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Risk assessment of compounds that could impair the aquatic environment

support for the Swedish Environmental Protection Agency
in their work to propose new priority substances within
the water Framework Directive

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Preface

This report on risk assessment was performed at the department of environmental toxicology, Uppsala University, on behalf of the Swedish Environmental Protection Agency. Due to today's massive use of chemicals, risk assessment and risk characterisation is an important task. I would like to thank you Axel Hullberg, Mikaela Gönczi, Britta Hedlund and Jonas Rodhe at the Environmental Assessment Department, Monitoring and Environmental impacts units, at the Swedish Environmental Protection Agency, for guidance and support. I also would like to thank Jan Örberg at the department of environmental toxicology at Uppsala University for for supervision and comments during the preparation of his report. Thank you all!

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Sammanfattning

En förvånansvärt liten andel av kemikalier i produkter som vi dagligen använder, t.ex. krämer och plaster, har blivit ekotoxikologiskt testade. Denna del utgör bara 5 % i Sverige. Den här studien gällande riskbedömningar för ett antal utvalda ämnesgrupper, utfördes som en del i arbetet att ge förslag på riskämnen för vattenmiljöer som bör prioriteras inom EU. De ämnen som har riskbedömts här är två tennföreningar: dibutyltenn (DBT) och monobutyltenn (MBT), som används som tillsatser i plast och som dessutom kan bildas genom nedbrytning av tributyltenn (TBT), den antiinflammatoriska läkemedelssubstansen diclofenac, aktiv substans i t.ex. läkemedlet Voltaren, samt myskämnet tonalide, ofta använt i parfymer, krämer och rengöringsmedel. Utifrån ekotoxikologisk data beräknades Predicted No Effect Concentration (PNEC). Measured Environmental Concentrations (MEC) erhölls från publicerade arbeten. Riskkvoter erhölls genom att beräkna MEC/PNEC.

Resultatet av studien visar på låga riskkvoter för MBT (under eller strax över 1), i allmänhet mycket låga (< 0,1) för tonalide (endast två värden över 1; 1,94 och 14,3), låga till höga för DBT (< 1 till > 10) och höga, över 10, för diclofenac. Detta visar på att MBT sannolikt inte innebär någon risk för den akvatiska miljön medan eventuellt tonalide och framförallt DBT och diclofenac utgör en hög risk för akvatiska miljöer. Risken för marina miljöer och särskilt för sedimentlevande organismer har dock inte blivit utvärderad i denna bedömning. Inte heller har risker för däggdjur och fåglar bedömts.

Summary

Surprisingly few chemicals in products that we are using in our daily life like lotions and plastics have been tested from an ecotoxicological point of view. In Sweden, this tested fraction is less than 5 %. This study about risk assessments of a couple of compounds was performed as a part of the process to propose new environmental disturbing compounds within aquatic environments which should be prioritised within the EU. The compounds that have been evaluated in this report are two tinorganic compounds: dibutyltin (DBT) and monobutyltin (MBT), often used as additives in plastics but can also be produced by degradation of tributyltin (TBT), the anti-inflammatory pharmaceutical diclofenac, the active substance in the medical product Voltaren among others, and finally the musk fragrance tonalide, commonly used in perfumes, lotions and household detergents. Predicted No Effect Concentrations (PNECs) were calculated from ecotoxicological literature data. Measured Environmental Concentrations (MECs) were were obtained from screening studies. Risk characterisation ratios (RCRs) were estimated by calculating MEC/PNEC.

The results of this study show very low RCRs for MBT (below and just above 1), in general very low (< 0.1) for tonalide (only two values above 1; 1.94 and 14.3), low to high for DBT (< 1 and >10) and above 10 for diclofenac. Thus, tonalide and especially DBT and diclofenac might pose a high risk to the aquatic (limnic) environment.

However, the risk to marine environments, especially to sediment living organisms has not been evaluated in this risk assessment. Nor either have the risks for mammals and birds been assessed.

Abbreviations and terms

Acute toxicity	Toxic effect after short-term exposure, often 24-96h
Aerobic degradation	Degradation with oxygen present
Anaerobic degradation	Degradation without oxygen
Base set	The basic requirement regarding ecotoxicity testing for risk assessment: at least one $LC_{50}/EC_{50}/IC_{50}$ for organisms representing three tropic levels, normally algae, *Daphnia* and fish.
BCF	Bioconcentration factor, calculated as concentration of a chemical in an animal divided by concentration of the same chemical in its food.
Bioaccumulation	The concentration of a chemical in an organism increases over time
Biodegradation	The process by which chemicals are degraded by organisms
Biomagnification	The increase in concentration of a substance, such as the pesticide DDT, that occurs in a food chain, also known as bioamplification or biological magnification.
Chronic toxicity	Toxic effects after long term exposure, often a whole life cycle of an organism. For aquatic organisms the exposure time varies between 72 h and six month depending on tested species.
dw	Dry weight
DBT	Dibutyltin
DBTC	Dibutyltin dichloride
DBTO	Dibutyltin oxide
EC_{50}	Effect concentration 50 %, the concentration estimated to cause effect in 50 % of the organisms or a 50 % change of the studied variable.
ECB	European Chemical Bureau
ELS	Early life stages
EPA	US Environmental Protection Agency
EUSES prediction	The fundamental principles and methodology of the EU technical guidance document (TGD) for risk assessment is implemented in the computer programme EUSES (European Union System for the Evaluation of Substances).
EQS	Environmental quality standard, most often the same as PNEC (See below)
FASS	Farmaceutiska Specialiteter i Sverige
FELS	Fish early life stage s
Henry´s law constant	The ratio between vapor pressure of a chemical and its solubility in water (Pa* m^3/mol).

IC_{50}	Inhibiting concentration 50 %, the concentration estimated to cause a 50 % decrease of the studied variable.
IVL	Swedish Environmental Research Institute
KEMI	Swedish Chemicals Agency
LC_{50}	Lethal concentration 50 %, the concentration estimated to cause death in 50 % of the tested organisms.
Log Kow	The logarithm octanol/water partition coefficient
LOEC	Lowest observed effect concentration
MBT	Monobutyltin
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NSAID	Non-steroid anti-inflammatory drugs
PCP	Personal care products
pKa	Dissociation constant
PNEC	Predicted no effect concentration
RAF	Risk assessment factor
RCR	Risk characterisation ratio
REACH	Registration, evaluation, authorisation and restrictions of chemicals
STP	Sewage treatment plant
TGD	Technical guidance document
WFD	Water framework directive
WHO	World health organisation
WSP	Williams Sayles partnership, a global company that provides management and consultancy services.
ww	Wet weight

Introduction

Background

Surprisingly few chemicals present in products that we are using in our daily life like shampoo, clothes, plastics and toys have been ecotoxicological tested. Only less than five percent of the chemicals have been tested properly [1]. Until 1 June 2007, when the European Unions (EUs) new chemical legislation about registration, evaluation, authorisation and restriction of chemicals (REACH) came into force, there was no distinct legislation for the chemistry industry regarding toxicity tests of chemicals before use. REACH will be completely into force in 2022 and is based on the principal that the producers, importers and distributers have the responsibility for providing information on human- and environmental effects. According to the legislation all chemicals in levels over one ton per year, produced or imported, shall be registered and tested [2]. In EU many of these are classified as persistent and bioaccumulating. Over 11 000 chemicals are registered at the Swedish Chemical Agency. These chemicals are ingredients in over 70 000 products [2]. Within EU over 100 000 different chemicals are available on the market and every year new chemicals are produced [1, 3].

The European Water Framework Directive (WFD) came into force in 2000 and its aim is to improve and protect inland and coastal waters, make the use of water sustainable, create better habitats for wildlife that lives in and around water and create a better quality of life for everyone [9]. In that work Environmental Quality Standards (EQS) are often used to specify the maximum permissible concentration of a potentially hazardous chemical in different environmental compartments, usually in water [10] .

In the WFD 33 substances are selected as “priority substances”. The member states have to progressively reduce pollution from these substances. In a new directive (still under negotiations when this report is written) regulating the priority substances in more detail, EU common Environmental Quality Standards (EQS) values are set. In the WFD it is also required that the Commission shall review the list of priority substances at least every forth year. A new proposal from the Commission will be presented in the end of 2008. For this work the Commission asked all member states to report monitoring data on hazardous substances that can be of concern. Out of this the Commission presented a list “Proposal for a manageable list of substances”, of around 200 substances which are found in the environment in several member states. Further work and discussions to agree on new priority substances and their EQS will follow during 2008 and further on. In Sweden the Swedish Environmental Protection Agency (EPA) has the responsibility for this work.

Aims

The purpose of this report is to make risk assessments and derive EQSs and risk characterisations of four selected substances found in the environment. These risk assessments will be a support for the Swedish Environmental Protection Agency in their work to propose new priority substances to be added to the 33 ones already present at the priority list of the WFD.

Selection of substances

The starting point for the selection was the proposed list of 200 substances from the Commission. Also anthropogenic chemicals not on that list but which have been found in aquatic environments in Sweden have been considered. The Swedish EPA has suggested the following chemicals to be of particular interest:

- Musk compounds (musk xylene, tonalide, galaxolide, traseolide)
- Flame retardants (Hexabromocyclododecane (HBCD) and tetrabrombisphenol A (TBBPA))
- Tin organic compounds (except for tributyltin (TBT) which is already a prioritised substance in the WFD)
- Parmaceuticals (diclofenac, ketoprofen, ibuprofen, naproxen, ethinylestradiol)

Out of this list four chemicals were selected to be further investigated in this report. These chemicals were two tinorganic compounds: dibutyltin (DBT) and monobutyltin (MBT), one pharmaceutical compound: diclofenac and one musk compound: tonalide (AHTN).

Method for risk assessment

Log Kow, Henry´s law constant, biodegradation, bioaccumulation, short and long term toxicity, predicted no effect concentration (PNEC) and predicted or measured environmental concentration (PEC and MEC) are all important variables used in the risk assessment and classification of chemicals. MEC and PNEC (often the same as the EQS), are the variables used for the risk characterisation. The process of the environmental risk assessment is illustrated in figure 1.

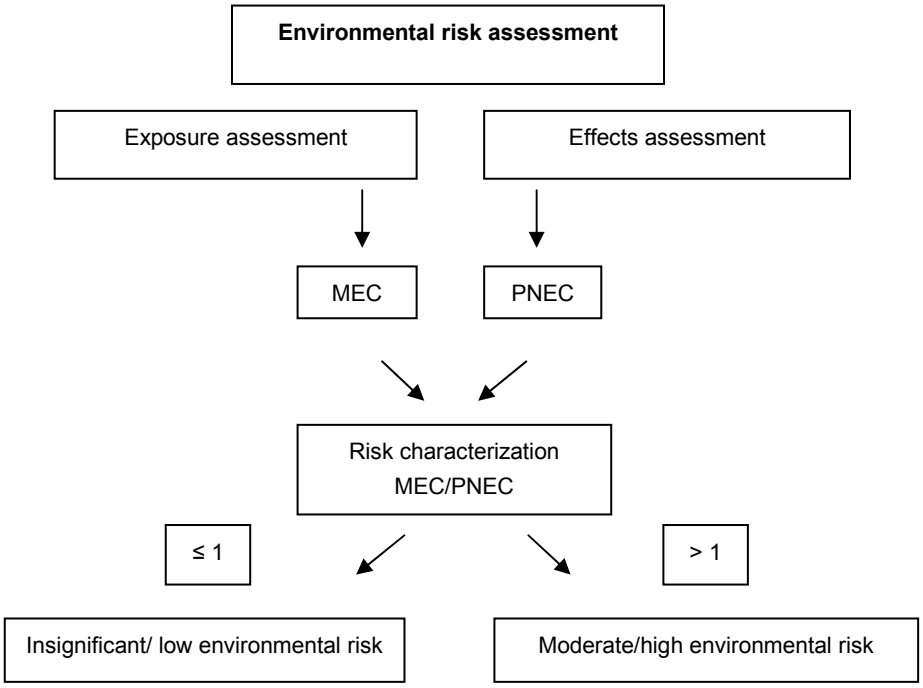


Figure 1. Environmental risk assessment of chemicals. MEC = Measured Environmental Concentration. PNEC = Predicted No Effect Concentration.

Log Kow

The partition coefficient between octanol and water (log Kow) is a measure of the hydrophobicity of a chemical and gives also information about the bioavailability of the chemical. In general, the bioavailability of a compound is directly related to its hydrophobicity.

Henry's law constant

The Henry's law constant is calculated as the quote between vapor pressure of a chemical and its solubility in water ($Pa \cdot m^3/mol$) [4]. Low values indicate that the concentration of the substance is much higher in the water than in the atmosphere. A high value is instead indicating that the concentration of the chemical is higher in the atmosphere than in the water [5].

Biodegradation

Biodegradation is the process by which organic chemicals are broken down by enzymes produced by living organisms. According to the criteria used for

classification and labeling of chemicals within EU a chemical with a degradation rate ≥ 70 % within 28 days is readily degradable [6].

Bioaccumulation

Bioaccumulation of a chemical occurs when the absorption rate is greater than the elimination rate. For substances with potential for bioaccumulation, long term and delayed effects are plausible [7]. The bioconcentration factor ((BCF, concentration in an aquatic organism/concentration in water) describes the ability of chemicals to accumulate in aquatic organisms [8]. Log Kow >3 indicates a potential for bioaccumulation [7].

Classification

Classification on the basis of environmental effects is made in accordance to the classification and labelling guideline provided by European Chemicals Bureau (ECB) [6]. For this classification experimental data for aquatic toxicity, degradation, log Kow and BCF are used. Risk phrases and the corresponding criteria for substances dangerous for the aquatic environment are shown in table 1.

Table 1. Risk phrases and the corresponding criteria for substances dangerous for the aquatic environment [6].

Risk phrase	Criteria		
R50 – Very toxic to aquatic organisms		96 h LC_{50} (fish) 48 h EC_{50} (*Daphnia*) 72 h IC_{50} (algae)	≤ 1 mg/l
R51 – Toxic to aquatic organisms	1 mg/l <	96 h LC_{50} (fish) 48 h EC_{50} (*Daphnia*) 72 h IC_{50} (algae)	≤ 10mg/l
R52 – Harmful to aquatic organisms	10 mg/l <	96 h LC_{50} (fish) 48 h EC_{50} (*Daphnia*) 72 h IC_{50} (algae)	≤ 100 mg/l
R53 – May cause long term adverse effects in the environment	The substance is not readily degradable (< 70 % degraded within 28 days), or the log P_{ow} ≥ 3 (unless the BCF ≤ 100)		

Short term toxicity

Measures of short term (24 to 96 h) toxicity are effect concentration 50 % (E(I)C_{50}) and lethal concentration 50 % (LC_{50}). The so called base set for testing aquatic toxicity is three tests conducted on algae, *Daphnia* and fish, respectively [7].

Long term toxicity

A measure of long term toxicity is no observed effect concentration (NOEC). For tests to be considered as long term they need to cover a full life cycle of algae and *Daphnia* or cover critical developmental early or juvenile stages. Fish tests considered as long term have to cover important life stages such as the fish early

life stage (FELS) toxicity test with exposure times of up to 60 days [7]. Criteria for long term tests for algae, *Daphnia* and fish are shown in table 2.

Table 2. Criteria for long term tests for algae, *Daphnia* and fish, in accordance to the technical guidance document (TGD) [7].

	Time of exposure	***Type of test***	***Comment***
Algae	72h	Growth inhibition test	Considered as a chronic test because of the short life cycle of algae
Daphnia	21days	Reproduction	21 days do not cover a full life cycle, but it covers the sensitive reproduction stage and is therefore considered a long term study.
Fish	60 days	Fish early-life stages (FELS)	Growth and survival of rainbow trout (Oncorhynchus mykiss)
	30 days	Fish early-life stages (FELS)	Growth and survival of warm water fish, e.g. zebra fish (Danio rerio).
	3-10 days	Short term toxicity test on early life stages (ELS), test on embryo and sac-fry stages	Only considered as a long term test if the tested compound has a log Kow< 4
	≥ 28 days	Juvenile growth test	Only considered as a long term test if the tested substance has a log Kow < 5

Predicted No Effect Concentration (PNEC)

Calculations of PNEC are made according to the standard given in technical guidance document (TGD): the lowest toxicity measure is divided by a so called risk assessment factor (RAF) [7]:

PNEC = (LC_{50}, EC_{50}, IC_{50} or NOEC)/Risk assessment factor

The study used should preferable be a long term study, resulting in a long term NOEC. If the toxicity measures available originate from short term toxicity tests (LC_{50}, EC_{50}) the RAF used is higher than if using toxicity measures from long term studies (table 3). Though, when the ratio between acute and chronic toxicity measures is low and does not guarantee appropriate protection against acute effects, the PNEC should be based on acute toxicity data [7].

Table 3. Risk assessment factors used for calculation of predicted no effect concentration (PNEC). The base set includes at least one LC_{50}/EC_{50} /IC_{50} for organisms representing three trophy levels, normally algae, Daphnia and fish.

	Risk assessment factor
Base set	*1000*
Base set + one chronic NOEC from a study on fish or *Daphnia*	*100*
Base set + two chronic NOECs from studies on organisms representing different trophic levels	*50*
Base set + three chronic NOECs from studies on algae, *Daphnia* and fish	*10*

Risk characterisation

According to TGD the risk characterisation ratio (RCR) is calculated by:

MEC/PNEC or
PEC/PNEC

The threshold values for the RCR are presented in table 4.

Table 4. Threshold values for the risk characterisation ratio (RCR) [7].

RCR	Interpretation
< 0.1	Insignificant environmental risk
0.1 – 1	Low environmental risk
1 – 10	Moderate environmental risk
> 10	High environmental risk

If this RCR exceeds 1 it should be understood as a warning and a signal for action to be taken.

Tinorganic compounds

General introduction

Tinorganic compounds are characterized by a Sn^{4+} ion to which one to four organic ligands are attached. They are classified according to the type of organic ligands and there are four series of tinorganic compounds depending on the number of organic ligands. These mono-, di, tri and tetra series have the general formula $RnSnX_4$-n where R is an alkyl or aryl group, often a butyl, octyl or phenyl group and X is a singly charged anion or an anionic group, often chloride, fluoride, oxide, hydroxide, carboxylate or thiolate (figure 2) [13]. The carbon-tin bond is strong while the bond to the anionic ligand is not why the compounds easily dissociate [14]. The cation may form dissolved complexes with e.g. chloride in seawater. Tinorganic compounds are hydrophobic and this hydrophobicity increases with increased length of the carbon chain and number of alkyl groups [15].

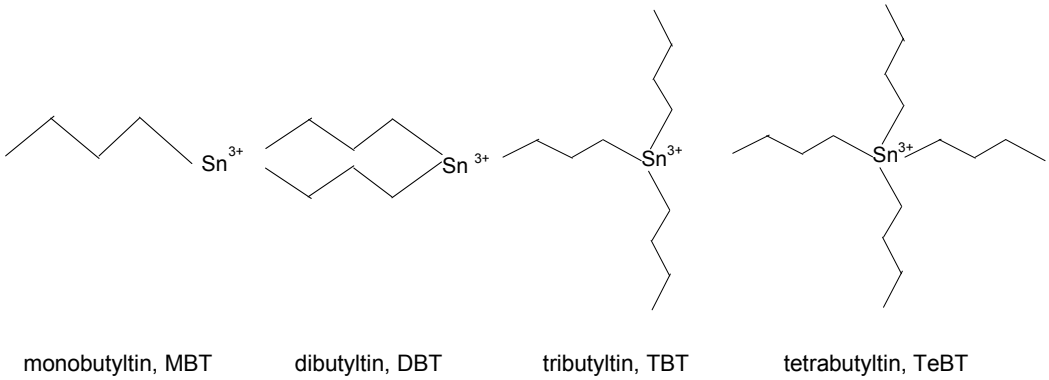


Figure 2. An example of a group of tinonorganic compounds: butyltin [16].

Tinorganic compounds have shown to be more toxic than inorganic tin compounds [13]. The reason for this is that the organic compounds have higher bioavailability as indicated by a higher log Kow [17]. Tinorganic compounds have been detected in a wide range of consumer products as food plastics, plastic toys and clothes [14]. They are one of five groups of substances that have been chosen by the Swedish Environmental Protection Agency to be continuously monitored because of their capability to cause adverse effects in the environment [18].

Compounds that have been found in the environment are tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), monoochtyltin (MOT), diochtyltin (DOT) and triphenyltin (TPT) [15]. The most frequently found tinorganic compounds are the butyltins (tri, di and mono), octyltins and phenyltins. Methyltins are another group of organotin compounds which can be produced naturally in the environment by microorganisms. Phenyltin is mostly occurring in marine environments where big ships are trafficking. Octyltins are most common in sewage and urban day water and in certain urban sediments. Butyltins are found in both marine environments and in urban areas [14].

The use of tinorganic compounds

According to KEMIs product register 240 tonnes tinorganic compounds were used in Sweden 2004 (figure 3). About half of this amount is made up by butyltins. On top of this a lot of these compounds are present in different imported products. In 2004, according to KEMIs product register, the Swedish use of TBT, DBT and MBT was 0.4, 145, and 4 tonnes, respectively. 16 tonnes of tinorganic compounds were imported to Sweden in 1994, of which about 5 tonnes were used as additives in paint, varnish and glue. The rest was used by the plastic industry, primary as additives in transparent polyvinyl chloride PVC [19]. The average content of tinorganic compounds in PVC as stabilisers are about 1% [2]. For the distribution and use of tinorganic substances, see figures 3 and 4.

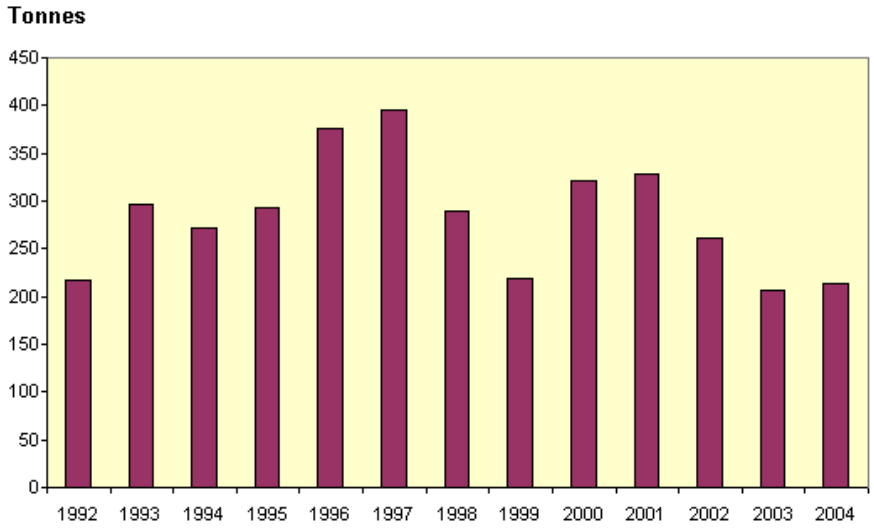


Figure 3. The use of tinorganic compounds (primary as stabilisers in plastics) in Sweden between 1992 and 2004 [20].

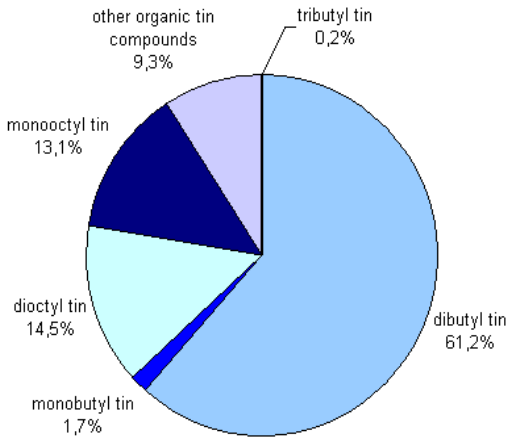


Figure 4. Distribution of the tinorganic substances used in Sweden 2004 [20].

Another well known use of tinorganic compounds is as biocides in antifouling paints. Biocidal products containing TBT have been used since the 1960s [19]. This use has harmed the aquatic environment by causing mussel larvae mortality, oyster shell malformation [21] and masculinising female snails at levels as low as 5 ng/l [22]. The use of TBT in antifouling paint was forbidden in Sweden in 1989

[23] for use on boats shorter than 25 m. In 2003 there were further restrictions for bigger boats taken and the European Union Antifouling System Convention (AFS-convention) for control of harmful antifouling system came into force. Since January 1, 2008 boats still using this type of antifouling paints are forbidden to use European harbours [24], but still TBT is widely spread both in sediments and water [19]. Although the use of TBT in Sweden has been forbidden for the last 20 years, a report made at the University of Stockholm show high concentrations of TBT in the surface sediment layer in the areas nearby a boat launching place in a marina [23].

Environmental fate

Tinorganic compounds leak out to the environment from antifouling paints and PVC plastic, mostly into water [25]. When released into water tinorganic compounds adsorbs strongly to sediments and particulate matter. Tinorganic compounds concentrate in the lipophilic surface film of the water [14] and are naturally biodegraded by sequential dealkylation, a process where the carbon-tin bond are broken and derivatives are formed. The biodegradation of butyltins occur in the stepwise order of TBT dealkylating into DBT, which in turn dealkylates into MBT [15]:

TBT→ DBT→ MBT→ Sn^{4+}

The derivates are more readily degraded than the parent substance [26]. Dealkylation can occur both by photolysis and via enzymatic reactions [15]. Hydrolytic degradation occurs under extreme conditions such as pH below 1 and above 13. The degradation of tinorganic compounds is more rapid in fresh water than in sea water [27]. The half-lives may be as short as one week in water exposed to sunlight and several months or longer in sediment [15]. Some tinorganic compounds have high potential for bioaccumulation [28].

Toxicity

The toxic effects of tinorganic compounds are connected to a high bioavailability and a slow biodegradation [29]. The toxicity increases dramatically in the order: MBT<DBT<TBT for certain endpoints, e.g. endocrine disruption [15]. The toxicity varies depending on the length of the alkyl- and aryl groups [19].

DBT and MBT are the most common tin organic compounds found in Swedish surface waters, effluents from sewage treatment plants, sewage sludge and biota and therefore this report will focus on these compounds. TBT is already included as a priority substance within EU.

Dibutyltin (DBT)

General information

There are a number of different DBT compounds, but all have the general formula R_2SnX_2 in common. DBT is the tinorganic compound that is most frequently found in the Swedish environment. DBT is also formed in the environment by dealkylation of TBT, where DBT is the first step in this process:

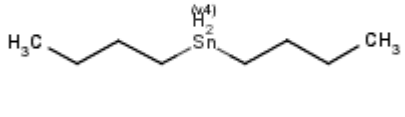
TBT→ DBT→ MBT→ Sn^{4+}

Field of application

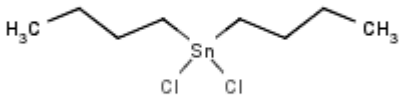
Dibutyltins are mostly used as stabilizers in PVC plastics but also as additives for fast drying in paints, in sealants and in plastic material made for food packing [28]. About 95 tonnes raw material of DBT compounds were imported to Sweden in 2005. About 90 tons were used in the plastic and paint industry. On top of this 16 tones were imported in chemical products, mostly as stabilizers and catalytic agents [12].

Physical and chemical properties

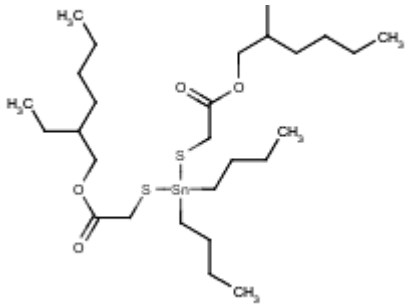
At room temperature DBT is colorless or white and has a scentless crystalline face. There are different forms of dibutyltins (figure 5) but all studies used in this report have been performed with the DBTC. Therefore all data in table 5 refer to dibutyltin dichloride.



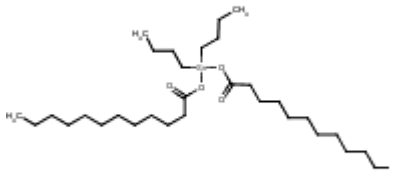
Dibutyltin (anion)
CAS number: 1002-53-5



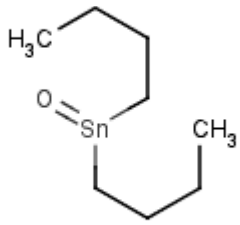
Dibutyltin dichloride
CAS number: 683-18-1



Dibutyltinbis (2-ethylhexyl mercaptoacetate)
CAS number: 10584-98-2



Dibutyltin dilaurat
CAS number: 77-58-7



Dibutyltin oxide
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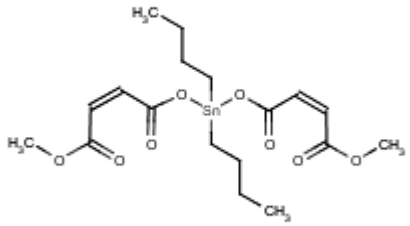
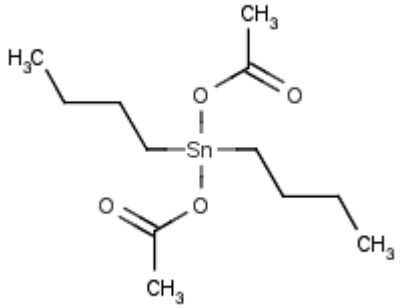


Dibutyltin methyl maleate
CAS number: 15546-11-9



Dibutyltin diacetate
CAS number: 1067-33-0

Figure 5. Different types of dibutyltins [17].

Table 5. Physical and chemical properties of dibutyltin dichloride.

		Reference
CAS-No.	683-18-1	[17]
Synonyms	Di-n-butyltin dichloride Dibutyldichlorostannane Dibutyldichlorotin Dibutylstannium dichloride Dibutyltin chloride Dichlorodibutylstannane Dichlorodibutyltin	[17]
Empirical formula	$C_8H_{18}Cl_2Sn$	[17]
Molecular weight (g/mol)	303.83	[30]
Solubility in water (mg/l)	33 (temp. not stated)	[31]
	92 (at 20°C)	[17]
Vapour pressure (Pa)	0.15 (temp. not stated)	[31]
	0.16 (at 20°C, pH 2.5)	[32]
Log Kow	0.97-1.89	[33, 17, 31]
Henry`s law constant (Pa×m^3/mol)	0.152-1.38	[32, 31, 34]

Mode of action and toxicity to aquatic organisms

One important mechanism behind the toxicity of DBT is its inhibition of oxidative phosphorylation and the subsequent decrease in ATP production [36]. DBT is acutely toxic to aquatic organisms [37], reprotoxic to mammals [15] and immunotoxic to fish after repeated exposure [38]. Some studies have shown developmental effects on mammals [39]. DBT has been shown to inhibit NH_4^+ uptake and growth of heterotrophic nitrifying bacteria [40].

Results from base set tests (72 h tests on algae, 48 h tests on *Daphnia* and 96 h tests on fish) and also results from other relevant short term toxicity tests are presented in table 6. In table 7 results from long term toxicity tests are presented. Results from studies with not specified endpoints have been excluded from the tables. All studies have been conducted with the dibutyltin dichloride (DBTC), or else the results have been recalculated to represent DBTC.

Table 6. Summary of results from short term toxicity test with dibutyltin dichloride on aquatic organisms. * Converted from the tested substance to the dibutyltin dichloride (DBTC) by calculating: (concentration of the tested substance (mg/l) * molecule weight for DBTC (g/mole))/molecule weight for tested substance (g/mole)

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Ankistrodesmus falcatus	growth rate	24h	EC_{50} = 17.4	[41]
Molluscs				
Crassostrea gigas	mortality	48h	LC_{50} = 0.17	[47]
Crustaceans				
Daphnia magna	immobilization	24h	EC_{50} = 0.3	[42]
Daphnia magna	immobilization	24h	EC_{50} = 0.9	[43]
Daphnia magna	immobilization	48h	EC_{50} = 0.02-1.8	[44, 45, 46]
Fish				
Oryzias latipes	mortality	48h	LC_{50} = 0.9-11 *	[48]
Leuciscus idus	mortality	48h	LC_{50} = 0.6	[42]
Leuciscus idus	mortality	48h	LC_{50} = 0.9	[42]
Oncorhynchus mykiss	mortality and behaviour	14 days	NOEC = 0.005	[49]
		14 days	LC_{100} = 40	[48]
Marine organisms (diatoms)				
Skeletonema costatum	growth rate	72h	EC_{50}= 0.09-0.1	[50]
Thalassiosira pseudonana	growth rate	72h	EC_{50}= 0.32-0.46 *	[50]

Table 7. Summary of results from long term toxicity test with dibutyltin dichloride on aquatic organisms. * Converted from the tested substance to the dibutyltin dichloride (DBTC) by calculating: (concentration of the tested substance (mg/l) * molecule weight for DBTC (g/mole))/molecule weight for tested substance (g/mole).

Species	*End-point*	*Time of exposure*	*Result (mg/l)*	*Reference*
Algae				
Scenedesmus subspicatus	growth rate	72h	NOEC = 0.8 *	[51]
Scenedesmus subspicatus	growth rate	72h	EC_{50} = 3.6 *	[51]
Skeletonema costatum	growth rate	72h	EC_{50} = 0.04	[50]
Scenedesmus acutus	growth rate	96h	EC_{50} = 0.0167	[52]
Platymonas sp.	growth rate	96h	$EC_{50} = 7.7 * 10^{-5}$	[53]
Scenedesmus obliguus	growth rate	96h	$IC_{50} = 8.10^{-5}$	[53]
Molluscs				
Mytilus edulis	mortality and larvae development	28 days	NOEC = 0.002	[54]
		28 days	LOEC = 0.020	[54]
Crassostrea gigas	mortality	49 days	LC_{50} = 0.1	[55]
Crustaceans				
Daphnia magna	survival and reproduction	21 days	NOEC = 0.015	[46]
Fish				
Poecilia reticulata	mortality, growth and behaviour	3 months	NOEC <0.32	[56]
		28 days	NOEC = 1.8	[56]
Oryzias latipes	mortality, growth and behaviour	28 days	NOEC = 1.8	[56]
Ocorhynchus mykiss	growth and mortality	110 days	LOEC = 0.24	[49]

Degradation

No data of half-lives in surface water have been found. A safety half life for risk assessment has been set to 150 days by WHO [14]. A half life of 150 days means that DBT is not readily degradable [6]. Half lives of DBT in activated sludge have been estimated to 3.6-5.5 days [13]. Half life in soil has been measured to 120 days [14].

Bioaccumulation

According to Tsuda DBT has potential for bioaccumulation [33,38]. In a review by WHO the measured BCF of DBTC was found to be 136 in the freshwater fish carp (*Cyprinus carpio L*) [36]. DBT has been found to accumulate in the kidney of freshwater clam (*Anodonta sp.)* [57]. BCFs for DBT in muscle, vertebra, liver and kidney tissue in carp (*Carassius carassius grandoculis*) were 12, 46, 135 and 61, respectively [33].

Biomagnification

No data have been found regarding biomagnification of DBT.

Classification

DBT is very toxic to aquatic organisms (EC_{50}, 48 h, *Daphnia*: 0.02 mg/l) and not readily degradable. In addition DBT has a potential for bioaccumulation. Therefore DBT is classified as dangerous for the environment and assigned the risk phrases R50/53 (table 1).

Environmental fate

For dibutyltin, measured concentrations in seawater reflect the use of tributyltin as a marine antifoulant rather than use of dibutyltin in plastics [14]. If released into fresh water or sea water DBT is expected to adsorb to suspended solids and sediment. If released to soil, it will adsorb to organic carbon and clay. Volatilization from soil surfaces is not expected to be an important process [16], though it still can be transported long distances via air and therefore occur in remote places. According to a study made by Mester & Sturgeon in 2002, release to atmosphere may be underestimated [57].

Environmental levels

Swedish screening programs have shown that DBT is one of the most common tin organic compound in the environment [15]. Sources for DBT are degradation of TBT, industrial point sources, diffuse urban leakage from day water and household and industry sewage water [28]. Municipal waste water has shown to contain considerably quantities of DBT [13].

In a Swedish screening study made in 2006 surface water contained 0.01-5.8 ng DBT/l and incoming waste water contained 5-120 ng/l [15]. The highest levels in surface water were found in Stockholm (3.2-11 ng/l), in the Viskan river and in the lakes downstream of Borås (5.8-13 ng/l) [28].

Levels of 1.1 ng/g ww in perch in areas around Stockholm were found to be much higher than in perch from other location [28]. DBT levels in surface water and in blue mussel (*Mylilus edulus*) collected close to a marina on the Swedish west coast were 0.40-0.77 ng/l and 25-370 ng/g dw, respectively [15]. DBT levels in surface water and in herring (*Clupeidae sp.)* and salmon (*Salmonidae sp*.) from the Baltic coast outside Umeå were 4.4±2.4 ng/l and 1.0-3.6 ng/g dw, respectively [15]. A Canadian study found maximum levels of 15.7 µg/l in fresh water and 1.3 µg/l in coastal water [58]. According to screening studies made in Europe the maximum concentration of DBT in the environment was 810 ng/l in sea water from marinas in the Netherlands [59].

In Sweden, the highest levels in sediment were found in Stockholm (30-300 ng/g dw) and the levels were also high in the Viskan river and in the lakes downstream of Borås (4-78 ng/g dw) [28].The highest level in sludge from a Swedish municipal sewage treatment plant was 2200 µg/kg dw [15]. A recent report from Stockholm University found 400 – 1400 µg DBT/kg dw in surface sediment from a marina indicating that the use of this compound has not stopped [23]. According to the Oslo-Paris convention (OSPAR)* the highest concentration where no ecotoxicological effects are assumed to appear is 0.05 µg/kg dw in sediment. The ecotoxicological safety limit for sediment, the ecological acceptable concentration, is set to 1 ng/l [62].

PNEC in fresh water

The lowest toxicity measure found in the literature was a 96h EC_{50} (growth rate) of $7.7x10^{-2}$ µg/l for the algae *Platymonas sp.* This measure is much lower than any of the long term toxicity measures. Therefore this 96h EC_{50} will be used for the risk assessment, although it is not a long term NOEC [7]. Since there are other long terms NOECs from organisms representing three trophic levels, a RAF of 10 will be used, resulting in a PNEC as low as $7.7x10^{-3}$ µg/l. If a NOEC would have been available from this study, the PNEC would have been even lower.

A PNEC of 1.5 µg/l have been proposed by WHO (2006) based on 21 days NOEC (*Daphnia,* survival and reproduction) of 0.015 mg/l, using a risk assessment factor of 10. A report prepared for the European Commission in 2005 presented a PNEC value in freshwater for DBT of 400 ng Sn/l (1.02 µg DBT/l) [31].

Due to lack of data a PNEC for sea water was not possible to derive. However, the acute toxicity to marine organisms seems to be similar to the acute toxicity to fresh water organisms. Therefore $PNEC_{sea\ water}$ would probably be close to $PNEC_{fresh\ water}$.

Proposed Environmental Quality Standard (EQS)

Since PNEC is $7.7x10^{-3}$µg/l the EQS value is $7.7x10^{-3}$µg/l.

Risk characterisation

The RCR calculated for different environmental compartments are shown in table 8.

Table 8. Risk characterisation ratios (RCR) for aquatic environments of dibutyltin dichloride. Predicted no effect concentration (PNEC) = $7.7x10^{-3}$µg/l. MEC = measured environmental concentration (mean or range).

Environmental compartment	MEC (µg/l)	RCR (MEC/PNEC)
Fresh water (Viskan river, Sweden)	0.013	1.69
Fresh water (Mälaren, Stockholm, Sweden)	0.0058-0.011	0.750
Waste water (Stockholm, Sweden)	0.12	15.6
Industrial storm waters (Sweden)	0.002-18	0.260-2340
Landfill leachates (Sweden)	0.003-0.5	0.390-64.9

The RCRs presented in the table above are in a wide range (varies between < 1 and >10) and therefore, depending on the actual site, DBT poses a low to a high risk to the aquatic environment. It is worth to note that the RCRs are all based on the highest measured levels of DBT.

In contrast, RCRs between 0.0013 and 0.53 indicating insignificant risk to aquatic organisms is obtained when using the PNEC of 1.5 µg/l proposed by WHO

The risk to marine environments, especially to sediment living organisms have not been evaluated in this risk assessment. Nor either have the risks for mammals and birds been assessed.

Comments

Because DBT is a degradation product from TBT it is difficult to perform a reliable risk assessment only on the current use of the compound [14]. Further investigations are needed to confirm the high levels of 1400 µg/kg dw DBT in sediment measured in a marina in Stockholm close to the launching area. Some data used in this assessment originate from studies performed by chemical companies and are therefore not available to the scientific community. However, the quality of these studies have been evaluated and considered acceptable by expert committees at WHO [14] and INERIS [34]. The studies of concern are the references: [45, 46, 47 & 51].

Monobutyltin (MBT)

General information

The general formula for monobutyltin (MBT) is $RSnX_3$. MBT is a tinorganic compound used in small quantities in Sweden, but fairly high levels are found in the nature probably because MTB is formed during degradation of TBT and DBT. According to a screening study made 2006 this compound was one of the most common tinorganic compounds found in the aquatic environment [15].

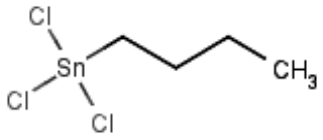
Field of application

The use of MBT is similar to that of DBT. MBT is e.g. used as stabilizers in PVC plastics, as catalysts in the production of plastics and in glass coating [14]. According to the Swedish Chemicals Agency 4.02 tones MBT was used in 1994, which is 1.7 percent of the total use of tinorganic compounds (figure 4) [20].

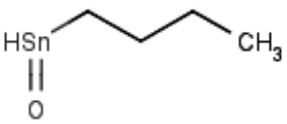
Physical and chemical properties

At room temperature MBT is a colourless liquid [16]. There are different MBT compounds (figure 6) but all studies in this report have been performed with the monobutyltin trichloride. Therefore all data in table 9 refer to monobutyltin trichloride.

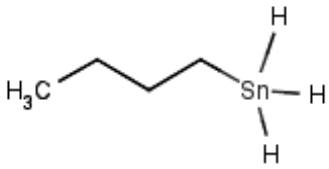
Monobutyltin ion
CAS-number: 78763-54-9



Monotributyltin trichloride
CAS-number: 1118-46-3



Monobutyltin oxide (Butyloxostannane)
CAS-number: 51590-67-1



Monobutyltin hydride
CAS-number: 2406-65-7

Figure 6. Structural formulas of different monobutyltin compounds.

Table 9. Physical and chemical properties of monobutyltin trichloride.

		Reference
CAS-No.	1118-46-3	[17]
Synonyms	Mono-n-butyltin trichloride	[17]
Empirical formula	C_4-H_9-Cl_3-Sn	[17]
Molecular weight (g/mol)	282.17	[16]
Solubility in water (mg/l)	8200 (at 25°C)	[14]
Vapour pressure (Pa)	5.84 (at 25°C)	[14]
Log Kow	0.18 , 0.41	[14, 16, 17]
Henry´s law constant ($Pa \times m^3/mol$)	1. 201	[16]

Mode of action and toxicity to aquatic organisms

In heterotrophic nitrifying bacteria MTB has been found to inhibit NH^{4+} uptake and nitrogen oxidation [40]. The acute toxicity of MTB to microorganisms in activated sludge is lower than that of other tinorganic compounds [64].

Results from base set tests (72 h tests on algae, 48 h tests on *Daphnia* and 96 h tests on fish) and also results from other relevant short term studies are presented in table 10. In table 11 results from long term studies are presented. Results from studies with not specified end-points are excluded from the tables. All studies have been conducted with the monotributyltin trichloride.

Table 10. Summary of results from short term toxicity test with monobutyltin trichloride on aquatic organisms.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Ankistrodesmus falcatus	growth rate	24 h	EC_{50} = 59,4	[41]
Crustaceans				
Daphnia magna	immobilization	24h	EC_{50} = 49	[43]
Daphnia magna	immobilization	24h	EC_{50} = 25	[66]
Fish				
Oryzias latipes	mortality	48h	LC_{50} = 38	[48]

Table 11. Summary of results from long term toxicity test with monobutyltin trichloride on aquatic organisms.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Platymonas sp.	growth rate	96 h	EC_{50} = 323	[53]
Scenedesmus obliquus	growth rate	96 h	EC_{50} = 336	[53]

Degradation

MBT is the final biodegradation product from sequential dealkylation of TBT and DBT. MBT is then degraded to the anion and finally to the tin ion. This stepwise process results in a reduction of toxicity [65, 67]. The biodegradation process occurs both during anaerobic and aerobic conditions. Some studies claim that the anaerobic degradation is slow and others that it is more rapid than aerobic degradation [26]. Degradation in surface water conducted according to OECD

guideline regarding inhibition of respiration has been found to be 69 % in 28 days [14, 68]. Thus MBT is not readily degradable.

Bioaccumulation

BFCs in carp (*Carassius carassius*) were found to be 2 in muscle, 126 in liver, 50 in kidney and 126 in gall bladder [33].

Biomagnification

No data have been found regarding biomagnification of MBT.

Classification

The 48h LC_{50} in fish is 38 mg/l. A probable 96h LC_{50} would be >10 mg/l. MBT is harmful to aquatic organisms and not readily degradable. Therefore MBT is classified as dangerous for the environment and assigned the risk phrases R52/53 (table 1).

Environmental fate

If released into fresh water or sea water and also in soil, MBT is expected to dissociate to the mono-n-butyltin cation which easily adsorbs to suspended solids and sediment [16, 70]. Also in moist air MBT is expected to dissociate to the mono-n-butyltin cation [16]. In air MBT is expected to exist only as a vapour [16].

Environmental levels

From a Swedish screening study made in 2006 the highest levels of MBT in fresh water were found in Eskilstuna (14-23 ng/l) and in the Viskan river and lakes downstream Borås (2.5-11 ng/l). Lower levels were found in Stockholm (2.0-2.9 ng/l) [15]. The same study showed levels in landfill leachates and storm waters from industrial sites to be 7-50 ng/l and 30-9600 (median 100) ng/l, respectively. MBT in waste water from municipal sewage treatment plants in Sweden were found in levels of 6-81 ng/l, with the highest levels in the Stockholm industrial areas [15]. According to Mersiowsky et al. the highest level of MBT in landfill leachates was as high as 2 µg/ l in Italy [127].

Levels of MBT in brackish and in sea waters in Sweden were found to be 0.005-6 ng/l with the highest levels measured in Norrby, Umeå, Stockholm city and close to a marina on the Swedish west coast [15]. Levels of MBT in herring (*Clupeidae sp.*) and salmon (*Salmonidae sp*.) collected from the Baltic coast outside Umeå were 0.3-0.6 ng/g dw [15]. Maximum concentration in sea water from marinas in the Netherlands was 0.310 µg Sn/l (0.74 µg MBT/l) [71]. MBT levels in blue mussel (*Mylilus edulus*) and in surface water from the Swedish west coast were found to be 26-600 ng/g dw and 3.8-5.9 ng/l, respectively [15].

MBT is the most common tinorganic compound found in sediments. This is not very surprising considering it being a dealkylation compound of dibutyltin (DBT) and tributyltin (TBT) [15]. A recent report from Stockholm university showed levels of MBT to be 200-1000 µg/kg dw in the surface sediment from a marina in Stockholm [23].

PNEC in fresh water

Few long term studies and only six short term studies of the toxicity of MBT to aquatic organisms were found. The lowest toxicity measure found in the literature was a 24 h EC_{50} (immobilization) of 25 mg/l for *Daphnia magna*. In the absence of long term tests for the three trophic levels a risk assessment factor of 1000 is used. This results in a PNEC of 25 µg/l, which is the same as the one presented by Mersiowsky in 2000 [127].

Due to lack of data $PNEC_{sea\ water}$ is not possible to derive.

Proposed Environmental Quality Standard (EQS)

Since PNEC is 25µg/l, the EQS is 25 µg/l.

Risk characterisation

The RCR calculated for different environmental compartments are shown in table 12.

Table 12. Risk characterisation ratios (RCR) for aquatic environments of monobutyl trichloride. Predicted no effect concentration (PNEC) = 25 µg/l, MEC = measured environmental concentration (mean or range).

Environmental compartment	MEC (µg/l)	RCR (MEC/PNEC)
Fresh water (Eskilstuna, Sweden)	0.023	$9.2*10^{-4}$
Brackish water (Norrby, Umeå)	0.006	$2.4*10^{-4}$
Sea water (marina, Netherlands)	0.31	0.0124
Waste water (Sweden)	0.006-0.081	$2.4*10^{-4}$ – 0.0032
Industrial storm waters (Sweden)	0.03-9.6 (median 0.1)	0.0012-0.38
Landfill leachates (Sweden)	0.007-0.05	$2.8*10^{-4}$ - 0.002
Landfill leachates (Italy)	2	0.08

The RCRs presented in the table above are all very low and only one ratio concerning aquatic environments is > 0.1 (0.31). Thus, according to these data MBT poses an insignificant risk to aquatic environments in Europe. It is worth to note that the RCRs are all based on the highest measured levels.

Low RCRs between 0.00004 and 0.03 have also been presented in a study by WHO [14].

The risk to marine environments, especially to sediment living organisms have not been evaluated in this risk assessment. Nor either have the risks for mammals and birds been assessed.

Comments

Though all studies made on MBT indicate low toxicity, too few studies on effects on different organisms have been performed in order to make a reliable risk assessment. Further investigations are needed to confirm the high levels of 1000 µg/kg dw DBT in surface sediment measured in a marina in Stockholm close to the launching area.

Some data used in this assessment originate from a study performed by a chemical company and is therefore not available to the scientific community. However, the quality of this study has been evaluated and considered acceptable by expert committees at WHO [14] and INERIS [34]. The study of concern is the reference: [66].

Pharmaceutical substances

General introduction

Pharmaceuticals have frequently been found in surface water, effluent water from municipal sewage treatment plants (STPs), ground water and drinking water [72]. Since large amounts of pharmaceutical products are used today there is a growing concern that these compounds may pose a risk to both human health and to the environment [73]. A screening study for pharmaceuticals made in German rivers and lakes found lipid regulators like bezafibrate and gemfibrozil, the non steroidal anti-inflammatory drugs (NSAIDs) diclofenac, ibuprofen, indometacine, naproxen and phenazone, the beta blockers metoprolol and propranolol and the antiepileptic drug carbamazepine [74]. Other pharmaceutical compounds like etinylestradiol, betaestradiol, eston, ciprofloxacin, linezolid and triclosan have been found in landfill sites [75].

After numerous studies and indications of high levels of pharmaceuticals in the environment, some regulations regarding pharmaceuticals were taken in 1995 according to the EU Directive 92/18 EEC and the corresponding "Note for Guidance". The EU guideline (Directive 2001/83/EC) specifies that a medical product for human use must be accompanied by an environmental risk assessment [77, 78]. A Swedish study made in 2006 estimated RCR for some often used pharmaceuticals which all indicated a low risk for the environment [75].

The use of pharmaceuticals

The pharmaceutical industry is a fast growing sector all over the world. In the U.S. the pharmaceutical industry has been growing twice as fast as the rest of the economy [79]. In Europe about 3000 different substances are used in human medicine and on top of this there are other compounds used in veterinary medicine [72]. In Sweden about 1200 pharmaceutical active substances in 7600 different products were in use in 2004 [80].

Environmental fate

Waste water treatment plants are not designed to handle pharmaceuticals and many of these compounds are not/incompletely removed during the cleaning process. Thus, effluent water from waste water treatment plants is an important route for pharmaceuticals to the aquatic environment. Pharmaceuticals may also enter ground water via leakage from waste disposal sites (figure 7). The pathways of pharmaceuticals from use to finally ending up in drinking water are shown in figure 7. Drug residues found in the aquatic environment usually occur in mixtures with other contaminants. Thus, scientific assessment of risk to aquatic life should consider this complex exposure situation [88].

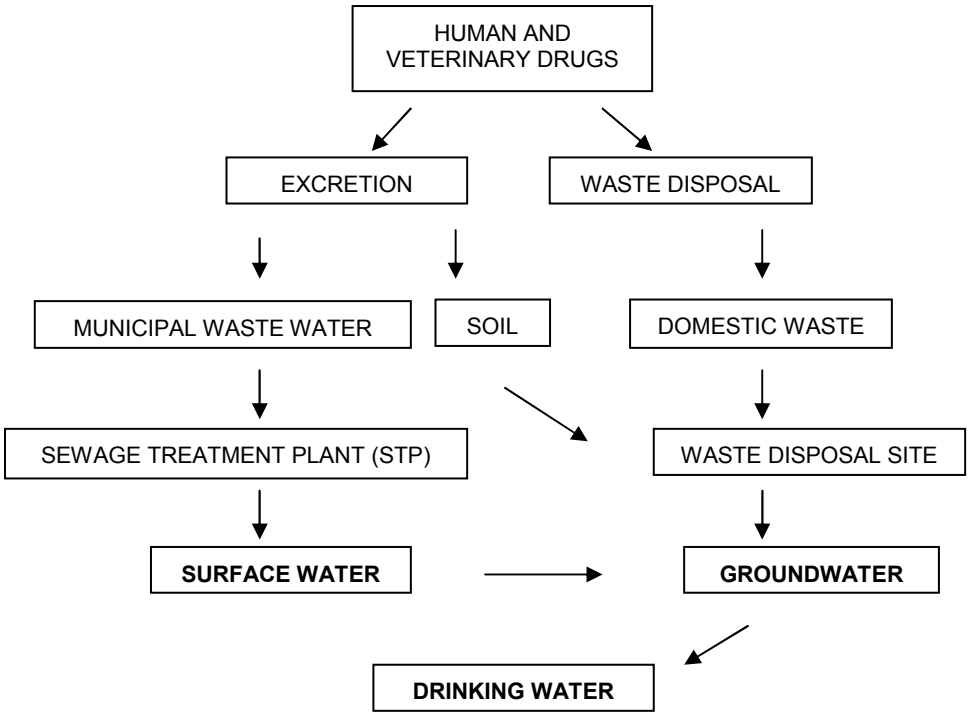


Figure 7. The pathways of pharmaceuticals from use to finally ending up in drinking water. Adapted from [82, 83].

Toxicity

Since pharmaceutical substances are intended to have biological effects, these easily can have effects on non-target organisms in the environment at low concentrations [84]. Risk assessments of a number of hormonal and anti-inflammatory pharmaceutical compounds have found that adverse effects on aquatic organisms cannot be excluded [75].

Diclofenac is one of the most common NSAID found in the Swedish environment and therefore this report will focus on this compound.

Diclofenac

General information

Diclofenac is a no steroidal anti-inflammatory drug used to reduce pain and inflammation [85]. This compound has been detected in surface water, ground water and drinking water and is one of the most common pharmaceuticals present in the water cycle [82].

Field of application

Diclofenac has anti-inflammatory, analgesic and antipyretic properties. The substance is used in pharmaceuticals used against headache, fever, cold, muscle pain, migraine, back pain and bone-ache [85]. The compound is used both as a human drug as well as a veterinary drug. In Germany 75 tons per year were used in 2002 and in Sweden the use was about 4 ton in 2002. Diclofenac is in use in 38 different pharmaceutical products in Sweden [95] and is the active substance in pharmaceuticals such as Voltaren, Solaraze, Eeze, Diclofenac Tratiopharm and Arthrotec forte.

Physical and chemical properties

At room temperature the substance is a crystalline solid [16]. In addition to the free acid diclofenac (CAS nr: 15307-86-5) there are two salts: diclofenac sodium (CAS Nr: 15307-79-6) and diclofenac potassium (CAS Nr: 15307-81-0) (figure 8). Physical and chemical properties are shown in table 13.

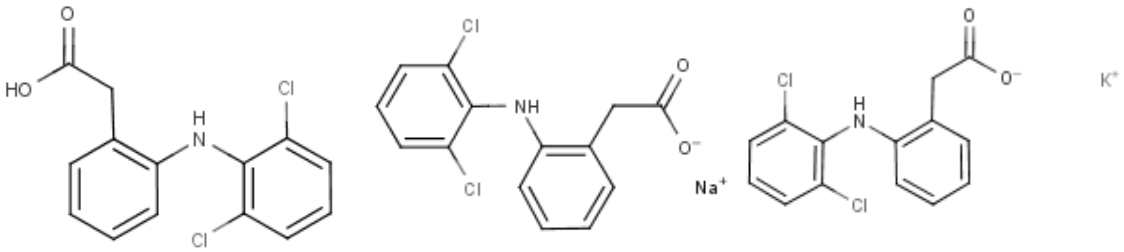


Figure 8. Structural formula of the free acid diclofenac (CAS-no: 15307-86-5) (to the left) and the salts diclofenac sodium (CAS-nr: 15307-79-6) (in the middle) and diclofenac potassium (CAS-nr: 15307-81-0) (to the right) [17].

Table 13: Physical and chemical properties of diclofenac sodium.

		Reference
CAS-No.	15307-79-6	[17]
Synonyms	(o-(2,6-Dichloroanilino)phenyl)acetic acid monosodium salt, Allvoran, Batafil, Anthraxiton, Kriplex, Ortofen, Sodium (o-((2,6-dichlorophenyl)amino)phenyl)acetate, Solaraze, Voltaren	[17]
Empirical formula	C_{14}-H_{10}-Cl_2-N-Na-O_2	[17]
Molecular weight (g/mol)	318.14	[16]
Solubility in water (mg/l)	2130-2430 (at 25°C)	[17]
Vapour pressure (Pa)	6.33×10^{-12} (at 25°C)	[17]
Log P_{ow}	0.7	[17]
Henry`s law constant ($Pa \times m^3/mol$)	$0.83\text{-}0.94 \times 10^{-9}$	[17]

Mode of action and toxicity to aquatic organisms

In mammals diclofenac inhibits prostaglandin synthesis via inhibition of cyclooxygenase 1 and 2 [16]. Prostaglandins are important regulators of renal blood flow and nefrotoxic effects of diclofenac are probably due to impaired control of renal blood flow [87]. The mode of action is considered to be the same in mammals and in aquatic animals like fish [88].

Results from base set tests (72 h tests on algae, 48 h tests on *Daphnia* and 96 h tests on fish) and also results from other relevant short term studies are presented in table 14. In table 15 results from long term toxicity tests are presented. Results from studies with not specified end-points are excluded from the tables.

Table 14. Summary of results from short term toxicity test with diclofenac sodium on aquatic organisms.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Microtox (bacteria)				
Vibrio fischeri	luminescence	30 min	EC_{50} = 11.45	[84]
Vibrio fischeri	luminescence	15 min	EC_{50} = 13.5	[79]
Vibrio fischeri	luminescence	15 min	EC_{50} = 13.7	[79]
Algae				
Desmodesmus subspicatus	growth rate	72h	EC_{50} = 71.9	[88]
Rotifers				
Brachionus calyciflorus	reproduction	48h	NOEC = 12.5 LOEC = 25	[84]
Crustaceans				
Daphnia magna	immobilization	48h	EC_{50} = 224.3	[84]
Cerodaphnia dubia	immobilization	48h	EC_{50} = 22.7	[84]
Daphnia magna	immobilization	48h	EC_{50} = 68	[88]
Cerodaphnia dubia	reproduction	7 days	NOEC = 1 LOEC = 2	[84, 89]
Fish				
Danio rerio	hatching time	96h	LOEC = 1	[90]
Oncorhynchus mykiss	cytopathology	48h	LOEC = 1	[91]
Salmo trutta f. fario	Histopathology	21 days	LOEC = 0.05	[92]

Table 15. Summary of results from long term toxicity test with diclofenac sodium on aquatic organisms. * According to TGD [7] these 28 days cytopathology tests does not fully meet the criteria for a long term study. However, in the present risk assessment this study is considered as a long term test.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Pseudokirchneriella subcapitata = *S. capricornutum*	growth rate	96h	NOEC = 10 LOEC = 20	[84]
Lemna minor	growth rate	7days	EC_{50} = 7,5	[88]
Fish				
Danio rerio	embryos and larvae mortality	10 days	LOEC = 4 NOEC = 8	[84] [84]
Oncorhynchus mykiss	histopathology (liver, kidney and gills)	28 days*	LOEC = 0.005	[93]
Oncorhynchus mykiss	cytopathology (liver, kidney and gills)	28 days*	LOEC = 0.001	[91]

The drug have anti-inflammatory, analgesic and antipyretic properties and the compound has been known for causing adverse effects on the kidney and liver in mammals and fish [75]. The compound has been found to cause a drastic decrease of the Oriental white-backed vulture (*Gyps bengalensis)* population in Asia. This species was used to be one of the most common raptors in India and Pakistan before the decrease of more than 95 % of the population, with start in the 1990s. The decrease was later found to be due to the birds eating pharmaceutical treated livestock. A level of 0.07 µg/g in the kidney was found to cause renal failure in the bird [94]. Developmental toxicity has been tested on zebra fish *(Danio rerio)*. A delay in the hatching time was found among zebra fish embryos exposed to 1000 or 2000 µg diclofenac/l (table 15). Cytopathology studies on rainbow trout found protein accumulation in the tubular cells and vesiculation of the endoplasmatic reticulum in the kidney. In the liver the cellular compartmentation was impaired and in the gills necrotic pillar cells and hypertrophied chloride cells were found after exposure to 1 µg/l (table 15) [91].

Degradation

Diclofenac was found not to be degraded in municipal wastewater treatment plants and none of the tested plants was able to remove diclofenac from wastewater [81]. Another study supports this result by showing 95% of the initial concentration remaining after treatment in a pilot sewage plant and in a biofilm reactor [96]. Heberer also found the removal of diclofenac to be low [82, 98]. Yet another study found diclofenac not to be eliminated in sewage treatment plants. The effluent concentration was found to be about 95% of the concentration in the incoming water [96]. In several sewage treatment plants the levels of diclofenac were higher in the effluent compared to the influent, possibly due to deconjugation of the metabolite diclofenac 1-O-acyl glucuronide [75]. On the other hand, other studies show up to 75 % removal of diclofenac in sewage treatment plants [83, 97].

Hydrolysis is not expected to be an important degradation process [16]. Direct photolysis is the predominant removal process in fresh water, with a half-life of 8 days [16]. In water, soil and sediment the half lives have been estimated to 38 days, 75 days and 340 days, respectively [75]. Degradation of 55.5% in 28 days in fresh water has been found in another study [85]. Thus, diclofenac is not readily degradable.

Bioaccumulation

Schwaiger found accumulation of diclofenac in all organs examined in rainbow trout when using concentrations in the ambient water of 1-500 µg/l. BCFs in different organs were 12-2732 in the liver, 5-971 in the kidney, 3-763 in the gills and 0.3-69 in the muscle, after 28 days exposure. The highest BCFs were found at a concentration in the ambient water of 1 µg/l [93]. This study may indicate a potential for bioaccumulation.

Biomagnification

No data have been found regarding biomagnification of diclofenac.

Classification

Diclofenac is harmful to aquatic organisms (EC_{50}, 48 h, *Daphnia*: 22.7 mg/l) and not readily degradable (half life: 38 days). Therefore diclofenac is classified as dangerous for the environment and assigned the risk phrases R52/53 (table 1).

Environmental fate

In water, diclofenac is expected to adsorb to suspended solids and sediment [16]. In soil, diclofenac is expected to adsorb to solids and therefore has a low mobility [16]. If released into air the compound will exist in both the vapour and particulate form. The vapour phase will be degraded by reaction with hydroxyl radicals. The half-live for this reaction is estimated to 2 hours [100].

Environmental levels

Diclofenac is one of the most frequent detected pharmaceuticals in environmental water samples [16] and concentrations in effluents in French, Greek, Italian and Swedish sewage treatment plants monitored February-March in 2001, varied between not detectable levels and 45 µg/l, with the highest level found in Italy, Naples. The median level was 0.29 µg/l [74]. The highest effluent levels in Sweden in 2006 were found in the treatment plants Ryaverken in Göteborg and Källby in Lund. The levels were 0.19 and 0.16 µg/l, respectively [75]. In 2004 no detectable level of diclofenac was found in Ryaverken in Göteborg [76]. The average concentrations of diclofenac in influent water of several Swedish sewage treatment plants have been found to be 0.37µg/l with the highest level of 0.7 µg/l [75]. Levels of diclofenac in sewage water from Swedish hospitals were found to be 0.12-2.2 µg/l [76]. Levels between 1 and 2 µg/l have been found in German streams receiving effluent water from sewage treatment plants [97]. A Swiss study found the highest levels in a lake in August and October to be 0.145 and 0.140 µg/l,

respectively. Another Swiss study found levels up to 2.940 µg/l in influents and effluents of sewage treatment plants. A background concentration from a nearby lake was 0.002µg/l [81].

PNEC in fresh water

The lowest LOEC obtained (1 µg/l) was from a 28 days study showing cytological alternations in liver, kidney and gill of rainbow trout [91]. Results from standardized long term tests were only available for algae and fish (embryo and larvae mortality, zebra fish) but not for *Daphnia*. According to TGD [7] the 28 days cytopathology test on rainbow trout, mentioned above, is not a standardized test and does not fully meet the criteria for a long term study. However, the studied cells appear to be very sensitive to diclofenac exposure and the results must be taken into account. In the present risk assessment this study is considered as a long term test. To convert this LOEC of 1µg/l to NOEC a safety factor of 10 is used giving a NOEC = 0.1 µg/l. The RAF to be used is 50 (base set + NOEC from two long term tests). This results in a PNEC of 0.002 µg/l.

The consequences of the cytological alternations in the fish are difficult to estimate. If strictly following TGD [7] the assessment should have been based on the short term 7 days NOEC (reproduction) of 1 mg/l for *Cerodaphnia dubia* (table 15) using a RAF of 50, resulting in a PNEC of 20 µg/l which is 20 times higher than the level causing cytological alterations in liver, kidney and gill of rainbow trout.

Carlsson et al. derived a PNEC of 0.01 µg/l based on the result from the same 28 days cytological study as the present study [86]. However, the RAF used in their study was 10 instead of 50. According to TGD to use a RAF of 10 data from the base set + long term NOECs from tests on species representing three trophic levels are required [7]. In this case long term NOEC from tests on Daphnia is missing. A PNEC as high as 100 µg/l have been calculated by Fent, IVL, and the Swedish Medical Products Agency [72, 75, 89]. They have all based their calculations on the 7 days NOEC (reproduction) of 1 mg/l for *Cerodaphnia dubia* (table 15). According to TGD [7] this test does not meet the criteria for a long term test and therefore a RAF of 50 (base set + NOEC from two long term tests) instead of 10 should have been used giving a PNEC of 20 µg/l.

Due to lack of data $PNEC_{sea\ water}$ is not possible to derive.

Proposed Environmental Quality Standard (EQS)

Since PNEC is 0.002 µg/l the EQS value is 0.002 µg/l.

Risk characterisation

The RCR calculated for different environmental compartments are shown in table 16.

Table 16. Risk characterisation ratios (RCR) for aquatic environments of diclofenac. Predicted no effect concentration (PNEC) = 0.002 µg/l, MEC = measured environmental concentration (mean or range). STP = sewage treatment plant.

Environmental compartment	MEC (µg/l)	RCR (MEC/PNEC)
Fresh water (Switzerland)	0.145	73
Streams close to STP (Germany)	1-2	500-1000
STP effluents (Naples, Italy)	45	22 500
STP effluents (Italy)	5.45	2725
STP influent and effluent (Switzerland)	0.489-4.89	245-2445
STP influent (Sweden)	0.7	350
Hospital waste water (Sweden)	0.12-2.2	60-1100

The RCRs presented in the table above are all very high (>10) and therefore diclofenac pose a high risk to the aquatic environment. It is worth to note that the RCRs are all based on the highest measured levels.

In contrast, Carlsson found a RCR of 4.8, based on the PNEC of 0.01 µg/l and a $PEC_{fresh\ water}$ of 0.048 µg/l [86], indicating diclofenac to pose a moderate risk to aquatic organisms. Other studies have presented RCRs showing diclofenac to pose an insignificant risk to aquatic organisms [75, 80, 81].

The risk to marine environments, especially to sediment living organisms have not been evaluated in this risk assessment. Nor either have the risks for mammals and birds been assessed.

Comments

The calculation of $PNEC_{fresh\ water}$ in the present study is based on results from a 28 days study showing cytological alternations at low exposure levels (LOEC = 1 µg/l) in liver, kidney and gill of rainbow trout. The significance of these cytological alterations is, at present, difficult to assess. In order to confirm these findings and to investigate consequences of the cytological alterations more studies are needed.

Musk substances

General introduction

Musk fragrances have been used for a long time in perfumes and the natural musk fragrance was originally extracted from glands of the musk ox and musk deer (*Moschus moschiferus)* [107]. Since the natural musk is a very expensive product, synthetic musks have been produced since the beginning of the twentieth century. The synthetic musks can be divided into four groups: polycyclic musks, nitro musks, macrocyclic musks and alicyclic musks [108, 126]. The first synthetic musks to be produced were the nitro musks [108]. The most common nitro musk compounds are musk xylene, musk ketone and musk ambrette. The polycyclic musks were introduced in the 1950s [108]. The two most produced polycyclic musks are galaxolide and tonalide. Other polycyclic musks are celestolide, phantolide and traseolide [110]. The chemical structures of nitro musks and polycyclic musks are shown in figure 9. The third and fourth classes of musk compounds are the macrocyclic and the alicyclic musks. These groups are new substances and the alicyclic musks were recently introduced to the market [110, 126].

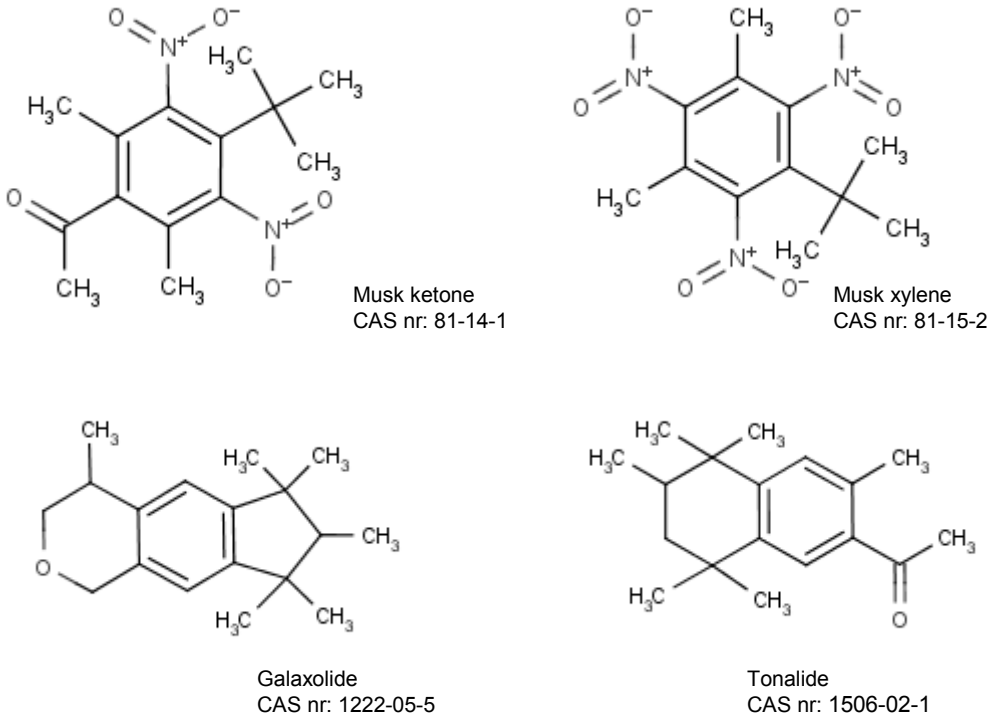


Figure 9. Examples of different musk compounds: the nitro musks; musk ketone and musk xylene and the polycyclic musks; galaxolide and tonalide.

The regulations to follow for cosmetic and hygienic products are stated in chapter 14 in the Swedish Environmental Code. [111].

The use of musk substances

Musks are often components in soaps, detergents and cleaning products as well as in perfumes, body creams, lotions and deodorants [112]. These products are often named personal care products which also include fragrances, perfumes and sunscreen agents. In personal care products musk levels can be up to 4 mg/g [113]. The world production of musk compounds are about 6000-8000 ton per year, of which Sweden use about 4-5 ton [114]. About 2000 ton polycyclic musk compounds, especially galaxaxolide and tonalide, are used in products produced in Europe every year [115]. Due to toxic properties, the nitro musks have been phased out and replaced by the polycyclic musks in many products. In the US the use of nitro musk compounds is still high [116].

Environmental fate

The occurrence of nitro musks in the aquatic environment was first reported in 1981 [117]. A problem with many chemicals present in personal care products is that they end up in the sewage system and they are poorly removed in sewage treatment plants (STPs) [112]. Studies have shown that effluents from sewage treatment plants are a major source of contamination and that the levels in water and biota depend on the distance from STPs [110]. Thus, effluent water from STPs is an important route for musk substances to the aquatic environment similar to that of pharmaceuticals.

Toxicity

The toxic effects of musk compounds are connected to a slow biotic degradation and a high potential for bioaccumulation [110]. Musks have a low acute toxicity but are carcinogenic in mice [114]. Polycyclic musk compounds have in various bioassays shown antiestrogenic activity [121]. However, their impacts as endocrine disruptors are regarded as low [122]. In the limited number of studies on early life stages of fish and amphibians no effects of these compounds have been found [124, 11].

Tonalide is one of the most frequently detected polycyclic musk compounds in the European and also in the Swedish environment and therefore this report will focus on this compound.

Tonalide

General information

Tonalide, 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphtahalene is a synthetic polycyclic musk fragrance [123]. Together with the compound galaxolide, tonalide is the most frequently detected polycyclic musk compound in water and biota in Sweden and in other parts of Europe [113].

Field of application

Tonalide is commonly used as a fragrance ingredient in household product such as detergents and washing powder and in personal care products as perfumes, cosmetics, lotions and shampoo. The use of tonalide is increasing, since musk xylene and musk keton and other nitro musks are today being replaced by tonalide and other polycyclic musk compounds [123]. In 2000 358 tons were used in Europe, where about one fourth was used in detergents [124].

Physical and chemical properties

At room temperature tonalide exists as solid granules [32]. The chemical structure and properties of tonalide (CAS nr: 1506-02-1) are shown in figure 10 and table 17.

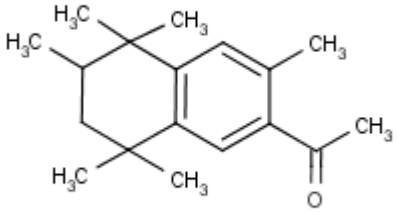


Figure 10. The structural formula of tonalide (CAS nr: 1506-02-1) [17].

Table 17: Physical and chemical properties of tonalide.

		Reference
CAS-No.	1506-02-1	[17, 109]
Synonyms	Acetyl hexamethyl tetralin, AHTN, AHMT, Musk tonalid, Tentarome, Tetralide, Tonalid, Tonalide, Fixolide	[17, 22]
Empirical formula	C_{18}-H_{26}-O	[16]
Molecular weight (g/mol)	258.4	[16]
Solubility in water (mg/l)	1.25 (at 25 ˚C)	[16]
Vapour pressure (Pa)	0.068 (at 25 ˚C)	[16]
Log Kow	5.4-6.35	[16, 32, 108]
Henry`s law constant ($Pa \times m^3/mol$)	14.1-37.1	[16, 124]

Mode of action and toxicity to aquatic organisms

No information in the literature regarding mechanisms behind toxic effects of tonalide has been found. However, *in vitro*, tonalide exhibits antiestrogenic activity via binding to the estrogen receptor ß (IC_{50}: 1.9 µM) [121]. Also *in vivo,*

*(*transgenic zebrafish (*Danio rerio*)) tonalide has been found to be antiestrogenic [27]. Though, anti-estrogenic effects of tonalide have been considered relatively low [27].

Results from base set tests (72 h tests on algae, 48 h tests on *Daphnia* and 96 h tests on fish) and also results from other relevant short term toxicity tests are presented in table 18. In table 19 results from long term toxicity tests are presented. Results from studies with not specified end-points are excluded from the tables.

Table 18. Summary of results from short term toxicity test with tonalide on aquatic organisms. * According to TGD [7] the exposure time of a long term test on cold water fish must exceed 60 days.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Pseudokirchneriella subcapitata	Growth rate	72h	EC_{50} = 0.468	[124, 125]
Pseudokirchneriella subcapitata	Growth rate	72h	EC_{50} > 0.835	[32, 125]
Crustaceans				
Nitocra spinipes	mortality	96h	LC_{50} = 0.61	[106]
Acartia tonsa	mortality	48h	LC_{50} = 0.71	[120, 124]
Fish				
Danio rerio	heart rate	48h	NOEC = 10 LOEC = 33	[103, 32]
Lepomis macrochirus	Growth	21 days*	NOEC = 0.067	[123]
Lepomis macrochirus	mortality	21 days*	NOEC = 0.089 LC_{50} = 0.314	[63, 86]] 124]
Pimephales promelas	Growth, development	36 days*	NOEC = 0.035	[32, 99]
Benthic organisms				
Chironomus riparius	mortality	96h	NOEC >0.5	[11, 32]
Marine mussel				
Mytilus californianus	inhibition of efflux transporters	24h	IC_{50} = 2.05	[32, 122]

Table 19. Summary of results from long term toxicity test with tonalide on aquatic organisms.

Species	End-point	Time of exposure	Result (mg/l)	Reference
Algae				
Pseudokirchnella subcapitata	growth rate	72h	NOEC = 0.204	[125]
Pseudokirchnella subcapitata	growth rate	72h	NOEC = 0.374	[123]
Crustaceans				
Daphnia magna	reproduction	21 days	NOEC = 0.196 EC_{50} = 0.244	[32, 35, 123] [32, 35, 123]
Fish				
Brachydanio rerio	growth, development	34 days	NOEC = 0.035	[32, 63]

The most sensitive tested organism during short term exposure is the algae *Pseudokirchneriella subcapitata* with a 72h EC_{50} (growth rate) of 0.468 mg/l (table 18). The most sensitive tested organisms for long term exposure are 21-36 days NOEC (mortality, growth, development) of 0.035 mg/l for the fishes *Brachydanio rerio* and *Pimephales promelas* (table 19).

Degradation

According to Hazardous Substances Data Base biodegradation is not regarded as an important elimination process for tonalide [16]. Measured experimental half-lives of 1873 days in fresh water microcosms at low total fungal biomass and 38 days at higher fungal activity were found by Martin et al. in 2003 [65]. In 2004 DiFrancesco found the concentration in soil after one year to be 14-55 percent of the initial concentration of 17-51 µg/ dw, which results in half-lives between 307 and 584 days [105]. Thus, tonalide is not readily degradable.

Bioaccumulation

Tonalide has shown to accumulate in several aquatic organisms, such as fish, mussel and river otter (*Lutra lutra*) [101]. BCFs of 597 and 600 for the bluegill sunfish (*Lepomis macrochirus*) and zebrafish have been found [108, 119]. BCFs for tonalide in different animal species from a pond of a municipal sewage treatment plant in Schleswig-Holstein, Germany were found to be 2400-40 000 [118], with the highest values found in zebra mussel (*Dreissena polymorpha*), tench (*Tinca tinca*) and crucial carp (*Carassius carassius*). Lower values were found in rudd (*Scardinus erythrophthalmus*) and eel (*Anguilla anguilla*). [108].

Biomagnification

No data have been found regarding biomagnification of tonalide.

Classification

Tonalide is very toxic to aquatic organisms (EC_{50}, 72 h, *Pseudokirchneriella subcapitata*: 0.468 mg/l) and is not readily degradable. It has a high potential for bioaccumulation (BCF: 600-40 000). Tonalide is therefore classified as dangerous for the environment and assigned the risk phrases R50/53 (table 1).

Environmental fate

A wide range of studies made show that synthetic musks are widespread in marine and freshwater environments [67, 69, 108, 122]. In water tonalide is expected to adsorb to solids and sediments and in soil the substance is expected to be immobile (K_{OC} = 6,309-63,000) [16, 123]. Adsorption to soil is expected to reduce volatilization [16]. The photolysis of tonalide is fairly rapid with a half-live estimated to 22 hours [16]. Biodegradation however, is not regarded as an important elimination process [16].

Environmental levels

A Nordic screening study showed tonalide together with galaxolide to be present in higher concentrations in the environment than other musk compounds [109]. The

highest levels found were in waters and sludge from STPs and in fish from sewage ponds. The levels in water and biota varied depending of the distance to sewage treatment plants [108].

In Sweden, levels in effluents from sewage treatment plants were in one study found to range from 0.042 to 0.104 µg/l, with the highest level in effluents from Gässlösa STP in Borås [67]. In another study Paxeus found levels between 0.5 and 50 µg/l in effluents from Swedish STPs [60]. The levels of tonalide in sewage sludge presented in a Nordic screening study ranged between 70 and 3600 µg/kg dry weight [109]. In areas in the Ruhr district in Germany the mean level in the surface water was found to be 10 ng/l [115]. The highest level in influent water to a German STP was found to be 0.58 µg/l while the effluent water had levels up to 0.24 µg/l [115]. The highest level in recipients of STPs in Germany was found to be 1.9 µg/l [118].

Levels in fish in the Swedish environment have been found to be 9-367 ng/g lipid, with the highest levels found in bream (*Albramis brama*) in the Viskan river [110]. In biota from a pond of a German municipal sewage treatment plant Gatermann found levels of 3-45 µg/g lipid [118]. Eels (*Anguilla anguilla*) had the lowest concentrations of 2.6 µg/g lipid. Also in rudd (*Scardinius erythrophthalmus*) the concentration was low while tench (*Tinca tinca*), crucian carp (*Carassius carassius*) and zebra mussel (*Dreissena polymorpha*) had concentrations up to 45 µg/g lipid. Levels in animals living in sea water were found to be low, sometimes below detection limit [101].

PNEC in fresh water

Apparently, fish are the most sensitive aquatic organisms to tonalide. Two different studies on growth and development in *Brachydanio rerio* (exposure time: 34 days) and *Pimephales promelas* (exposure time: 36 days) resulted both in a NOEC of 0.035 mg/l [32, 63]. Long term NOECs were available for three tropic levels and therefore a risk assessment factor of 10 was used. This results in a PNEC of 3.5 µg/l.

Balk (1999) and the organisation Human & Environmental Risk Assessment on ingredients of Household Cleaning Products (HERA) (2004) also suggested a PNEC of 3.5 µg/l based on three long term tests (*Pseudokirchneriella subcapitata* 72h NOEC, *Daphnia magna* 21 days and *Lepomis macrochirus* 21 days) [123, 124]. According to TGD [7] the fish test does not meet the criteria for a long term test and therefore a higher RAF should have been used.

Due to lack of data a PNEC in sea water was not possible to derive.

Proposed Environmental Quality Standard (EQS)

Since PNEC is 3.5 µg/l the EQS value is 3.5 µg/l.

Risk characterisation

The RCR calculated for different environmental compartments are shown in table 20.

Table 20. Risk characterisation ratios (RCR) for tonalide in aquatic environment. Predicted no effect concentration (PNEC) = 3.5 µg/l, MEC = measured environmental concentration. STP = sewage treatment plant.

Environmental compartment	MEC (µg/l)	RCR (MEC/PNEC)
STP effluent (Berlin, Germany)	6.8	1.94
STP effluents (Sweden)	50	14.3
Fresh water (Ruhr, Germany)	0.010	0.029
STP effluent (Germany)	0.240-1.9	0.069-0.54
STP effluent (USA)	1.555	0.44

The RCRs presented in the table above are low in most cases (mostly <0.1 but one >10) and therefore tonalide pose an insignificant to high risk to the aquatic environment. It is worth to note that the RCRs are all based on the highest measured levels.

Balk (1999) found RCRs of 0.086 [123], indicating a low risk to aquatic organisms.

The risk to marine environments, especially to sediment living organisms have not been evaluated in this risk assessment. Nor either have the risks for mammals and birds been assessed.

Comments

Many studies on environmental levels of tonalide have been performed but very few on effects on different organisms. More standardized long term studies and studies regarding biomagnification are needed.

Some data used in this assessment originate from studies performed by chemical and/or pharmaceutical companies and are therefore not available to the scientific community. However, the quality of these studies have been evaluated and considered acceptable by expert committees at WHO [14] and INERIS [34]. The studies of concern are the references: [63, 86, 99, 119 & 125].

Conclusions

The results of this study show very low RCRs for MBT (below and just above 1), in general very low (< 0.1) for tonalide (only two values above 1; 1.94 and 14.3), low to high for DBT (< 1 and >10) and above 10 for diclofenac. Thus, tonalide and especially DBT and diclofenac might pose a high risk to the aquatic (limnic) environment. In many instances more standard tests according to the technical guidance document are requested, as well as studies regarding biomagnification. Another importance to take in consideration is the fact that compounds found in the aquatic environment often occur as mixtures, not as single contaminants. Thus, scientific assessment of risk to aquatic life should consider this complex exposure situation [88]. However, the risk to marine environments, especially to sediment living organisms has not been evaluated in this risk assessment. Neither have the risks for mammals and birds been assessed.

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