of mitotane is considered to be moderate, as limited empirical data were identified.

9.3 Characterization of risk to human health

No classifications or assessments of the health effects of mitotane by national or international regulatory agencies were identified. Available information indicates that mitotane is not likely to be genotoxic. On the basis of the empirical health effects data for mitotane, adrenal glands were identified as a target tissue.

The lowest effect level reported in the health effects database for mitotane was 0.6 mg/kg-bw per day and was based on the presence of Leydig cell tumours (LCTs) in the testis of male rats after one year of exposure. This report is inconsistent with other studies (because of possible contamination of mitotane and/or strain used), and there is uncertainty associated with interpretation of the relevance of effects given a lack of mode-of-action data. However, information in the literature suggests that human Leydig cells are quantitatively less sensitive than rat cells to chemically induced LCTs. Therefore, the lowest level at which adverse effects were observed was 4 mg/kg-bw per day, these effects were atrophy of the adrenal glands in dogs.

A comparison between the lowest level associated with an adverse effect in experimental animals exposed to repeated oral doses of mitotane (4 mg/kg-bw per day) and the upper-bounding estimate of general population exposure from environmental sources (0.022 µg/kg-bw) results in a margin of exposure (MOE) of approximately 180 000. This margin is considered adequate to address uncertainties in the health effects and exposure databases.

In Canada, mitotane is used only as an oral chemotherapeutic agent in the treatment of cancer of the adrenal glands (DPD 2010). Direct exposure from use of mitotane as a therapeutic product is addressed under the *Food and Drug*

Regulations (Canada 1978), and exposure to the general public from products available to consumers is not expected.

9.4 Uncertainties in evaluation of risk to human health

This screening assessment does not include a full analysis of the mode of induction of effects of mitotane. Onlyinformation is available concerning the potential toxicity of mitotane following oral and dermal exposure, and no inhalation studies were identified. Thus, critical effect levels determined in this screening assessment are associated with uncertainty due to limitations in the toxicity database; there are uncertainties in the interpretation of the biological significance of effects, including uncertainties in the interpretation of intraspecies and interspecies variation. Some health effects data identified were tested with an unspecified isomer of mitotane that may represent the commercial product containing 90% of the isomer *p,p'*-DDD and only 5% to 8% *o,p'*-DDD (mitotane).

Due to limited empirical health effects data and use of modelling, confidence in the determination of critical health effects is moderate.

Uncertainty in exposure to mitotane from environmental media and food in Canada is moderate to high, as limited empirical data were available with which to derive exposure estimates. Consumer products are not expected to be a source of exposure to mitotane. The only reported use of mitotane is as an oral chemotherapeutic agent. Therefore, the general population is not expected to be exposed to mitotane via consumer products.

10. Conclusion

Considering all available lines of evidence presented in this screening assessment, there is risk of harm to organisms, but not to the broader integrity of the environment from mitotane. It is concluded that mitotane meets the criteria under paragraph 64(*a*) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. However, it is concluded that mitotane does not meet the criteria under paragraph 64(*b*) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends.

On the basis of the information presented in this screening assessment, it is concluded that mitotane does not meet the criteria under paragraph 64(*c*) of CEPA 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 concluded that mitotane meets one or more of the criteria set out in section 64 of CEPA.

Additionally, mitotane has been determined to meet the persistence and bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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Appendix A. PBT model input summary tables

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

Model input parameters	**EPI Suite (all models, including AOPWIN, KOCWIN, BCFBAF, BIOWIN and ECOSAR)**
SMILES code	ClC(Cl)C(c1ccc(Cl)cc1)c2ccccc2Cl
Molecular weight (g/mol)	320.05
Melting point (°C)	77
Boiling point (°C)	NA
Data temperature (°C)	NA
Vapour pressure (Pa)	2.51E-3
Henry's law constant ($Pa \cdot m^3/mol$)	8.035
log K_{aw} (dimensionless)	NA
log K_{ow} (dimensionless)	6.22
K_{ow} (dimensionless)	NA
log K_{oc} (L/kg)	NA
Water solubility (mg/L)	0.1
log K_{oa} (dimensionless)	NA

Abbreviations: Kaw, air–water partition coefficient; Koa, octanol–air partition coefficient; Koc, organic carbon–water partition coefficient; Kow, octanol–water partition coefficient; SMILES, simplified molecular input line entry system; NA, not applicable

Table A-2. PBT model input summary table for fate modelling

Model input parameters	STP (1) ASTreat (2) SimpleTreat (3) (required inputs are different, depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	Arnot-Gobas BCF/BAF Model
SMILES code	NA	NA	NA
Molecular weight (g/mol)	320.05	320.05	NA
Melting point (°C)	NA	77	NA
Boiling point (°C)	NA	NA	NA
Data temperature (°C)	NA	20	NA
Vapour pressure (Pa)	2.51E-3	2.51E-3	NA
Henry's law constant ($Pa \cdot m^3/mol$)	8.035	8.035	NA
log K_{aw} (dimensionless)	NA	NA	NA
log K_{ow} (dimensionless)	6.22	6.22	6.22
K_{ow} (dimensionless)	NA	NA	NA
log K_{oc} (L/kg)	NA	NA	NA
Water solubility (mg/L)	0.1	0.1	0.1
log K_{oa} (dimensionless)			NA
Soil–water partition coefficient (L/kg)[a]	NA	NA	NA
Sediment–water partition coefficient (L/kg)[a]	NA	NA	NA
Suspended particles–water partition coefficient (L/kg)[a]	NA	NA	NA
Fish–water partition coefficient (L/kg)[b]	NA	NA	NA
Aerosol–water partition coefficient (dimensionless)[c]	NA	NA	NA
Vegetation–water partition coefficient (dimensionless)[a]	NA	NA	NA
Enthalpy (K_{ow})	NA	NA	NA
Enthalpy (K_{aw})	NA	NA	NA
Half-life in air (days)	NA	59.1	NA
Half-life in water (days)	NA	4320	NA
Half-life in sediment (days)	NA	38900	NA

Model input parameters	STP (1) ASTreat (2) SimpleTreat (3) (required inputs are different, depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	Arnot-Gobas BCF/BAF Model
Half-life in soil (days)	NA	4320	NA
Half-life in vegetation (days)[d]	NA	NA	NA
Metabolic rate constant (1/day)	NA	NA	0.0155
Biodegradation rate constant (1/day or 1/h) – specify	0.0001 h^{-1}	NA	NA
Biodegradation half-life in primary clarifier ($t_{½\text{-}p}$) (h)	10000	NA	NA
Biodegradation half-life in aeration vessel ($t_{½\text{-}s}$) (h)	10000	NA	NA
Biodegradation half-life in settling tank ($t_{½\text{-}s}$) (h)	10000	NA	NA

Abbreviations: BCF, bioconcentration factor; Kaw, air–water partition coefficient; Koa, octanol–air partition coefficient; Koc, organic carbon–water partition coefficient; Kow, octanol–water partition coefficient; SMILES, simplified molecular input line entry system; NA, not applicable

[a] Derived from log Koc.
[b] Derived from BCF data.
[c] Default value.
[d] Derived from half-life in water.

Appendix B. Upper-bounding estimates of mitotane exposure to the general population of Canada from environmental media

Table B-1. Upper-bounding estimates of mitotane exposure to the general population of Canada from environmental media

Estimated intake (µg/kg-bw per day)	Ambient air[i]	Indoor air[j]	Drinking water[k]	Food/ Beverages[l]	Soil[m]	Total intake
0-6 months[a,b,c] – breast fed	3.85×10^{-5}	2.70×10^{-5}	0.00	0.00	1.6×10^{-3}	1.63×10^{-3}
0-6 months[a,b,c] – formula fed	3.85×10^{-5}	2.70×10^{-5}	2.13×10^{-5}	0.00	1.6×10^{-3}	1.65×10^{-3}
0-6 months[a,b,c] – not formula fed	3.85×10^{-5}	2.70×10^{-5}	1.64×10^{-3}	0.00	1.6×10^{-3}	1.64×10^{-3}
0.5-4 years[d]	8.2×10^{-6}	5.78×10^{-5}	9.03×10^{-6}	2.00×10^{-3}	2.58×10^{-3}	2.27×10^{-2}
5-11 years[e]	6.43×10^{-6}	4.50×10^{-5}	7.10×10^{-6}	1.52×10^{-2}	8.39×10^{-4}	1.61×10^{-2}
12-19 years[f]	3.66×10^{-6}	2.56×10^{-5}	4.04×10^{-6}	9.89×10^{-2}	2.02×10^{-4}	1.01×10^{-2}
20-59 years[g]	3.14×10^{-6}	2.20×10^{-5}	4.04×10^{-6}	6.69×10^{-3}	2.02×10^{-4}	6.89×10^{-3}
60+ years[h]	2.73×10^{-6}	1.91×10^{-5}	4.44×10^{-5}	4.06×10^{-3}	1.67×10^{-4}	4.25×10^{-3}

[a] No information on mitotane in breast milk was identified.

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

[c] For exclusively formula-fed infants, intake from water is synonymous with intake from food. No data on concentrations of mitotane in formula were identified for Canada.

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

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

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

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

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

[i] Based on the maximum concentration of mitotane detected (110 pg/m^3) in air collected from 72 cm above agricultural soils in southern Ontario (Kurt-Karakus et al. 2006). Canadians are assumed to spend 3 hours outdoors each day (Health Canada 1998).

[j] No reported data for the concentration of mitotane in indoor air were identified. The concentration for ambient air (110 pg/m^3) reported by Kurt-Karakus et al. (2006) was used as a surrogate for indoor air. Canadians are assumed to spend 21 hours indoors each day (Health Canada 1998).

[k] No data for mitotane in drinking water were identified. As a surrogate, the maximum concentration of 0.0002 µg/L reported for mitotane in surface water in the Okanagan Valley and Fraser Valley, Canada between 2003, and 2005 was used (Environment Canada 2009a).

[l] There were no reported data for the concentration of mitotane in foods in Canada. As a surrogate, the maximum reported concentration of mitotane in fish collected from Great Lakes influenced rivers in the USA (81.7 µg/kg wet weight; Giesy et al. 1994) and the maximum concentration reported in cheese from Spain (78 µg/kg; Bentabol and Jodral 1995) were used. Amounts of foods consumed on a daily basis by each age group are described by Health Canada (1998).

[m] Based on the reported concentration of mitotane (400 ng/g) detected in agricultural soils in southern Ontario (Kurt-Karakus et al. 2006).

Appendix C. Summary of health effects information for mitotane

The following acronyms apply to Appendix C.

LD50 = median lethal dose
LOAEL = lowest-observed-adverse-effect level
LOEL = lowest-observed-effect level

Appendix C-1. Experimental animals and *in vitro*

Acute toxicity:

Lowest oral LD_{50} (rat, mouse) > 4000 mg/kg-bw (Gaines 1969; RTECS 2009).
Other oral LD_{50} (guinea pig) > 5000 mg/kg-bw (RTECS 2009).
Lowest dermal LD_{50} (rabbit) > 1200 mg/kg-bw (Lehman 1951).

Short-term repeated-dose toxicity:

Lowest oral LOAEL = 4 mg/kg-bw per day based on gross atrophy of the adrenal glands and degeneration of the cells of its inner cortex in dogs exposed via the diet to *o,p'*-DDD for four days (number and sex of animals and other doses used were not specified in secondary source) (Cueto and Brown 1958).

Other oral LOAEL = 50 mg/kg-bw per day based on decrease in contractile force of the heart and plasma volume in dogs exposed orally to *o,p'*-DDD for 14 days (breed and sex not specified; no further details provided). According to the author, the reduction of plasma volume may have been caused by a loss of fluid from intravascular compartment and was not caused by a release of histamine (Cueto 1970).

Other oral LOAEL = 121 mg/kg-bw per day based on atrophy of both the adrenal cortex and the thymolymphatic organs in Sprague-Dawley male rats (20/group) exposed via the diet to 0 or 121 mg/kg-bw per day *o,p'*-DDD for 16 days. Diminished body weights as well as the weights in the thymus, spleen and adrenal glands were observed. There was minimal fatty infiltration of the liver. No changes could be detected in the kidneys, heart and lungs. The numbers of plaque-forming cells (PFC) and rosette-forming cells (RFC) in the spleen and thymus were lower compared to controls. One group receiving protein-deficient diet (referred as malnourished rats) showed diminution of plasma corticosteroid concentration and less impairment of the immune response. The number of PFC and RFC in the spleen and thymus of the *o,p'*-DDD treated malnourished rats were almost equal to control (Hamid et al. 1974).

Other oral LOAEL = 138.5 mg/kg based on damage to the zona fasciculata and reticularis in dogs (4) exposed orally to 138.5 mg/kg-bw/day (capsule) of *o,p'*-DDD for 10 days. Plasma levels of cortisol were decreased and a decreased

response to ACTH stimulation was also observed. In one animal, there was hemorrhage, invasion by lymphocytes, and necrosis of the adrenal cortex. No changes in the spleen and the liver were seen (Kirk et al. 1974).

Subchronic toxicity

Lowest oral LOAEL = 0.6 mg/kg-bw per day based on Leydig cell tumours reported in the testis of rats exposed orally to *o,p'*-DDD for 285–348 days (other doses not specified; no further details provided) (Lacassagne 1971).

Oral LOAEL = 50 mg/kg-bw per day based on adrenocortical necrosis in mongrel dogs (10 animals, sex of animals not specified) orally exposed to 50 mg/kg-bw/day of *o,p'*-DDD tablets for 36–150 days. Necrosis involved primarily the zona fasciculata and zona reticularis although the zona glumerulosa was partially or completely necrotic. Minute foci resembling hemorrhagic lesions were noted on the retina of five treated dogs; however, no retinal lesions were identified (Kirk and Jensen 1975).

Dermal LOAEL = 200 mg/kg-bw per day based on severe effects (but no deaths) in rabbits exposed dermally to 0, 200 or 400 mg/kg-bw per day of TDE (isomer not specified) for 90 days. Dose of 400 mg/kg-bw was lethal to all rabbits after 6 days (Lehman 1951).

Chronic toxicity/ carcinogenicity

Lowest Oral LOAEL = 5 mg/kg-bw based on tissue damage in rats exposed via the diet to 0, 5 or 20 mg/kg-bw per day of TDE (isomer not specified) for 104 weeks. Liver enlargement was observed at the highest dose (Lehman 1952).

Other oral LOAEL = 50 mg/kg-bw based on atrophy of adrenal cortex and fatty liver in dogs exposed via the diet to 0, 50 or 80 mg/kg-bw per day of TDE (isomer not specified) until death (last death occurred at day 990 for the highest dose) (Lehman 1952).

Developmental toxicity

Groups of 13 female Sprague-Dawley rats were bred and exposed by gavage to 0 or 10 mg/kg-bw per day of *o,p'*-DDD during gestation days 15 to 19. Pups derived therefrom were used in two experiments. All animals were weaned at 21 days of age. Maternal toxicity of mitotane was not assessed in this study.

Developmental LOEL = 10 mg/kg-bw per day based on slight, but significant delay (2 days) in vaginal opening of offspring derived from Sprague-Dawley dams. No significant effects on body weight, weight of ovaries and pituitary or estrous cycle were observed in offspring at 49, 111 and 209 days of life.

From the same study, female rats derived from the mating (12/group) were treated subcutaneously with 0 or 1 mg/kg-bw *o,p'*-DDD on second, third and fourth day of life. They showed persistent vaginal estrus by 209 days of age and absence of corpora lutea by 258 days of age (Gellert and Heinrichs 1975).

Reproductive toxicity

No reproductive toxicity studies were identified.

Genotoxicity and related endpoints: *in vivo*

Unscheduled DNA Synthesis:
Positive: in lung of C57B1 mouse; single intraperitoneal injection at 0, 100 or 500 mg/kg-bw. Negative results were observed in the liver (Lund et al. 1990).

Genotoxicity and related endpoints: *in vitro*

Gene mutation
Negative: *Salmonella typhimurium*, strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation (Mortelmans et al. 1986).
Negative: *Salmonella typhimurium*, strains TA98 and TA100 with mouse lung and liver S9 metabolic activation (Lund et al. 1990).

Host-mediated assay
Negative: *Salmonella typhimurium his* G46 without metabolic activation (unspecified isomer of TDE) (Buselmaier et al. 1972).

Chromosomal aberration (Cytogenetic assay)
Negative: Chinese hamster ovary (CHO) cells with or without metabolic activation (Galloway et al. 1987).
Positive: kangaroo rat cells without metabolic activation (not tested with metabolic activation) (Palmer et al. 1972).

In vitro transformation assay
Negative: mouse embryo cells without metabolic activation (unspecified isomer of TDE) (Langenbach and Gingell 1975).

Unscheduled DNA synthesis assays (UDS)
Negative: Rat, mouse and hamster hepatocytes without metabolic activation (hepatocytes capable of metabolic activation; unspecified isomer of TDE) (Maslansky and Williams 1981).
Negative: Rodent hepatocytes without metabolic activation (Lund et al. 1990).

Sister chromatid exchange:
Negative: Chinese hamster cells with and without metabolic activation (Galloway et al. 1987).

Sensitization and irritation
No sensitization or irritation studies were identified.

Appendix C-2. Human studies – clinical studies

Side effects in patients treated with mitotane were observed at doses between 110 and 140 mg/kg-bw. Significant anorexia and nausea and central nervous system depression varying from lethargy to somnolence were observed in patients treated with mitotane (sex and number of patients were not specified in secondary source). The compound did not produce any detectable injury to the liver, kidney or bone marrow. Toxic effects cleared when dosing was discontinued. The testes (including Leydig cells) were not investigated (Bergenstal et al. 1960).

Large doses of mitotane (1000 to 12 000 mg/kg-bw/day) induced fatigue, nausea, anorexia, vomiting and diarrhoea in patients with Cushing's syndrome and adrenocortical carcinoma (sex and number of patients were not specified in secondary source) and exposed at doses of 1000 to 12 000 mg per day for up to 34 months. The testes (including Leydig cells) were not investigated (Hoffman and Mattox 1972; Luton et al. 1979). The symptoms disappeared soon after administration of the drug ceased or when the dosage was reduced (Perevodcikova et al. 1972).