OSPAR COMMISSION

Background Document on Clotrimazole (2013 update)

Hazardous Substances Series

2013

OSPAR Convention

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. The Contracting Parties are Belgium, Denmark, the European Union, Finland, France, Germany, Iceland, Ireland, Luxembourg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Convention OSPAR

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. Les Parties contractantes sont l'Allemagne, la Belgique, le Danemark, l’Espagne, 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, la Suisse et l’Union européenne.

Acknowledgement

This report has been prepared by Ms Mélissa Dallet, France as lead country.
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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.

We estimate that approximately 25 tonnes of clotrimazole are brought on the European market each year (worst case scenario). 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 under REACh regulation (E.C., 2006). The persistence (P) criterion is therefore fulfilled according to European and OSPAR criteria (DT50 $\geq$ 50 days). The estimated bioconcentration factor (BCF) value depends on the method used (no experimental data available). BCF is between 610 and 1290. From these BCF we can conclude that Bioaccumulation (B) criterion is not fulfilled according to REACh criteria (BCF$\geq$2000) but it is according to OSPAR criteria (BCF$\geq$500). Crustacean is the most sensitive trophic level, a long-term toxicity data of 0,00827 mg/l is reported. The toxicity (T) criterion is therefore fulfilled according to REACh (NOEC $\leq$ 0.01 mg/L) and OSPAR criteria (NOEC $\leq$ 0.1 mg/L). 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 the P and T criteria are considered as fulfilled in regards of the available data (according to REACh and OSPAR criteria) and the B criteria is only fulfilled according to OSPAR criteria (BCF$\geq$500). But, for each criterion, recommendations have been made for further testing which would allow strengthening the PBT assessment. At present there is 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 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 new data will be available.

Récapitulatif

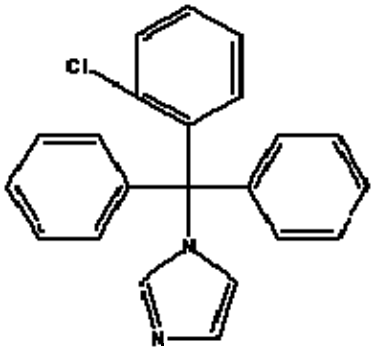
Le clotrimazole (N° CAS 23593-75-1) a été inscrit à OSPAR 2002 sur la liste OSPAR des produits chimiques devant faire l'objet de mesures prioritaires. Le clotrimazole est un produit pharmaceutique. Sa principale application est le traitement des infections fongiques dermatologiques et gynécologiques.

On estime qu'environ 25 tonnes de clotrimazole sont mises sur le marché Européen chaque année (pire cas). La principale source potentielle de clotrimazole dans l'environnement tient aux rejets des stations municipales d'épuration, 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 demi-vie du clotrimazole devrait être supérieure à 60 jours (durée à partir de laquelle une substance est considérée comme persistante selon la réglementation Européenne REACh (E.C., 2006)). Le critère de persistance (P) est donc rempli selon les critères de REACh et OSPAR (DT50 $\geq$ 50 days). La valeur de BCF calculée (aucune donnée expérimentale n'étant disponible) varie en fonction de la méthode utilisée. Le BCF est compris entre 610 et 1290. Le critère de bioaccumulation (B) n'est donc pas rempli selon les critères de REACh (BCF$\geq$2000) mais il l'est selon les critères d'OSPAR (BCF$\geq$500). Le niveau trophique le plus sensible est constitué par les crustacés, pour lesquels on signale des données de toxicité sur le long terme de 0,00827 mg/l. Par conséquent, le critère de la toxicité (T) est rempli que l'on se réfère aux valeurs limites établies par REACh (NOEC $\leq$ 0.01 mg/L) ou OSPAR (NOEC $\leq$ 0.1 mg/L). 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 les critères P et T sont considérés comme remplis au regard des informations disponibles (selon les critères fixés par l'Europe et OSPAR) et le critère B est seulement rempli selon les critères d'OSPAR. Cependant, pour chacun des paramètres, des recommandations ont été faites concernant les tests à réaliser permettant de renforcer cette évaluation. A 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 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 nouvelles données seront disponibles.

I. Environmental properties



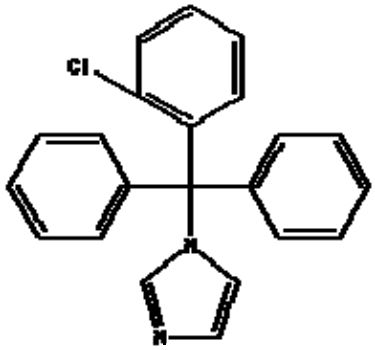
Clotrimazole (CAS n°- 23593-75-1) was first synthesised in 1969. Chemical name of the substance is 1-(2-chloro-phenyl)diphenylmethyl-1H-imidazole). Molecular formula is $C_{22}H_{17}ClN_2$ and the molecular weight is 344.8 $g.mol^{-1}$.

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 Guidance for implementation of REACh (E.C., 2006) 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)

Chemical name	Clotrimazole
Synonymes (other names)	*1-(o-Chlorophenyldiphenylmethyl)imidazole* *1-(o-Chlorotrityl)imidazole* *1-[(2-Chlorophenyl)diphenylmethyl]-1H-imidazole* *BAY 5097*
Chemical Abstracts Service Registry Number (CAS RN)	23593-75-1
Molecular formula	$C_{22}H_{17}ClN_2$
Code SMILES	Clc1ccccc1C(c2ccccc2)(c3ccccc3)n4ccnc4
Molecular structure	

I.1.1. Melting point

Melting point values of clotrimazole between 141 and 145°C (Hoogerheide et Wyka, 1982) and between 147 and 149 (HSDB, 2003) were reported. 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 $2.84.10^{-07}$ Pa. The vapour pressure of clotrimazole is therefore very low and the value of $3.31.10^{-07}$ 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. Other data were found : 3-8 mg/L at 23 °C (Buchana *et al.,* 2007) (experimental data), < 5 mg/L at 23 °C (Fligge and Schuler, 2006) (experimental data), <10 mg/L at 25 °C (Hoogerheide and Wyka, 1982) (experimental data).

I.1.5. Henry's law constant

The Henry's law constant of clotrimazole is reported as $3.16.10^{-3}$ $Pa.m^3$/mole at 25°C (modelled – HENRYWIN-EPI Suite4.1.)

I.1.6. Partition coefficients

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.The higher temperature (40°C) was used for both the test and the reference substance and it may be assumed, although not ascertained, that the influence of the temperature on the calculated log K_{ow} remain limited and lies within the range of the error of the method. 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 (KOWWIN- EPI Suite 4.1.).

We can see that the difference between the measured and predicted values is very large. It must be stressed that the OECD guideline (GL 117) by Reverse Phase HPLC by itself is also an estimation method. For confirmation of the experimental Kow it would thus be preferable to investigate the log K_{ow} using slow-stirring method (OECD 123) which is recommended for hydrophobic compounds.

As no other information is available on the log Kow of the substance, the value determined according to the OECD guideline (GL 117) (Erstling et Jungheim, 2003) is preferred rather than the QSAR value and the **log Kow of 4.1 will be used in the risk assessment.**

The Log Koc (organic carbon-water partition coefficient L/kg) of clotrimazole is reported as 3.5 (calculated with KOCWIN – EPI Suite 4.1. from log Kow of 4.1).

I.1.7. 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] (Pa)	$3.31.10^{-07}$
Henry's law constant ($Pa.m^3$/mole)	$3.16.10^{-3}$ (25°C) (modelled –HENRYWIN-EPI Suite4.1.)
Partition coefficient octanol-water[1] (Log Kow)	4.1
Water solubility[1] (mg/l at 25°C)	0.49
Log Koc (organic carbon-water partition coefficient –L/kg)	3.5 (KOCWIN – EPI Suite 4.1. from log Kow of 4.1)
PKa (acid dissociation constant – dimensionless)	5.21 (25°C - Experimental)[4]

I.2. Classification

Clotrimazole is not listed in the annexe I of the directive 67/548/EEC (E.C, 1967) and the annexe VI of the regulation (EC) No 1272/2008 (E.C., 2008a).

1 Measured value

2 Calculated value

3 Wiczling et al., 2006

Substances not listed either individually or in group entries must be self-classified.

Clotrimazole is classified as a dangerous substance within the meaning of the regulation (EC) No 1272/2008 (E.C, 2008a). The classification is:

Acute Tox. 4	H302 (harmful if swallowed)
Aquatic Acute 1	H400 (very toxic to aquatic organisms)
Aquatic Chronic 1	H410 (may cause long-term adverse effects in the aquatic environment)

I.3. Environmental fate and behavior

I.3.1. Persistence

Hydrolysis

The abiotic degradation of clotrimazole was tested at a range of environmentally relevant pH values (4, 7 and 9) according to the OECD guideline 111 (Erstling, 2001). The substance is not hydrolysed at pH 9 at 50°C in the screening test but shows some degradation at lower pH. 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 in this test. However, (Hoogerheide et Wyka, 1982) confirm that the substance is stable in alkaline medium and report that clotrimazole hydrolyses in acidic medium to (o-chlorophenyl)-diphenylmethanol and imidazole.

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 for a simulation in May under optimal conditions. No information on the degradation products is available.

Photolysis does not significantly contribute to the overall disappearance of the substance.

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

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. In order to know if degradation products were produced during the bioassay, it would have been interested to make measurements and identify potential residues.

Kahle *et al.* (2008) performed a biodegradation test of clotrimazole in activated sludge and obtained no degradation of the substance following 24-hour incubation with a concentration of 250 ng CLO/L; as a positive control ibuprofen was completely degraded within 6 hours.

In an investigation of the occurrence of selected pharmaceuticals in the Tyne River (United Kingdom), Roberts and Thomas (2006) detected clotrimazole in 100% of the surface water samples they collected. They interpreted this result as an expression of the persistence of the compound in water.

Furthermore, QSAR estimation (US-EPA, 2003) indicates that the substance is not readily biodegradable (Biowin 1 : linear biodegradation probability = 0.4732; Biowin 2 : Non-linear biodegradation probability = 0.1162, Biowin 3 : Survey model – Ultimate biodegradation = 2.0624, Biowin 4 : Survey model – Primary biodegradation = 3.0412, Biowin 5 : MITI linear biodegradation probability = -0.1003, MITI non-linear = biodegradation probability = 0.0054). A majority of QSAR (Quantitative Structure Activity Relationships) modelled estimations of biodegradability suggest that the fungicide does not biodegrade fast.

From the information available on the biodegradability, clotrimazole is considered not readily biodegradable, but the kinetic and the extent for primary degradation cannot be quantified.

ENVIRON, on behalf of Bayer Health Care, proposed to OSPAR to conduct a higher tier biodegradation test which provides additional information concerning the persistency criteria of clotrimazole. To be relevant this study should also provide confirmation of the identity of transformation products and an evaluation of the toxicity, persistency and bioaccumulation properties of these transformation products.

I.3.2. Environmental fate

a) Distribution

The very low modelled vapour pressure and Henry's Law constant of clotrimazole indicate that this antifungal product will be essentially non-volatile under atmospheric conditions. The pK_a value of 5.21 implies that at pH 6, 14% of CLO will be protonated in natural waters. More alkaline waters (e.g., pH 7 to 9) will see nearly all of the substance present in the un-ionized form.

If clotrimazole is released to the atmosphere, its non volatility will cause the substance to leave air to partition into soil. If it is released to surface water, about 35% and 65% of the drug will partition to water and sediment, respectively. If it is released to soil, CLO will remain entirely in soil (Environmental simulations with the Equilibrium Criterion (EQC) model (Mackay et al., 1996).

b) Bioaccumulation

Clotrimazole must be accumulated in fungi to be effective (i.e. Mode of action) (Vanden Bossche *et al.,* 2003).

It has been known for many years that biotransformation and (mono-oxygenase) enzyme induction modulate bioaccumulation of xenobiotics in mammals and fish. Information is available in the literature on the metabolisation of clotrimazole in human and rats' bodies. A study performed by Duhm et *al.,* (1974) revealed that clotrimazole is rapidly biotransformed to inactive metabolites, resulting in a nearly complete elimination (i.e., 97%) 48 hours after intravenous or oral administration. This result is explain by the fact that in rats, CLO was able to bind to transcription factors regulating CYP induction (Dickins, 2004), and to increase gene expression coding for enzymes involved in drug and steroid metabolism (Sahi *et al.,* 2009).

Contrary, studies performed on another mammal, bird, fish, and freshwater crustacean showed that clotrimazole is generally an inhibitor of these same P450 enzymatic systems involved in drug and steroid metabolism (Navas *et al.,* 2004). In addition to that, imidazole-containing compounds are known for their specific inhibition of cytochrome P450 biotransformation reactions (Olkowski *et al.* 1998).

From these results we can conclude that the metabolic rate of clotrimazole is low in non-human organisms other than the rat.

No experimental values for bioconcentration factor (BCF) or Bioaccumulation factor (BAF) are found in the available published literature. So these values for clotrimazole were calculated by QSARs.

<u>BCF value estimated by the Veith *et al.,* 1979 method (QSAR)</u>

A bioconcentration factor (BCF) for fish can be estimated according to the following equation developed by Veith *et al.,* 1979 as reported in the Guidance for the implementation of REACh (chapter R.7.c : Endpoint specific guidance) (E.C., 2008b). The partition coefficient of test substance as determined before is 4.1.

$$\text{Log } BCF_{fish} = 0.85 \text{ . log } Kow - 0.70$$

$$\text{Log } BCF_{fish} = 2.785$$

$$\boldsymbol{BCF_{fish} = 610}$$

BCF and BAF values estimated by the Arnot-Gobas methods (QSAR)

This QSAR focuses on the fish trophic levels of aquatic food webs, accounting for chemical uptake from water and food, and considering elimination pathways including metabolic transformation. The biotransformation rate constants are defined by default by the model.

The partition coefficient of test substance as determined before is 4.1.

Arnot-Gobas BCF and BAF methods (Including biotransformation rate estimates):

Estimated Log BCF (Upper trophic) = 2.985 (BCF = **966** L/ kg ww)

Estimated Log BAF (Upper trophic) = 3.002 (BAF = 1006 L/ kg ww)

Arnot-Gobas BCF and BAF methods (Assuming a biotransformation rate of zero):

Estimated Log BCF (Upper trophic) = 3.111(BCF = **1290** L/ kg ww)

Estimated Log BAF (Upper trophic) = 3.527 (BAF = 3369 L/ kg ww)

A biotransformation rate of 0 day has been considered given the uncertainty of what this metabolic rate should be for fish. To be the most protective the result obtained with the metabolic rate of 0 should be chosen.

We can see that the BCF value obtained depends on the calculation method used. **It would be more interesting to determine the BCF in fish experimentally.**

I.4. 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.4.1. Water organisms

a) Acute toxicity

Acute toxicity to Algae

A study was performed by Bruns (2003b) to assess adverse effects of clotrimazole on the planktonic freshwater algal species *Desmodesmus subspicatus* over several generations. The study was conducted in accordance with EEC Methods for determination of Ecotoxicity Annex to Directive 92/69/EEC part C Method 3 “Algal inhibition test” which is in most parts equivalent to the OCDE Guideline for testing of Chemicals No. 201 “Algal, Growth Inhibition Test”.

The effects measured were the growth and the growth rate of the algal population exposed to 6 test concentrations (3 replicates per concentration) and one control (6 replicates). The concentrations tested were 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mg/L. The cell densities were measured at 24 hour intervals. During the test a temperature range of 21 – 25 °C was maintained in the test vessels and continuous uniform illumination was provided in the spectral range 400 to 700 nm. 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. Measured concentrations ranged from

73.3 –91.5 % of nominal values at 0 hours, and from 65.3-104 % of nominal values at 72 hours. This test is considered valid.

E_bC_{50} (72h) = 0.098 mg/L (0.063 mg/L – 0.169 mg/L)

E_rC_{50} (72h) = 0.268 mg/L (0.186 mg/L – 0.532 mg/L)

Acute toxicity to Crustacean

Caspers and Müller (1994) report an EC_0 = 0.025 mg/l and an EC_{100} = 0.1 mg/l on *Daphnia magna* after an exposure of 48 hours. The EC_{50} was not calculated in the study only the geometrical mean of the EC_0 and the EC_{100} 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 EC_0 and EC_{100} are respectively of 0.008 and 0.033 mg/L after 48 hours of exposure and a statistical evaluation leads to an EC_{50} (48h) = 0.02 mg/l (95% C.I: 0.018 – 0.022 mg/L).

EC_{50} (48h) = 0.020 mg/L

(to be considered with caution, calculated as the geometrical mean of the EC_0 and the EC_{100})

In view of the uncertainty related to the tested concentration this value is considered valid with restriction.

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 are 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 EC_{20} = 0.29 mg/l as well as an LC_{50} > 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 study was conducted in accordance with EEC Methods for determination of Ecotoxicity Annex to Directive 92/69/EEC part C Method 1 "Acute toxicity for fish" which is in most parts equivalent to the OCDE Guideline for testing of Chemicals No. 203 "Fish, acute toxicity test".

Groups of ten fish of the recommended size were exposed to a limit test concentration of nominally 0.5 mg/L of clotrimazole dissolved in water (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. During the test, a temperature range of 20-24 °C was maintained in the test vessels. The hardness of the dilution water used was 251.7 mg/L CaCO3. pH and oxygen values are measured at the beginning of the test and every 24 hours. One test per concentration was performed and one control (with 10 animals per test). At the highest test concentration 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) (effects: number of dead and the incidence of sub-lethal effects). Therefore the test results in an LC_0

(96 hours) ≥ 0.278 mg/L for *Brachydanio rerio*. The results are all expressed as measured concentrations. Measured concentrations correspond to 62.8 % of nominal values at 0 hours, to 54.7 % of nominal values at 24 hours, to 56.1 % of nominal values at 48 hours, to 53.3 % of nominal values at 72 hours and 51.1 % of nominal values at 96 hours.

LC_0 (96h) ≥ 0.278 mg/L

b) Chronic toxicity

Chronic toxicity to algae

In the study of Bruns (2003b) presented above, 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

Porsbring *et al.* (2009) evaluated the toxicity of clotrimazole to marine periphytic communities dominated by diatoms. Natural marine microalgal communities (periphyton) were exposed to concentration series of clotrimazole over 4 days. 50 pmol/L clotrimazole caused a concentration-dependent accumulation of C14α-methylated sterol precursors, which coincided with a decrease in algal-specific C14-desmethyl sterols. This indicates an inhibition of algal 14α-demethylase already at environmental concentrations. A clotrimazole concentration of 500 pmol/L reduced total sterol content to 64% of control level. Community chlorophyll “a” content was affected by clotrimazole with first reductions becoming visible at 500 pmol/L (= 0.17 µg/L). Concentration of 10-100 nmol/L and higher caused large reductions in community growth, and changes community profiles in a concentration-dependent monotonous manner. These effects are not tested in single species tests and we don’t have endpoint results (EC50, NOEC). However, given the fact that clotrimazole is a fungicide these communities might be very sensitive (more than green algae) as a consequence of the mode of action of clotrimazole. This point would merit to be studied in more detail with realization of single tests species for example. Before that, this result can be not used to calculate the PNEC but it should be taken into account for “T” criteria evaluation.

Chronic toxicity to crustacean

The chronic toxicity of clotrimazole to *Daphnia magna* was assessed according to the OECD test guideline 211 (Caspers, 2004). The effects on the reproductive output of this species were observed after a 21 days exposure period to a range of 5 test concentrations (0,32, 1, 3,2, 10 and 32 µg/l of clotrimazole) and one control with 10 replicates per concentration. A semi-static system was used with a renewal of the test media three times a week. The hardness of the dilution water ranged from 260.61 – 269,54 mg:L CaCO3 during the test and a temperature range of 18 – 22 °C is maintained in the test vessel, with a maximum temperature fluctuation of +/- 2°C in each individual test. Test vessels were not aerated during the test. A photoperiod of 8 hours darkness and 16 hours light was maintained. Feeding oh the *Daphnia* with living cells of *Desmodesmus subspicatus* was preferably done daily but at least three times a week (corresponding to media change). After a 21 d exposure period, the total number of living offspring produced per parent animal alive at the end of the test was assessed. 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 (nominal) = 0.00827 mg/l (measured)

LOEC (21d)reproduction = 0,032 mg/l (nominal) = 0.02879 mg/l (measured)

Chronic toxicity to fish

A sub-chronic study was performed to assess the effects of prolonged exposure of clotrimazole on the growth of juvenile rainbow trout (*Oncorhynchus mykiss)* under semi-static conditions (Bruns, 2003c). The study was conducted in accordance with the OECD Guideline for testing of Chemicals No. 215 "Fish, juvenile Growth test".

Batches of 16 juvenile fish in exponential growth were, after being weighed, exposed to a range of concentrations, nominally, 0.001, 0.0032, 0.01, 0.032 and 0.1 mg/L of clotrimazole dissolved in water and one control (16 juvenile fish per test and control) .The numbers of juvenile fish and the mortality were recorded daily throughout the whole test period. Tank average specific growth rate was measured after 14d and 28d of exposure. The total hardness of the dilution water measured at test start in the control and the highest test concentration ranged from 273 – 282 mg/L CaCO3. During the test a temperature range of 12.5 – 16 °C was maintained in the test vessels.

The effects measured were the mortality of fish, behavioural abnormalities and fresh weight of juvenile fish after an exposure of 28 days to clotrimazole. Measured concentrations range from 75.5 – 139.1 % of nominal values at 0 hours, and from 40.0 – 84.4 % of nominal values after 24 hours of exposure. 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 (measured concentration)

c) Endocrine disruptor effects

Azole fungicides are able to interfere with the biosynthesis of fungal biosteroids and inhibit ergosterol biosynthesis. At the molecular level, these agents inhibit C-14 demethylase, an enzyme (cytochrome P450 monooxygenase) required along the pathway of ergosterol production. This mechanism of action is responsible for undesirable secondary effects of clotrimazole in non target organisms.

Indeed, 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.

A study reports the *in vitro* effects of clotrimazole tested on brain and ovarian microsomal aromatase activity of the rainbow trout (*Onchorynchus mykiss*). It is shown that clotrimazole is able to inhibit brain and ovarian aromatase activities in a dose dependent manner. The respective EC50 values are for ovarian and brain: EC50 = 16.10^{-9} M and EC50 = 11.10^{-9} M (Brion *et al.*, 2006). Monod *et al.* (1993) reports also inhibition of microsomal aromatase in rainbow trout due to clotrimazole (IC50 = 5.10-7M). Several recent studies in male fish highlight the inhibition of various cytochrome P450 enzyme activities by clotrimazole. In zebrafish, clotrimazole has an impact on the gonadal steroidogenesis (inhibition of the 11-ketotestosterone (a potent androgen in fish) testicular release (Hinfray *et al.,* 2011 and Baudiffier *et al.,* 2012) and is able to affect testicular physiology and raised further concern about the impact of clotrimazole on reproduction (Baudiffier *et al.,* 2013).

The consequence of the inhibition of clotrimazole on key enzymes involved in sex steroid hormone synthesis will likely be adverse effects on fertility, sexual behaviour and reproductive organ development.

d) 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	measured (1)	(Bruns, 2003b)
Crustacean	*Daphnia magna*	static	EC50 (48h) (Immobilization)	**0.02**	Nominal, derived from geometric mean of an EC_0 and EC_{100} (2)	(Caspers et Müller, 1994 ; Bruns, 2004)
	Penaeus monodon (marine shrimp)	Static	96 hours (Survival)	1	(0% survival for nauplii and zoea stages) (1)	(Lio-Po and Sanvictores, 1986)
Fish	*Brachydanio rerio*	Semi static	LC50 (96h) EC_{20} (96h)	>0.29 0.29	measured (2)	(Caspers et Müller, 1994)
	Brachydanio rerio	Semi static	LC0 (96h)	> 0.278	measured (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	Measured conc. (1)	(Bruns, 2003b)
Crustacean	*Daphnia magna*	Semi static	NOEC (21j)	0.01 **0.00827**	Nominal conc. (1) measured (1)	(Casper, 2004)
Fish	*Oncorhynchus mykiss*	Semi static	EC_{10} (28 d)	0.025	Nominal conc. Effects measured were mortality, behavioural abnormalities and fresh weight of juvenile fish (1)	(Bruns, 2003c)

1: valid without restriction
2: valid with restriction.

I.4.2. Sediment-dwelling organisms

No ecotoxicological data on benthic organisms are available.

I.4.3. Terrestrial compartment

No ecotoxicological data on soil organisms are available.

I.4.4. Ecotoxicity to STP

Several ecotoxicological data are available on sewage sludge 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 10000 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 EC_{50} value of 13.0 mg/l. The results of the controls performed at the concentration of clotrimazole of 10000 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 > 10000 mg/l. The test will be considered valid with restriction.

EC_{50} (30 min) > 10000 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 M26A[2]). The incubation time 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.5. Toxicological properties

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

I.5.1. 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

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

2 Methods for determining bactericidal activity of antimicrobial agents; NCCLS-Guideline, M26A, Vol.19 No 18,1999, ISBN 1-56238-384-1

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. The nitrogen atom of the imidazole heterocycle binds to the iron atom of the cytochrome P450 where an oxygen molecule would normally bind. At the same time, the lipophilic moiety of the fungicide binds to the region of the enzyme where the C-14 sterol would normally approach (Roberts and Hutson, 1998).

I.5.2. 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.5.3. 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.5.4. 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.5.5. Pharmacokinetics of clotrimazole.

A lot of information are available in the literature on the metabolisation of clotrimazole in human and rats. 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 (nearly complete elimination (i.e., 97%) 48 hours after intravenous or oral administration). The main metabolites found are represented in figure 1.

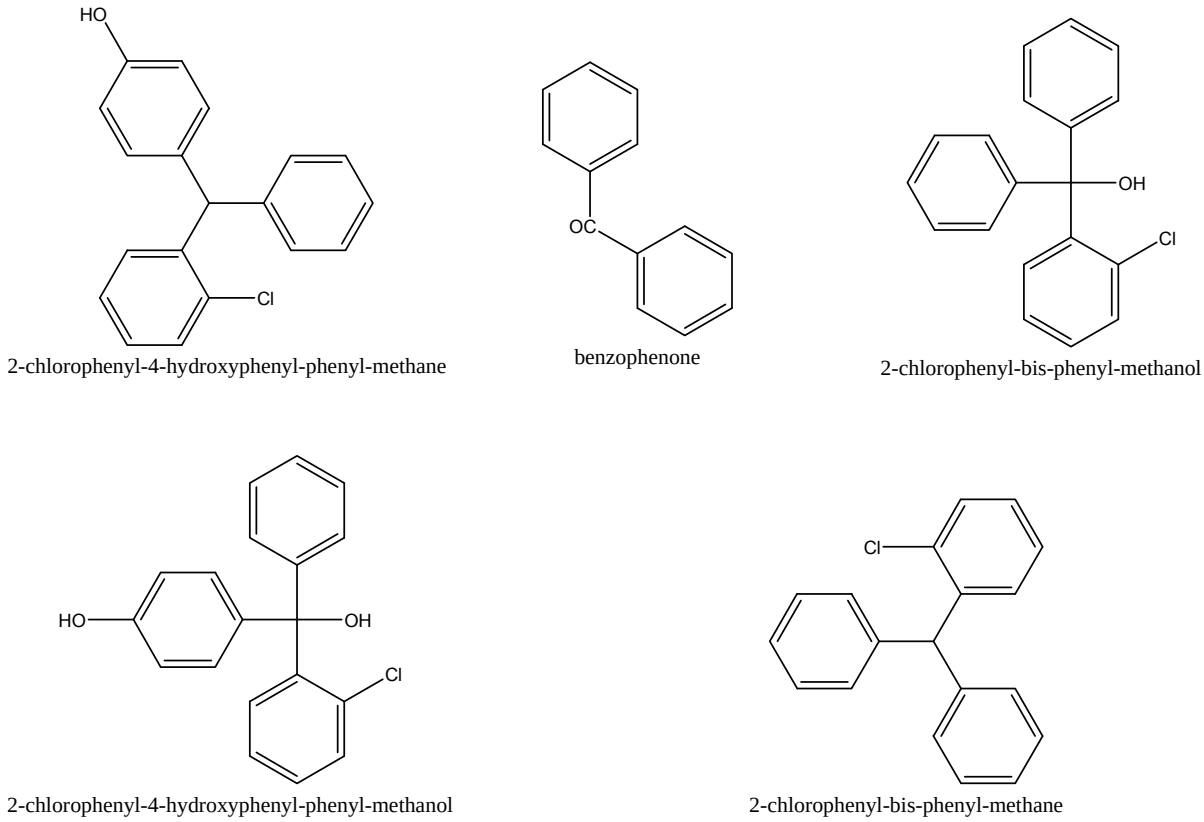


Figure 1: Main metabolites of clotrimazole

I.5.6. 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.6. PBT assessment.

I.6.1. 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. **In conclusion, with the current level of knowledge, the P criterion should be considered as fulfilled (according to OSPAR and European REACh criteria).**

ENVIRON, on behalf of Bayer Health Care, proposed to OSPAR to conduct a higher tier biodegradation study which provides additional information concerning the persistency criteria of clotrimazole. To be relevant this study should also provide confirmation of the identity of transformation products and an evaluation of the toxicity, persistency and bioaccumulation properties of these transformation products.

I.6.2. Bioaccumulation criterion

The estimated BCF value depends on the method used (no experimental data available). BCF is between 610 and 1290. From these BCF we can conclude that Bioaccumulation (B) criterion is not fulfilled according to REACh criteria (BCF≥2000) but it is according to OSPAR criteria (BCF≥500). **Experimentally determined BCF value is needed to conclude whether the B criterion is fulfilled.**

I.6.3. Toxicity criterion

The lowest NOEC is reported for crustacean (NOEC (21d) = 0,00827 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, under REACH or NOEC less than 0.1 under OSPAR. Results obtained in a sub chronic test in fish are already close to the T criterion (EC_{10} (28d) = 0.025 mg/L measured concentration). There is also a study on field communities (marine microalgal communities (periphyton)), which shows effects far below the T criterion. These are not tested in single species tests. However, given the fact that this is a fungicide they might be very sensitive (more than green algae) as a consequence of the mode of action of clotrimazole. Further, some recent studies show that clotrimazole has endocrine disrupting effects in fish. **The T criterion will therefore be considered as fulfilled (according to OSPAR and European REACH criteria).**

I.6.4. Conclusion of the PBT assessment

The P and T criteria are considered as fulfilled in regards of the available data (according to European and OSPAR criteria). The B criterion is only fulfilled according to OSPAR criteria (BCF≥500).

For each criterion, recommendations have been made for further testing which would allow strengthening the PBT assessment.

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

II.1. Production

Several companies offer clotrimazole products on the European market one of which is Bayer. Bayer produces clotrimazole active ingredient at one production site in the EU (Spain). The finished products (creams, vaginal tablets, solution, spray and powder) are made at several European formulation sites in Spain, Germany and other European countries. The amount of clotrimazole active ingredient brought on the European market by Bayer was about 10 in 2007 and about 9-10 tonnes in 2010. The UK (about 3 tonnes), Italy and Spain (about 2 tonnes) are the most important markets for Bayer.

The total amount of clotrimazole active ingredient sold by other companies on the European markets is estimated to be 10 to 15 tonnes per year.

These figures indicate that clotrimazole use in Europe remained stable with only small changes compared to figures reported earlier to OSPAR.

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 is 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.

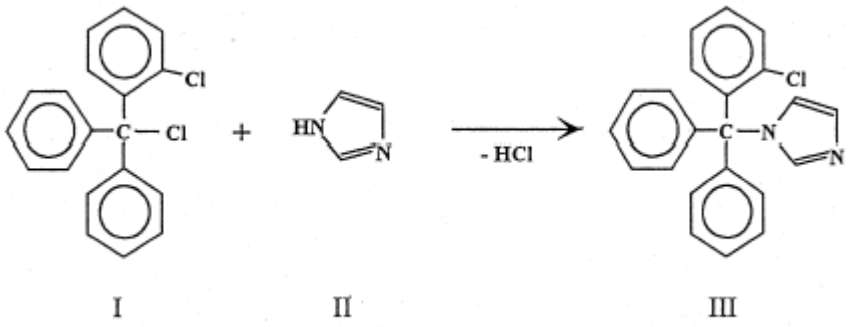


Figure 2: Reaction scheme (I: o-Chlorotritylchloride, II: Imidazole, III: clotrimazole)

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.

With regard to total market volume of clotrimazole products (packages sold), Germany and the UK have the biggest markets in Europe.

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 Cream 5 g	0.5
Gyno Canesten 3 Vaginal Cream 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 Cream	 0.5 0.2
Gyno Canesten Combi: - 3 Tablets - 20 g Cream	 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 Cream 100 g	1
Canesten Cream 50 g	0,5
Canesten Cream 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

Several companies offer clotrimazole products on the European market. In the only Bayer production site in the EU (Spain), 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

Formulation

Several companies offer clotrimazole products on the European market one of which is Bayer. Bayer produces clotrimazole active ingredient at one production site in the EU (Spain). On this site, 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.

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. 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 EUSES 2.1.1 default values were used only for the private use step.

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

Life cycle stage	Estimated local release (kg/d)	Estimated regional release (kg/d)	Estimated continental release (kg/d)
Production	-	-	-
Formulation	-	-	-
Private use	0 to air $3.42.10^{-3}$ to waste water	0 to air 1.71 to waste water 0 to surface water 0 to industrial soil 0 to agricultural soil	0 to air 15.4 to waste water 0 to surface water 0 to industrial soil 0 to agricultural soil

III.2. Aquatic compartment

III.2.1. Inland environment

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 2.1.1. The exposure scenario has been performed on the basis of the estimated amount of clotrimazole active ingredient brought on the European market (25 tonnes/years). Production and formulation steps are not considered because as it says before, at these steps releases to the different environmental compartments should be negligible and will be set to 0. It was assumed that the substance was administered externally as only release of metabolites is expected after oral administration (worst case scenario).

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

Life cycle	PEC_{water} (mg/L)	$PEC_{sediment}$ (mg/kg wwt)	PEC_{STP} (mg/l)
Private use	$1.51.10^{-04}$	$4.77.10^{-03}$	$1.43.10^{-3}$

Calculation of $PEC_{regional}$ and $PEC_{continental}$:

The regional and continental PEC calculated by EUSES 2.1.1 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)	$8.37.10^{-6}$	$1.26.10^{-6}$
Sediment (mg/kg wwt)	$3.97.10^{-4}$	$5.98.10^{-5}$
Air (mg/m^3)	$4.25.10^{-8}$	$2.47.10^{-8}$

III.2.2. Marine environment

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 REACh guidance (C.E, 2006) to apply a dilution factor 10 times higher than in the freshwater environment due to tidal influences.

Table 9: PEClocal for the marine environment

Life cycle	PEC_{water} (mg/L)	$PEC_{sediment}$ (mg/kg wwt)
Private use	$1.79.10^{-5}$	$5.66.10^{-4}$

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

The calculation was made by EUSES 2.1.1 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)

	$PEC_{regional}$	$PEC_{continental}$
Seawater (mg/L)	$8.14.10^{-7}$	$3.82.10^{-8}$
Marine Sediment (mg/kg wwt)	$3.24.10^{-5}$	$1.52.10^{-6}$

Monitoring in the freshwater and marine 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).

A recent study is available on the occurrence of human pharmaceuticals compounds in UK estuaries 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).

There is no routine monitoring of clotrimazole releases to air or water, but the occurrence of clotrimazole in water has been investigated in projects in the UK and Germany. Clotrimazole was not detected in any of the -3 samples in a German survey for pharmaceutical substances in the river Elbe in 1999 and 2000. The detection limit of the analytical method was 2.5 ng/l (Wiegel *et al.*, 2003). Conversely samples were taken in 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) (Thomas and Hilton, 2006).

In a further UK survey in 2004 of the wastewater treatment plant effluent and surface waters of the lower river Tyne, clotrimazole was detected in all the samples (Roberts and Thomas, 2006). Waste water treatment was shown to decrease, but not completely eliminate, CLO inputs into receiving water bodies (Kahle *et al.,* 2008; Peschka et al., 2007; Roberts and Thomas, 2006). These studies show that clotrimazole may reach the marine environment even after passage through a tertiary wastewater treatment plant. Further studies conducted by Bayer in 2006 showed that clotrimazole can hardly be detected in rivers in Germany and the Tyne estuary due to its high adsorption potential to particulate matter (Peschka et al., 2007).

The study by Peschka et al. yielded the following results:

- Analytical methods based on gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry were developed with limits of quantification down to 5 and 1 ng/l, respectively.
- Formerly reported differences in monitoring results in the UK and Germany (see above) can be explained by differences in the analytical method. Due to its relatively high Kow, Clotrimazole adsorbs easily to glass walls of analytical apparatuses and filter residues in which case recovery rates are low. Suitable analytical methods were developed to solve that problem. Particularly, acidification of aqueous samples to pH 2 prior to extraction is a contribution to good recovery rates. In an intercalibration exercise both methods were compared.
- The tendency of Clotrimazole to adsorb on mineral surfaces impedes not only the analysis at trace concentrations but also hinders the application of usual degradation tests. It is recommended that biodegradation tests be performed at low concentrations relevant in the environment.

The results showed that the improved method was able to find very low levels of analyte in samples where formerly no analyte could be found. Intercalibration proved that both analytical methods, employed by Knepper (Europa University of Applied Science Fresenius, Germany) and by Roberts (Centre for Environment, Fisheries and Aquaculture Science, CEFAS / UK) respectively, are valid. However, certain precautionary measures need to be taken to avoid loss of analyte during sampling, storing and preparation of the analyte.

After re-analyzing samples monitoring results in Germany and the UK showed consistent levels of clotrimazole in samples from different water bodies in both countries more or less influenced by waste water treatment effluent. Generally, levels in rivers are in the range of several nanograms per liter, WWTP effluents show higher levels but still in the lower nanogram range (< 100 ng/l).

Conclusions

Additional monitoring studies in Germany and the UK confirm previously found levels in the environment (water and sediment) in the low nanogram per liter range. These results indicate stable, very low levels in the aquatic environment over several years.

Discharges into waste water are linked to the use of clotrimazole by patients to cure e.g. topical fungal infections. Recent figures on clotrimazole marketing also indicate an extremely low and stable level of potential discharge into the environment.

The effort of detecting clotrimazole in the environment in a reproducible and reliable way proved to be high. Reliable and highly sensitive analytical methods are needed in monitoring studies.

III.2.3. Terrestrial compartment

Local, regional and continental estimated concentrations in soil.

Predicted concentrations of clotrimazole in soil have been calculated using EUSES 2.1.1 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.

Levels in soil

No monitoring data of clotrimazole are available.

Table 11: Local PEC for the soil compartment

Life cycle	$PEC_{agricultural\ soil}$ (mg/kg wwt)	$PEC_{grassland}$ (mg/kg wwt)
Private use	$7.86.10^{-3}$ (average over 30 days) $7.79.10^{-3}$ (average over 180 days)	$2.61.10^{-3}$ (average over 180 days)

Table 12: Regional and continental PEC for the soil compartment

	$PEC_{regional}$	$PEC_{continental}$
Agricultural soil (mg/kg wwt)	$1.49.10^{-4}$	$1.78.10^{-5}$
Natural soil (mg/kg wwt)	$5.24.10^{-6}$	$3.05.10^{-6}$
Industrial soil (mg/kg wwt)	$5.24.10^{-6}$	$3.05.10^{-6}$

III.2.4. Atmosphere

Local, regional and continental estimated concentrations in air

Predicted concentrations of clotrimazole in the atmosphere have been calculated using EUSES 2.1.1 for the local, regional and continental scenarios. The estimated PECs are reported in Table 13 and 14.

Table 13: Local PEC for the atmospheric compartment

PEClocal$_{air}$(mg/m^3)	**Private use**	5.85.10^{-8}

Table 14: Regional and continental PEC for the atmospheric compartment

	PEC$_{regional}$	**PEC$_{continental}$**
Air (mg/m^3)	4.25.10^{-8}	2.47.10^{-8}

Monitoring in the atmosphere

No atmospheric monitoring data of clotrimazole are available.

III.2.5. Secondary poisoning

Predicted concentrations in fish for both the inland and the marine environment are reported in Table 15.

Table 15: Estimated concentrations in freshwater and marine fish and marine predators

Life cycle	PECfish$_{freshwater}$ (mg/kg)	PECfish$_{marine}$ (mg/kg)	PECtop-predator (mg/kg)	PEC earthworms from agricultural
Private use (EUSES	0.0486	5.7.10^{-3}	1.54.10^{-3}	0.0219

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) = 0,00827 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 guidance for the implementation of REACh (Chapter R.10 : Characterisation of dose-response for environment) (E.C., 2008c). This results in:

PNECfresh water = 0,00827/10 = 0,000827 mg/l

PNECfresh water = 0,8 µg/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 guidance for the implementation of REACh (Chapter R.10 : Characterisation of dose-

response for environment) (E.C., 2008) an assessment factor of 100 will be applied to the NOEC value, which gives:

PNECmarine water = 0,08 µg/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*). 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. As recommended in the guidance for the implementation of REACh (Chapter R.10 : Characterisation of dose-response for environment) (E.C., 2008), an assessment factor of 10 can be applied to this value which gives a PNEC for the micro-organisms of 1000 mg/l.

PNECmicro-organisms = 1000 mg/l

IV.1.2. Sediment

There are no studies available on sediment-dwelling organisms. As recommended in the guidance for the implementation of REACh (Chapter R.10: Characterisation of dose-response for environment) (E.C., 2008), in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNECsed may be provisionally calculated using the equilibrium partitioning method (EPM). This method uses the PNECwater for aquatic organisms and the suspended matter/water partitioning coefficient as inputs.

It has to be considered that the equilibrium partitioning method may result both in an overestimation or underestimation of the toxicity to benthic organisms. Therefore this method can only be used as rough screening to decide whether sediment toxicity tests with benthic organisms are required.

In the partitioning method, it is assumed that the:

- sediment-dwelling organisms and water column organisms are equally sensitive to the chemical;
- concentration of the substance in sediment, interstitial water and benthic organisms are at thermodynamic equilibrium: the concentration in any of these phases can be predicted using the appropriate partition coefficients;
- sediment/water partition coefficients can either be measured or derived on the basis of a generic partition method from separately measurable characteristics of the sediment and the properties of the chemical.

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 ($m^3.m^{-3}$)

$Fwater_{ssusp}$: 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})

PNECfreshwater sed = (36,4/1150) ×0.8.10-3 × 1000 = 0,0253 mg/kg wet weight

PNECfreshwater sed = 25.3 µg/kg wwt

PNECmarine sed = (36.4/1150) ×0.08.10-3 × 1000 = 0,00253 mg/kg wet weight

PNECmarine sed = 2.53 µg/kg wwt

IV.2. Terrestrial compartment

There are no studies available on soil organisms. As recommended in the guidance for the implementation of REACh (Chapter R.10 : Characterisation of dose-response for environment) (E.C., 2008, 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 $K_{soil\text{-}water}$ = 42.08 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})

PNECsoil = (42/1700) ×0.8.10-3 × 1000 = 0,0197 mg/kg wet weight

PNECsoil = 19.7 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 ; as recommended in the technical guidance for deriving Environmental Quality Standards (E.C., 2011).

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 as recommended in the technical guidance for deriving Environmental Quality Standards (E.C., 2011) we propose to apply an assessment factor of 90 on this NOEC. Thus:

PNECoral = 2.78 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 guidance for the implementation of REACh regulation (E.C., 2006).

V.1. Inland environment

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.

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.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.3. Marine environment

Water

The PEC/PNEC ratios on the local and regional scale were determined and are below 1. The conclusion is that there is at present no risk for organisms living in the marine environment due to the use of clotrimazole.

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.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.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 1). Therefore it seems that there is no risk for the atmospheric compartment due to an exposure to clotrimazole.

V.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 do not indicate a risk of secondary poisoning due to exposure to clotrimazole in the environment.

V.7. Conclusion

The risk assessment indicates that there is at present no risk for the environment over the use of clotrimazole. We have to keep in mind that worst case scenario was used to calculate the PEC (The exposure scenario has been performed on the basis of the estimated amount of clotrimazole active ingredient brought on the European market (25 tonnes/years) and It was assumed that the substance was administered externally as only release of metabolites is expected after oral administration) but worst case scenario was not used to calculate the PNEC in water. Indeed, there is a study on field communities (marine microalgal communities (periphyton)), which shows effects below the data used to determine the PNEC in water. This result was not used because effects were not tested in single species tests and we don’t have endpoint (EC50, NOEC). But this result is far below the others results and so need to be confirmed with other tests.

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.

The P and T criteria are considered as fulfilled in regards of the available data (according to REACh and OSPAR criteria). The B criteria is only fulfilled according to OSPAR criteria (BCF≥500).

The estimated PEC/PNEC ratios for clotrimazole for the marine environment are all below 1. 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, formulation 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."

Information given by Bayer:

OSPAR has recommended that, to enhance environmental protection, 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".* This text has been included in the EMEA Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (Doc. Ref. EMEA/CHMP/SWP/4447/00, chapter 6): "Medicines should not be disposed of via wastewater or household waste. Ask your pharmacist how to dispose of medicines no longer required. These measures will help to protect the environment." The company formulating clotrimazole (Bayer) started to include the text of the EMEA Guideline in their product information leaflets for clotrimazole in EU countries.

Council Regulation (EEC) No 2309/93 and Directive 2001/83/EC regulate the authorization of marketing of pharmaceuticals and require 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.

December 2006, the EMEA “Guideline on Environmental Risk Assessment of Medicinal Products for Human Use” has entered into force. Since then, Bayer has already conducted an ERA on a new clotrimazole formulation according to this generally acknowledged methodology. According to the Risk Assessment, which went through Tier A and B assessment standards, no risk for the different environmental compartments could be identified.

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/CE 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.

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 the estimated amount of clotrimazole active ingredient brought on the European market. 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.

Action in the EC

Contracting Parties who are also EC member States 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.

Action in OSPAR

OSPAR should re-evaluate the risks posed by clotrimazole releases when new data will be available. Any associated measures which might be justified in the light of new findings should be addressed through the background document review process.

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Appendix 1: Updated factsheet of clotrimazole

	NAME	**1H-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-**		**VERSION: 2013-04-15**
	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*	2.84E-07 3.31E-07	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
		0%	Kahle *et al*. (2008) – biodegradation test in activated sludge, no degradation of the substance following 24 hours	not readily biodegradable
3.4	*Halflife*			

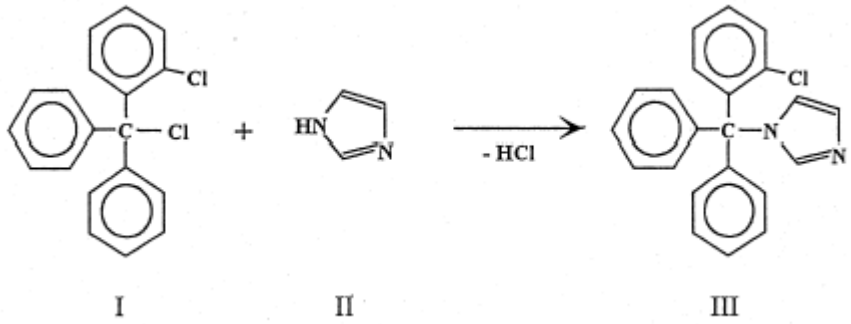
3.5	*Inherent Biodegradability*			
3.6	*Biodeg-QSAR*	0,4732	QSAR-DK: BIOWIN1	not readily biodegradable (50-70%)
3.6		2,0624	QSAR-DK: BIOWIN3	
3.6			QSAR-DK: Interpretation of BIOWIN1and BIOWIN3	not Inherently biodegradable (20-70%)
3.6		-0,1003	QSAR-DK:Environ.Tox.Chem. 18(8): 1763-1768. Environ.Tox.Chem. 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
		966	Arnot-Gobas BCF and BAF methods (Including biotransformation rate estimates) (calculated with the logKow of 4,1)	
		1290	Arnot-Gobas BCF and BAF methods (Including biotransformation rate estimates of 0) (calculated with the logKow of 4,1)	
	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.	
5.2	*Chronic toxicity algae NOEC, mg/l*	0,017	Bruns (2003) - Clotrimazol: Alga growth inhibition test. Bayer AG. Leverkusen, Germany. 1253 A/03 A1.	
		0.000172	Porsbring, T., H. Blanck, H. Tjellstrom, and T. Backhaus T. (2009) - Toxicity of the pharmaceutical clotrimazole to marine microalgal communities. Aquat. Toxicol. 91: 203-211.	These effects are not tested in single species tests and we don't have endpoint results (EC50, NOEC). However, given the fact that clotrimazole is a fungicide these communities might be very sensitive (more than green algae) as a consequence of the mode of action of clotrimazole. This point would merit to be studied in more detail with realization of single tests species for example. Before that, this result can be not used to calculate the PNEC but it should be taken into account for "T" criteria evaluation.

5.3	*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.	
5.4	*Chronic toxicity daphnia NOEC, mg/l*	0.00827	Casper (2004). Clotrimazole: Daphnia magna reproduction test. Bayey AG. Leverkusen, Germany. 1253 A/03 DL.	
5.5	*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.	
5.6	*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.	
5.7	*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)
5.8	*Aquatic toxicity - other species*			
	HUMAN TOXIC PROPERTIES			
6.1	*Acute toxicity*			
6.2	*Carcinogenicity*		No evidence of carcinogenic potential	
6.3	*Chronic toxicity*			
6.4	*Mutagenicity*		No sign of mutagenic potential	
6.5	*Reprotoxicity*	C+	QSAR-DK:	a C+ sign indicate prediction for reprotox, T+ indicate positive test
			The consequence of the inhibition of clotrimazole on key enzymes involved in sex steroid hormone synthesis will likely be adverse effects on fertility, sexual behaviour and reproductive organ development.	
	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



I II III

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.

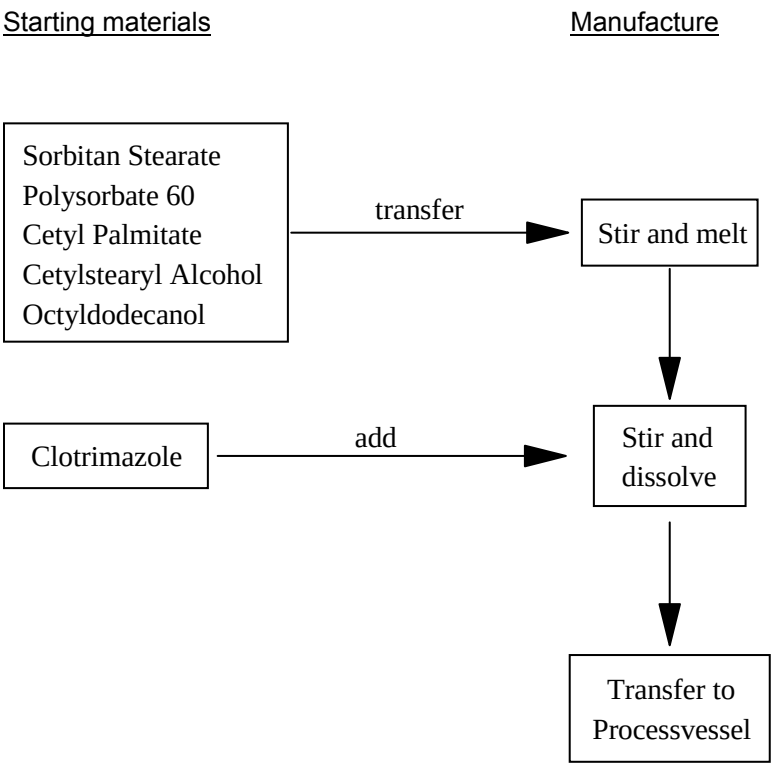
Product (III) crystallises after cooling and is separated from the mother liquor and washed.

The crystalline material is dried in a suitable dryer.

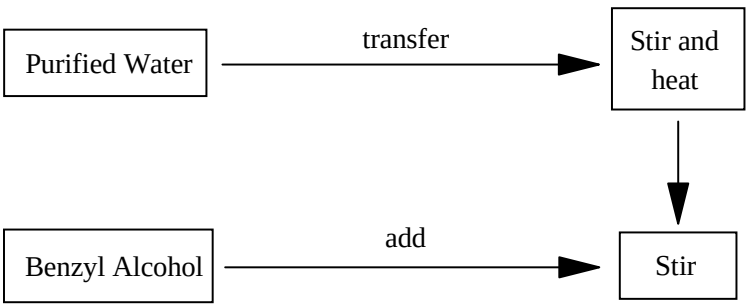
Batches which do not meet the specification are reprocessed by dissolving in the presence of charcoal, filtration, crystallisation, isolation and drying.

Appendix 3: Manufacturing description of CANESTEN Cream 1%

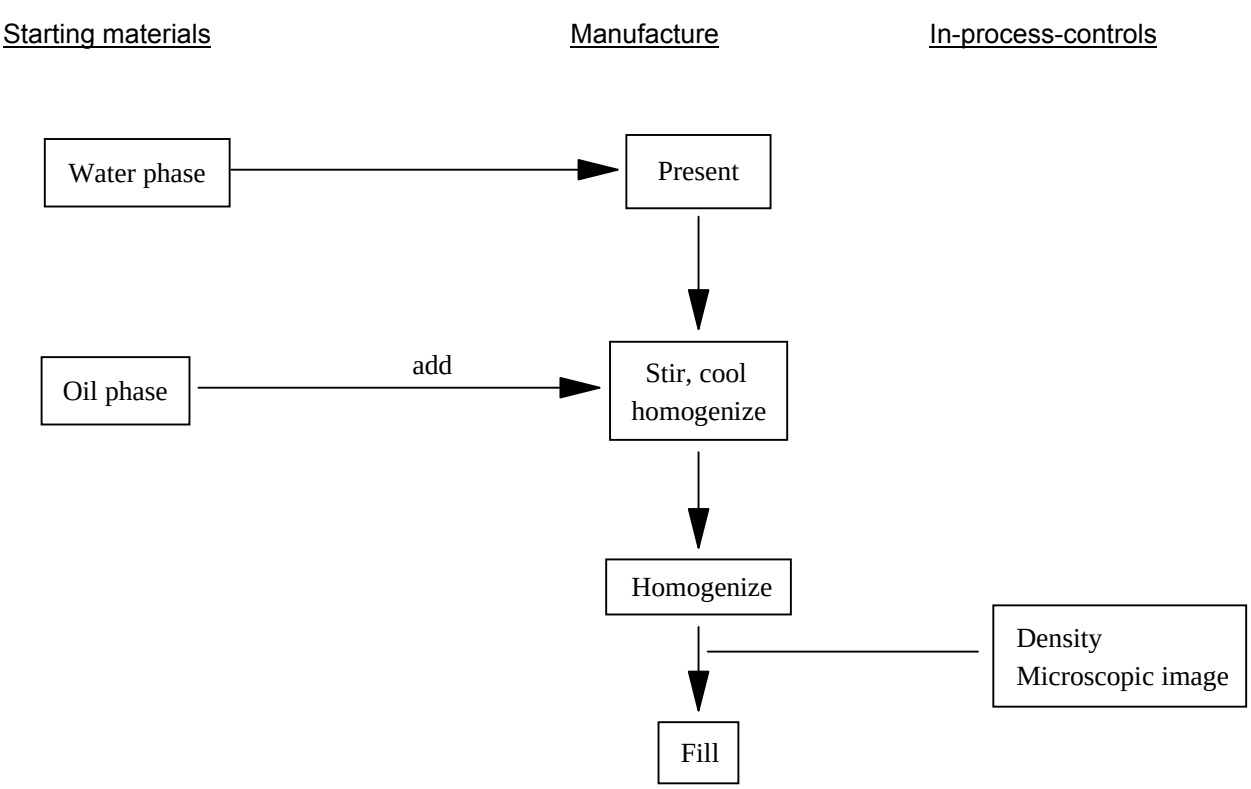
A. Flowchart Oil phase



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

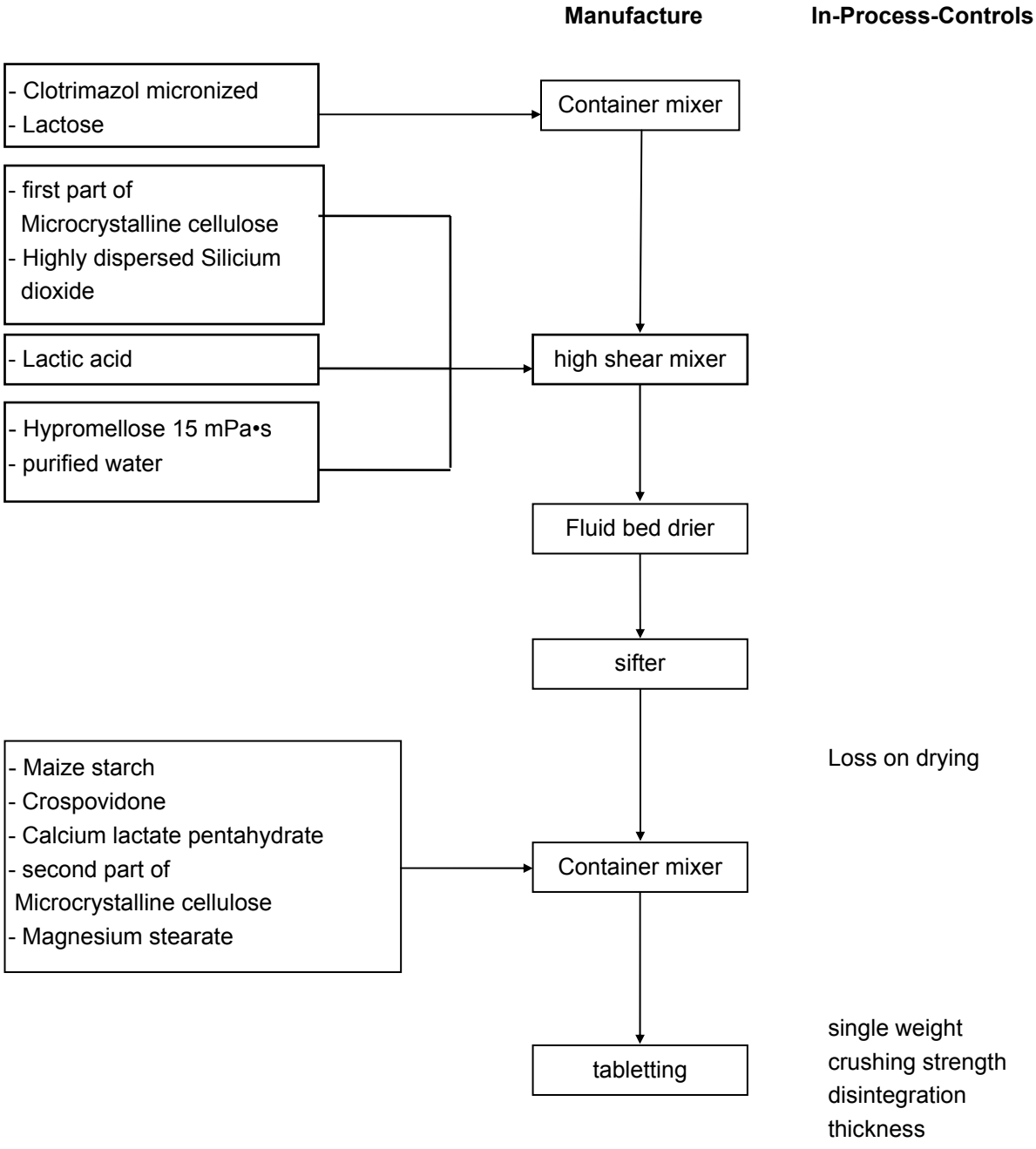
Transfer Purified Water to a stainless steel vessel fitted with stirrer whilst stirring and add Benzyl Alcohol.

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**
	Not relevant	Not relevant	0.18
Regional freshwater	0.01		
Local freshwater sediment	**Production**	**Formulation**	**Private use**
	Not relevant	Not relevant	0.18
Regional freshwater sediment	0.0156		
Local marine water	**Production**	**Formulation**	**Private use**
Scenario EUSES 2.1	Not relevant	Not relevant	0.22
Regional marine water	0.01		
Local marine sediment	**Production**	**Formulation**	**Private use**
Scenario EUSES 2.1	Not relevant	Not relevant	0.22
Regional marine sediment	0.0128		
Freshwater predators	**Production**	**Formulation**	**Private use**
	Not relevant	Not relevant	0.0175
Marine predators	**Production**	**Formulation**	**Private use**
Scenario EUSES 2.1	No relevant	Not relevant	$2.05.10^{-3}$
Marine top-predators	**Production**	**Formulation**	**Private use**
Scenario EUSES 2.1	Not relevant	Not relevant	$5.53.10^{-4}$
Soil organisms local	**Production**	**Formulation**	**Private use**
	Not relevant	Not relevant	0.39
Soil organisms regional	7.10^{-3}		
Micro-organisms in STP	**Production**	**Formulation**	**Private use**
	Not relevant	Not relevant	$1.43.10^{-6}$

Appendix 6: 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 and T fulfilled, B fulfilled but borderline). However, clotrimazole is not a PBT chemical according to the EU REACh PBT criteria (E.C., 2006) and the result of the 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 and 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 European 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 European 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.
Maritime area:	
Concentrations in water	- Where available, data will be compiled.

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