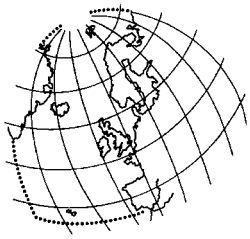
Hazardous Substances Series

Clotrimazole



OSPAR Commission
2005 Update

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was opened for signature at the Ministerial Meeting of the former Oslo and Paris Commissions in Paris on 22 September 1992. The Convention entered into force on 25 March 1998. It has been ratified by Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland and the United Kingdom and approved by the European Community and Spain.

La Convention pour la protection du milieu marin de l'Atlantique du Nord-Est, dite Convention OSPAR, a été ouverte à la signature à la réunion ministérielle des anciennes Commissions d'Oslo et de Paris, à Paris le 22 septembre 1992. La Convention est entrée en vigueur le 25 mars 1998. La Convention a été ratifiée par l'Allemagne, la Belgique, le Danemark, la Finlande, la France, l'Irlande, l'Islande, le Luxembourg, la Norvège, les Pays-Bas, le Portugal, le Royaume-Uni de Grande Bretagne et d'Irlande du Nord, la Suède et la Suisse et approuvée par la Communauté européenne et l'Espagne.

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Contents

Executive Summary

Clotrimazole (CAS No. 23593-75-1) was included on the OSPAR List of Chemicals for Priority Action at OSPAR 2002. Clotrimazole is a pharmaceutical. Its main use is for treatment of dermatological and gynaecological fungal infections.

There is only one producer and one production site in the European Union (Spain). Approximately 10 tonnes are produced in the EU each year, and almost the same quantity is imported. The main potential source of clotrimazole to the environment is discharges from municipal waste water treatment plants as a result of waste water from households.

The substance is non biodegradable according to the results of a biodegradability test and estimation of the QSAR data. The half-life of clotrimazole in the environment is thus expected to be more than 60 days, which is the cut-off value in the EU Technical Guidance Document (TGD). The persistence (P) criterion is therefore fulfilled. Based on the experimentally established value for the octanol-water partition coefficient (log K_{ow} 4,1) and an estimated bioconcentration factor for fish (BCF 610), it is concluded that the TGD bioaccumulation (B) criterion is not fulfilled. The most sensitive trophic level is Crustacean where a long-term toxicity data of 0,01 mg/l is reported. The toxicity (T) criterion is therefore fulfilled. Risk assessment based on calculation of the ratio Predicted Environmental Concentration over Predicted No Effect Concentration (PEC/PNEC) indicates that there is at present no risk both for freshwater organisms and for organisms living in the marine water column. The overall conclusion is that clotrimazole is not a PBT substance according to the EU TGD criteria and, that there is at present no risk for the marine environment due to the production and use of clotrimazole.

At present there is no need for OSPAR to propose measures for the reduction of discharges of clotrimazole. The actions recommended are: Contracting Parties who are also EU Member States should support the ongoing development of the Risk Assessment Review and provide new information, if available, on exposure, discharges, emissions and losses, which would enable the PEC/PNEC ratios to be refined; OSPAR should re-evaluate the risks posed by clotrimazole releases when further information has been collected; Users should be informed by package leaflets about environmentally safe ways of disposal of medicines that are no longer required. OSPAR should communicate this Background Document to the European Commission and to other appropriate international organisations which deal with hazardous substances to promote action to take account of this Background Document in a consistent manner.

A monitoring strategy for clotrimazole is attached to this background document.

Récapitulatif

Le clotrimazole (N° CAS 23593-75-1) a été inscrit à OSPAR 2002 sur la liste OSPAR des produits chimiques devant faire l'objet de mesures prioritaires. Le clotrimazole est un produit pharmaceutique. Sa principale application est le traitement des infections fongiques dermatologiques et gynécologiques.

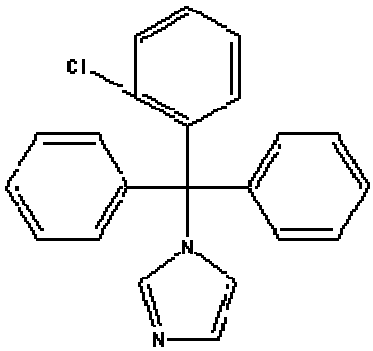
Il n'existe dans l'Union européenne (en Espagne) qu'un seul fabricant, et un seul site de production. Environ 10 tonnes sont fabriquées tous les ans dans l'Union européenne, et un volume presque identique à celui-ci est également importé. La principale source potentielle de clotrimazole dans l'environnement tient aux rejets des stations municipales d'épuration des eaux usées, ceci en raison des eaux usées des ménages.

Selon les résultats d'un test de biodégradabilité et de l'estimation des données QSAR, cette substance n'est pas biodégradable. Par conséquent, dans l'environnement, la demie-vie du clotrimazole devrait être supérieure à 60 jours, ce qui constitue la valeur de coupure indiquée dans le Document d'orientation technique de l'Union européenne (DOT). Le critère de persistance (P) est donc rempli. Sur la base de la valeur, obtenue expérimentalement, du coefficient de partage octanol-eau (log K_{ow} 4,1) et d'un coefficient estimé de bio-concentration chez le poisson (BCF 610), il est conclu que le critère de bio-accumulation (B) du DOT n'est pas rempli. Le niveau trophique le plus sensible est constitué par les crustacés, pour lesquels on signale des données de toxicité sur le long terme de 0,01 mg/l. Par conséquent, le critère de la toxicité (T) est rempli. L'évaluation des risques, basée sur le calcul du ratio entre concentration environnementale prédite et concentration prédite sans effet (PEC/PNEC) indique que pour l'heure, il n'y a pas de risque tant pour les organismes vivant en eau douce que pour les organismes vivant dans la colonne d'eau de mer. La conclusion générale est que le clotrimazole n'est pas une substance PBT selon les critères du DOT de l'Union européenne, et que, à l'heure actuelle, la fabrication et l'utilisation du clotrimazole ne présentent pas de risque pour le milieu marin.

Pour l'heure, il n'y a pas lieu, pour OSPAR, de proposer des mesures visant à réduire les rejets de clotrimazole. Les actions recommandées sont les suivantes : il conviendrait que les Parties contractantes qui sont également des Etats membres de l'Union européenne apportent leur appui à l'élaboration, qui est en cours, du réexamen de l'évaluation des risques, et communiquent de nouveaux éléments d'information, si elles en disposent, sur l'exposition, les rejets, les émissions et les pertes, qui permettraient de raffiner les ratios PEC/PNEC ; il conviendrait qu'OSPAR réévalue les risques que présentent les émissions de clotrimazole lorsque de plus amples renseignements auront été réunis ; il conviendrait que les utilisateurs soient informés, par des brochures, des moyens environnementalement sûrs d'éliminer les produits médicaux qui ne sont plus nécessaires. Il conviendrait enfin qu'OSPAR communique le présent document de fond à la Commission européenne ainsi qu'à d'autres organisations internationales compétentes traitant des substances dangereuses, ceci afin de favoriser la prise en compte du présent document de fond dans des conditions cohérentes.

Une stratégie de surveillance sur le clotrimazole est jointe à ce document de fond.

I. Environmental properties



Clotrimazole (CAS n°- 23593-75-1) was first synthesised in 1969. Chemical name of the substance is 1-(2-chloro-phenyl)diphenylmethyl-1H-imidazole). Molecular formula is $C_{22}H_{17}ClN_2$ and the molecular weight is 344,8 g.mol-1.

Clotrimazole is an inhibitor of ergosterol biosynthesis and as such it has many ecotoxicological properties in common with a range of fungicides used in agriculture. Clotrimazole is a broad-spectrum antimycotic agent effective against pathogenic dermophytes, yeasts and several species of *Candida*, *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia.*

This chemical was selected as a priority substance according to the DYNAMEC criteria mainly based on QSAR data. In this background document, revision of data and application of the risk assessment based on the criteria outlined in the Technical Guidance Document (E.U., 2003) have led to the following assessment of the substance.

All the physico-chemical, ecotoxicological or toxicological data are reported in the clotrimazole factsheet available in Appendix 1.

I.1. General substance information (physico-chemical properties)

I.1.1. Melting point

Melting point values of clotrimazole between 141 and 145°C were reported (Hoogerheide et Wyka, 1982). No information on the method used to determine these values is available.

I.1.2. Boiling point

No experimental data on the boiling point of clotrimazole is available. QSAR data can be calculated from the US EPA EPI suite model (US-EPA, 2003). The calculated QSAR value is 494,52°C.

I.1.3. Vapour pressure

Only calculated values are available. A value of 3,31E-07 Pa is reported from the calculation program SRC-MPBP (Meylan, 1994). The EPI suite model reports a similar calculated vapour pressure of 2,84E-07 Pa. The vapour pressure of clotrimazole is therefore very low and the value of 3,31E-07 Pa will be used in the risk assessment.

I.1.4. Water solubility

The water solubility of clotrimazole is reported as 0,49 mg/l in different test reports (Bruns, 2003b; Bruns, 2003a; Bruns, 2003c). No information on the method used to determine this value is available.

I.1.5. Partition coefficient

The log K_{ow} was determined according to the OECD guideline (GL 117) by Reverse Phase HPLC (Erstling et Jungheim, 2003). The buffer pH value was 8 and the temperature of the column was of 40°C.
The pH value was chosen according to the OECD guideline recommendation for weak bases. In this case the test must be performed with the non-ionised form of the test substance which can be achieved by performing the test at least one pH unit above the pKa value (6,12 for clotrimazole).

The OECD guideline presumably refers to 25°C. However, the influence of the higher temperature (40°C) on the calculated log K_{ow} lies within the range of the error of the method and was used for both the test and the reference substance. The test is therefore considered valid.

The partition coefficient determined by this method is 4,1. A QSAR value of 6,26 is also available (US-EPA, 2003). As experimental values are preferred rather than calculated one the log Kow of 4,1 will be used in the risk assessment.

I.1.6. Summary

The physico-chemical properties of clotrimazole are reported in Table 1.

Table 1. Physico-chemical properties of clotrimazole

Properties	Value
Molecular weight (g/mol)	344,84
Melting point[1] (°C)	141 – 145
Boiling point[2] (°C)	494,52
Vapour pressure[2] (hPa)	3,31E-05
Partition coefficient octanol-water[1] (Log Kow)	4,1
Water solubility[1] (mg/l at 25°C)	0,49

I.2. Classification

Clotrimazole is classified as a dangerous substance within the meaning of directive 67/548/EEC. The classification is:

- Xn: harmful and N: dangerous for the environment;
- The risk phrases assigned to clotrimazole are:
  - R22: harmful if swallowed;
  - R50/53: very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

I.3. Degradation

I.3.1. Abiotic degradation

a) Hydrolysis

The abiotic degradation of clotrimazole was tested at several pH values (4, 7 and 9) according to the OECD guideline 111 (Erstling, 2001). The substance is not degradable at pH 9 at 50°C. The reported half-lives, based on linear regression following a first order concentration-time-law, are respectively 242 days and 20 days at pH 7 and 4 at 25°C.

No information regarding the degradation products is available at these two pHs. However, (Hoogerheide et Wyka, 1982) report that the substance is stable in alkaline medium and hydrolyses in acidic medium to (o-chlorophenyl)-diphenylmethanol and imidazole.

b) Photolysis

The photodegradation of clotrimazole in water was determined according to the method developed by the ECETOC "Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water of clotrimazol" (Hellpointer, 2002). The quantum yield of direct photodegradation was determined using a polychromatic light. A degradation of approximately 40% of clotrimazole was measured by HPLC-UV during a maximum irradiation period of 500 minutes. The mean quantum yield calculated (arithmetic mean of the two experiments) was of Φ = 0,000305. The environmental half-lives were assessed thanks to two different arithmetic models (GC SOLAR and Klöppfer modelling). The resulted half-lives are in a range between 3 to 310 days depending on the sunlight conditions. The 310 days value was obtained with the Klöpffer modelling under the following conditions: December with a maximum amount of clouds. The mean value for December with the same model is 63 days. The 3 days value was obtained in May under optimal conditions. No information on the degradation products is available.

1 Measured value

2 Calculated value

Due to its low vapour pressure clotrimazole is not expected in the atmosphere and therefore the photodegradation in this compartment was not studied.

I.3.2. Biodegradation

The biodegradability of clotrimazole (Bayer, 1994) was tested in a carbon dioxide evolution test (OECD 301B). The substance was tested at concentrations of 20 mg/l during 28 days. The test substance is not biodegradable under the test conditions. As no other information is available on the biodegradability of the substance, clotrimazole will be considered not readily biodegradable. However due to the chemical structure of this compound the release of CO_2 by biodegradation is not expected in 28 days. In order to know if degradation products were produced during the bioassay, it would have been interested to make measurements and identify potential residues in the sludge.

Furthermore, QSAR estimation (US-EPA, 2003) indicates that the substance is not readily biodegradable (non linear model prediction = 0,4732; MITI non linear model prediction = 0,0054 and ultimate biodegradation timeframe prediction = 2,0624).

I.4. Bioaccumulation

The partition coefficient of test substance as determined before is 4,1.

A bioconcentration factor (BCF) for fish can be estimated according to the following equation as reported in the TGD (E.U., 2003):

$$\text{Log } BCF_{fish} = 0{,}85 \text{ . log } Kow - 0{,}70$$

$$\text{Log } BCF_{fish} = 2{,}785$$

$$BCF_{fish} = 610.$$

I.5. Aquatic toxicity

Ecotoxicological data on clotrimazole are available on algae (*Desmodesmus subspicatus*), micro-organisms (*Pseudomonas putida, Vibrio fischeri*), crustacean (*Daphnia magna*) and fish (*Brachydanio rerio*, *Oncorhynchus mykiss*).

I.5.1. Water organisms

a) Acute toxicity

Acute toxicity to Algae

Bruns (2003b) assessed the toxicity of clotrimazole to *Desmodesmus subspicatus* after an exposure time of 72 hours according to the method described in Directive 92/69/EEC, C3. The effects measured were the growth and the growth rate of the algal population exposed to 0,01, 0,02, 0,04, 0,08, 0,16 and 0,32 mg/l. The EC_{50} determined respectively for growth and growth rates were of 0,098 (CL95%: 0,063 – 0,169) mg/l and 0,268 (CL95%: 0,186 – 0,532) mg/l. The results are all expressed as measured concentrations.

$$E_bC_{50} \text{ (72h)} = 0{,}098 \text{ mg/l}$$

$$E_rC_{50} \text{ (72h)} = 0{,}268 \text{ mg/l}$$

Acute toxicity to Crustacean

Caspers and Müller (1994) report an EC0 = 0,025 mg/l and an EC100 = 0,1 mg/l on *Daphnia magna* after an exposure of 48 hours. The EC50 was not calculated in the study only the geometrical mean of the EC0 and the EC100 is given and results in a concentration of 0,05 mg/l. The test concentrations were 0,007, 0,013, 0,025, 0,05 and 0,1 mg/l. As no analytical method was available for the determination of concentrations as low as 0,05 mg/l, the results are expressed only as nominal concentrations. It is also mentioned that due to the low water solubility of the substance, the mixture was stirred three hours and filtrated before being used. However there is no information on the possible loss of the chemical and on the real concentrations tested. On the basis of recent ecotoxicological studies performed on fish and algae (Bruns, 2003b; Bruns, 2003a), it seems that the water solubility of the test item under exposure conditions is about 0,35 mg/l. Therefore, the nominal concentrations of the study with *Daphnia magna* were recalculated based on a stock solution of 0,35 mg/l instead of 1 mg/l (Bruns, 2004). This results in the following test item concentrations: 0,0021, 0,004, 0,008, 0,017, 0,033 mg/l. The EC0 and EC100 are respectively of 0,008 and 0,033 mg/l after 48 hours of exposure and a statistical evaluation leads to an EC50 (48h) = 0,02 mg/l (95% C.I: 0,018 – 0,022 mg/l).

$$EC_{50} \text{ (48h)} = 0{,}020 \text{ mg/l}$$

This test is considered valid with restriction and will be used in the derivation of the PNEC for the aquatic compartment.

Acute toxicity to fish

Caspers and Müller (1994) report data on the acute toxicity of clotrimazole to *Brachydanio rerio*. The method used is similar to the one described under Directive 92/69/EEC, C1. Only one concentration was tested at the limit of the water solubility of the substance (0,5 mg/l). The fish were tested in semi-static conditions and the concentrations of clotrimazole were measured during the whole test at the start, after 24 hours and after additional 24 hours. The respective values varied between 0,26 and 0,30 mg/l. The arithmetic mean of these measured concentrations is 0,29 mg/l and the results of the test will be expressed according to this test concentration.

No fish died during the test, however, after 96 hours exposure two fish showed abnormal swimming behaviour. Hence an EC20 = 0,29 mg/l as well as an LC50 > 0,29 mg/l can be derived.

$$LC_{50}\ (96h) > 0{,}29\ mg/l$$

$$EC_{20}\ (96h) = 0{,}29\ mg/l$$

A recent study reports also results (Bruns, 2003a) on *Brachydanio rerio*. The method used is similar to the one described under Directive 92/69/EEC, C1. A limit test was realised at the limit of the water solubility of the substance (0,49 mg/l according to the data of the sponsor of the substance). A preliminary test at the limit of water solubility of the substance revealed that it was not possible to achieve a higher concentration than 0,3 mg/l. Clotrimazole had no effect on fish after 96 hours of exposure to 0,278 mg/l (arithmetic mean of two analytical values measured at 96 hours). Therefore the test results in an LC_0 (96 hours) $\geq$ 0,278 mg/l for *Brachydanio rerio*. This value will be used preferably in the determination of the PNEC.

$$LC_0\ (96h) \geq 0{,}278\ mg/l$$

b) Chronic toxicity

Chronic toxicity to algae

In the study of Bruns (2003b) presented in section I.5.1.a), the author reports a NOEC (72 hours) of 0,017 mg/l for both endpoints (growth and growth rate). The results are expressed as measured concentrations. Therefore:

$$NOEC\ (72h) = 0{,}017\ mg/l$$

Chronic toxicity to crustacean

The chronic toxicity of clotrimazole to *Daphnia magna* was assessed according to the OECD test guideline 211 [Caspers, 2004 #115]. The effects on the reproductive output of this species were observed after a 21 days exposure period to a range of concentrations (0,32, 1, 3,2, 10 and 32 µg/l of clotrimazole). A semi-static system is used with a renewal of the test media three times a week. The statistically derived NOEC and LOEC obtained after 21d of exposure are given below. The results are expressed as nominal concentrations. The recovery rates for the 10 µg/l and 32 µg/l range from 82,7 – 109,3% of the nominal values. Therefore these results are considered valid. However it must be underlined that for lower test concentrations (0,32, 1 and 3,2 µg/l) recovery rates range from 43,8 – 631% of the nominal values. The only explanation given is the fact that chemical analysis is difficult at these trace levels, however no reason was proposed for the extreme variation of the recoveries.

$$NOEC\ (21d)_{reproduction} = 0{,}010\ mg/l$$

$$LOEC\ (21d)_{reproduction} = 0{,}032\ mg/l$$

Chronic toxicity to fish

The sub-chronic toxicity of clotrimazole to *Oncorhynchus mykiss* was assessed according to the OECD Guide-line 215 (Bruns, 2003c). The effects measured were the mortality of fish, behavioural abnormalities and fresh weight of juvenile fish after an exposure of 28 days to clotrimazole. The tested concentrations were 0,001, 0,0032, 0,01, 0,032 and 0,1 mg/l. One fish died after 28 days at the nominal exposure concentration of 0,1 mg/l. The statistically derived NOEC obtained on growth data is 0,025 mg/l expressed as mean measured concentrations. The NOEC was determined as an EC10.

$$EC_{10}\ (28d) = 0{,}025\ mg/l$$

c) Summary

Acute and chronic toxicity results are reported respectively in Table 2 and Table 3.

Table 2. Acute toxicity of clotrimazole to aquatic organisms

Organisms	Species	Test type	Endpoint	Value (mg/l)	Comments (reliability)	Reference
Algae	*Desmodesmus subspicatus*	static	E_bC50 (72h) E_rC50 (72h)	0,098 0,268	1	(Bruns, 2003b)
Crustacean	*Daphnia magna*	static	EC50 (48h)	0,02	2	(Caspers et Müller, 1994; Bruns, 2004)
Fish	*Brachydanio rerio*	Semi static	LC50 (96h) EC_{20} (96h)	>0,29 0,29	2	(Caspers et Müller, 1994)
	Brachydanio rerio	Semi static	LC0 (96h)	> 0,278	1	(Bruns, 2003a)

Table 3. Chronic toxicity of clotrimazole to aquatic organisms

Organisms	Species	Test type	Endpoint	Value (mg/l)	Comments (reliability)	Reference
Algae	*Desmodesmus subspicatus*	static	NOEC (72h)	0,017	1	(Bruns, 2003b)
Crustacean	*Daphnia magna*	Semi static	NOEC (21d)	0,01	1	[Caspers, 2004 #115]
Fish	*Oncorhynchus mykiss*	Semi static	EC_{10} (28 d)	0,025	1	(Bruns, 2003c)

1: valid without restriction

2: valid with restriction.

I.5.2. Sediment-dwelling organisms

No ecotoxicological data on soil organisms are available.

I.5.3. Terrestrial compartment

No ecotoxicological data on benthic organisms are available.

I.5.4. Ecotoxicity to micro-organisms

Several ecotoxicological data are available on micro-organisms. The effects of clotrimazole on micro-organisms are tested on organisms in activated sludge (OECD guideline 209) and on two bacterial species, *Pseudomonas putida* and *Vibrio fischeri.*

The effects of clotrimazole on activated sludge from domestic sewage treatment plant were studied (Caspers et Müller, 1994). Effects were determined in a respiration inhibition test according to the OECD guideline 209 ("Activated sludge. Respiration inhibition test"). The nominal concentrations tested were 100, 1000 and 10 000 mg/l. No inhibition of the respiration was measured after 30 minutes of exposure.

The test control performed with a reference substance (3,5-dichlorophenol) yielded an EC50 value of 13,0 mg/l. The results of the controls performed at the concentration of clotrimazole of 10 000 mg/l are not reported. Only one value is reported for the control at 0 mg/l of clotrimazole, therefore there is no way to know if the difference between the two recommended controls is less than 15%.

No inhibition of the respiration rate was observed within the tested concentration range. The EC50 is therefore assessed to be > 10 000 mg/l. The test will be considered valid with restriction.

EC_{50} (30 min) > 10 000 mg/l

The effect of clotrimazole was also tested on two bacterial strains *Pseudomonas putida* and *Vibrio fischeri*, a marine species (Brötz-Oesterhelt et Sauer, 2003). The Minimal Inhibitory Concentration (MIC) (lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye) was determined in two tests according to different methods, the broth microdilution method (NCCLS guideline, M7-A5[1]) and the agar dilution method (NCCLS guideline M26-A[1]). The incubation time

[1] Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; NCCLS-Guideline, M7-A5, Vol.20, No 2 ISBN 1-56238-394-9

after inoculation of the tested substance was of 18h. The tested concentrations were of 0,00024 to 32 mg/l and 0,00097 to 32 mg/l respectively in the broth microdilution and the agar dilution method.

By both methods, no growth inhibition was observed up to the solubility limit of the substance in broth. The MIC is thus recorded as >32 mg/l.

A time-kill study was also conducted on these two bacterial strains. The tested concentrations were from 1 to 32 µg/mL and culture samples were taken after 1, 2, 4, 6 and 24h for *P. putida* and 1, 2, 4, 6, 8 and 24h for *V. fischeri*. No inhibitory effect on the growth or survival of both strains was detected.

I.6. Toxicological properties

Under the marketing authorisation of medicinal products (MAM) for human use procedure, a dossier for clotrimazole was submitted. The MAM was valid. A summary of the results of the different relevant toxicological studies submitted is available and reported in this document.

I.6.1. Mode of action

Clotrimazole is a potent antimycotic that acts against fungi by inhibiting ergosterol synthesis, which in turn leads to structural and functional impairment of the cytoplasmic membrane. The primary mode of action of clotrimazole is therefore damage of the cell membrane, which causes leakage of intracellular phosphorus compounds with a concomitant breakdown of cellular nucleic acids and potassium efflux. After exposure of the organisms to the drug the onset of these events is rapid and extensive and causes a time-dependent and concentration-dependent inhibition of fungal growth.

On the molecular basis, clotrimazole interferes with the cytochrome P450 dependent 14-α-demethylation of lanosterole or 24-methylendihydrolanosterole, which is the main step in biosynthesis of ergosterole. Ergosterole is an important sterol in fungi and responsible for fungal cellular integrity. The following accumulation of 14-α-methylsterole is regarded as basis of pharmaceutical activity.

In fish and other vertebrates, the effect of different imidazole fungicides as potent inhibitors of cytochrome P450s is well known (Mason et al, 1985), (Monod *et al.*, 1993). This is especially the case of the P450 aromatase which is a key steroidogenic enzyme that catalyses the conversion of androgens to estrogens which are key regulators in sexual differentiation and development in vertebrates. Recent results report the *in vitro* effect of clotrimazole tested on brain and ovarian microsomal aromatase activity of the rainbow trout (*Onchorynchus mykiss*). The respective EC50 values are for ovarian and brain: EC50 = 16.10^{-9}M and EC50 = 11.10^{-9}M (Hinfray *et al.*, 2004). Monod *et al.* (1993) reports also inhibition of microsomal aromatase in rainbow trout due to clotrimazole (IC50 = 5.10^{-7}M).

I.6.2. Reproduction studies

Reproduction toxicity studies were conducted using oral route of clotrimazole administration in mice, rats and rabbits. At 50 mg/kg of clotrimazole in the diet of rats, neonatal survival was reduced. However dietary doses up to and including 25 mg/kg did not impair the development of pups. Doses up to and including 50 mg/kg did not affect fertility.

I.6.3. Mutagenicity studies

The mutagenic potential of clotrimazole was assessed in three in vitro and three in vivo assays. There was no sign of mutagenic potential of clotrimazole in all tests.

I.6.4. Carcinogenic potential

The carcinogenic potential of clotrimazole has been investigated in a rat 78-week study. There was no evidence of carcinogenic potential at doses up to and including dietary concentrations equivalent to 150 mg/kg/day.

I.6.5. Pharmacokinetics of clotrimazole

A lot of information are available in the literature on the metabolisation of clotrimazole in human and rats bodies. Duhm *et al.* (1974) in a study on the pharmacokinetics of clotrimazole ^{14}C in human and rats report that the drug is readily absorbed, distributed and excreted as inactive metabolites by way of the liver and bile. The main metabolites found are represented in figure 1.

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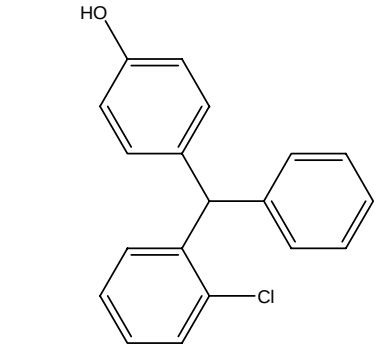
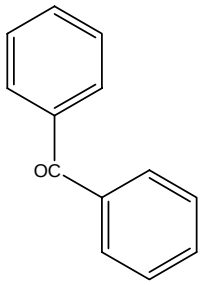
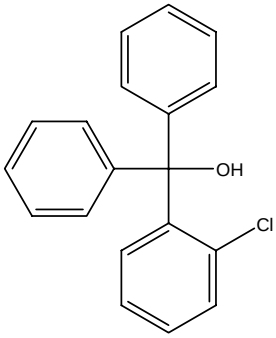
[1] Methods for determining bactericidal activity of antimicrobial agents; NCCLS-Guideline, M26-A, Vol.19 No 18, 1999, ISBN 1-56238-384-1



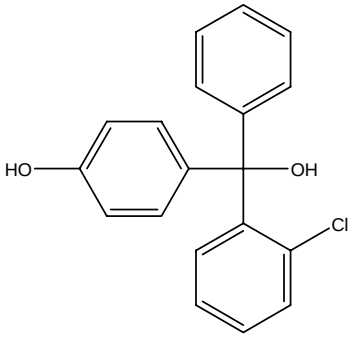
2-chlorophenyl-4-hydroxyphenyl-phenyl-methane



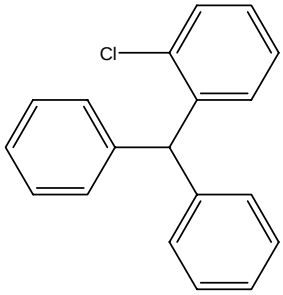
benzophenone



2-chlorophenyl-bis-phenyl-methanol



2-chlorophenyl-4-hydroxyphenyl-phenyl-methanol



2-chlorophenyl-bis-phenyl-methane

Figure 1: Main metabolites of clotrimazole

I.6.6. Summary

Reproduction studies showed no effects on fertility, or signs of embryo-, fetotoxicity, or teratogenicity at low concentrations. The maternal organism was affected in high dosages only.

Clotrimazole is a non-mutagenic and is of no genotoxic hazard for man. There is no evidence of carcinogenicity of clotrimazole.

I.7. PBT assessment

I.7.1. Persistency criterion

The substance is non biodegradable in regards of the biodegradability screening test and the QSAR data. The half-life of clotrimazole in the environment is thus expected to be more than 60 days. The P criterion is therefore fulfilled. Pharmacokinetics data in human and rats suggest that this chemical may be biodegraded in metabolites, without adverse activity, in bodies. With the present knowledge it is difficult to extrapolate pharmacokinetics data from mammals or human bodies to the fate of a chemical in the environment under different conditions. In conclusion the P criterion is fulfilled.

I.7.2. Bioaccumulation criterion

Based on a log Kow of 4,1 and a calculated BCF of 610, the B criterion is not fulfilled according to the EU TGD criterion (E.U., 2003).

I.7.3. Toxicity criterion

The most sensitive NOEC is reported for crustacean (NOEC (21d) = 0,01 mg/l for *Daphnia magna*). The T-criterion is fulfilled when the substance has a chronic NOEC less than 0,01 mg/l or when the substance has CMR properties or a potential for endocrine disrupting effects. Long-term ecotoxicity results on crustacean are at the limit of the trigger value of 0,01 mg/l. Therefore it is difficult to conclude on the non-fulfilment of the T criterion. The T criterion will therefore be considered as fulfilled.

I.7.4. Conclusion of the PBT assessment

Only the B criterion is clearly not fulfilled. The P and T criteria are fulfilled in regards of the available data.

II. Information on sources of clotrimazole (production and uses)

II.1. Production

There is only one producer of clotrimazole in the EU and only one site of production. The amount of active ingredient produced has decreased during the last years and is now in the range of 10 tons/year. Almost the same quantity of clotrimazole is imported.

Clotrimazole is synthesised by the reaction of o-chlorotritylchloride with imidazole suspended in acetone (Hoogerheide et Wyka, 1982), in a reaction vessel. Typically this synthesis is performed in batch. After addition of triethylamine, the liquid is heated under return flow to complete the synthesis. Activated charcoal is then added and the precipitated triethylammonium chloride are filtered on a stirred pressure filter. They are then washed with acetone. The filtrates are then collected in a reactor vessel for crystallisation. The crystallised product obtained (clotrimazole) is then separated from the mother liquors and washed with acetone and demineralised water. After a step of decolourization (with activated charcoal) and another step of crystallisation, the crystallised product is washed and dried. The yield in this synthesis is solvent-dependent (reactions in solvents with high dielectric constants give the higher yields). The synthetic pathway to clotrimazole is represented in figure 2 and a complete description of the process is available in Appendix 2.

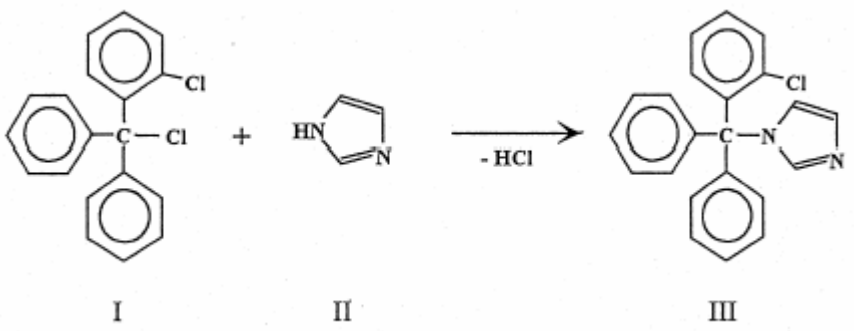


Figure 2: Reaction scheme (I: o-Chlorotritylchloride, II: Imidazole, III: clotrimazole)

The final product undergoes quality controls in order to meet the specification of the producer. The final product is then transported to formulation site.

II.2. Use

Clotrimazole is an antimycotic drug used in the treatment of dermatomycosis and vaginal mycosis. This active ingredient is marketed as a generic under different trade names by numerous companies. It is mainly used as topical drug for the cure of dermatological and gynaecological fungal infections in humans. Clotrimazole is also active against trichomoniasis and infections with gram-positive and certain anaerobic bacteria.

On the basis of the sales data, Germany and UK have the most important market. The total amount of clotrimazole (as active ingredient) on the market in 2002 was 3,7 t/year for UK and 2,8 t/year for Germany.

The products that can be found on the market in Germany are reported in Table 4 and Table 5.

Table 4. Clotrimazole formulated products (Bayer Health Care) used in the treatment of vaginal mycosis

Product	Clotrimazole quantity per unit [g]
Gyno Canesten 1 Vaginal Creme 5 g	0,5
Gyno Canesten 3 Vaginal Creme 20 g	0,4
Gyno Canesten 1 Vaginal Tablet 1St	0,5
Gyno Canesten 3 Vaginal Tablet 3 St	0,6
Gyno Canesten Combi: - 1 Tablet - 20 g Creme	 0,5 0,2
Gyno Canesten Combi: - 3 Tablets - 20 g Creme	 0,6 0,2

The usual mode of administration is one vaginal tablet or the content of one applicator of vaginal cream per day during 1, 3 or 6 days.

Table 5. Clotrimazole formulated products (Bayer Health Care) used in the treatment of dermatomycosis

Product	Clotrimazole quantity per unit [g]
Canesten Powder 30 g	0,3
Canesten Creme 100 g	1
Canesten Creme 50 g	0,5
Canesten Creme 20 g	0,2
Canesten lsg 100 ml	1
Canesten lsg 50 ml	0,5
Canesten lsg 20 ml	0,2
Canesten Pumpspray 30 ml	0,3

Cream and solution are respectively applied thinly 2 to 3 times a day and rubbed in. A ribbon of the cream of ½ cm or a few drops of the solution are enough for treating an area of about the size of a hand.

For the application by spraying the product is applied thinly 2 to 3 times a day with 2 depressions of the spray head. When using the powder, it is recommended to dust the relevant area with the product 2 to 3 times a day.

III. Concentrations in the environment

III.1. Exposure assessment

III.1.1. Release from production

There is only one site of production of active ingredient in the European Union. The synthesis of active ingredient is performed in closed systems. During the synthesis of clotrimazole, vapours of organic solvents may be emitted as well as residues (mother liquor, filter residue, discharge from wet scrubber). However, all the residues are incinerated. This is the case for the mother liquor and for the filter residue which are treated before with water and alkali together with separated triethylamin. The aqueous phase and discharge from wet scrubber are incinerated too.

Thus, releases during the production are not expected in normal situation and will be set to 0.

III.1.2. Release from use

a) Formulation

Today there is only one site of formulation in the EU. Clotrimazole is mainly formulated as cream and tablets. A complete description of the formulation processes is reported in Appendix 3 and Appendix 4. During the drying steps there might be releases to the air compartment, however air-filtration systems are available in the whole formulation processes (initial weight, granulation, compaction). Separated dusts are collected and incinerated as well as waste from granulate material and broken tablets. It is also ruled that product-dusts are sucked or swept before wet-cleaning for after being incinerated.

Therefore the releases to the different environmental compartments should be negligible and will be set to 0.

b) Private use

Clotrimazole is only used as topical drug in the treatment of dermatological and gynaecological fungal infections in humans. As said before the products may be administered by tablets, spray, powder or cream and solution. The main potential source of clotrimazole to the environment seems to be the private use of clotrimazole.

A study on the pharmacokinetics properties of clotrimazole ^{14}C after oral administration in rats and human (Duhm *et al.*, 1974) showed that almost all ^{14}C activity is released in faeces and urine (in rats more than 90 % in the faeces and between 2 and 4% in the urine). The radioactivity is mainly released as inactive metabolites (reported in figure 1) and only traces of unchanged clotrimazole were found.

Absorption of clotrimazole through intact skin in humans was generally negligible when either 1% cream or 1% solution was applied in conjunction with occlusive dressings.

Therefore, almost all the clotrimazole will be washed into the waste water by normal body hygiene procedures. The release to the waste water from the municipal waste water treatment plant is as a consequence the main pathway of clotrimazole to the environment.

Moreover, as the vapour pressure of clotrimazole is really low, the release of this substance to the atmosphere is not expected.

III.1.3. Summary of release estimates

The default TGD values corresponding to the IC5 (personal/domestic) / UC41 (pharmaceuticals) were used to estimate the releases from production, formulation and private use of clotrimazole.

For the production step and formulation, releases to the environment are not expected. Therefore the TGD default values were used only for the private use step. The market data from UK were used as a realistic worst case for the private use of clotrimazole.

The releases estimates for each step at the different scales (local, regional, continental) are reported in Table 6.

Table 6. Summary of environmental release estimates of clotrimazole (worst case UK)

Life cycle stage	Estimated local release (kg/d)	Estimated regional release (kg/d)	Estimated continental release (kg/d)
Production	0	0	0
Formulation	0	0	0
Private use	0 to air 5,48E-03 to waste water	0 to air 2,74 to waste water 0 to industrial soil	0 to air 17,8 to waste water 0 to industrial soil

III.2. Aquatic compartment

III.2.1. Inland environment

a) Calculation of PEC_{local}

The PEC local for the different steps of the life cycle of clotrimazole are reported in Table 7. The calculations were made with EUSES 2. For the private use, the tonnage data from UK were used as a worst case and it was assumed that the substance was administered externally as only release of metabolites is expected after oral administration.

Table 7. PEClocal for surface water, microorganisms (STP) and sediment for production, formulation and private use of clotrimazole

Life cycle	PEC $_{water}$ (mg/l)	PEC $_{sediment}$ (mg/kg wwt)	PEC_{STP} (mg/l)
Production	1,30E-05	4,12E-04	0
Formulation	1,30E-05	4,12E-04	0
Private use	1,88E-04	5,93E-04	1,75E-03

b) Calculation of $PEC_{regional}$ and $PEC_{continental}$

The regional and continental PEC calculated by EUSES 2 for freshwater (surface water and sediment) are reported in Table 8.

Table 8. Regional and continental PEC for freshwater (surface water and sediment)

	$PEC_{regional}$	$PEC_{continental}$
Surface water (mg/l)	1,30E-05	9,52E-06
Sediment (mg/kg wwt)	6,21E-04	4,54E-04

c) Monitoring in the freshwater environment

Clotrimazole concentration was measured in the effluent of the sewage treatment plant of a former production plant from 1994 to 2002 (Bald, 2002). The concentration was always found to be under the limit of detection (0,02 mg/l) even when the clotrimazole was found in the influent of the STP. The highest influent concentration reported was 0,67 mg/l (this concentration was only measured once).

In addition an independent study of ARGE (Arbeitgemeinschaft für die Reinhaltung der Elbe) measured in 1999 and 2000 the concentration of different pharmaceutical substances in the Elbe and the Saale river in Germany (Wiegel *et al.*, 2003). Clotrimazole was not detected in any of the measured points. The detection limit of the analytical method was 2,5 ng/l.

III.2.2. Marine environment

a) Calculation of PEC_{local}

Clotrimazole is mainly used as private or personal use. Therefore the releases of this chemical will be mainly to municipal sewage treatment plant and then there is a need to calculate a PEC local for the marine environment as sewage treatment plants may be located on coasts. The formulation and production sites in the EU are not located on the sea. Thus only a PEC local for private use will be calculated. It is recommended in the TGD (E.U., 2003) to apply a dilution factor 10 times higher than in the freshwater environment due to tidal influences. However this additional dilution factor should not be used when there is low tides or currents conditions (e.g. Baltic or Mediterranean seas). Therefore depending on the location two different PEC values, reported in Table 9, may be considered. First a PEC value corresponding to the value reported in EUSES 2 with a dilution factor of 100 taking into account the tidal influence and then another PEC value for seas with low tide using a dilution factor of only 10.

Table 9. PEClocal for the marine environment

Life cycle	PEC $_{water}$ (mg/l)	PEC $_{sediment}$ (mg/kg wwt)
Private use (EUSES 2)	2,31E-05	7,32E-04
Private use (low tide)	2,08E-04	6,57E-03

b) Calculation of $PEC_{regional}$ and $PEC_{continental}$

The calculation was made by EUSES 2 which integrates the marine environment. The results of the calculation are reported in Table 10.

Table 10. Regional and continental PEC for marine water (surface water and sediment)

	$PEC_{regional}$	$PEC_{continental}$
Surface water (mg/l)	2,63E-06	1,08E-07
Sediment (mg/kg wwt)	1,05E-04	4,32E-06

c) Monitoring in the marine environment

A recent study is available on the occurrence of human pharmaceuticals compounds in UK estuaries (Thomas et Hilton, in press). Surface water samples were collected in October and November 2002 from five UK estuaries (Tyne, Tees, Mersey and Thames) and analysed for the presence of 14 pharmaceutical compounds among which clotrimazole. Clotrimazole was the most frequently detected (present in 59% of all collected samples) at a maximal concentration of 22 ng/l and a median concentration of 7 ng/l. The compounds were analysed using liquid chromatography coupled to electrospray mass spectrometry or tandem mass spectrometry. Analysis followed the extraction and pre-concentration of the samples by solid phase extraction (SPE), after the addition of a surrogate standard (^{13}C-phenacetin). The concentrations of clotrimazole calculated by EUSES2 (23 ng/l) are similar to the upper measured values in the UK estuaries (22 ng/l).

III.2.3. Terrestrial compartment

a) Local, regional and continental estimated concentrations in soil

Predicted concentrations of clotrimazole in soil have been calculated using EUSES 2 for the local, regional and continental scenarios. The estimated PEC are reported in Table 11. Clotrimazole can enter the soil compartment by the landspreading of municipal WWTP sludge. Due to a relatively high Kow, clotrimazole may adsorb on the sludge and thus be released in the soil when spread.

b) Levels in soil

No monitoring data of clotrimazole are available.

Table 11. Local, regional and continental PEC for the soil compartment

PEClocal$_{soil}$ (mg/kg wwt)	**Production**	6,31E-07
	Formulation	6,31E-07
	Private use	0,0154
PECregional$_{soil}$ (mg/kg wwt)	4,34E-05	
PECcontinental$_{soil}$ (mg/kg wwt)	2,80E-05	

III.2.4. Atmosphere

a) Local, regional and continental estimated concentrations in air

Predicted concentrations of clotrimazole in the atmosphere have been calculated using EUSES 2 for the local, regional and continental scenarios. The estimated PECs are reported in Table 12.

Table 12. Local, regional and continental PEC for the atmospheric compartment

PEClocal$_{air}$ (mg/m^3)	**Production**	1,57E-11
	Formulation	1,57E-11
	Private use	2,02E-11
PECregional$_{air}$ (mg/m^3)	1,57E-11	
PECcontinental$_{air}$ (mg/m^3)	7,51E-12	

b) Monitoring in the atmosphere

No atmospheric monitoring data of clotrimazole are available.

III.2.5. Secondary poisoning

Predicted concentrations in fish have been calculated for both the inland and the marine environment. PEC values are reported in Table 13.

The concentrations in fish are calculated from the concentrations in the surface water and the BCF (QSAR data = 610 l/kg) according to the equations for predators (fresh and marine waters) and top-predators (marine water) reported in the TGD (E.U., 2003).

Freshwater and marine predators

$$PEC_{oral,\ predator} = (0{,}5\ PEC_{local} + 0{,}5\ PEC_{regional}) \cdot BCF_{fish} \cdot BMF$$

Marine top-predators

$$PEC_{oral,\ top\text{-}predator} = (0{,}1\ PEC_{local} + 0{,}9\ PEC_{regional\ seawater}) \cdot BCF_{fish} \cdot BMF_1 . BMF_2$$

$PEC_{oral\ ,\ predator}$: concentration in the food of the predator ($mg.kg^{-1}$)
$PEC_{oral\ top\text{-}predator}$: concentration in the food of the top-predator ($mg.kg^{-1}$)
PEC_{local}: annual average predicted local environmental concentration ($mg.l^{-1}$)
$PEC_{regional}$: predicted regional environmental concentration ($mg.l^{-1}$)
$PEC_{regional\ seawater}$: predicted marine regional environmental concentration ($mg.l^{-1}$)
BCF_{fish}: bioconcentration factor (1300 $l.kg^{-1}$)
BMF_1: biomagnification factor in fish (1)
BMF_2: biomagnification factor in predator (1)

Table 13. Estimated concentrations in freshwater and marine fish and marine predators

Life cycle	$PECfish_{freshwater}$ (mg/kg)	$PECfish_{marine}$ (mg/kg)	PECtop-predator (mg/kg)
Production	7,95E-03	1,61E-03	1,61E-03
Formulation	7,95E-03	1,61E-03	1,61E-03
Private use (EUSES2)	0,0612	7,86E-03	2,86E-03
Private use (low tide)	0,0612	6,41E-02	1,41E-02

IV. Effects assessment

IV.1. Aquatic compartment

IV.1.1. Water column

Acute as well as chronic ecotoxicity values are available on freshwater organisms for three trophic levels (algae, crustacean and fish). *Daphnia magna* seems to be the most sensitive species on the basis of the short-term and long-term ecotoxicity results with a NOEC (21d) = 0,01 mg/l. Therefore, for the determination of the Predicted No Effect Concentration (PNEC) for aquatic organisms, an assessment factor of 10 is applied on the lowest NOEC obtained on daphnia as recommended in the TGD. This results in:

$$PNEC_{fresh\ water} = 0{,}01/10 = 0{,}001\ mg/l$$

$$PNEC_{fresh\ water} = 1\ \mu g/l$$

Regarding the PNEC for the marine compartment, no ecotoxicological results on marine organisms are available (with the exception of the data reported on the marine bacteria *Vibrio fischeri*). Therefore, according to the TGD an assessment factor of 100 will be applied to the NOEC value, which gives:

$$PNEC_{marine\ water} = 0{,}1 \mu g/l$$

There is also toxicity data available for micro-organisms in domestic sewage sludge as well as data on microorganisms (*Pseudomonas putida* and *Vibrio fischeri*). Data available on micro-organisms are considered as less relevant and will not be used for the determination of the PNEC for micro-organisms in a STP as recommended in the TGD (E.U., 2003). No effect on the respiration rate was observed at the concentrations tested which gives an EC50 >10 000 mg/l. As no effects were observed the value of 10 000 mg/l can be considered as a NOEC. An assessment factor of 10 can be applied to this value which gives a PNEC for the micro-organisms of 1000 mg/l.

$$PNEC_{micro\text{-}organisms} = 1000\ mg/l$$

IV.1.2. Sediment

There are no studies available on sediment-dwelling organisms. In the absence of any ecotoxicological data, the PNEC may be calculated using the equilibrium partitioning method from the PNEC for aquatic organisms and the sediment-water partition coefficient.

The suspended matter-water partition coefficient can be calculated from the following equation:

$$K_{susp-water} = Fwater_{susp} + Fsolid_{susp} \cdot \frac{Kp_{susp}}{1000} \cdot RHOsolid$$

with $K_{susp\text{-}water}$: partition coefficient suspended matter - water ($m^3.m^{-3}$)
$Fwater_{susp}$: volume fraction water in suspended matter (0,9 $m_{water}^3.m_{sed}^{-3}$)
$Fsolid_{susp}$: volume fraction solids in suspended matter (0,1 $m_{solid}^3.m_{sed}^{-3}$)
$Kp_{susp} = Foc_{susp} . Koc$
Kp_{susp}: partition coefficient solid-water in suspended matter (141,9 l/kg)
Foc_{susp}: weight fraction of organic carbon in suspended matter (0,1 $kg_{oc}.kg_{solid}^{-1}$)
Koc: partition coefficient organic carbon-water (1419 l/kg)
logKoc = 0,52 logKow + 1,02 = 3,15
RHOsolid: density of the solid phase (2500 $kg_{solid}.m_{solid}^{-3}$)

Then $K_{susp\text{-}water} = 0,9 + 0,1 \times (0,1 \times 1419)/1000 \times 2500 = 36,4\ m^3.m^{-3}$

And $$PNECsed = \frac{K_{susp-water}}{RHOsusp} \times PNEC_{water} \times 1000$$

With *RHOsusp*: bulk density of wet sediment (1150 $kg.m^{-3}$)

$PNEC_{freshwater\ sed} = (36,4/1150) \times 1.10^{-3} \times 1000 = 0,0316$ mg/kg wet weight = 31,6 µg/kg wwt
$PNEC_{marine\ sed} = (66,8/1150) \times 0,1.10^{-3} \times 1000 = 0,0058$ mg/kg wet weight = 5,8 µg/kg wwt

IV.2. Terrestrial compartment

There are no studies available on soil organisms. In the absence of any ecotoxicological data, the PNEC may be calculated using the equilibrium partitioning method from the PNEC for aquatic organisms and the soil-water partition coefficient.

The soil-water partition coefficient can be calculated from the following equation:

$$K_{soil-water} = Fair_{soil} \cdot K_{air-water} + Fwater_{soil} + Fsolid_{soil} \cdot \frac{Kp_{soil}}{1000} \cdot RHOsolid$$

with $K_{soil\text{-}water}$: partition coefficient soil water ($m^3.m^{-3}$)
$Fwater_{soil}$: volume fraction water in soil (0,2 $m_{water}^3.m_{soil}^{-3}$)
$Fair_{soil}$: volume fraction air in soil (0,2 $m_{water}^3.m_{soil}^{-3}$)
$K_{air\text{-}water}$: partition coefficient air water (9,38E-08 $m^3.m^{-3}$)
$Fsolid_{soil}$: volume fraction solids in soil (0,6 $m_{solid}^3.m_{soil}^{-3}$)
$Kp_{soil} = Foc_{soil} . Koc$
Kp_{soil}: partition coefficient solid-water in soil (28,38 l/kg)
Foc_{soil}: weight fraction of organic carbon in soil (0,02 $kg_{oc}.kg_{solid}^{-1}$)
Koc: partition coefficient organic carbon-water (1419 l/kg)
logKoc = 0,52 logKow + 1,02 = 3,15
RHOsolid: density of the solid phase (2500 $kg_{solid}.m_{solid}^{-3}$)

Then $K_{soil\text{-}water} = 42,77\ m^3.m^{-3}$

And $$PNECsoil = \frac{K_{soil-water}}{RHOsoil} \times PNEC_{water} \times 1000$$

With *RHOsoil*: bulk density of wet soil (1700 kg.m^{-3})

$$PNEC_{soil} = (48,2/1700) \times 1.10^{-3} \times 1000 = 0,0283 \text{ mg/kg wet weight} = 28,3 \text{ µg/kg wwt.}$$

IV.3. Atmosphere

The methodology used for effects assessment (and therefore the risk characterisation) of chemicals in water and soil cannot be applied yet in the same manner to the atmosphere. Hence, no PNEC for the air compartment will be estimated and only a qualitative risk assessment will be performed. Only abiotic effects could be estimated as no toxicological results are available on animal or plant species. There is no evidence of an influence of clotrimazole on global warming or ozone depletion/formation in the stratosphere. The vapour pressure of clotrimazole is really low and therefore high concentrations of this chemical in the atmospheric compartment is not expected.

IV.4. Secondary poisoning

IV.4.1. Oral toxicity studies

Several oral toxicity studies are available. Most of them are not the result of a dietary exposure. The most sensitive NOAEL was reported by Lorke (1970) in a study on fertility and general procreative ability of rats.

In this study 10 male and 20 female rats were fed a food mixture contaminated by: 0, 5, 10, 25 and 50 mg/kg body weight for 10 weeks. After mating the author followed the behaviour and development of pregnant females and later the development of the young (observed for 4 weeks).

The results showed that the female treated at 50 mg/kg body weight reared a significantly smaller number of young. However, the weights of the young raised corresponded to the weights of the control.

Hence a NOAEL of 25 mg/kg body weight.

$$NOEC_{mammal,food\_chr} = NOAEL \,.\, CONV_{mammal} = 250 \text{ mg/kg food}$$

$CONV_{mammal}$: conversion factor from NOAEL to NOEC (10 kg bw.d.kg$_{food}^{-1}$)

IV.4.2. PNECoral

A 10 weeks NOEC on mammal is available. At 250 mg/kg food there were no deleterious effects on either rats fertility or the general procreation ability of the animals. Therefore we propose to apply an assessment factor of 90 on this NOEC. Thus:

$$PNECoral = 2,78 \text{ mg/kg food.}$$

V. Risk assessment

A summary of the PEC/PNEC ratios are reported in Appendix 5 for the different environmental compartments of concern. The results are discussed in the following sections. In addition to the risk assessment performed on the basis of the Technical Guidance Document (E.U., 2003), a risk assessment for clotrimazole was also performed according to the note for guidance on environmental risk assessment of medicinal products for human use (EMEA., 2003). The results of this risk assessment is in Appendix 6.

V.1. Aquatic compartment

V.1.1. Inland environment

a) Water

The PEC/PNECs ratios reported in Appendix 5 are all below 1. Therefore there is at present no risk for organisms living in freshwater due to the use of clotrimazole.

b) Sediment

As no ecotoxicological data are available on sediment dwelling organisms, the PEC/PNEC ratios reported were determined on the basis of the partition coefficient and the ecotoxicological data available for the organisms living in the water column. Therefore the risk ratios for the benthic organisms are the same as those for the organisms living in the water column.

V.1.2. Micro-organisms in the STP

The PEC/PNEC ratio reported for micro-organisms living in a STP is less than 1 and therefore there is at present no risk for these organisms due to exposure to clotrimazole.

V.1.3. Marine environment

a) Water

The PEC/PNEC ratios on the local and regional scale were determined. On the local scale two scenarios were used taking into account the tidal influence that can be different depending on the considered marine location. The local PEC/PNEC ratios are all below 1 except in the low tide scenario for the private use of the substance (PEC/PNEC = 2,08). However, this scenario is a worst case scenario using only default values and as the risk ratio is really close to 1 we may be considered that due to the high uncertainties around the calculation the risk can be considered negligible. The conclusion is that there is at present no risk for organisms living in the marine environment due to the use of clotrimazole.

b) Sediment

As no ecotoxicological data are available on marine sediment dwelling organisms, the PEC/PNEC ratios reported were determined on the basis of the partition coefficient and the ecotoxicological data available for the organisms living in the water column. Therefore the risk ratios for the benthic organisms are the same as those for the organisms living in the water column.

V.1.4. Terrestrial environment

The PEC/PNEC ratios reported in Appendix 5 are all below 1 therefore there is at present no risk for organisms living in the soil due to the use of clotrimazole.

V.1.5. Atmosphere

Due to the low vapour pressure of clotrimazole the presence of this compound in the atmosphere is not expected. This assumption is supported by the really low concentrations of clotrimazole calculated in the atmospheric compartment (Table 12). Therefore it seems that there is no risk for the atmospheric compartment due to an exposure to clotrimazole.

V.1.6. Secondary poisoning

The risk characterisation was realised for both predators of freshwater and marine waters as well as marine top-predators. The PEC/PNEC ratios reported in Appendix 5 do not indicate a risk of secondary poisoning due to exposure to clotrimazole in the environment.

VI. Desired reduction and identification of possible measures

VI.1. Achieving the desired reduction

VI.1.1. OSPAR targets

In 2002, Clotrimazole was included in the OSPAR list of chemicals for priority action. The OSPAR objective with regard to hazardous substances is to continuously reduce discharges, emissions and losses with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances. Every endeavour will be made to move towards the target of cessation discharges, emissions and losses of hazardous substances by the year 2020 (OSPAR, 1998).

At OSPAR 2002, the guidance on the role of marine risk assessment, which gives, in particular advice on the urgency of taking measures based particularly on the PEC/PNEC ratios and the PBT properties of the chemicals (cf. Annex 6 of OSPAR 2002 Summary Record) was adopted by OSPAR. We have attempted to apply this guidance to the document and reached the following conclusions. However, these conclusions are considered to be provisional, and could change in the light of further information.

Clotrimazole cannot be considered a PBT chemical as defined in the TGD (E.U., 2003). The estimated PEC/PNEC ratios for clotrimazole for the marine environment are all below 1 except in the low tide scenario for the private use of the substance in water and sediment (PEC/PNEC = 2,08).

Only default values were used in the calculations of emissions from private use in the different compartments. The low tide scenario is considered as a worst case scenario and due to the high uncertainties around the calculated values and the relatively low risk ratio (2,08), the risks may be considered negligible. The conclusions are therefore that there is at present no risk for the environmental compartments including the marine compartment due to the use of clotrimazole.

VI.1.2. OSPAR's role in achieving the desired targets

The results of the risk assessment of the clotrimazole indicates that there is at present no risk for the marine environment due to the use of clotrimazole.

Only risks from production steps and use of the substance were assessed. However, another source of clotrimazole to the environment is through disposal of unused pharmaceuticals. Due to a lack of information and/or scenario this step was not assessed in the document. In order to enhance environmental protection, it is therefore recommended that – even for medicinal products that do not require special disposal measures – package leaflets (patient information leaflets) should include the following general statement:

"Medicines no longer required should not be disposed of via wastewater or the municipal drainage system. Return them to a pharmacy or ask your pharmacist how to dispose of them in accordance with the national regulations. These measures will help to protect the environment."

VI.2. Identification of possible measures

VI.2.1. Review of existing OSPAR, EU and national measures

No measures have been taken to date in any of these forums. Under the EU marketing authorisation of medicinal products (MAM) for human use procedure, a dossier for clotrimazole was submitted. However it was only recently stated by Council Directive 2001/83/EC that an application for the marketing authorisation for a medicinal product for human use shall be accompanied by an environmental risk assessment. 2001/83/EC requires the applicant to indicate any potential risks exhibited by the medicinal product for the environment. It should be noted that 2001/83/EC relates to those risks to the environment arising from use, storage and disposal of the medicinal product and not to those arising from synthesis and manufacture of the product.

VI.2.2. Choice for actions

a) General considerations

The risk assessment indicates that there is at present no risk for the environment over the use of clotrimazole.

The exposure scenario has been performed on the basis of market data from one producer, however clotrimazole is a generic medicinal product and the data from private use might be higher which can explain why relatively high concentrations of the chemical were measured in UK estuaries.

b) Action in the EC

Contracting Parties should support the ongoing development of the RAR and provide new information, if available on exposure and discharges, emissions and losses which would enable the PEC/PNEC ratios to be refined.

To support this process and ensure that the information available in this background document and the conclusions reached by OSPAR are generally taken into account in the approach of the European Community, OSPAR should communicate this document to the European Commission in relation with the recent Council Directive 2001/83/EC on the use of environmental risk assessment in the marketing authorisation of medicinal product for human use.

c) Action in OSPAR

OSPAR should re-evaluate the risks posed by clotrimazole releases when further information has been collected. Any associated measures which might be justified in the light of new findings should be addressed through the background document review process.

In order to enhance environmental protection, it is therefore recommended that – even for medicinal products that do not require special disposal measures – package leaflets (patient information leaflets) should include the following general statement:

"Medicines no longer required should not be disposed of via wastewater or the municipal drainage system. Return them to a pharmacy or ask your pharmacist how to dispose of them in accordance with the national regulations. These measures will help to protect the environment."

VI.2.3. Action in other forums

To ensure that the information in this background document can be considered in the context of other international agreements which deal with hazardous substances, and with which Contracting Parties are associated, OSPAR should send copies of this background document to the appropriate bodies dealing with those agreements and invite Contracting Parties who are parties both to OSPAR and those other

agreements to promote action to take account of this background document by those other international bodies in a consistent manner.

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Appendix 1: Updated factsheet of clotrimazole

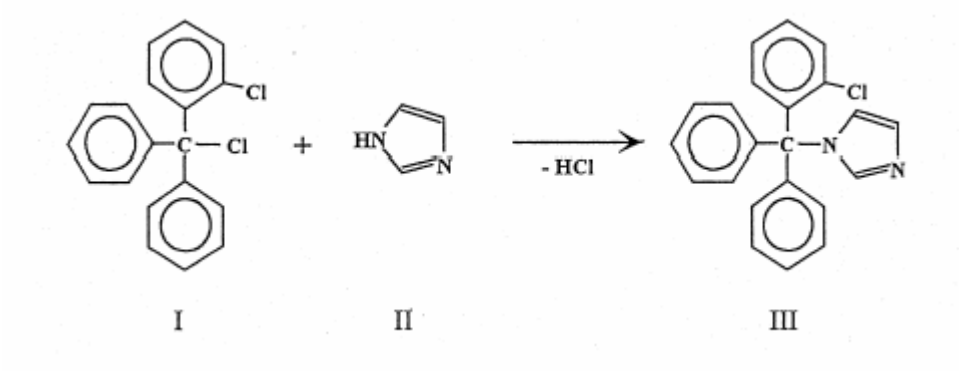
	NAME	**1H-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-**		**VERSION: 2002-07-18**
	IDENTIFICATION			
1.1	*CasNo*	23593751		
1.2	*EINECS/ELINCS*	245-764-8		
1.3	*Synonym*	clotrimazole		
1.4	*Group/Function*	Pharmaceutical		
1.5	*Initial selection*	**PBT QSAR-DK(V),**		
1.6	*Prioritised for action*	Date: OSPAR 2002; Lead Country: France; Background document: OSPAR 2004		
	Parameter	**Value**	**Source/Reference**	**Remarks**
	PHYSICAL/CHEMICAL PROPERTIES			
2.1	*Molecular weight, g/mole*	344,85	QSAR-DK:	
2.2	*Water solubility, mg/l*	298E-04	QSAR-DK: EPIWIN 3.02	
2.2		117E-04	IUCT QSAR Fraunhofer	
		490E-03		
2.3	*Vapour pressure, Pa*	284E-09 3,31E-09	QSAR-DK: EPISUITE program MpBpVp v1.40 SRC-MPBP 5meylan, 1994)	
	ABIOTIC/BIOTIC DEGRADATION PROPERTIES			
3.1	*Abiotic OH-oxidation t½ d*	231E-03	QSAR-DK: EPIWIN 3.02	
3.2	*Photolysis t½d*	3-310	Hellpointer (2002) Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water of clotrimazol. Bayer AG Leverkusen, Germany, MR 307/02	310 d in December under cloudy conditions 3 d in May
	Hydrolysis t½d	242E-00	Erstling (2001) - Abiotic degradation of clotrimazol. Bayer AG. Leverkusen, Germany. G01/0133/00LEV.	pH7, 25°C
		200E-01	Erstling (2001) - Abiotic degradation of clotrimazol. Bayer AG. Leverkusen, Germany. G01/0133/00LEV.	pH4, 25°C, the substance is not degradable at pH9
3.3	*Ready Biodegradability*	0%	Bayer (1994) - CO2-Entw.Test. Bayer AG. 33/00LEV.	not readily biodegradable
3.4	*Halflife*			
3.5	*Inherent Biodegradability*			
3.6	*Biodeg-QSAR*	0,4732	QSAR-DK: BIOWIN1	not readily biodegradable (50-70%)
3.6		2,0624	QSAR-DK: BIOWIN3	
3.6			QSAR-DK: Interpretation of BIOWIN1 and BIOWIN3	not Inherently biodegradable (20-70%)

3.6		-0,1003	QSAR-DK:Environ.Tox.Chem. 18(8): 1763-1768. Environ.Tox.Chem. 19(10): 2478-2485. (Syracuse version of H. Loonen's Simca Fragment linear MITI model.)	not readily biodegradable (20-50%)
3.6		0,0054	QSAR-DK:Environ.Tox.Chem. 18(8): 1763-1768. Environ.Tox.Chem. 19(10): 2478-2485.(Syracuse version of H. Loonen's Simca Fragment non-linear MITI model)	not readily biodegradable (20-50%)
	BIOACCUMULATION/ BIOCONCENTRATION			
4.1	*logKow*	6	QSAR-DK: EPIWIN 3.02	high potential for bioaccumulation
		4,1	Erstling and Jungheim (2003) - Partition coefficient (n-Octanol/Water). Bayer AG. Leverkusen.	Used in the PBT and risk assessment
4.2	*Bcf*	13183	QSAR-DK: EPIWIN 3.02 calculated with log Kow of 6	very high bioconcentration factor
		610	calculated with the logKow of 4,1	Low bioconcentration factor. Used in the PBT and risk assessment
	AQUATIC TOXIC PROPERTIES			
5.1	*Acute toxicity algae IC50, mg/l*	0,098	Bruns (2003) - Clotrimazol: Alga growth inhibition test. Bayer AG. Leverkusen, Germany. 1253 A/03 A1.	
	Chronic toxicity algae NOEC, mg/l	0,017	Bruns (2003) - Clotrimazol: Alga growth inhibition test. Bayer AG. Leverkusen, Germany. 1253 A/03 A1.	
5.2	*Acute toxicity daphnia EC50, mg/l*	0,022	Caspers and Müller (1994) - Untersuchungen zum ökologischen verhalten von Canesten wirkstoff. Bayer, AG. Leverkusen, Germany. 463A/94B.	
5.3	*Acute toxicity fish LC50, mg/l*	>0,29	Caspers and Müller (1994) - Untersuchungen zum ökologischen verhalten von Canesten wirkstoff. Bayer, AG. Leverkusen, Germany. 463A/94B	
	Acute toxicity fish LC0, mg/l	>0,278	Bruns (2003) - Clotrimazol: Acute fish toxicity. Bayer AG. Leverkusen. Internal report. 1253A/03F.	
5.4	*Chronic toxicity daphnia NOEC, mg/l*	0,01	Caspers (2004) – clotrimazol: *Daphnia magna* reproduction test. Bayer AG Leverkusen, Germany. Internal Report 1253 A/03 DL	
5.5	*Chronic toxicity fish NOEC, mg/l*	0,025	Bruns (2003) - Clotrimazol: Fish, Juvenile Growth Test. Bayer AG. Leverkusen, Germany. Internal report. 1253 A/03 FF.	
5.6	*Aquatox-QSAR*	0,0213	QSAR-DK: Fish NOEC, Lethal Body Burden NOEC mg/l (A:C ratio 10:1) for fish based on EPIWIN 3.02 BCF	Very toxic (<0,1 mg/l)
5.7	*Aquatic toxicity - other species*			
	HUMAN TOXIC PROPERTIES			
6.1	*Acute toxicity*			
6.2	*Carcinogenicity*			
6.3	*Chronic toxicity*			
6.4	*Mutagenicity*			

6.5	*Reprotoxicity*	C+	QSAR-DK:	a C+ sign indicate prediction for reprotox, T+ indicate positive test
	EXPOSURE			
7.1	*Production Volume*		Bayer	10-50 t
7.2	*Use/Industry Category*	PUBLIC DOMAIN , PHARMACEUTICALS		Source: IUCLID
7.3	*Use in articles*			
7.4	*Environm.Occur. Measured*			(Compartment)
7.5	*Environm.Occur. Modelled*			(Compartment)
8	**EU-LEGISLATION**			
8.1	*Dir 67/548/EEC (Classification)*	R3,22,50,53	:Annex1, Dir 67/548/EEC	
8.2	*Reg 793/93/EEC (Existing substances)*			
8.3	*Dir 2000/60/EEC (WFD)*			
8.4	*Dir 76/769/EEC (M&U)*			
8.5	*Dir 76/464/EEC (water)*			
8.6	*Dir 91/414/EEC (ppp)*			
8.7	*Dir 98/8/EEC (biocid)*			
9	**ADDITIONAL INFORMATION**			
9.1	*Hazard assessment-OECD*			
9.2	*Other risk assessments*			

Appendix 2: Detailed description of the process used for production of clotrimazole

1. Reaction scheme



2. Production

For a typical batch, a reactor is charged with:

- Imidazole (II)
- o-Chlorotritylchloride (I)

These reagents are added in a suitable solvent (e.g. acetone)

The reagents and the solvent are filled into a vessel. The reagents will react at an elevated temperature to product (III) in presence of an organic amine (e.g. triethylamine).

The reaction mixture is stirred in the presence of charcoal. Tryethylammonium hydrochloride and other residues are separated by filtration, the residues are separated by filtration. The residues are then washed with solvent and the filtrates are collected and combined.

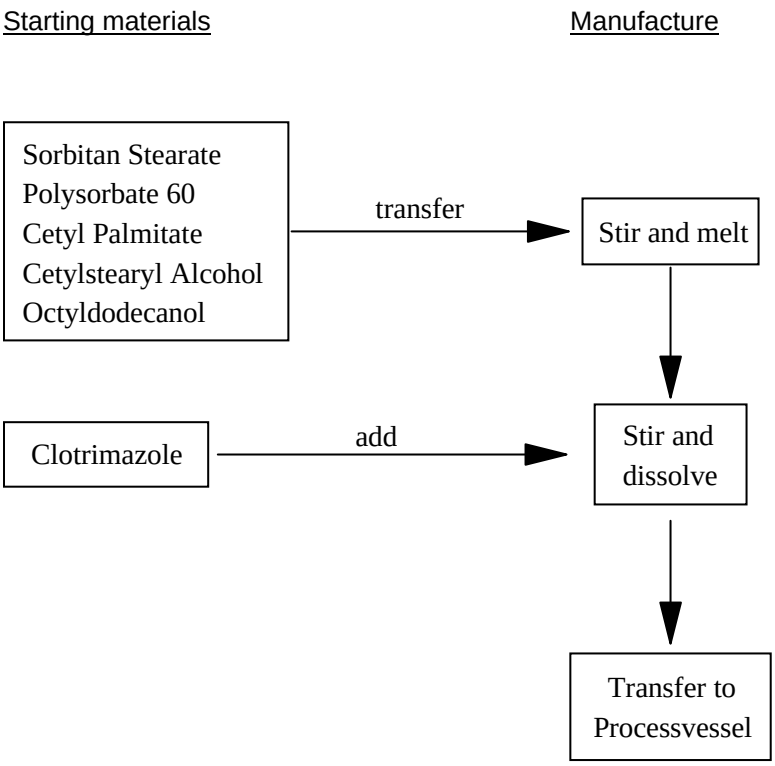
Product (III) crystallises after cooling and is separated from the mother liquor and washed.

The crystalline material is dried in a suitable dryer.

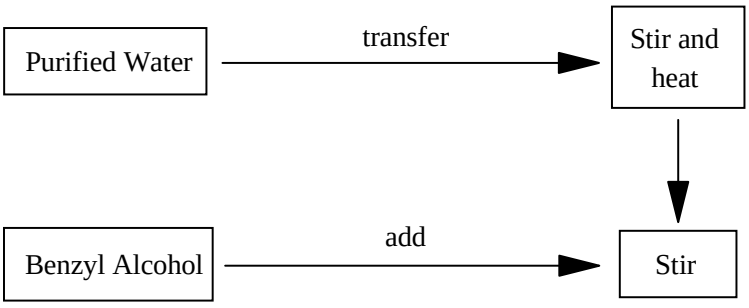
Batches which do not meet the specification are reprocessed by dissolving in the presence of charcoal, filtration, crystallisation, isolation and drying.

Appendix 3: Manufacturing description of CANESTEN Cream 1%

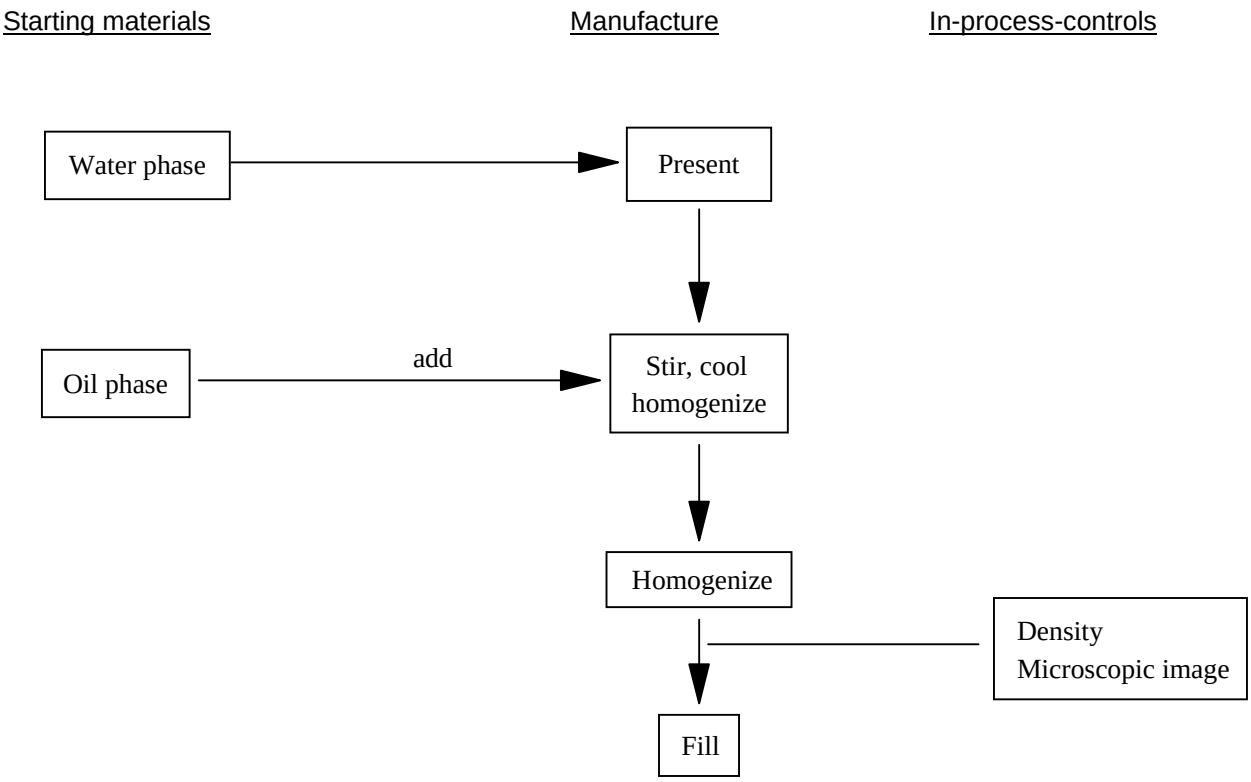
A. Flowchart Oil phase



A. Flowchart Water phase



A. Flowchart Process phase



B. Manufacturing

Oil phase

Under stirring, melt Sorbitan Monostearate, Octyldecanol, Cetostearyl Alcohol, Polysorbate 60 and Cetyl Palmitate in a stainless steel vessel fitted with a stirrer.

Then add Clotrimazole and dissolve.

Water Phase

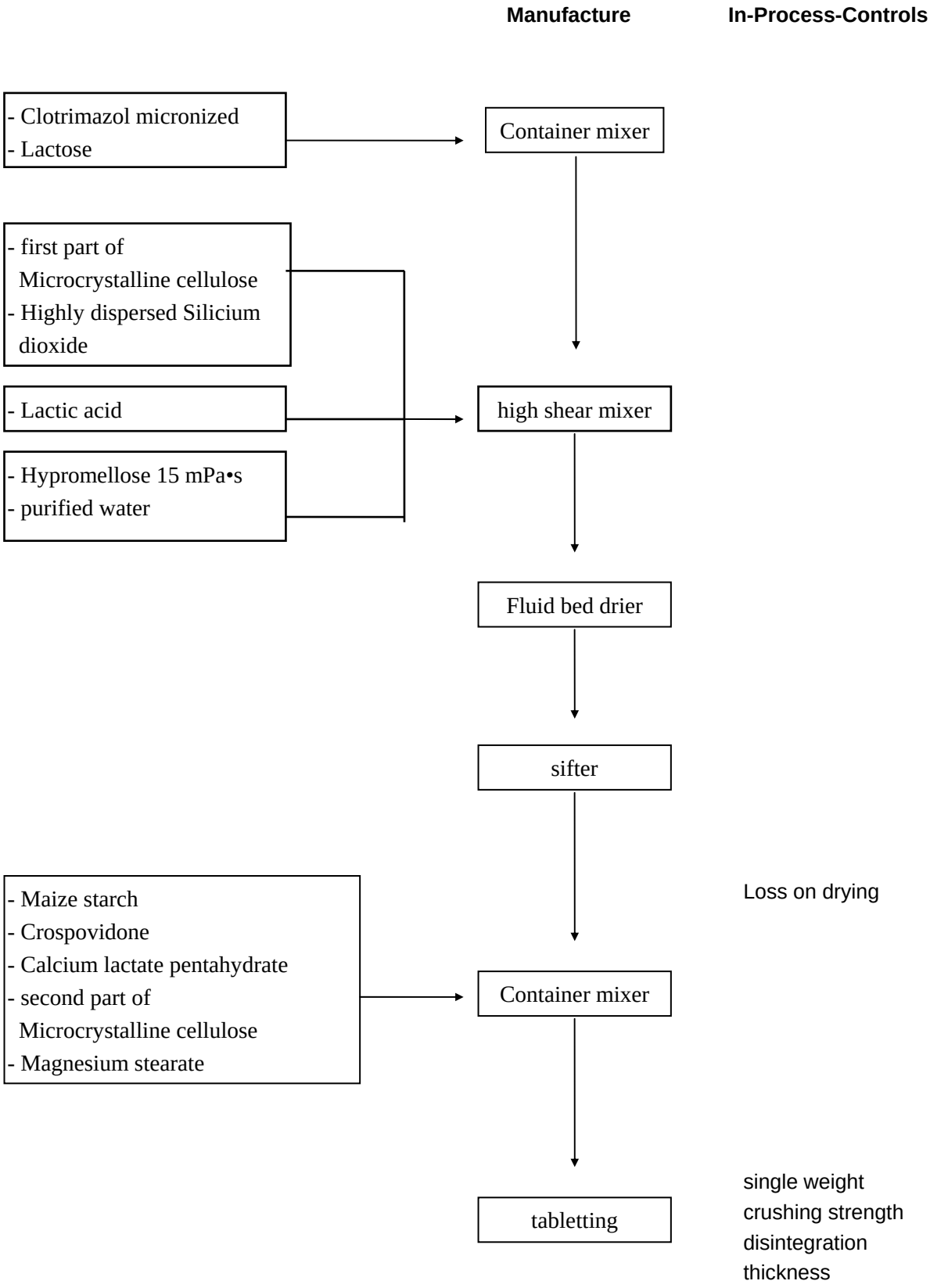
Transfer Purified Water to a stainless steel vessel fitted with stirrer whilst stirring and add Benzyl Alcohol.

Process Phase

In a stainless steel vessel fitted with stirrer, add the above mentioned oil phase to the above-mentioned water phase via a sieve and de-aerate in a vacuum. Subsequently, cool whilst stirring, homogenising at the beginning of this cooling process in a vacuum. Then homogenising in a vacuum. Perform in-process controls (microscopic image and density) and subsequently fill into suitable transport containers, whilst stirring, with the homogeniser operating at reduced speed.

Appendix 4: Manufacturing process description of CANESTEN Vaginal tablets 0,5g

A. Flowchart



B. Manufacture

Clotrimazole micronized, lactose, first part of microcrystalline cellulose, highly dispersed silicium dioxide and lactic acid are granulated in a high shear mixer with a suspension made of hypromellose 15mPa • s and water. The granulate is dried in a fluid bed drier and subsequently discharged into suitable container through an oscillator sieve. After adding the post mix (maize starch, crospovidone, calcium lactate pentahydrate, magnesium stearate and second part of microcrystalline cellulose) the ingredients are mixed in the container.

The final blend is pressed to tablets with a weight of 1,500 mg.

Appendix 5: Summary of the PEC/PNEC ratios

	PEC/PNEC ratio		
Local freshwater	**Production**	**Formulation**	**Private use**
	1,3E-02	1,3E-02	1,88E-01
Regional freshwater	1,3E-02		
Local freshwater sediment	**Production**	**Formulation**	**Private use**
	1,3E-02	1,3E-02	1,88E-01
Regional freshwater sediment	1,97E-02		
Local marine water	**Production**	**Formulation**	**Private use**
Scenario EUSES 2	Not relevant	Not relevant	2,31E-01
Scenario (low tide)	Not relevant	Not relevant	2,08
Regional marine water	2,63E-02		
Local marine sediment	**Production**	**Formulation**	**Private use**
Scenario EUSES 2	Not relevant	Not relevant	1,26E-01
Scenario (low tide)	Not relevant	Not relevant	1,13
Regional marine sediment	1,81E-02		
Freshwater predators	**Production**	**Formulation**	**Private use**
	2,86E-03	2,86E-03	2,20E-02
Marine predators	**Production**	**Formulation**	**Private use**
Scenario EUSES 2	No relevant	Not relevant	2,83E-03
Scenario (low tide)	No relevant	Not relevant	2,31E-02
Marine top-predators	**Production**	**Formulation**	**Private use**
Scenario EUSES 2	Not relevant	Not relevant	1,03E-03
Scenario (low tide)	Not relevant	Not relevant	5,07E-03
Soil organisms local	**Production**	**Formulation**	**Private use**
	2,23E-05	2,23E-0	5,44E-01
Soil organisms regional	1,53E-03		
Micro-organisms in STP	**Production**	**Formulation**	**Private use**
	0	0	1,75E-03

Appendix 6: Environmental risk assessment of medicinal products for human use (CPMP/SWP/4447/00 draft July 2003)

Extracted from document CPMP/SWP/4447/00 draft July 2003. For more details: http://www.emea.eu.int/pdfs/human/swp/444700en.pdf

Introduction

Council Directive 2001/83/EC states that an application for the marketing authorisation for a medicinal product for human use shall be accompanied by an environmental risk assessment. 2001/83/EC requires the applicant to indicate any potential risks exhibited by the medicinal product for the environment. It should be noted that 2001/83/EC relates to those risks to the environment arising from use, storage and disposal of the medicinal product and not to those arising from synthesis and manufacture of the product.

1. Scope of the guidance

This note for guidance is applicable for medicinal products for human use that apply to Council Directive 2001/83/EC, which are intended to be placed on the market in the European Union, renewals of such products and type II variations in the case of a major increase of the use of the product.

This note for guidance is not applicable for medicinal products containing or consisting of Genetically Modified Organisms; applicants are referred to the note for guidance on *Environmental risk assessment for human medicinal products containing or consisting of GMOs* (CPMP/III/5507/94).

This note for guidance describes a two-phased environmental risk assessment of medicinal products when administered to patients.

2. General principles

Assessment of potential risks to the environment is a step-wise, phased procedure that may be terminated when sufficient information/data are available to either indicate that the medicinal products is unlikely to represent a risk to the environment or to identify and sufficiently characterise the potential risks. If relevant experimental data (e.g. metabolism) can be obtained from other parts of the dossier, these should be used in the assessment, and such studies therefore need not to be repeated. Existing information on synergistic effects should be included in the risk assessment. If, based on the available information and data, the applicant concludes that the medicinal product is unlikely to represent a risk to the environment and that therefore it would not be necessary to generate additional experimental data, the applicant should justify this decision. When the medicinal product exhibits potential risks to the environment, the applicant should propose appropriate precautionary and safety measures to be observed when the product is administered to patients and/or for the disposal of waste products. These measures should be included in the Summary of Products Characteristics (SPC).

Emphasis should be given to the parent compound and/or metabolite(s), as determined by human excretion profile, however the assessment should consider any substance of concern. Although most excipients can be described as inert, it is nevertheless possible that some may warrant attention in relation to their potential for harmful environmental effects. This should be discussed in the Environmental Risk Assessment report, where relevant.

The environmental risk assessment consists of two phases. The first phase (Phase I) assesses the exposure of the environment to the drug substance. Substances such as vitamins, electrolytes, amino acids can be exempted from further testing because they are unlikely to result in significant exposure of the environment and will consequently be of low environmental risk. A justification should be provided within expert report. Certain substances e.g. endocrine disrupters may need to be addressed irrespective of the quantity released into the environment.

In a second phase (Phase II) information about the physical/chemical, pharmacological and/or toxicological properties are obtained and assessed in relation to the extent of exposure of the environment. During the conduct of the tests required, the investigator shall consider whether further specific investigation on the fate and effects of the product on particular ecosystems is necessary. Phase II is divided in two parts, Tier A and Tier B.

Table 1. The phased approach in the environmental risk assessment

Stage in regulatory evaluation	Stage in risk assessment	Objective	Method	Test/data requirement
Phase I		Estimation of exposure	Action limits	No test requirement
Phase II Tier A	screening	Rapid prediction of risk	Risk assessment	Base set aquatic toxicology and fate
Phase II Tier B	Primary	Standard approach to ensure consistent decision making	Risk assessment	Extended data set on emission, fate and effects
	Secondary	Substance and site-specific refinement		Case-by-case; alternative approaches, TGD approach.

Tier A begins an evaluation of the possible fate and effects of the drug substance and/or major metabolites.

If within Tier A, no risk is detected, there is no need to proceed to Tier B. if a risk is detected, then the fate and effects of the medicinal product in the relevant compartment should be adequately investigated in Tier B.

Application to clotrimazole

First only a crude PEC for the aquatic compartment is calculated based on a worst case scenario. The initial calculation of PEC in surface water assumes:

- a percentage of the overall market penetration (market penetration factor: Fpen) within the range of existing medicinal products.
- The predicted amount used per year is evenly distributed over the year and throughout the geographic area
- The sewage system is the main route of entry of the medicinal product into the surface water
- There is no biodegradation or retention of the medicinal product in the sewage treatment plant (STP)
- Metabolism in the patient is not taken into account

The following formula should be used:

$$PEC_{SURFACE\ WATER}\ (mg/l) = (DOSEai \times Fpen) / (WASTEWinhab \times DILUTION \times 100)$$

DOSEai (mg.inh^{-1}d^{-1}): maximum daily dose of active ingredient consumed per inhabitant

Fpen (%) : percentage of market penetration

With $Fpen = (CONai \times 100) / (DOSEai \times inhabitants \times 365\ d/a)$

CONai (mg.a^{-1}): estimated consumption of active ingredient in geographic region per year based on statistics and/or epidemiological studies.

WASTEWinhab (L.inh^{-1}.d^{-1}): amount of wastewater per inhabitant per day (200 from TGD)

DILUTION: dilution factor (10 from TGD).

The equation can also be expressed as:

$$PEC_{SURFACE\ WATER}\ (mg/l) = CONai / (WASTEWinhab \times DILUTION \times inhabitants \times 365\ d/a)$$

One of the assumptions in the crude PEC calculation is that there is no metabolism of the active substance.

If the crude PEC value is < 0,01 µg/l and no other environmental concerns are apparent, it may be assumed that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients.

If the crude PEC value is > 0,01 µg/l a crude environmental effect analysis should be performed.

These action limits may not be universally applicable, e.g.:

- For substances with known suspected special ecotoxicological effects, lower action limits would be appropriate (oestrogens, genotoxic substances…)
- substances of known low ecotoxicological potential may warrant higher PEC action limits

For clotrimazole, the calculation can be made using the following values for the UK:

CONai (mg.a^{-1}): 3,7E09 (market data from UK)

WASTEWinhab (L.inh^{-1}.d^{-1}): 200 (from TGD as recommended in CPMP document)

DILUTION: 10 (from TGD for freshwater)

100 (from TGD for marine water)

Inhabitants: 5,9E07 (United Kingdom)

$$PEC_{SURFACE\ WATER\ fresh}\ (\mu g/l) = (3,7E12) / (200 \times 10 \times 5,9E07 \times 365) = 0,086$$

$$PEC_{SURFACE\ WATER\ fresh} = 86\ ng/l$$

$$PEC_{SURFACE\ WATER\ marine}(mg/l) = (3,7E12) / (200 \times 100 \times 5,9E07 \times 365) = 0,0086$$

$$PEC_{SURFACE\ WATER\ marine} = 8,6\ ng/l$$

As $PEC_{SURFACE\ WATER\ fresh}$ > 10 ng/l, a phase II environmental effect analysis should be performed. This phase II is close to the methodology of the TGD and therefore will normally lead to the same result.

$PEC_{SURFACE\ WATER\ marine}$ is less than 10 ng/l, therefore there is *a priori* no risk for the marine environment and the assessment should stop at this step. However the first step of the methodology proposed in the frame of the assessment of medicinal products should normally be applied only to fresh surface water in order to conclude on the need of further assessment or not. The PEC calculated for inland surface water in above the trigger value of 10 n/L and therefore there is a need for further assessment through the phase II.

Results of the risk assessment

When the possibility of environmental risks cannot be excluded, precautionary and safety measures may consist of, but are not be restricted to

- restricted clinical use (e.g. hospitals only)
- product labelling, SPC, PL, etc. for patient use, product storage and disposal.

Appendix 7: Monitoring Strategy for clotrimazole

1. As part of the Joint Assessment and Monitoring Programme (*reference number 2003-22*), OSPAR 2005 adopted a revised Agreement on Monitoring Strategies for OSPAR Chemicals for Priority Action (*reference number 2004-14)* to implement the following monitoring for tracking progress towards the objectives of the OSPAR Hazardous Substances Strategy (*reference number 2003-21*) with regard to clotrimazole. The monitoring strategy for clotrimazole will be updated as and when necessary, and redirected in the light of subsequent experience.

2. Clotrimazole is an antimycotic agent used as a topical drug for the cure of dermatological and gynaecological fungal infections in human.

3. Clotrimazole is considered a borderline chemical regarding OSPAR DYNAMEC PBT criteria (P fulfilled, B and T borderline). However, clotrimazole is not a PBT chemical according to the EU TGD PBT criteria and the result of the national risk assessment indicates that there is at present no risk for the environment due to the use of clotrimazole.

4. The main source of releases of clotrimazole to the environment is from the private use of the substance. Releases from the production and the formulation steps are considered negligible. Emissions are mainly to water, and clotrimazole is likely to reach the freshwater and/or marine waters through the releases of municipal and/or domestic as well as hospital sewage treatment plants' effluents. Emissions to air are considered negligible.

5. The Background Document reported measured levels of clotrimazole in several estuaries in the United Kingdom whereas the substance was not detected in the Elbe River in Germany. Analytical methodologies are therefore available.

6. Clotrimazole is neither on the EU Water Framework Directive list of priority substances (annex 10 of this directive) nor on the EPER list (IPPC Directive, Commission Decision 2000/479/EC, 17 July 2000). It is also not part of HARP-HAZ or of any national monitoring programmes or emission inventories.

7. In the light of the factors listed above and considering that there is at present no risk for the marine environment on the basis of current production and use data, it seems that there is no need to take actions to move towards a cessation of discharges, emissions and losses of clotrimazole. Therefore the main components of the monitoring strategy of clotrimazole should be as follows:

a. the main source of releases of clotrimazole to the environment is from the private use of the substance and its subsequent release into municipal and/or domestic sewage treatment plants. The monitoring strategy therefore includes a one off survey of domestic and hospital effluents;

b. the outcomes of the background document do not underline the need for actions towards a cessation of discharges, emissions and losses of clotrimazole at present. However the risk assessment was performed on the basis of current production and use data. Therefore in case of a change in the production and/or sales figures, the risk assessment should be reviewed as well as the subsequent monitoring strategy.

CLOTRIMAZOLE MONITORING STRATEGY	
Implementation of actions and measures	• Examination of progress in the implementation of regulations on marketing and/or use or emission and/or discharge which have been agreed, or are endorsed, by the Background Document.
Discharges and losses to water	• Recommendation of a general survey of domestic and hospital effluents.
Production/use/ sales/figures	• The lead country will update information on production, sales and use of clotrimazole during review of the Background Document. The next review is planned for 2007/08.
Maritime area:	
Concentrations in water	• Where available, data will be compiled.