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CONSIDERATION OF AN INITIAL PROPOSAL TO AMEND ANNEX 1 TO THE AFS CONVENTION TO INCLUDE CONTROLS ON CYBUTRYNE

Report presenting scientific evidence for the adverse effects of cybutryne to the environment

Submitted by Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, the United Kingdom and the European Commission

SUMMARY

Executive summary:	This document contains in detail all the scientific evidence that supports the co-sponsors' position that cybutryne can be associated with adverse effects to the environment and has to be included in annex 1 of the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001
Strategic direction:	Number to be assigned after A 30
High-level action:	Number to be assigned after A 30
Output:	Number to be assigned after A 30
Action to be taken:	Paragraph 2
Related documents:	International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001; resolution A.900(21); PPR 5/19 and MEPC 71/14

Introduction

1 The annex to this document provides detailed scientific evidence required by article 6 of the AFS Convention and is addressing the information required by annex 2 to the AFS Convention.

Action requested of the Sub-Committee

2 The Sub-Committee is invited to consider the information provided in this document when considering the related document PPR 5/19.

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ANNEX

SCIENTIFIC EVIDENCE TO SUPPORT A PROPOSAL FOR AN AMENDMENT TO INCLUDE CYBUTRYNE INTO ANNEX 1 TO THE INTERNATIONAL CONVENTION ON THE CONTROL OF HARMFUL ANTI-FOULING SYSTEMS ON SHIPS, 2001

Table of contents

CHAPTER I

Characterization of the information which suggests that the anti-fouling system or its transformation products may pose a risk to human health or may cause adverse effects in non-target organisms at concentrations likely to be found in the environment

Cybutryne proved to be highly toxic to fish independently of whether marine or fresh water specie was tested ranging from an LC50 of 0.86 to 25 mg/L acutely and 4 to 170 µg/L chronically.

In the case of aquatic invertebrates, the 96 hours LC50 value of cybutryne towards the marine mysid shrimp *Mysidopsis bahia* was 0.48 mg/L. Most other acute LC50 data on marine crustaceans range between 0.556 and 6.03 mg/L. Acute toxicity to freshwater crustaceans are in the same range between 2.4 and 12 mg/L.

Data was also available for 3 more marine taxonomic groups: molluscs, echinoderms and cnidarians. Acute toxicity LC50's to echinderms and molluscs range between 1.54 and 6.03 mg/L, which is in the same range as the toxicity to crustaceans. The marine symbiotic dinoflagellates (algae) of the Cnidaria species *Acropora formosa* and *Seriatophora hystrix*, however, were much more sensitive showing 50% inhibition of photosynthesis at 0.0007 and 0.0009 mg/L after 10 hours exposure. Inhibition of photosynthesis has also been shown for the coral species *Madractis mirabilis* after exposure to 1 µg/L of cybutryne, and for zooxanthellae isolated from the same species effects was seen already at a concentration of 63 ng/L by *Owen et al (2003)*. Effects on isolated zooxanthellae have also been shown by *Owen et al. (2003)*. Zooxanthellae isolated from the coral species *Microprius mirabilis*, *Diploria strigosa* and *Favia framum* were affected after exposure to 2 µg/L cybutryne. Nevertheless, no toxicity values related to the cnidarian hosts are available, however, a reduction of calcification of the coral species *Galaxea fascicularis* has been shown after exposure to 10 µg/l (photosynthesis affected at 1 µg/L) in the study by *Sheikh et al. (2009)*, and for *M. mirabilis*, in the study by *Downs and Downs (2007)*, showed changes in expressions of proteins related to the cnidarian after exposure to 10 µg/L.

The 28-day NOEC based on growth/reproduction of cybutryne on the crustacean *Mysidopsis bahia* was 110 µg/L, which is of the same magnitude as the chronic toxicity observed for sheepshead minnow. Freshwater daphnids are less susceptible within a factor of 5 (i.e. NOECmortality, 30 day was 510 µg/L).

A great number of algal tests are available for cybutryne. The effects of cybutryne and its metabolite GS 26575 to growth inhibition of algae have been investigated in a marine and a freshwater diatom species. The effects on aquatic macrophytes were tested in two related freshwater species (*Lemna gibba* and *Lemna minor*). The toxicity in algae ranged between EC50 values of 0.12 to 12 µg/L as compared to the macrophyte EC50 values ranging between 1.65 and 8.1 µg/L. The algae *Navicula pelliculosa* appeared to be the most sensitive species showing effects on the growth with a 72 hours EC10 of 0.020 µg/l in the same range as the valid Skeletonema study (96 hours EC10 of 0.022 µg/L). Nevertheless, this value could be an underestimation due to large variability within the controls in the test. This value was used for the environment risk assessment under EU BPR.

In the review of the study by *Buma et al. (2009),* where also photosystem II (PSII) efficiency was tested as effect parameter, the extra ErC10 values for *Thalassiosira weissflogii* (diatom), *Emiliania huxleii* (cocolithophore), *Tetraselmis sp*. (green alga) and *Fibrocapsa japonica* (golden brown flagellate) support the values derived from Skeletonema and Navicula. The PSII efficiency, however, seemed to be a more sensitive effect parameter than growth rate (lowest

EC10 of 0.017 µg/L). At present, however, there is not enough knowledge concerning this effect parameter and therefore it cannot be used for risk assessment or labelling purposes.
The metabolite GS 26575 was shown to be less toxic to algae than cybutryne, the marine species (120 hour EC50 value of 16 µg/L) was considerably more sensitive than the freshwater species (120 hour EC50 value of 190 µg/L). However, these values are only indicative as exponential growth could not be determined in these studies.

Acute toxicity of cybutryne to sediment dwelling organisms was tested in a spiked-sediment test with the marine amphipod *Ampelisca abdita*. The NOEC was 44 mg/kg dry weight sediment and based on measured concentration in sediment. An EC50 of 0.04 mg/kg dry weight was determined for a brackish-freshwater amphipod (*Monoporeia affinis*) which showed a reduced burial in sediment when exposed to cybutryne.

In a chronic spiked-water test with the freshwater midge *Chironomus riparius*, cybutryne had no effect on mortality, emergence success and development rate. The number of midges emerging in the cybutryne treatments were not statistically different from the controls at concentrations of 0.3 µg /L.

Higher-tier tests were also conducted exposing natural phyto- and zooplankton communities under more realistic conditions for several weeks but did not result in significant adverse effects on functional parameters of algae and biomass of macrophytes and on the taxonomic abundance of phytoplankton, periphyton, zooplankton, macro invertebrates, eelgrass and marsh grass. Significant effects were seen only with phytoplankton until day 28 at nominal 400 ng/L (single sampling date) and 800 ng/L. Therefore NOEC microcosm study is nominal 400 ng/L (288 ng/L as mean measured concentration). Due to the experimental set-up of this microcosm study, there was an immigration of phyto- and zooplankton three times each week when 30% of the water was replaced with newly collected. Therefore, long-term effects on periphyton and zooplankton will have been masked. Therefore, the results of the study are considered to be those of a short-term exposure (that was repeated 31 times during the 70 day test). Two other more chronic micro/mesocosm studies provided NOECs of 16 ng/L and 186,000 ng/L, respectively.

A higher tier freshwater study is also available. In the indoor freshwater mesocosm study, fauna and flora naturally present in highly eutrophic but uncontaminated sediment from a lake near Brandenburg were treated once with cybutryne at a nominal concentration of 0.04, 0.2, 1 and 5 µg/L. Since chemical analysis showed dissipation of the active substance (less than 8% remained in the water column after 147 days), it was considered appropriate to use time weighted average (TWA) concentrations. The corresponding TWA concentrations are 0, 0.006, 0.031, 0.211and 1.425 µg/L. Periphyton and phyto- and zooplankton were regularly sampled during the 150 day test period. Additionally, macrophyte biomass was determined at test termination.

Differences in functional parameters, community structure, and taxonomic abundance of periphyton, phytoplankton and zooplankton and macrophyte abundance in macro-invertebrates between controls and cybutryne-treated microcosms were evaluated using appropriate univariate and multivariate statistical methods. The authors calculated EC10 and EC50 values, that are based upon nominal and TWA-based concentrations, of the different endpoints, which could not be evaluated during the EU BPR evaluation.

Both periphyton, zooplankton and macrophytes communities were directly affected by a single application of cybutryne. The periphyton chlorophytes were most susceptible, the lowest EC10 (nominal) of 0.01 µg/L occurred after 135 days for the chlorophytes (EC10 TWA 0.0005 µg/L). One more chronic freshwater micro/mesocosm study with phytoplankton derived a 24 d NOEC of 4 ng/L.

It is not possible to carry out statistical analysis on basis of the limited raw data submitted in addition to the study. Nevertheless the data presented in the report can be used for a whole evidence approach to the effect of cybutryne to the aquatic compartment or to assist in the determination of the assessment factor for the PNEC derivation.

Conclusion

The toxicity data with species from different phyla indicate that the primary producers, i.e. algae and aquatic macrophytes, are the most sensitive group of aquatic species. Since the mode of toxic action of cybutryne, like other triazine herbicides, is the inhibition of photosynthetic electron transport, this could be expected. The inhibition of the photosynthetic activity occurs in photo-system II (PSII), where the incorporation of CO_2 in organic molecules is inhibited, ultimately leading to an inhibition in growth. In standard laboratory tests the lowest 72 hour NOEC for cybutryne was observed with the freshwater diatom Navicula pelliculosa (NOEC 20 ng/L), while marine diatoms were slightly less susceptible: 120 hour NOEC 146 ng/L.

It can be concluded that cybutryne is highly toxic for primary producers and highly but less toxic towards most non-photosynthetic aquatic organisms, such as fish and invertebrates (NOEC 4-170 µg/L). An exception is the toxicity to the snail *Potamopyrgus antipodarum*, which appeared to be highly sensitive showing adverse effects even at the lowest concentration tested (50 ng/L).

The metabolite GS 26575 was less toxic towards fish and invertebrates (96 hour LC50 11 and 1.50 mg/L, respectively), and highly but slightly less toxic to marine algae (120 hour NOEC is 180 ng/L). Freshwater algae were much less susceptible: 120 hour NOEC was 77 mg/L.

In a microcosm study, in which natural marine algae, zooplankton, three macrophytes and macro-invertebrate communities were exposed to cybutryne under more realistic conditions for 10 weeks and where GS 26575 was the only formed metabolite, the lowest observed ecologically relevant NOEC for the most susceptible taxon (phytoplankton) under field conditions was 288 ng(cybutryne)/L. The results of an indoor freshwater mesocosm study has been used under the EU BPR evaluation to discuss the decision on pooling or not of data on freshwater and marine organisms. Furthermore, as no thorough statistical analysis was included in the report, this study could not be used to derive a PNEC freshwater.

The results from the test with the snail *Potamopyrgus antipodarum* study indicate that cybutryne is able to cause similar xeno-estrogenic effects as known endocrine disrupters such as Bisphenol A and Ethinylestradiol. The test results, however, cannot be used to identify cybutryne as an endocrine disrupter due to the fact that the molecular mode of action in snails is unknown. Further research would be needed to clarify this issue.

CHAPTER II

Material supporting the potential of the toxic components in the anti-fouling system, or its transformation products, to occur in the environment at concentrations which could result in adverse effects to non-target organisms, human health, or water quality

Cybutryne is considered not readily biodegradable based on a ready biodegradability study following the OECD 301 guidance (301B CO_2 Evolution).

Higher tier tests on the degradation of cybutryne in more realistic test systems (both for marine and freshwater) were conducted both under laboratory and field conditions. The findings from those tests are considered to give a comprehensive insight in the degradation behaviour of the substance and its transformation products in the aquatic environment.

A study on the aerobic aquatic metabolism of cybutryne in a marine and a freshwater system is available (*Schmidt and Head (1991)*). In this test, cybutryne was applied at an initial test substance concentration of 1.6 mg /L in water. Total recoveries of radioactivity ranged from 93.3 to 104.7% of initial measured dose. In the marine system, the test substance concentration decreased from 96.2% at day 0 to 85.6% at day 30. In the freshwater system the test substance concentration decreased from 99.0% at day 0 to 76.2% at day 30. However, the incubation period was too short to calculate a reliable degradation rate. In the marine system, 19% of the applied cybutryne was found in the water and 78% in the sediment by day 30. In the fresh water system, the equivalent ratios were 45% in water and 48% in the sediment at the end of the test period. In both test systems the metabolite N2-tert-butyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine (GS 26575) occurred at an amount <1% of initial measured dose, whilst non-extractable residues were at <7% of initial measured dose at the final sampling date. It should be mentioned that in other tests major metabolites of > 10% were observed.

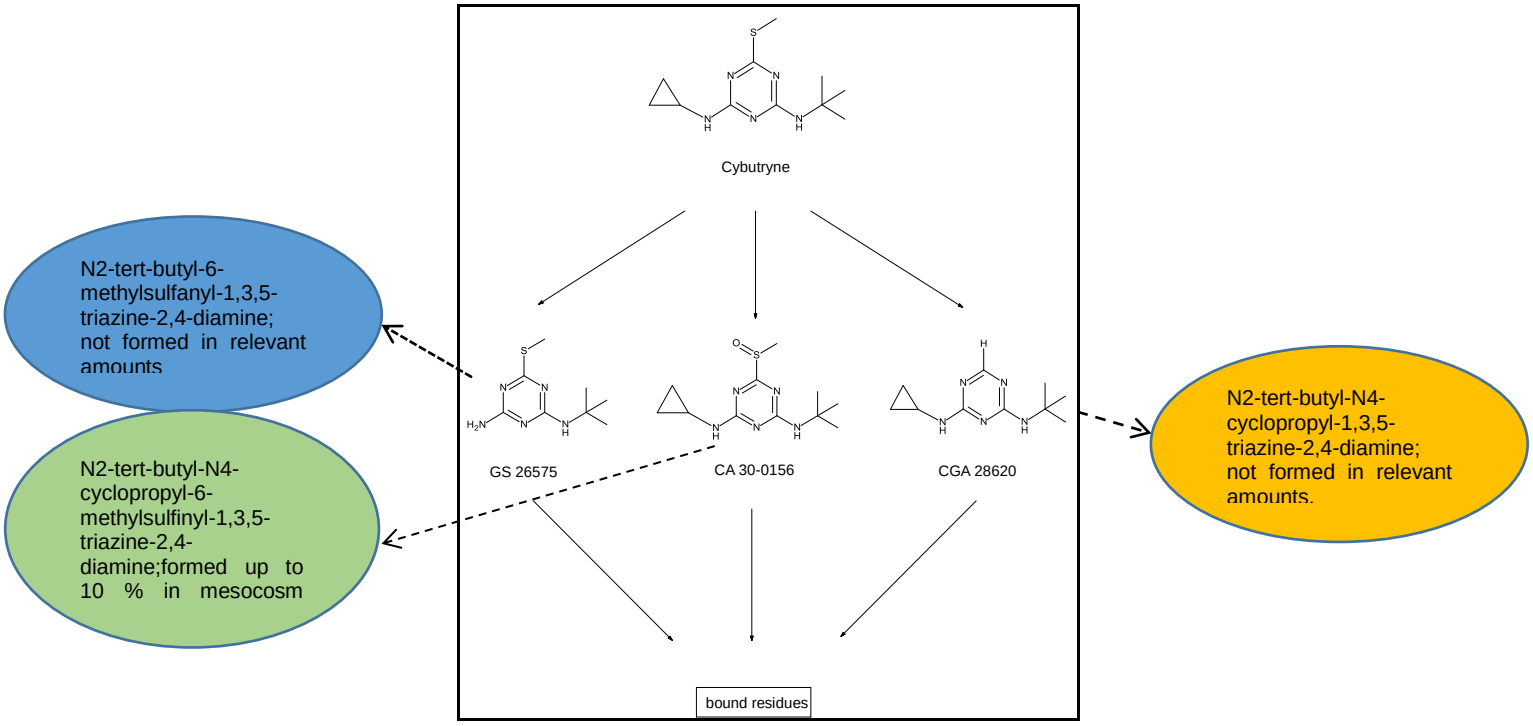

Figure 1: Presenting the proposed degradation pathway of cybutryne in water/sediment and mesocosm systems.

No significant degradation of cybutryne was found under anaerobic aquatic conditions during a 1-year period in the dark.

The dissipation of cybutryne from seawater and its potential distribution into sediment was measured in an outdoor microcosm under static conditions by *Hoberg et al. (1999a).* Cybutryne was applied at a nominal concentration of 560 ng/L. The test systems were exposed under natural climatic conditions for 22 weeks. The results showed that cybutryne dissipated from the microcosm water under the actual test conditions with a DT50 of approximately 22.5 days. The metabolite GS 26575 was the only metabolite found in the test system peaking at a maximum concentration of 150 ng/L after one month, then declining with approximately the same half-life as cybutryne (22.7 days). The maximum amount formed of metabolite GS 26575 was 26% in water and therefore this metabolite is considered to be a major and relevant metabolite in seawater-sediment systems.

Neither cybutryne nor GS 26575 was found in the sediment at concentrations exceeding the analytical detection limit of 260 ng/kg (cybutryne). The data obtained in this study, do not allow a conclusive estimate regarding the degradation rate of cybutryne in water/sediment systems. The application of unlabelled test substance invalidates closure of the mass balance, whereas the very low test concentration caused sediment concentrations for cybutryne and GS 26575 below the detection limit.

In an additional higher tier study by *Schmidt et al. (2005)* using a freshwater outdoor mesocosm under static conditions, cybutryne was applied in a single dosage. The test systems were exposed under natural climatic conditions for 315 days. Cybutryne dissipated from the microcosm water with a half-life of approximately 35 days. GS 26575 was the only metabolite found in the test system. The half-life of cybutryne for the total system was calculated to be 118 days, however this DT50 value should be considered as a best-case scenario since the concentration of cybutryne in biofilms, macrophytes and bound residues was not measured. Compared to the parent, the primary metabolite GS 26575 has to be considered more persistent in this study. The results of this study demonstrated that cybutryne and the major metabolite GS 26575 are persistent to biodegradation in both water and sediment systems. In addition, cybutryne has a high potential for accumulation in sediments. No DT50 values for GS 26575 in the water phase and cybutryne in the sediment could be calculated since the dataset was very limited.

In relation to abiotic degradation, a study by *Okamura et al. (1999)* is available in which hydrolysis was investigated using sea water, river water and buffered solutions (pH 5, 7, 9) according to ASTM guideline. No degradation was observed in any test solution even after one week at 50°C in the dark. Hence, cybutryne is considered hydrolytically stable.

When assessing the potential for photodegradation, continuous exposure to light for 15 days (equivalent to 30 days natural exposure) resulted in 7.8% degradation in sterile artificial seawater which was measured by HPLC-UV analysis and corrected for variation in dark controls. The photolysis of the test substance in sterile buffer was very slow resulting in 4.1% degradation under continuous exposure to light for 15 days.

The potential for adsorption and desorption was studied in four soils and two sediments according to the guideline OECD 106. The arithmetic mean Koc value of 895 L/kg was obtained from the results from five different soils, classifying cybutryne as having a low mobility potential in soil.

The potential accumulation of radiolabelled cybutryne in marine sediment was evaluated in an outdoor microcosm simulating shallow marine ecosystems. The nominal target cybutryne concentration was 200 ng/L. Three times per week, approximately 30% of the test water was

replaced with fresh unfiltered seawater, and fresh stock solution of the test substance was simultaneously added to keep the concentration close to nominal. The test systems were exposed under natural climatic conditions for 10 weeks. Water and sediment were sampled at regular intervals, and the total 14C-residue concentrations were measured. Concentration factors at each sampling interval were calculated relative to the mean 14C-concentration in water at the same interval. No time-dependent increase was observed and the concentrations were statistically similar indicating no unacceptable accumulation of residues with time for this compartment.

Due to the fact that sorption data (Koc) has only been studied at concentrations which are not fully relevant in the marine environment, severe underestimation of the risk to sediment dwelling organisms from exposure via suspended matter may have been considered. Therefore further studies on sorption at environmentally relevant conditions would be useful in order to fully understand the fate and behaviour of cybutryne and its main metabolites in the marine environment.

The uptake and elimination of radiolabelled cybutryne residues in the marine sheepshead minnow (*Cyprinodon variegatus*) was investigated under flow-through conditions. The mean steady-state bioconcentration factor (BCF) in whole fish was approximately 250 L/kg wet weight for sheepshead minnow. After transfer to uncontaminated medium, 50% of the total 14C-residues (parent compound including possible metabolites) present in fish on the last day of exposure was depurated within 3 days. According to these findings, cybutryne is considered to have a moderate potential for accumulation in fish, and residues are rapidly depurated if fish are transferred to fresh, uncontaminated medium.

Bioconcentration of [14C]-Cybutryne residues in sea lettuce, *Ulva lactuca*, a marine macro algae, was investigated under flow-through conditions. The mean steady-state tissue concentration for 14C-residues in the algal plant was 0.038 µg/kgwet weight, establishing a BCF of 5200 L/kgwet weight. After transfer to uncontaminated medium, approximately 76% of the 14C-residues (parent compound including possible metabolites) present in the macro algae on the last day of exposure were depurated within 21 days, resulting in a depuration half-life of 9.2 days. According to these findings, cybutryne is considered to accumulate in sea lettuce but residues are rapidly depurated if the macro algae are transferred to fresh, untreated medium.

Potential bioconcentration of radiolabelled cybutryne in communities was evaluated in outdoor microcosms simulating shallow marine ecosystems. The nominal target cybutryne concentration was 200 ng a.s./L. Three times per week, approximately 30% of the test water was replaced with fresh unfiltered seawater, and fresh stock solution of the test substance was simultaneously added to keep the concentration close to nominal. The test systems were exposed under natural climatic conditions for 10 weeks. Water as well as phytoplankton, periphyton, macrophytes and macro invertebrates were sampled at regular intervals, and the total 14C-residue concentrations were measured. Bioconcentration factors at each sampling interval were calculated relative to the mean 14C-concentration in water at the same interval. The geomean concentration of 14C-residues in water was 168 ng/L. The BCF values determined in biota were highly variable showing no trend for accumulation in particular groups of organisms and no increase in accumulation with time except for the marsh grass *Spartina alterniflora* (with a highest observed BCF of 177 L/kg wet weight at 70 days). Bioconcentration did also not reach steady state in phytoplankton but observations indicate that a high BCFSS value (BCFSteady State) can be expected from the data (reached values up to 1878 L/kg wet weight within the test period), although these values were probably biased due to technical problems (low biomass). The average BCFSS value for periphyton of 231 L/kg wet weight was clearly lower in comparison with eelgrass (*Zostera marina*) which had an average BCFSS value of 505 L/kg wet weight. For sea lettuce only one BCF could be derived in one replicate

at day 70 indicating a BCF of 1651 L/kg wet weight. The latter value is considerably lower than the BCF of 5200 L/kg wet weight derived in a laboratory study.

It should be noticed that the BCFs for phytoplankton, marsh grass and sea lettuce are only indicative due to technical problems with sampling, the time dependent increase in BCF and only one data point, respectively.

In macro invertebrate species highest BCFSS values were 110 L/kg wet weight for oyster (*Crassostrea virginica*; suspension feeder) and 307 L/kg wet weight for amphipods (*Leptocheirus plumulosus*; surface deposit feeder). The latter values can be taken as an indication that food-chain transfer resulting in biomagnification is not an apparent concern, since the BCFs in algae and plants were below the 2000 L/kg wet weight trigger (482-1397) and higher than the BCFs in the herbivorous organisms.

Information on the bioaccumulation of major metabolite GS 26575 is lacking, but on basis of in-silico estimation the logKow is 2.73 indicating that there is potential for bioaccumulation.

Conclusion

Cybutryne is not readily biodegradable, and also the abiotic degradation of cybutryne in seawater is very slow. The dissipation from the seawater compartment in a microcosm study however was found to be relatively fast (DT50: 23 days). The major degradation product of cybutryne in the seawater compartment is GS 26575, which has the same dissipation DT50 as its parent compound. The dissipation DT50 for cybutryne in freshwater was calculated to be approximately 35 days. No reliable degradation rates are at present available for the sediment compartment or the whole water/sediment system.

Cybutryne has a moderate potential to accumulate in fish (BCF 250 L/kg), with an elimination half life of less than 3 days. The accumulative potential in macro algae was relatively high (BCF 5,200 L/kg) with an elimination half-life of cybutryne of 9.2 days. The bioconcentration in a microcosm test demonstrated that cybutryne does not bioconcentrate in periphyton, rooted plants and macro invertebrates, and that biomagnification does not play a significant role on the parent compound.

CHAPTER III

An analysis of the association between the anti-fouling system, the related adverse effects and the environmental concentrations observed or anticipated

Cybutryne is a booster biocide used as an additive in antifouling paints for protection against "soft fouling", e.g. due to algae. It is used in conjunction with copper, which controls "hard fouling", e.g. by barnacles. The biocidal product that was supported by industry during the EU BPR process was AlphaGen 20 Series from Sigma Coatings. Cybutryne is estimated to have around 20% share of the market for commercial vessels (*d'Arcy (2005)*).

According to the notifier, the formulated product AlphaGen 20 Series is manufactured in the EU at a single facility, at which extensive measures are taken to prevent release of the active substance into the environment.

In addition, according to the notifier, the application and removal of formulated paint on commercial vessels in Europe was normally performed by professional users, and extensive measures were also taken at this stage of the lifecycle to reduce or eliminate environmental releases.

The expected routes of environmental exposure are therefore limited to releases into marine waters. The main route of entry will be from leaching out of paint during the service life of commercial vessels. A second potential route of entry would be from application and removal of antifouling paint.

Konstantinou and Albanis (2004)[1] have made a review of environmental levels of cybutryne in marine waters and freshwaters as well as sediments in a number of countries, as reported in the literature (European countries included in the review were: the United Kingdom, France, Spain, Greece, the Netherlands, Switzerland, Germany, Portugal and Sweden). In the UK, cybutryne has been found in surveys including marinas, ports, coastal waters and estuaries and it was detected in both water column and sediment. Similar concentrations were reported in the Mediterranean Sea in Spain (Catalonia and southeast coast), France (Cote d'Azur, Riviera) and Greece (main ports and marinas such as Piraeus, Thessaloniki, Patras and Igoumenitsa). Other areas of Europe where the occurrence of cybutryne has been reported include West Coast of Sweden, Stockholm Archipelago, Sweden; Sas van Gent and Schaar van Ouden, Netherlands; Baltic and North Sea, Germany; Western Sheldt, Netherlands. The report additionally indicates that fishery harbours can also be a significant source of contamination. Fishing boats require heavy applications of antifouling paint to prevent the growth of fouling organisms, due to the faster wear off of the antifouling chemicals through their heavy use. The complete overview of this study is included below in annex IV and contains areas exposed to pleasure crafts and commercial vessels.

Cybutryne has been measured in screening studies in the Swedish environment by *Woldegiorgis et al. (2007)* and *Kaj et al. (2010).* Cybutryne has also been studied in the Swedish marina Bullandö; in this study it was shown that water concentrations of cybutryne were higher during the summer (Swedish Chemical Agency, 2006).

In 2008, the Swedish Chemical Agency did measurements in sediments from 16 stations in the outer sea (Baltic and North Sea). These accumulating sediments are representative of the different sub-basins and probably represent use on commercial vessels, but some part

[1] Konstantinou I.K. and Albanis T.A. (2004) Review article, Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environmental International* 30: 235-248

probably comes from other use like pleasure crafts and sewage treatment plants. Sediment concentrations ranged between <0.061 ng/g up to 2.2 ng/g which all exceeds the predicted no effect concentrations (PNEC) used in the EU BPR risk assessment.

Three samples with exceptionally high concentrations were found in several stations in the Swedish waters; station SE-5 NE Gotska Sandön, SE-6 The Fårö deep (Fårödjupet) and SE-8 The Landsort deep (Landsortsdjupet). NE Gotska Sandön and Fårö deep are stations in the middle of the Baltic. The Landsort deep is somewhat closer to the coast but still is an off shore sediment. There are a few commercial harbours on the Swedish coast but also lots of marinas for pleasure crafts as well as natural harbours and pleasure craft areas. It is very difficult to link these stations and the concentrations of cybutryne found on them, with the type of boats and use of cybutryne (pleasure or commercial ships). However, as the monitoring stations are off shore stations, it can be argued that they would be more closely linked to commercial vessel traffic as people with pleasure crafts tend to spend most of their time close to the coast. At least the Landsort deep and the Fårö deep are probably close to shipping lanes.

The German Federal Environment Agency (UBA Umweltbundesamt, 2010) conducted a screening and monitoring study on cybutryne, and its metabolite GS 26575. During the screening, only single or few samples were taken at each location, mainly during the summer season. In the monitoring study on the other hand, samples were taken over the year. Data from the freshwater and marine datasets are given in the table below.

Cybutryne concentrations in the whole dataset, including also 4 marine locations and 4 industrially influenced sites, were between 2 ng/L and 25630 ng/L with a mean of 168 ng/L (n=218). Concentrations of the metabolite GS 26575 were in the reduced dataset, i.e. freshwater locations and non-industrial influenced locations, between 2 ng/L and 604 ng/L with a mean of 22 ng/L (n=210). In the monitoring data, where measurements were made over the year, GS 26575 mean concentrations were similar to cybutryne concentrations. The mean concentration of GS 26575 in the whole dataset, including marine sites and industrially influenced sites was lower than cybutryne concentration (3.2 ng/l compared to 168 ng/l, n=218). The report nevertheless did not discuss whether the concentrations found could be due to commercial or recreational ships.

There is also one available study on cybutryne in surface waters in the south coast of UK by *Cresswell et al., (2006)*[2]*.* The study showed how the national banning of cybutryne in UK in 2001 caused the concentrations of cybutryne in sea water to decrease in four years but still, at the time of the study, levels above the PNEC were measured. It is unclear whether the cybutryne remaining after years of banning was due to any ongoing use, to continued presence on pre-painted hulls, to continued use on non-UK vessels entering UK ports or simply due to the high persistence of the chemical in the environment. Most probably it is due to a combination of all these factors.

At present in many European member states, cybutryne is used on freshwater and marine commercial vessels and pleasure crafts, which may explain the high concentrations observed in the monitoring data. Known member states that revoked all cybutryne uses are UK (2001), Sweden (2004) and the Netherlands (2014). In Sweden, however, cybutryne is still on the market for other product types (37 products in 2012).

Monitoring data cannot exactly distinguish between different types of uses, but it is most likely that the measured concentrations are caused by the use in antifouling paints and not by other uses. At present it is not expected that the other biocidal uses of cybutryne will lead to high

[2] Cresswell T.,Richards JP, Glegg GA., Readman JW. (2006) The impact of legislation on the usage and environmental concentrations of Igrarol 1051 in UK coastal waters, Mar. Pollution Bulletin, Oct, 52(10) 1169-75

exposure to the marine environment and therefore shall not be considered responsible for the concentrations measured in marine waters. Especially because these monitoring data are measured at several marine locations also outside harbours and marinas, showing that the use on commercial vessels may have an important role. Therefore it cannot be guaranteed that the concentrations in marine waters will be of acceptable levels, even if the use of cybutryne is restricted to commercial marine vessels only.

When compared with other antifouling active ingredients, cybutryne has shown changes in species populations, significant adverse environmental impacts, under environmental conditions related to cybutryne use.

During the evaluation of cybutryne under the EU BPR the monitoring data was used to highlight that the calculated exposures through the common models and tools were reasonable or even underestimating the exposure to cybutryne.

Monitoring data indeed cannot exactly distinguish between different types of uses, but it is most likely that the measured concentrations are caused by the use in antifoulings and not by other uses. Especially because these monitoring data are measured at several marine locations, like surface waters in the south coast of UK, and estuaries, beaches and background locations in Sweden, it is shown that the use on commercial vessels may play an important role and it cannot be guaranteed that the PEC in marine waters will be of acceptable levels if the use of cybutryne is restricted to commercial marine vessels only.

Conclusion

The conclusions after the EU BPR process in relation to the environment were that exposure of cybutryne arising from the life-cycle of the product (application/removal phase losses and in-service losses) associated with commercial coastal and ocean-going vessels causes an unacceptable risk to marine water and sediment organisms. This is even the case when appropriate risk mitigation measures are taken into account during application, i.e. painting of the vessel, in a commercial harbour.

A further concern, however, originates from information derived from monitoring studies. Monitoring data from the United Kingdom, France, Spain, Greece, the Netherlands, Switzerland, Germany, Portugal and Sweden shows that cybutryne was detected in marine and fresh waters (coastal and/or transitional) at concentrations up to 1700 ng/L and in sediments at concentrations up to 42 ng/g (median 0.46 ng/g dry weight). These concentrations exceed the PNEC for the marine and freshwater environments indicating that adverse effects can be expected.

CHAPTER IV

References

Table 1: References for cybutryne for section characterization of the information which suggests that the anti-fouling system or its transformation products may pose a risk to human health or may cause adverse effects in non-target organisms at concentrations likely to be found in the environment

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source	Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
OECD 203	Rainbow trout, *Oncorhynchus mykiss*	*Freshwater*	Mortality / acute	Rufli, 1985	*EU CAR*	FIFRA 72-4	*Eisena bicyclis*	*Marine species*	Growth	Okamura et al. (2000b)	*WFD*
OECD 203	*Oncorhynchus mykiss*	*Freshwater*	Mortality	Okamura et al.(2002)	*WFD*	FIFRA 72-4	*Eisena bicyclis*	*Marine species*	Cell division	Okamura et al. (2000b)	*WFD*
FIFRA 72-3	Inland silverside, *Menidia beryllina*	*Marine*	Mortality / acute	Chandler, 1989	*EU CAR*	FIFRA 72-4	*Eisena bicyclis*	*Marine species*	Growth	Okamura et al. (2000b)	*WFD*
FIFRA 72-3	*Fundulus heteroclitus*	*Marine*	Mortality	Key et al. (2009)	*WFD*	FIFRA 72-4	*Eisena bicyclis*	*Marine species*	Growth	Okamura et al. (2000b)	*WFD*
FIFRA 72-4	Rainbow trout, *Oncorhynchus mykiss*	*Freshwater*	Growth / ELS	Cohle & Veltri, 1994 A7.4.3.2/02	*WFD*	FIFRA 72-4	*Emiliana huxleyi*	*Marine species*	Growth rate, PSII efficiency	Buma et al. (2009)	*WFD*
FIFRA 72-4	Sheepshead minnow, *Cyprinodon variegatus*	*Marine*	Growth / ELS	Sousa, 2001	*EU CAR*	FIFRA 72-4	*Emiliana huxleyi*	*Marine species*	Growth	Devilla et al. (2005)	*WFD*
FIFRA 72-4	Crustacea *Daphnia magna*	*Freshwater*	Mortality / acute	Okamura et al., 2000b	*WFD*	FIFRA 72-4	*Enteromorpha intestinalis*	*Marine species*	Growth	Scarlett et al. (1997)	*WFD*
FIFRA 72-4	Crustacea *Daphnia magna*	*Freshwater*	Mortality	Vial (1990)	*WFD*	FIFRA 72-4	*Enteromorpha intestinalis*	*Marine species*	Photosynthesis	Scarlett et al. (1997)	*WFD*
FIFRA 72-4	Crustacea *Daphnia pulex*	*Freshwater*	Mortality / acute	Okamura et al., 2000b	*WFD*	FIFRA 72-4	*Fibrocapsa japonica*	*Marine species*	Growth rate, PSII efficiency	Buma et al. (2009)	*WFD*
FIFRA 72-4	Crustacea *Daphnia magna*	*Freshwater*	Immobilisation	Fernandez-Alba et al. (2002)	*WFD*	FIFRA 72-4	*Fucus serratus*	*Marine species*	zygote germination (area)	Braithwaite and Fletcher (2005)	*WFD*

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
FIFRA 72-4	Crustacea *Thamnocepharus platyurus*	*Freshwater*	Mortality / acute	Okamura et al. (2000b)	*WFD*
FIFRA 72-3	Mysid shrimp, *Mysidopsis bahia*	*Marine*	Mortality / acute	Hoberg, 1986	*EU CAR*
FIFRA 72-3	Eastern oyster	*Marine*	Larval development / acute	Surprenant, 1986 *(not evaluated)*	*WFD*
FIFRA 72-3	Ascidia, *Ciona intestinalis*	*Marine*	Embryogenesis	Bellas (2006)	*WFD*
FIFRA 72-3	Cnidaria, *Acropora formosa*	*Marine*	Photosynthesis of symbiotic dinoflagellates	Jones and Kerswell (2003)	*WFD*
FIFRA 72-3	Cnidaria *Seriatophora hystrix*	*Marine*	Photosynthesis of symbiotic dinoflagellates	Jones and Kerswell (2003)	*WFD*
FIFRA 72-3	Crustacea *Nitocra spinipes*	*Marine*	Mortality	Karlsson et al. (2006)	*WFD*
FIFRA 72-3	Crustacea, *Palaemonetes pugio*	*Marine*	Larval mortality	Key et al. (2008)	*WFD*
FIFRA 72-3	Crustacea *Palaemonetes pugio*	*Marine*	Adult mortality	Key et al. (2008)	*WFD*
FIFRA 72-3	Crustacea *Balanus albicostatus*	*Marine*	Mortality	Khandeparker et al. (2005)	*WFD*
FIFRA 72-3	Crustacea *Artemia salina*	*Marine*	Mortality	Bakoulia et al. (2002)	*WFD*
FIFRA 72-3	Echinodermata, *Paracentrotus lividus*	*Marine*	Embryogenesis	Bellas (2006)	*WFD*
FIFRA 72-3	Echinodermata, *Paracentrotus lividus*	*Marine*	Growth	Bellas (2006)	*WFD*
FIFRA 72-3	Mollusca, *Mytilus edulis*	*Marine*	Embryogenesis	Bellas (2006)	*WFD*
FIFRA 72-4	*Fucus vesiculosus*	*Marine species*	fertilization	Andersson (1995)	*WFD*
FIFRA 72-4	*Hormosira banksii*	*Marine species*	photosynthesis	Seery et al. (2006)	*WFD*
FIFRA 72-4	*Navicula forcipata*	*Marine species*	Growth	Gatidou and Thomaidis (2007)	*WFD*
FIFRA 72-4	*Porphyra yezoensis*	*Marine species*	growth	Okamura et al. (2000b)	*WFD*
FIFRA 72-4	*Porphyra yezoensis*	*Marine species*	lethality	Okamura et al. (2000b)	*WFD*
FIFRA 72-4	*Porphyra yezoensis*	*Marine species*	germination	Okamura et al. (2000b)	*WFD*
FIFRA 72-4	*Skeletonema costatum*	*Marine species*	growth	Zhang et al. (2008)	*WFD*
FIFRA 123-2	*Skeletonema costatum*	*Marine species*	Growth inhibition	Hughes & Alexander, 1993a	*EU CAR*
FIFRA 123-2	*Tetraselmis sp.*	*Marine species*	Growth rate, PSII efficiency	Buma et al. (2009)	*WFD*
FIFRA 123-2	*Thalassiosira pseudonana*	*Marine species*	Growth	Zhang et al. (2008)	*WFD*
FIFRA 123-2	*Thalassiosira weissflogii*	*Marine species*	Growth rate, PSII efficiency	Buma et al. (2009)	*WFD*
FIFRA 123-2	cyanobacteria *Synechococcus sp.*	*Marine species*	Growth	Devilla et al. (2005)	*WFD*
FIFRA 123-2	Inflated duckweed, *Lemna gibba*	*Freshwater*	Growth inhibition	Hughes & Alexander, 1993e	*EU CAR*
FIFRA 123-2	*Lemna gibba*	*Freshwater*	Growth	Okamura et al. (2000)	*WFD*

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
FIFRA 72-3	Mollusca, *Ilyanassa obsoleta*	*Marine*	Adult mortality	Finnegan et al. (2009)	*WFD*
FIFRA 72-3	Mollusca, *Ilyanassa obsoleta*	*Marine*	Larval mortality	Finnegan et al. (2009)	*WFD*
FIFRA 72-4	*D. magna*	*Freshwater*	Survival, growth, reproduction	Putt, 1999a	*EU CAR*
FIFRA 72-4	*Lymnaea peregra*	*Freshwater*	Mortality	Morley et al., 2004	*EU CAR*
FIFRA 72-4	*Physa fontinalis*	*Freshwater*	Mortality	Morley et al., 2004	*EU CAR*
OECD 2010	*Lymnaea stagnalis* *	*Freshwater*	Reproduction	Habekost, 2010	*EU CAR*
OECD 2010	*Potamopyrgus antipodarum* *	*Freshwater*	Reproduction	Oehlmann & Ziebart, 2011	*EU CAR*
FIFRA 72-4	Mysid shrimp, *Mysidopsis bahia*	*Marine*	Growth / reproduction	Boeri & Ward, 1991	*EU CAR*
FIFRA 72-4	mollusc *Ilyanassa obsoleta*	*Marine*	Mortality	Finnegan et al. (2009)	*WFD*
FIFRA 72-4	*Chlamydomonas intermediata*	*Freshwater*	Growth	Berard et al.(2003)	*WFD*
FIFRA 72-4	freshwater algae *Chlorella vulgaris*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Chlorella vulgaris*	*Freshwater*	Growth	Nyström et al. (2002)	*WFD*
FIFRA 123-2	*Lemna minor*	*Freshwater*	Growth	Okamura et al. (2000)	*WFD*
FIFRA 123-2	*Potamogeton pectinatus*	*Marine*	Dry weight	Hall et al. (1999a)	*WFD*
FIFRA 123-2	*Ruppia maritima*	*Marine*	Growth	Hall et al. (1999a)	*WFD*
FIFRA 123-2	*Zostera marina*	*Marine*	Photosynthetic efficiency	Chesworth et al., 2004	*EU CAR*
FIFRA 123-2	*Zostera marina*	*Marine*	Photosynthesis and growth	Scarlett et al. (1999)	*WFD*
OPPts 850.1735	Marine amphipod, *Ampelisca abdita*	*Marine*	Mortality / acute	Putt, 1999b	*EU CAR*
OPPts 850.1735	Brackish-freshwater amphipod,*Monoporeia affinis*	*Freshwater*	Avoidance response (reduced burial in sediment)	Eriksson Wiklund et al. (2009)	*WFD*
OECD 219	Midge, *Chironomus riparius*	*Freshwater*	Development, Emergence / chronic sediment-water test	Luit, 2000	*EU CAR*
OECD 219	Midge, *Chironomus riparius*	*Freshwater*	Development / emergence	Desmares-Koopmans (1997)* evaluated by KEMI (1998)	*WFD*
Marine microcosms	Phytoplankton	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Pigments (chlorophyll)	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Pigments (phaeophytin)	*Marine*		Hoberg, 2004	*EU CAR*

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
FIFRA 72-4	*Closterium ehrenbergii*	*Freshwater*	Growth	Okamura et al. (2000b)	*WFD*
FIFRA 72-4	*Closterium ehrenbergii*	*Freshwater*	Embryogenesis	Okamura et al. (2000b)	*WFD*
FIFRA 123-2	*Navicula pelliculosa*	*Freshwater*	Growth inhibition	Hughes & Alexander, 1993b	*EU CAR*
FIFRA 72-4	*Navicula accomoda*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Navicula accomoda*	*Freshwater*	Growth	Nyström et al. (2002)	*WFD*
FIFRA 72-4	*Nitszchia sp.*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Nitszchia sp.*	*Freshwater*	Growth	Nyström et al. (2002)	*WFD*
FIFRA 72-4	*Pseudokirchneriella subcapitata*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Scenedesmus acutus*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Scenedesmus vacuolatus*	*Freshwater*	Reproduction	Arrhenius et al. (2006)	*WFD*
FIFRA 72-4	*Scenedesmus vacuolatus*	*Freshwater*	Reproduction	Neuwoehner et al. (2008)	*WFD*
FIFRA 72-4	*Scenedesmus vacuolatus*	*Freshwater*	Photosynthesis	Neuwoehner et al. (2008)	*WFD*
FIFRA 72-4	freshwater algae *Selenastrum capricornutum*	*Freshwater*	Growth	Fernandez-Alba et al. (2002)	*WFD*
Marine microcosms	Photosynthesis/respiration	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Taxonomic abundance	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Periphyton	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Pigments (chlorophyll)	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Pigments (phaeophytin)	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Photosynthesis/respiration	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Taxonomic abundance	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Zooplankton	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Eelgrass, *Zostera marina*	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Marsh grass, *Spartina alterniflora*	*Marine*		Hoberg, 2004	*EU CAR*
Marine microcosms	Macro invertebrates	*Marine*		Hoberg, 2004	*EU CAR*
Pilot study. Only one exposure concentration and two replicates	Plankton, macrophytes and macro-invertebrates	*Marine*		Giddings (2002)	*WFD*
Marine, photosynthesis; 21 days	Periphyton	*Marine*		Dahl and Blanck (1996)	*WFD*

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
FIFRA 72-4	*Selenastrum capricornutum*	*Freshwater*	Growth	Okamura et al. (2003)	*WFD*
FIFRA 72-4	*Selenastrum capricornutum*	*Freshwater*	Cell number-area	Okamura et al. (2000a)	*WFD*
FIFRA 72-4	*Selenastrum capricornutum*	*Freshwater*	Cell number-growth rate	Okamura et al. (2000a)	*WFD*
FIFRA 72-4	*Staurastrum sebaldii*	*Freshwater*	Growth	Berard et al. (2003)	*WFD*
FIFRA 72-4	*Ceramium tenuicorne*	*Marine species*	Growth	Karlsson et al. (2006)	*WFD*
FIFRA 72-4	*Chaetocerus gracilis*	*Marine species*	Growth	Koutsaftis et al. (2006)	*WFD*
FIFRA 72-4	*Dunaliella tertiolecta*	*Marine species*	Growth	DeLorenzo and Serano (2006)	*WFD*
FIFRA 72-4	*Dunaliella tertiolecta*	*Marine species*	Growth	Gatidou and Thomaidis (2007)	*WFD*
No specific protocol	*coral zooxanthellae*	*Marine species*	Acute toxicity	Owen, Knap, A., Ostrander, N., Carbery, K. (2003)	Bull. Environ. Contam. Toxicol. 70 : 541-548
Indoor freshwater mesocosm study	Periphyton	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	Chlorophytes (day 135)	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	Zooplankton (day 78)	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	Cyclopoid copepods	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	Macrophyte biomass (day 150)	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	*Myriophyllum verticillatum*	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	Filamentous algae	Freshwater		Schmidt et al., 2007	*EU CAR*
Indoor freshwater mesocosm study	*Potamogeton nodosus*	Freshwater		Schmidt et al., 2007	*EU CAR*
Phytoplankton; 24 d Bray-curtis index	*Phytoplankton*	Freshwater		Nyström et al. 2002	*WFD*

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source	Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
No specific protocol	*coral Madracis mirabilis*	*Marine species*	Short-term cellular toxicological responses	Downs, C., Downs, A. (2007)	Arch. Environ. Contam. Toxicol. 52 : 47-57						

Table 2: References for GS 26575 for section (b) characterization of the information which suggests that the anti-fouling system or its transformation products may pose a risk to human health or may cause adverse effects in non-target organisms at concentrations likely to be found in the environment

Guideline / test method	Species	Habitat	Endpoint / type of test	Reference	Source
Not specified	*Daphnia magna*	*Freshwater*	Mortality / acute	Okamura et al., 2000b	*EU CAR*
Not specified	*Daphnia pulex*	*Freshwater*	Mortality / acute	Okamura et al., 2000b	*EU CAR*
Not specified	*Crustacea Thamnocepharus platyurus*	*Freshwater*	Mortality / acute	Okamura et al. (2000b)	*EU CAR*
FIFRA 72-3	Mysid shrimp, *Mysidopsis bahia*	Marine/estuarine	Mortality / acute	Cafarella, 1999b	*EU CAR*
Not specified	Crustacea *Artemia salina*	Marine	Mortality / acute	Okamura et al. (2000b)	*EU CAR*
FIFRA 72-3	Sheepshead minnow, *Cyprinodon variegatus*	Freshwater	Mortality / acute	Cafarella, 1999a A7.4.1.1/03	EU CAR
FIFRA 123-2	Freshwater diatom, *Navicula pelliculosa*	*Freshwater*	Growth inhibition	Hoberg, 1999b	*EU CAR*
FIFRA 123-2	Marine diatom, *Skeletonema costatum*	Marine	Growth inhibition	Hoberg, 1998a	*EU CAR*

Table 3: References for fate and behaviour in section material supporting the potential of the toxic components in the anti-fouling system, or its transformation products, to occur in the environment at concentrations which could result in adverse effects to non-target organisms, human health, or water quality

Guideline/ test method	Test type	Test parameter	Reference	Source
OECD 301B	Ready Biodegradability	CO_2 - evolution	Baumann, 1987	*EU CAR*
US EPA, subdivision N, 162-3	Anaerobic 25°C darkness	Degradation DT50 in marine water	Schmidt, 1992a	*EU CAR*
US EPA, 162-1	Soil degradation (aerobic)	Degradation DT50 in soil	Schmidt, 1992b	*EU CAR*
US EPA, subdivision N, 161-1	Hydrolysis artificial seawater	Hydrolysis DT50	Head & Schmidt, 1991	*EU CAR*
US EPA, subdivision N, 161-2	Phototransformation in water	Photolyisis DT50	Doyle, 1991	*EU CAR*
US EPA, subdivision N, 163-1	Adsorption and desorption in soil	Koc	Williams & Hargadine, 1990	*EU CAR*
Tailored study design	Fate and effect studies	Koc, DT50	Schmidt et al. (2005)	*EU CAR*
Tailored study design	Degradation	Degradation DT50 in marine water	Hoberg et al. 1999a	*EU CAR*
ASTM guideline	Photodegradation in water		Okamura et al. 1999	*EU CAR*

Table 4: References for bioaccumulation in section (c) material supporting the potential of the toxic components in the anti-fouling system, or its transformation products, to occur in the environment at concentrations which could result in adverse effects to non-target organisms, human health, or water quality

Guideline / test method	Species	Exposure days / type	Reference	Source
OECD 305E	Sheepshead minnow, *Cyprinodon variegatus*	35 / flow-through	Dionne, 1991a	*EU CAR*
Based on OECD 305	Green macro algae, *Ulva lactuca*	25 / flow-through	Hoberg, 2001	*EU CAR*
Fate and bioaccumulation in marine microcosms	Phytoplankton, Periphyton, Eel grass Zostera marina, Marsh grass Spartina alterniflora, Sea lettuce Ulva lactuca, Oyster Crassostrea virginica,Amphipods Leptocheirus plumulosus	7 weeks	Sousa, 2005	*EU CAR*
Field, fate and effect studies – Outdoor fate study	Chara vulgaris, Lymnea stagnalis	315 days	Schmidt et al., 2005	*EU CAR*
Field, fate and effect studies – Indoor fate and effect study	Myriophylum.verticillatum, Chara vulgaris, P.nodosa, Filamentous algae, Lymnea stagnalis	150 days	Schmidt et al., 2005	*EU CAR*

Table 5: References for concentrations of cybutryne in water, sediment, WWTP effluent and biota samples for section (d) an analysis of the association between the anti-fouling system, the related adverse effects and the environmental concentrations observed or anticipated.

Compartment	Concentration	Reference
Freshwater	< 0.3 – 1.6 ng/L	Woldegiorgis *et al.* (2007)
	2.5-260 ng/L	Konstantinou and Albanis (2004)
	2 – 1725 ng/L (mean 41 ng/L n=210, samples from 86 locations)	UBA Umweltbundesamt (2010)
	3 – 30 ng/L (mean values from eight locations, samples taken over the year, n=8-12/location)	UBA Umweltbundesamt (2010)
	22 ng/L (maximum of the annual average by station) 58 ng/L (maximum of analyses)	UBA Umweltbundesamt (2010)
Marine waters (coastal and/or transitional)	< 1-36 ng/L (n=14 samples) in surface waters in the south coast UK	Cresswell *et al.* (2006)
	< 6 – 170 ng/L In the marina < 5 – 14 ng/L In the bay, outside the marina < 4 – 42 ng/L Natural harbour < LOQ (6 – 15 ng/L) Background location	KEMI (2006)
	< 1 – 1700 ng/L Marinas, ports, estuaries and beaches	Konstantinou and Albanis (2004)
	95-296 ng/L 4 marinas at the German Baltic Sea coast	UBA Umweltbundesamt (2010)
WWTP effluent	< 0.3 – 11 ng/L	Woldegiorgis *et al.* (2007)
Sediment	< 0.001 – 20 ng/g dw	Woldegiorgis *et al.* (2007)
	< 0.2 – 1010 ng/g dw	Konstantinou and Albanis (2004)
	Swedish lakes and rivers: 0.26 – 9.1 ng/g dw, median: 2.3 ng/g dw Above LOQ (0.050 ng/g dw) in 7/12 samples Swedish Baltic and west coasts-marinas, coastal areas and open sea background stations: 0.07-42 ng/g dw, median 0.46 ng/g dw Above LOQ (0.05-0.070 ng/g dw) in 56/87 samples	Kaj et al. (2010)
Biota	< 1 ng/g ww fish and mussels	Woldegiorgis *et al.* (2007)
	< 2 – 96 ng/g dw bladderwrack	KEMI (2006)