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From: Secretary-General of the European Commission,  
signed by Mr Jordi AYET PUIGARNAU, Director

date of receipt: 29 October 2018

To: Mr Jeppe TRANHOLM-MIKKELSEN, Secretary-General of the Council of  
the European Union

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Subject: COMMISSION STAFF WORKING DOCUMENT For the Council Shipping  
Working party IMO - Union submission to be submitted to the 6th session  
of the Sub-Committee on Pollution Prevention and Response (PPR 6) of  
the IMO in London from 18 – 22 February 2019 concerning information  
presenting scientific evidence for the adverse effects of cybutryne to the  
environment

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Delegations will find attached document SWD(2018) 457 final.

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Brussels, 29.10.2018  
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**COMMISSION STAFF WORKING DOCUMENT**

**For the Council Shipping Working party**

**IMO - Union submission to be submitted to the 6th session of the Sub-Committee on  
Pollution Prevention and Response (PPR 6) of the IMO in London from 18 – 22  
February 2019 concerning information presenting scientific evidence for the adverse  
effects of cybutryne to the environment**

**COMMISSION STAFF WORKING DOCUMENT**  
**For the Council Shipping Working party**

**IMO - Union submission to be submitted to the 6th session of the Sub-Committee on Pollution Prevention and Response (PPR 6) of the IMO in London from 18 – 22 February 2019 concerning information presenting scientific evidence for the adverse effects of cybutryne to the environment**

**Purpose**

The document in Annex contains a draft Union submission to the Sub-Committee on Pollution Prevention and Response, which will hold its sixth session from 18 – 22 February 2019 (PPR 6), concerning information presenting scientific evidence for the adverse effects of cybutryne to the environment, in relation to the comprehensive proposal to amend the Anti-Fouling Systems Convention (AFS 2001) to include in Annex 1 controls on cybutryne, as well as consequential revision of relevant guidelines. It is hereby submitted to the appropriate technical body of the Council with a view to achieving agreement on transmission of the document to the IMO prior to the required deadline of 16 November 2018<sup>1</sup>.

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products<sup>2</sup> establishes a harmonised system in the EU concerning the placing on the market and use of biocidal active substances and biocidal products. In particular, it aims at establishing at Union level a list of active substances which may be used in biocidal products. Pursuant to Article 9 of Regulation (EU) No 528/2012, decisions to approve or ban an active substance are adopted at EU level by the Commission. The non-approval Decision (EU) 2016/107<sup>3</sup> was adopted to ban cybutryne for use in antifouling paints, and antifouling paints containing cybutryne cannot be placed on the market as from 17 February 2017 nor used in the EU as from 17 August 2017. The said draft Union submission therefore falls under EU exclusive competence.

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<sup>1</sup> The submission of proposals or information papers to the IMO, on issues falling under external exclusive EU competence, are acts of external representation. Such submissions are to be made by an EU actor who can represent the Union externally under the Treaty, which for non-CFSP (Common Foreign and Security Policy) issues is the Commission or the EU Delegation in accordance with Article 17(1) TEU and Article 221 TFEU. IMO internal rules make such an arrangement absolutely possible as regards existing agenda and work programme items. This way of proceeding is in line with the General Arrangements for EU statements in multilateral organisations endorsed by COREPER on 24 October 2011.

<sup>2</sup> OJ L 167, 27.6.2012, p. 1.

<sup>3</sup> OJ L 21, 28.1.2016, p. 81.

## ANNEX

SUB-COMMITTEE ON POLLUTION  
PREVENTION & RESPONSE  
6<sup>th</sup> session  
Agenda Item 19

PPR 6/INF  
[Date]

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### **CONSIDERATION OF A COMPREHENSIVE PROPOSAL TO AMEND ANNEX 1 TO THE AFS CONVENTION TO INCLUDE CONTROLS ON CYBUTRYNE**

**Information presenting scientific evidence for the adverse effects of cybutryne to the  
environment**

**Submitted by the European Commission on behalf of the European Union**

#### **SUMMARY**

*Executive summary:* This document contains the elements for a comprehensive proposal to amend the Anti-Fouling Systems Convention (AFS 2001). The proposal refers the documented scientific evidence understood to include all the elements required for a comprehensive proposal as listed in Annex 3 of the AFS Convention.

*Strategic Direction, if applicable:* 2

*Output:* 2.19

*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), MEPC 71/14, PPR 5/19, PPR 5/INF.9, MEPC73/INF.10, PPR6/\*\*

#### **Introduction**

1 The annex to this document provides detailed scientific evidence required by article 6 of the AFS Convention by addressing all the elements for a comprehensive proposal contained in Annex 3 of the Convention.

#### **Action requested of the Sub-Committee**

2 The Sub-Committee is invited to consider the information provided in this document when considering document PPR 6/19\*.

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# 1. Required Elements for the Comprehensive proposal in order to include cybutryne in Annex I of the AFS Convention (PART I)

## 1.1. Developments in the data cited in the initial proposal

In Chapter IV of the Annex of document PPR5-INF.9, a number of references were included suggesting that cybutryne used as biocide in antifouling systems is posing a risk to non-target organisms at concentrations lower than those actually detected in the marine environment. The analysis and data supporting the negative effects of cybutryne were not challenged and therefore remain valid.

Furthermore, additional information supporting the initial proposal and highlighting the need to control cybutryne by adding cybutryne to Annex 1 of the AFS Convention has been included.

## 1.2. Findings from the categories of data set out in paragraphs 3(a), (b) and (c) of Annex 3 of the AFS Convention, as applicable, depending on the subject of the proposal and the identification or description of the methodologies under which the data were developed

The requirements for this section are covered in detail in section 3.

## 1.3. A summary of the results of studies conducted on the adverse effects of the anti-fouling system

The toxicity data of 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<sub>2</sub> 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) and a NOEC of 22 ng/L for the marine algae *Skeletonema costatum*.

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 main metabolite M1 (GS 26575) was less toxic towards fish and invertebrates (96 hour LC<sub>50</sub> 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 far much less susceptible: 120 hour NOEC was 77 µg/L.

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.

Cybutryne and its main metabolite M1 (GS 26575) have also been found to be persistent or very persistent in water and sediment therefore causing a long term effects to the marine ecosystem.

For humans, cybutryne is a skin sensitiser. In general, it is rather difficult to set a clear dose-response for skin sensitiser; safe use conditions and concentration are quite often difficult to foresee and regulate. To minimise the risk of a sensitisation reaction, risk mitigation measures which prevent the skin exposure, such as gloves and coverall, must be put in place. In addition, the human health risk assessment during the EU assessment showed that personal protective equipment (impermeable coverall and gloves) should be applied for professional users also to protect for potential systemic effects. In the case of spray application, a double coverall would be needed to ensure a safe use and it can questioned whether a worker would wear a double coverall in warm climate conditions.

Relevant residues are not expected in matrices for human consumption (drinking water or fish/seafood). Nonetheless, a reverse reference scenario was performed to calculate the amount of fresh fish eaten by a person every day of his life before filling up the ADI and it can be concluded that there is no appreciable risk to human health.

#### **1.4. If any monitoring has been conducted, a summary of the results of that monitoring, including information on ship traffic and a general description of the area monitored**

Extensive monitoring of cybutryne levels in water and sediments has been conducted in Europe, Asia, North America and the Caribbean. The body of scientific literature examining cybutryne concentrations in territorial waters shows that in most areas, the levels detected far exceed those considered safe for the environment.

Especially in South-East Asia, concentrations exceeding 1000 ng/L have repeatedly been found. In Malaysia in 2013, concentrations of up to 2021 ng/L were detected in open ports, with open waters still containing up to 624 ng/L of the substance ([Ali et al., 2013](#)). These are levels that have been shown to be highly harmful to marine organisms and that completely interrupt the photosynthesis processes of a large variety of algae, on which most marine organisms directly or indirectly depend on for food.

While concentrations in East Asia were not as high as in Singapore ([Basheer et al 2002](#)) and Malaysia, levels were nonetheless in a range that can be considered harmful for the marine environment. Concentrations above 200ng/L were repeatedly found in Japanese waters. In the Republic of Korea ([Lee et al., 2016](#)) elevated sediment concentrations were detected, confirming the tendency of cybutryne to persist in the environment and accumulate in sediment.



There are scarce data on Africa, with the only monitoring having been performed in the open seas surrounding the Zanzibar coral reefs of the United Republic of Tanzania. The levels were not elevated beyond concentrations considered harmful for the marine environment. However, the samples were taken in an area particularly sensitive to Photosynthesis inhibitors such as cybutryne, and show the even outside of the main seafaring lanes and harbours, the substance is still present in considerable amounts. Regarding the Arabian Sea and the Persian Gulf Region, the only monitoring data available are from the Iranian Port of Bushehr, where levels that endanger phytoplankton species were found. None of the samples taken exceeded 100ng/L.

In North America, levels have been shown to be high enough to be harmful to algae and other marine organisms on both the Eastern and the Western Coast of the United States. While not exceeding 1000 ng/L as in South East Asia, levels were regularly found to be above 100 ng/L, exceeding 300 ng at times.

Another region where cybutryne concentrations have repeatedly been shown to be very high is the Caribbean. In the waters surrounding the U.S. Virgin Islands, levels were found to exceed 800 ng/L, a concentration that is above the LD<sub>50</sub> of the majority of algae tested and can be assumed to negatively affect phytoplankton abundance.

Country and region	Type of port	Conc. Water (cybutryne, ng/L)	M1 Conc. Water (ng/L)	Conc. Sediment (ng/g dry)	Author, year	Journal
Singapore	Ports	2800-4000			<a href="#">Basheer et al, 2000</a>	Marine Pollution Bulletin
Singapore	open sea	below detection			<a href="#">Basheer et al, 2000</a>	Marine Pollution Bulletin
Iran	Bushehr port surroundings	0-63.4			<a href="#">Saleh et al, 2013</a>	Marine Pollution Bulletin
United Republic of Tanzania,	Zanzibar coral reefs	1.35-15.44			<a href="#">Sheikh et al, 2015</a>	Marine Pollution Bulletin
United States, Hawaii	Oahu marinas and harbours (Hawaii)	0-283			<a href="#">Knutson et al, 2011</a>	Ecotoxicology
United States, California	Ports and marinas	1.7-339	1.35-74.4		<a href="#">Hall Jr. et al, 2006</a>	Marine Pollution Bulletin
Malaysia	commercial cargo ports and fishing ports	6-2021			<a href="#">Ali et al, 2013</a>	Marine Pollution Bulletin
Malaysia	open sea	8-624			<a href="#">Ali et al, 2013</a>	Marine Pollution Bulletin
United States (California)	marinas and small ports	23-304			<a href="#">Shapoznikova et al, 2007</a>	Marine Pollution Bulletin
United States, Florida	Florida Keys	10.6-144.2			<a href="#">Owen et al, 2002</a>	Marine Pollution Bulletin
United States, Bermuda	Florida Keys	3.1-234.7			<a href="#">Owen et al, 2002</a>	Marine Pollution Bulletin

United States, Puerto Rico	Ports and marinas	1-32.7			<a href="#">Carbery et al, 2006</a>	Marine Pollution Bulletin
United States, US Virgin Islands	Ports	5-825			<a href="#">Carbery et al, 2006</a>	Marine Pollution Bulletin
Japan, Hiroshima	Fishery harbours and ports	10-148			<a href="#">Okamura et al, 2003</a>	Marine Pollution Bulletin
Japan, Yamaguchi	Fishery harbours and ports	14-157			<a href="#">Okamura et al, 2003</a>	Marine Pollution Bulletin
Japan, Hyogo and Wakayama	Fishery harbours	13-262			<a href="#">Okamura et al, 2003</a>	Marine Pollution Bulletin
ROK	Fishing ports, ports and shipyards			1-11.5	<a href="#">N.S. Kim et al., 2015</a>	Marine Pollution Bulletin

Table 1.4-1: List of monitoring results and publications from different regions world-wide.

Following this overview, below are described in more detail some of the research studies conducted in Asian and Oceanian waters, where the measured cybutryne concentrations dramatically exceeded the various limits set by different environmental protection agencies around the world.

These publications are publicly available and are among those included in the information document that was submitted during MEPC 73.

**Ali, Arifin, Sheikh et al, "Occurrence and distribution of antifouling biocide Irgarol-1051 in coastal waters of Peninsular Malaysia", *Marine Pollution Bulletin*, 2013**

In this study samples were collected from different areas in the Port of Klang in Malaysia. In figures 1.4-1 to 1.4-3 the port areas from where the samples have been collected are presented. In figure 1.4-1 the zones for commercial, cargo ships and boats are presented.

Similar in figure 1.4-2, the area where oil tankers and cargo ships are berthing is presented and in figure 1.4-3 the port area where container ships, passenger ships and small touristic ships are berthing is presented.

Figure 1.4-1: Sampling points in the port of Klang where the commercial and cargo area is located and the concentration of cybutryne in these points measured (ng/L) Source: (Hassan Rashid Ali, 2013).

Figure 1.4-2: Sampling points in the port of Klang where the oil tankers and cargo area is located and the concentration of cybutryne in these points measured (ng/L) Source: (Hassan Rashid Ali, 2013).

Figure 1.4-3: Sampling points in the port of Klang where the passenger jetty, the flour container area and the tourist and residential jetty are located and the concentration of cybutryne in these points measured (ng/L) Source: (Hassan Rashid Ali, 2013).

In figure 1.4-4 the concentrations of cybutryne for the port areas are presented for the months from November 2011 to April 2012 which is the period the sampling period took place. Furthermore, in order to correlate the number of ships in the port and the levels of

cybutryne measured the traffic of the vessel in the port areas are presented in figure 1.4-5. These traffic data are available from the official website of the port of Klang.

A higher number of ships was expected to trigger higher values of cybutryne to be measured. This correlation could be observed for the period from November 2011 to December 2011, during which the measured levels of cybutryne increased. However, for the period January to February 2012 the decrease in the number of ships that were calling the port did not affect the rising values of cybutryne that were measured during this period. The values of cybutryne detected were higher at the end of the test period in April 2012 with the number of ships calling in March 2012 increasing and decreasing again in April 2012. The number of ships calling the port could affect the concentration of cybutryne detected in the sample, however, it is not the only factor that is affecting the measurements since also other parameters like currents, dilution effects, water depth are factors that are affecting the measurements especially if the size of a port is large. This was one of the reasons that cybutryne and also other antifouling agents were easier to detect in smaller fishing harbours and harbours for smaller boats.

The paper included also sampling points from other areas on the Malaysian peninsular such as Johor, Kenaman, Redong Island, and the Bidong Island. The results from the collection points from these areas can be found in Annex 2.

Figure 1.4-4: Irgarol 1051 concentrations for different sampling points Source: (Hassan Rashid Ali, 2013).

Figure 1.4-5: Total number of ship calls during the sampling period.

**Seongeon Lee et al. "Determination of the concentrations of alternative antifouling agents on the Korean coast" ,*Marine Pollution Bulletin*", 2016**

In this paper the research team took samples from harbours located on the Korean Coast to find the contamination from antifouling agents for the period 2006 to 2013. The study was initiated for identifying the increase of the alternative antifouling agents that were used after the ban of TBT in 2008. Cybutryne was one of these. Samples were taken from 9 harbours and five of them were categorized as big harbours. The study correlated the total number of ships that were incoming and outgoing to the ports. In figure 6 the tonnage from the sampling ports from 2006-2013 are presented. The mean concentrations for cybutryne for the same period for these harbours was increased by 650% and on the same period an increase of 50% in the Gross tonnage was recorded for these ports which indicates the effect that ships had in the ports monitored from 2006 to 2013. Furthermore, an even more significant increase (around 996%) was recorded for fish harbours, likely to have been caused by an increased number of fishing vessels visiting the port of Sokcho.

Figure 1.4-6: Sampling positions in the ports that were categorized as big harbours and the gross tonnage increase from 2006-2013. (Source: Seongeon Lee et al. 2016)

**Basheer, Tan and Lee, "Organotin and Irgarol-1051 contamination in Singapore coastal waters", *Marine Pollution Bulletin*, 2002**

This study completed along and off the coast of Singapore in October and November 2000. Cybutryne was one of the antifoulings that was detected in high quantities in 13 out of 26 locations where samples were taken. Figure 1.4-7 presents these locations including also the detected quantities that were measured.

Figure 1.4-7: Sampling points in the coast of Singapore including the concentration of cybutryne

The study concluded that the concentrations of cybutryne are extremely high and that the area is suffering from increased levels of cybutryne.

**Kroon, F.J., Berry, K.L.E., Brinkman, D.L., Davis, A., King, O., Kookana, R., Lewis, S., Leusch, F., Makarynsky, O., Melvin, S., Müller, J., Neale, P., Negri, A., O'Brien, D., Puotinen, M., Smith, R., Tsang, J., van de Merwe, J., Warne, M., Williams, M. (2015). Identification, impacts, and prioritisation of emerging contaminants present in the GBR and Torres Strait marine environments. Report to the National Environmental Science Programme. Reef and Rainforest Research Centre Limited, Cairns (138pp.).**

From this report it was identified that antifouling paints containing booster biocides including cybutryne were detected in seagrass tissue in four out of five locations that were sampled along the Great Barrier Reef (GBR) coast in 1997 and the concentrations that were detected were considered to be potentially toxic. It has to be highlighted that these concentrations have been detected despite the fact that cybutryne is not registered for use as an antifouling substance in Australia.

The main sources of antifouling components in the GBR and TS (Torres Strait) marine ecosystems were reported to be mainly large commercial, military and recreational vessels, commercial fishing vessels and tourist boats. In the years 2012-2013 almost 11000 movements of large commercial vessels were monitored in the area and more than 87% of 4440 vessels berthing arrived at four larger ports: Gladstone, Hay Point, Cairns and Townsville. This number increased to 11417 movements comprising 2910 large commercial ships in 2013-2014. The projected increase in the ship traffic in the coming years combined with the projected increase in the vessel size will increase the likelihood of cybutryne release along the shipping lanes in the marine environments of the GBR and TS regions.

Furthermore, MEPC 68 approved the resolution MEPC 268(68) in which the areas of GBR and TS have been recognized as PSSA (Particularly Sensitive Sea Areas). In figure 1.4.8-3 the traffic density of the specific areas can be seen. This combined with the findings of increased levels of antifouling substances including cybutryne is in line with the conclusion that commercial shipping is a source of antifouling components to be found in the area.

Figure 1.4-8: Chart with the proposed PSSA including AIS data (Source: MEPC.268(68))

In the EU one of the countries that introduced restrictions for the use of cybutryne was the UK. In the following paper the impact of legislation on the reduction of cybutryne to the marine environment in the UK waters is highlighted.

**Thomas et al, "The impact of legislation on the usage and environmental concentrations of Irgarol 1051 in UK coastal waters" *Marine Pollution Bulletin*, 2006**

The study presented measurements of cybutryne concentrations in the Brighton Marina, Southampton waters and from Plymouth. In total, samples were collected from 17 locations along the south coast of the UK and were screened for cybutryne with the methodology described in the paper. The cybutryne levels that were recorded after the restrictions were between 10 to 55% lower compared to the levels detected in previous studies before the legislative measures were in place as is illustrated in figures 1.4-9 a) to c). The measures that were taken by the authorities in the UK have successfully reduced the environmental concentrations of cybutryne to levels below 24 ng/l which are below the threshold proposed by the Dutch National Institute of Public Health and the Environment during the EU assessment.

Figure 1.4-9: Cybutryne concentrations in (a) Plymouth, (b) Brighton Marina and (c) Southampton water as were recorded for the period from 1995 to 2005, Source: Cresswell Thomas et al. 2006

**1.5. A summary of the available data on environmental concentrations developed through the application of mathematical models, using all available environmental fate parameters, preferably those which were determined experimentally, along with an identification or description of the modelling methodology.**

Cybutryne is a booster biocide used as an additive in antifouling paints for protection against "soft fouling", i.e. fouling due to algae. It is used in conjunction with copper, which controls "hard fouling", e.g. by barnacles.

In principle, any chemical risk assessment procedure should consider the full lifecycle of a given product, including manufacture, formulation, professional, and private uses, and service life and disposal. The potential impact on all relevant environmental compartments should be considered for any of life cycle stages, nevertheless it is not the intention of this work to cover the emissions caused during the manufacturing or formulation of the product.

The main route of entry will be from the leaching out of paint during the service life of commercial vessels and pleasure crafts, therefore the expected routes of environmental exposure are limited to releases into marine waters. Emissions to soil, to air or to sewage treatment plants due to the application of the product have not been considered. A second potential route of entry would be from application and removal of antifouling paint in port areas.

The releases have been calculated according to the Emission Scenario Document (ESD) for antifouling products (OECD, 2004) to estimate Predicted Environmental Concentrations (PEC) in marine water and sediment.

The actual work of producing the OECD model ESD was done by a consultant overseen by a Steering Group composed of regulators from different OECD countries, the European Commission and industry representatives.

The primary aim of the harmonised ESD is its use in risk assessments for notification and authorisation procedures in regulatory frameworks used in all OECD countries. The ESD is intended to be used for general risk assessment and explicitly not for site specific risk assessments. Its scope was intended to develop a methodology for determining the emission load or initial concentrations from the use of antifoulants. It is mentioned in the ESD that the determination of any PEC in the receiving environmental compartment and therefore any assessment of the environmental impact of antifoulants should be carried out according to the regional practices in the member states of the OECD. Thus, for example, in the European Union (EU) the Technical Guidance Documents or Technical Notes on Guidance under the Biocidal Products Regulation (BPR) have been employed to determine PEC values.

For the estimation of PECs, the daily emission loads estimated through the ESD were entered into the Marine Antifoulant Model to Predict Environmental Concentrations (MAMPEC). In order to model concentrations in surface water and suspended matter, MAMPEC version 3.0.1 (IVM & WL – Delft Hydraulics, 2008) was used.

MAMPEC is an integrated 2D hydrodynamical and chemical fate model, based on the Delft3D-WAQ and Silthar model and recognized and used by regulatory authorities and applicants in USA, EU and other OECD countries for antifouling substances, and by the IMO for ballast water discharges. The model is being developed and maintained by Deltares and the Institute for Environmental Studies with continuing support of the European Paintmakers Association (CEPE).

Originally developed to estimate PECs for the exposure assessment of antifoulants in harbours, rivers, estuaries and open water. MAMPEC is also being used for exposure assessment in freshwater systems and discharges of chemicals in ballast water. It is an easy-to-use and freely available mathematical model.

### **Fate and distribution in the environment for the model**

The fate of cybutryne in marine waters is characterised by a dissipation half-life of approximately 23 days in water/sediment studies and a mean sediment-water partition coefficient ( $K_{oc}$ ) of 895 L/kg. As cybutryne was shown to be persistent in aquatic environments and removal rates observed in fate studies showed dissipation rather than degradation, the degradation constant  $k$  in model calculations to predict the environmental concentration was set to 0 (1/d) in both the water and sediment compartment. For a detailed assessment of the environmental fate and behaviour of cybutryne please refer to section 3.1.

The major degradation product of cybutryne in marine waters is M1 (GS 26575). Since the effects of cybutryne on marine aquatic organisms were assessed using the results of a microcosm study (see section 3.1 of this document) in which M1 (GS 26575) was the only major formed metabolite observed, it is considered that the endpoint for cybutryne from this study also accounts for any potential ecological effects due to the presence of M1 (GS 26575). A separate risk assessment for the degradation product has been considered not necessary.



The leaching rates used for the calculations are further explained in section 3.1.3 of this document. As a conservative measure an average leaching rate for new building of 1.9 µg/cm<sup>2</sup>/day has been used for the calculations.

### Emission scenarios used

The Emission Scenarios Document for PT21 (OECD, 2004) specifies different scenarios for the service life of antifouling products on commercial vessels and pleasure craft, such as a commercial harbour scenario, wider environment (surrounding of commercial harbour), a shipping lane scenario, an EU marina and an open sea scenario. The calculated PEC in a marina will always be higher than the PECs in harbour, those will be higher than the PEC for the wider environment, which in turn will be higher than the expected concentration for a shipping lane, which in turn will be higher than the expected concentration in the open sea, because of the higher concentration of ships per m<sup>3</sup> water. The four scenarios indicated above are used to calculate PEC values for cybutryne in the following assessment.

Default values for the total underwater surfaces covered with antifouling paint are given in the OECD scenarios or in case of the marinas, explained in the document “Regional marina scenario: defining typical regional pleasure craft marinas in the EU for use in environmental risk assessment of antifouling products (University of Newcastle, 2013), based on the number of ships in various size categories, together with representative treated surface areas for each size category. The total surface area treated with a particular active substance is then calculated using the ‘application factor’, i.e. the fraction of ships treated with that active substance. By default an application factor of 0.9 was used in the calculations, this represents, as a worst-case scenario, the maximum market share of the active substance cybutryne in all antifouling products. In this case, refinement based on market share is not applicable given the considerable uncertainty on the use of cybutryne in other regions of the world and due to the availability of monitoring information to calibrate the model. Table 1.5-1 shows the emissions modelled using MAMPEC.

Scenario		PEC <sub>seawater</sub> (µg/L)	PEC <sub>suspended matter</sub> (mg/kg dwt)	PEC <sub>marine sediment 10 years</sub> (mg/kg dwt)
OECD-EU commercial harbour	In harbour (Realistic worst case)	<b>426</b>	<b>10.8</b>	0.145
	Outside harbour (surrounding waters) (Realistic worst case)	<b>12</b>	0.3	0.048
OECD-EU Marina	In marina	<b>340</b>	<b>8.64</b>	4.08
	Outside marina (surrounding waters)	<b>2.85</b>	0.07	0.03
OECD-EU Shipping Lane		1.78E-2	9.53E-4	8.66E-6
Default Open Sea		3.62E-4	1.94E-5	6.94E-7

Table 1.5-1. Summary of emissions calculated with MAMPEC

The concentrations estimated through the model exceed the concentrations that would not affect harbours and marinas, both within the harbours or marinas and their surrounding environments. The concentrations found correlate well with the concentrations being found through monitoring in EU harbours, when using a market penetration factor of 90%.

In the cases of shipping lanes and open sea, the concentrations obtained through the model are below those expected to cause negative effects on marine species. This is due to the high dilution of the substance in areas more open than the harbour. Nevertheless, as cybutryne accumulates in the environment, higher concentrations in sediment could be expected in the long term if the substance is in use continuously.

### **Emissions of cybutryne during service life in a commercial harbour and wider environment**

According to the Emission Scenario Document for PT21 (OECD, 2004, Table 0.4), a realistic worst-case scenario for the calculation of the release of an antifouling biocide in a commercial harbour is based on 24 ships at berth, with a combined treated surface area of 102,362 m<sup>2</sup>, and 2.8 moving ships, with a combined treated surface area of 8,530 m<sup>2</sup>. The total treated surface area available for leaching of the biocide is therefore 110,892 m<sup>2</sup>. Given an application factor of 0.9 and an average leaching rate of 1.9 µg/cm<sup>2</sup>/day, the emission of cybutryne in the commercial harbour service life scenario can be calculated in MAMPEC as 1,900 g/d.

The commercial harbour environmental settings are described by OECD (OECD 2004, Table 0.5). The standardised OECD commercial harbour is 5,000 m long, 1,000 m wide and 15 m deep, so has a water volume of 75×10<sup>9</sup> L. It is a dynamic system with a tidal height of 1.5 m.

In 2011 it was agreed at EU level to use the wider environment scenario with the dimensions as defined in MAMPEC for decisionmaking on the approval or non-approval of antifouling paints on commercial ships in EU. The calculations, however, indicate that the average cybutryne concentrations in the wider environment are about a factor of 35 lower than the average concentrations in the commercial harbour.

It should be noted that the average leaching rate over the whole service period is considered to overestimate the typical leaching rate in a commercial harbour situation. The leaching rate increases with the speed of a vessel and will be lower than average whilst at berth or manoeuvring slowly in a harbour.

For the purpose of the following calculations, all releases from application and removal are assumed to end up in a commercial harbour. The total annual releases into the commercial harbour are summarised in Table 1.5-2. For the realistic worst-case scenario the total release is 1112 kg active substance (active substance)/year, equivalent to an average release of 3.05 g per day. For the typical case scenario the total release is 797 kg/year, equivalent to an average release of 2.18 g per day. Further explanation of the input parameters for application and removal can be found in the ESD for PT1.



Emission scenario	Type of scenario	Daily emission [g/d]	Number of ships treated per year	Days per treatment period	Number of emission days per year	Total average annual release share [kg/yr]
Service life	-	1,900	-	-	365 *	694
New building, application	Realistic	10,750	2	1	2	19.4
	Typical case	2,304	2	1	2	4.1
M&R, application	Realistic	10,750	20	2	40	387
	Typical case	2,304	20	2	40	83
M&R, re-blasting	Realistic	7,371	20	1	2 **	13.3
	Typical case	1,069	20	1	18 **	17.3
Total	Realistic					1113
	worst-case					798
	Typical case					798

\* assumption of 90% market share was already included in the daily service life emission.

\*\* according to OECD (2004), 10% of ships are re-blasted each year and the remaining 90% are spot blasted

Table 1.5-2 Annual release of Cybutryne to a commercial harbour

A schematic presentation of above mentioned data is provided in Figure 1.5-1, which shows the frequency distribution of the daily release of active substance over a year.

Frequency distribution of daily release of Cybutryne to a commercial harbour

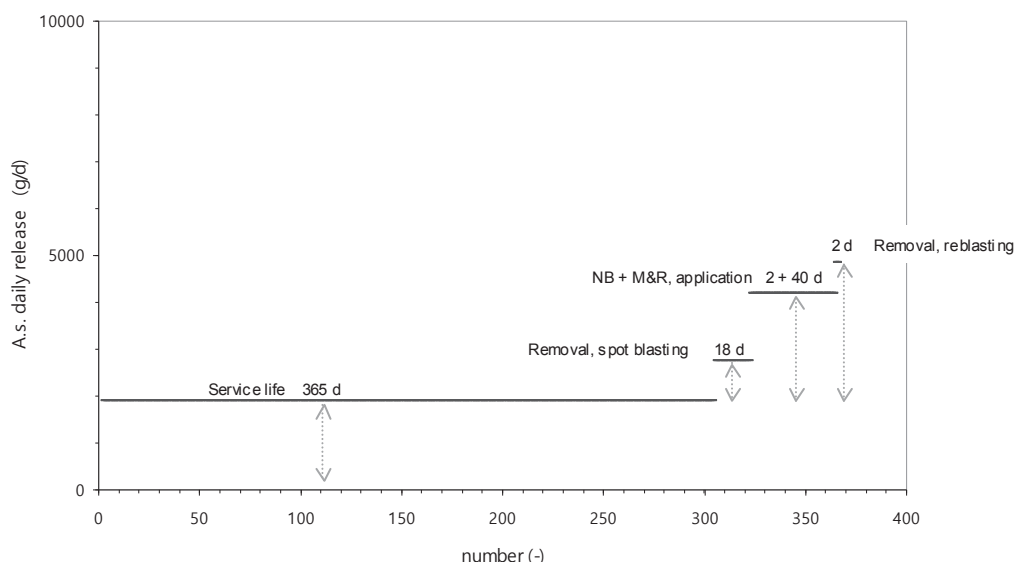


Figure 1.5-1 Frequency distribution of daily release of Cybutryne to a commercial harbour

Figure 1.5-1 shows a continuous release from boats that reside in the harbour (in service) and discontinuous releases from ship yards. The combined  $PEC_{\text{harbour}}$  consists of a  $PEC_{\text{in service}}$  plus a  $C_{\text{local}}$  for discontinuous releases. The  $C_{\text{local}}$  reflects emissions from paint application and paint removal in ship yards.

$$PEC_{\text{harbour}} = PEC_{\text{in service}} + C_{\text{local}}$$

Emissions from the application and removal scenarios are intermittent and are assumed to take place on 62 days per year.

The largest single daily emissions calculated for the application and removal scenarios, are due to removal from reblasting. These emissions account for 2,949 g/d for the realistic worst-case and 848 g/d for the typical case with a frequency of 2 days per year. It should be noted that for the paint removal scenarios, substantial emission reduction is possible by implementation of control measures and separation techniques that hold back paint particles from potential emission to surface water e.g. wash water can be treated/filtered on the yard before discharge or disposal, including processing of paint residues as hazardous waste.

The second large single day emissions are calculated for the application of antifouling paints in new building and maintenance & repair, accounting 10,750 g/d for the realistic worst-case and 2,304 g/d for the typical case with an occurrence of 42 events a year. As emphasised in the ESD, particulate emissions of antifoulants from application and removal life-stages will have different fate and behaviour properties compared to molecular emissions from the service-life stage, e.g. lower bioavailability and longer persistence. Paint droplets from overspray lost during application, are relatively large and dense, whereas due to the high volatility of the solvents the droplets undergo significant drying before they reach the surrounding water surface. Waste streams from high pressure water washing, hydro and abrasive blasting will contain active substance in paint flakes and paint dust as well as freely dissolved. Depending on control measures in place, particulate waste material may not be readily dispersed into the environment. However, there is no background documentation to support which fraction of active substance in waste streams from application and from removal can be held back by control measures and separation techniques. Due to the physical chemical characteristics and the active substance concentration in the paint particles, the RMS expects higher active substance emissions from paint droplets compared to paint flakes and dust derived from removal.

Therefore, the peak daily concentration due to emissions from application and removal scenarios, is calculated using the emissions from the from the paint application scenario with 42 emission days in new building and M&R. Considering the daily release of 1,900 g cybutryne per day from in service and 10,750 g cybutryne for the realistic worst-case and 2,304 g cybutryne for the typical case respectively from maintenance and repair causing local discharge into the standard commercial harbour, gives a combined peak emission of 12,646 g cybutryne per day for the realistic worst-case and of 4,200 g cybutryne per day for the typical case.

The average predicted peak concentrations for the dissolved fraction in harbour water calculated with MAMPEC are included in Table 1.5-3.

OECD Commercial harbour scenario	Type of scenario	PEC <sub>harbour, water</sub> [ng/L]	PEC <sub>harbour, freshly deposited sediment/suspended matter</sub> [ng/g dw]	PEC <sub>marine sediment</sub> years (mg/kg dwt)	10
In harbour	Realistic worst-case	426	10.8	5.11	
	Typical case	137	3.48	1.64	
Wider environment (surrounding harbour)	Realistic worst-case	12	0.3	0.145	
	Typical case	4	0.1	0.048	

Table 1.5-3 Average predicted water and freshly deposited sediment/suspended matter concentrations of Cybutryne for in service and M&R in a commercial harbour and the wider environment (surrounding harbour)

### Emissions of cybutryne during service life in a marina

During the EU submission, the use of Cybutryne containing antifouling paints was restricted by the manufacturer to commercial marine vessels (non-pleasure vessels), and the risk assessment for marinas was not performed. Nevertheless, for this report, the risk assessment for marinas has been included for completeness and in order to illustrate what would be the expected concentrations of cybutryne in such environments. Only the EU marina scenario present by default in the ESD and MAMPEC modelling has been used for illustrative purposes, but other marina characteristics could be considered if needed in the future.

The OECD ESD specifies that more research should be done with respect to boat characteristics in marinas in the OECD countries. Presently, different marina characteristics are used when registering antifouling products in the EU and other regions such as US and Australia. With respect to defining a set of standard parameters for use in risk assessments of antifouling for a specific marina there are options that should be considered. The dominant parameters which account for differences amongst all the marinas are tidal height, salinity and pH. The remaining parameters have much weaker associations with the canonical functions derived from the data and therefore play a far smaller role in defining which group a marina belongs to. An analysis of the marina dimensions revealed that there is very little difference in surface area per boat between the Atlantic, Mediterranean, Baltic and Baltic Transition region suggesting that irrespective of where the marina is located, marinas are designed according to similar rules governing how many vessels you can safely accommodate whilst accounting for practical requirements such as providing room for manoeuvre. This is largely down to the fact that there will be regulations (or codes of practice) dictating maximum occupancy rates for marinas and minimum space for individual vessels.

The predicted concentrations for cybutryne in marine water and sediment from emissions during service life were calculated with MAMPEC using the MAMPEC EU Marina model (OECD, 2004, Table 0.7). The results of the calculations are summarised in Table 1.5-4. The average concentration for the dissolved fraction calculated by MAMPEC is appropriate for the

purpose of risk assessment due to the worst-case assumptions in the associated scenarios (OECD, 2004).

The average concentration of cybutryne on suspended matter in the EU Marina is 8.64 ng/g dw. The average predicted concentrations in sediment, calculated with the default open sea scenario is calculated for 1, 2, 5 and 10 years after use (see Table 1.5-5). The concentration of the active substance in sediment is maximally equal to the suspended matter concentration. Increase of the active substance sediment concentration, does not reflect accumulation, but demonstrates the gradual displacement of non-exposed material in the modelled sediment compartment box. Data in relation to persistence and partition seem to indicate that cybutryne may accumulate in the sediment (see section 3.1).

OECD EU marina scenario	Type of scenario	PEC <sub>marina, water</sub> [ng/L]	PEC <sub>marina, freshly deposited sediment/suspended matter</sub> [ng/g dw]	PEC <sub>marine sediment 10 years</sub> (mg/kg dwt)
In marina	-	340	8.64	4.08
Wider environment (surrounding marina)		2.85	0.07	0.03

Table 1.5-4 Average predicted water and freshly deposited sediment/suspended matter concentrations of cybutryne in a EU marina and the wider environment (surrounding harbour)

The values obtained though the model exceed by far the concentrations that would have a significative negative effect in the algal marine communities. The calculations are also in line with the findings in monitoring data in the EU and other regions in the world.

### Emissions of cybutryne during service life in a shipping lane

A realistic worst-case scenario for calculation of the release of an antifouling biocide in a shipping lane is based on the characteristics described in the Emission Scenario Document for PT 21 (OECD, 2004). An application factor of 0.9 and a leaching rate of 1.9 µg/cm<sup>2</sup>/day have been used for the calculations in MAMPEC. The total emission is 587 g/d.

The predicted concentrations for cybutryne in marine water and sediment from emissions during service life were calculated with MAMPEC using the modified MAMPEC shipping lane scenario (OECD, 2004, Table 0.2 and 0.3). The results of the calculations are summarised in Table 1.5-5. The average concentration for the dissolved fraction calculated by MAMPEC is appropriate for the purpose of risk assessment due to the worst-case assumptions in the associated scenarios (OECD, 2004).

The average concentration of cybutryne on suspended matter in the shipping lane is 9.53E-4 ng/g dw. The average predicted concentrations in sediment, calculated with the OECD EU shipping lane scenario is calculated for 1, 2, 5 and 10 years after use. The concentration of the active substance in sediment is maximally equal to the suspended matter concentration.

OECD Shipping lane scenario	Type of scenario	PEC <sub>shipping lane, water</sub> [ng/L]	PEC <sub>shipping lane, freshly deposited sediment/suspended matter</sub> [ng/g dw]	PEC <sub>marine sediment 10 years</sub> (mg/kg dwt)
Shipping lane	-	1.78E-2	9.53E-4	8.66E-6

Table 1.5-5 Average predicted water and freshly deposited sediment/suspended matter concentrations of cybutryne for shipping lane

The concentration of cybutryne in freshly deposited sediment/suspended matter is taken as the PEC for shipping lane sediment (see table 1.5-5).

The concentrations obtained through the model are below those expected to cause negative effect in marine species. This is due to the high dilution of the substance in a more open area than the harbour. Nevertheless, as cybutryne accumulates in the environment, higher concentrations in sediment could be expected in the long term if the substance continues to be used.

### Emissions of cybutryne during service life in the open sea

A realistic worst-case scenario for calculation of the release of an antifouling biocide on open sea has been calculated using the defined parameters in the Emission Scenario document for PT21 products (OECD 2014). An application factor of 0.9 and a leaching rate of 1.9 µg/cm<sup>2</sup>/day has been used

The total emission is 12 g/d.

The predicted concentrations for cybutryne in marine water and sediment from emissions during service life were calculated with MAMPEC using the MAMPEC open sea scenario (OECD, 2004, Table 4.15 and 4.16). The results of the calculations are summarised in Table 1.5-6. The average concentration for the dissolved fraction calculated by MAMPEC is appropriate for the purpose of risk assessment due to the worst-case assumptions in the associated scenarios (OECD, 2004).

The average concentration of cybutryne on suspended matter in the open sea is 1.94E-5 ng/g dw. The average predicted concentrations in sediment, calculated with the default open sea scenario is calculated for 1, 2, 5 and 10 years after use (see Table 1.5-7). The concentration of the active substance in sediment is maximally equal to the suspended matter concentration. Increase of the active substance sediment concentration as shown in Table 1.5-7, does not reflect accumulation, but demonstrates the gradual displacement of non-exposed material in the modelled sediment compartment box. Monitoring data seem to indicate that cybutryne may accumulate in the sediment (see section 3.1).

OECD Open sea scenario	Type of scenario	PEC <sub>open sea, water</sub> [ng/L]	PEC <sub>open sea, freshly deposited sediment/suspended matter</sub> [ng/g dw]	PEC <sub>marine sediment 10 years</sub> (mg/kg dwt)
Open sea	-	3.62E-4	1.94E-5	6.94E-7

Table 1.5-6 Average predicted water and freshly deposited sediment/suspended matter concentrations of cybutryne for open sea

The concentrations obtained through the model are below those expected to cause negative effect in marine species. This is due to the high dilution of the substance in a more open area than the harbour. Nevertheless, as cybutryne accumulates in the environment, higher concentrations in sediment could be expected in the long term if the substance continues to be used.

#### 1.6. Evaluation of the association between the anti-fouling system in question, the related adverse effects and the environmental concentrations expected and observed.

As it has been described in different sections of this report, adverse effects of cybutryne have been shown in the laboratory for the standard species found in the marine compartment. The level of sensitivity depends on the phylum or species tested it has been shown that algae are the most sensitive taxonomic group with a NOEC of 0.022 µg/L for the marine algae *Skeletonema costatum*. According to current guidance's used in Europe for the environmental risk assessment of chemicals, in order to calculate the concentration that would not cause any effects in the environment, it is necessary to apply an assessment factor (AF) to the lowest concentration that causes an effect to any of the species tested in the lab (for further details see Guidance on the EU's Biocidal Products Regulation Volume IV Environment - Assessment and Evaluation (Parts B + C)). For this specific case, due to the availability of a large data set including fresh water and marine species, it was considered that an AF of 10 would be appropriate, providing therefore a **predicted no effect concentration of 2 ng/L**. According to this risk assessment methodology, this will essentially mean that any concentration above 2 ng/L in water would have a negative effect in algae species and consequently a potential effect in the ecosystems as algae are the pillar for the ecosystem structure and function as they provide the food base for most marine food chains.

As for corals, which are currently not integrated in common risk assessment methodologies, it has been shown that cybutryne may negatively affect coral species by inhibiting the photosynthesis of these organisms already at very low concentrations. Furthermore, cybutryne has been shown to reduce coral settlement.

On the other hand, concentrations well above 2 ng/L have been observed both in monitoring campaigns across the world (see section 1.4) and through modelling the concentrations by using the state of the art computational models (see section 1.5). Concentrations above 2 ng/L have been observed and modelled in harbours (inside and outside), marinas (inside and outside), and observed on the open sea even in regions where the substance has been banned or where there are no registered uses. This demonstrates that cybutryne is present at undesirable levels in water and sediment in many regions around the world causing an unacceptable risk for the marine compartment.

These concentrations exceeding the predicted no-effect concentrations and therefore resulting in unacceptable risk found in the marine compartment from commercial shipping led the EU and several other member states to restrict the use of cybutryne as an antifouling paint.

This report has compiled and summarised information from different sources with the aim of gathering all the necessary elements to show that cybutryne poses an unacceptable risk for the environment due to its negative effect on algae, which are key to maintaining the balance and the functioning of the marine ecosystem. In addition, negative effects have been observed in different coral species *in vitro* at very low concentrations. Although the risk estimation is proving difficult empirically due to the lack of agreed methodologies, this may pose an unacceptable risk to already endangered filae.

### **1.7. Qualitative statement of the level of uncertainty during the evaluation**

As explained in section 1.5 of this document the predicted environmental concentrations have been calculated using the MAMPEC model together with monitoring data collected from open literature and several other reports.

The use of any mathematical model involves assuming certain limitations both in the key parameters that are used as starting point, the calculation methods and the output of the model. Certain assumptions made for the estimation of cybutryne concentration in marine waters may incorporate uncertainty. Those assumptions which can entail higher uncertainty are described below.

In regards to the monitoring data, the only uncertainty may be associated with the extraction and analytical methods being used. It is not the intention of this work to challenge peer reviewed and published data therefore no uncertainty can be associated with the monitoring data.

The following parameters can be considered to carry uncertainty in the calculations:

#### **Degradation of cybutryne:**

MAMPEC allows the user to input different degradation rates (i.e. hydrolysis, photolysis and biodegradation) which should be obtained either by laboratory test or using different other methods (e.g. QSAR(s) or read-across). In the case of cybutryne, according to the data available for the substance (see section 3) it was decided to consider the substance as non-degradable both in water and in sediment. According to the studies, cybutryne is hydrolytically stable. With regard to photolysis, the studies show a very slow degradation of the compound, therefore it is assumed as zero. 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 into the degradation behaviour of the substance in the marine environment, and more simple tests are therefore not considered necessary. Cybutryne demonstrated to be persistent in aquatic environments and removal rates observed in fate



studies showed dissipation rather than degradation. Therefore the degradation constant  $k$  in model calculations to predict the environmental concentration was set to 0 (1/d) in both the water and sediment compartment.

The selection of these model inputs may lead to some overestimation of the concentration of cybutryne but it was not deemed as over conservative.

#### **Market share or application factor of cybutryne:**

MAMPEC allows the user to select an application factor. The figure specifies the fraction (%) of the ships painted with the specific cybutryne containing product and can be refined with the use of market figures. In this case seems impossible to model the market share figure for cybutryne globally. It is also considered that there will be regions where most of the ships have been treated with cybutryne in search for a worst case assumption. During the EU review of cybutryne, a substance manufacturer made a great effort to prove that the market penetration factor was far less than the 90% default value used for the evaluation. If the market penetration factor is lowered, the concentrations of cybutryne in the receiving compartments would decrease proportionality. One of the reasons why during the EU evaluation it was not considered appropriate to lower the market share value was because monitoring data from different coastal waters in EU was showing concentrations similar to those estimated when using the default value of 90 % as application factor in the model. In this report, the exposure is obtained both by calculations using mathematical models and also by monitoring information from coastal waters across the globe. Both figures show concentrations of cybutryne which exceed the concentrations which may cause a negative effect to the marine ecosystems therefore the refinement of market share seems not appropriate.

#### **Average concentrations taken instead of maximum:**

Average concentrations should be considered for regulatory purposes at the active substance approval stage in the EU. Maximum concentrations may also be considered when considering a restriction of cybutryne.

#### **Representativeness of the regional conditions used in the estimations:**

During the writing of this document, ECHA made a consultation to the OECD Working Group on Biocides during its annual meeting (Ireland during May-June 2018). The aim of the consultation was to collect feedback from the OECD members on the specific regulatory status of cybutryne in their countries, to gather available monitoring information for cybutryne and to know whether or not the scenarios chosen and described in the ESD for PT21 from 2004 are being used for regulatory purposes in the different member states. Australia, Canada and USA provided feedback to the questions made.

Canada noted that cybutryne is not found in any registered pesticide (including biocides) products in Canada. Cybutryne was registered as a material preservative in 1999 under the Importation for Manufacturing and Export Program (IMEP), which consists of the registration of a pest control product for importation into Canada solely for manufacturing and export. However, in 2013, this registration was discontinued by the registrant. Additionally, cybutryne has never been used as a formulant in any product registered under the Pest Control



Products Act. Canada does not have any monitoring data on cybutryne. In terms of the scenario being used, Canada stated that recent Health Canada re-evaluations of antifouling coatings were based on US EPA assessments. These assessments used the MAMPEC model, with modified scenarios based on the OECD emission scenarios. The OECD scenarios are typically considered to present a valid framework to determine environmental concentrations to determine risk to non-target species in Canada. They provide sound default values and calculations, and are generally adaptable to various environments. Health Canada has not, however, had the opportunity to compare the specified scenarios to actual data on Canadian marinas, so it is unknown whether the scenarios should be considered representative. In Canada's view other parameters presented in the OECD scenarios are considered adequate. The OECD ESD for Antifoulants provides a good basis for determining aquatic concentrations in various parts of the world and can be modified as needed to reflect local conditions and uses.

USA replied that EPA is aware of the OECD Emission Scenario Document (ESD), but based on need for more US specific scenarios for recreational boats in marinas, in 2016, EPA used MAMPEC and information on US marinas to build a saltwater marina scenario representative of US parameters. The EPA particularly focused on marinas for recreational boats.

Based on the responses received and considering that extensive monitoring data has been compiled, it was decided to include only the EU scenarios for the estimations. Specific conditions in regards to water characteristics and marinas and harbour dimensions and traffic have not been assessed due to the lack of data. The EU scenarios must then be considered with care in order to extrapolate to other regions and must be analysed in conjunction with monitoring data available. Nevertheless it can be considered a first tier approach.

#### **1.8. A recommendation of specific control measures to reduce risks associated with the antifouling system**

In this work it was highlighted from scientific studies that cybutryne is responsible for the adverse effects to the marine environment as it was repeatedly detected in levels exceeding safe environmental limits in many areas worldwide. Therefore, the indications that the substance is negatively affecting non-target marine organisms worldwide are a strong argument for globally banning cybutryne.

Antifouling systems containing cybutryne have been already regionally banned by many IMO member states and studies of the areas that were monitored recorded the reduction of the concentrations, which in some cases were reduced by 55% from what was initially measured ([Cresswell, 2006](#)). An example on how the legislative measures contributed to the reduction of cybutryne concentration has been reported in Plymouth (UK).

Therefore, the only effective control of the substance can be achieved by including cybutryne to Annex I of AFS Convention since it is the most effective option in order to control the production and distribution of the substance in order to eventually reduce unsafe concentrations of cybutryne.

**1.9. A summary of the results of any available studies on the potential effects of the recommended control measures relating to air quality, shipyard conditions, international shipping and other relevant sectors, as well as the availability of suitable alternatives.**

The control measures recommended above would only have marginal effects on international shipping, and no negative effects on any other sectors. There are a large number of booster biocides which serve as alternatives to cybutryne and which are similar in effectiveness and cost, but pose a far lower risk to the environment. The risk to human health of these substances is comparable to that of cybutryne, i.e. low. Particularly suitable replacements are DCOIT, dichlorofluanid, and zineb.

In an economic analysis prepared for New Zealand's Environmental Protection Authority (Denne and Hoskins, 2013), the effects of a ban on cybutryne and three other antifouling substances are modelled and found to be negligible. Paint costs were expected to rise by a maximum of 5 to 10 percent for a short period of time after the ban, then stabilising at levels similar to or below those before the exclusion of cybutryne from the list of approved substances. It is also noteworthy that there were no price hikes or difficulties associated with the EU decision to remove cybutryne from the list of accepted antifouling substances in the Union. Painting costs for shipowners did not increase as a consequence and the paint industry was able to cope very well with the ban in light of the range of paints based on other substances that seamlessly replaced cybutryne-containing products. This shows that suitable alternative paints are available already today, at costs that do not differ significantly from cybutryne-based products.

Painting costs are only a very small fraction of total operating costs in the large vessel market. Ships tend to be painted by commercial painters in dry docks. The cost associated with this operation is in the bigger part caused by the losses of profit of a ship in dry dock than with the actual cost of painting. Regarding commercial painting, about 20-30 percent of the cost of antifouling service is estimated to come from paint costs. The expected short-term rise in paint prices, if it happens at all, could therefore increase the cost of antifouling services by 0.2 to 0.9 percent (Denne and Hosins, 2013). Given that commercial vessels are usually only repainted every 2-3 years, only a small fraction of ships will be affected. In light of the fact that antifouling services in themselves make up less than 2 to 3 per mill of a vessel's operating costs, the possible short-term price increases by less than 1 percent provoked by a cybutryne ban would be insignificant.

Air quality is not directly affected by cybutryne, nor can it be expected that a ban of the substance alone would elicit any changes to air quality.

Similar to other antifouling systems, coatings containing cybutryne are applied on commercial ships and boats via spraying. The common methods of antifouling coating application are: the conventional air spray and the conventional airless spray. The second type of spraying is frequently used when applying marine coating in large volumes to large surface areas. For both methods the use of spray guns is required and the coating manufacturer provides the guidelines in order for the user to determine the film thickness he needs to apply per pass.

By using these two methods the entry of cybutryne into the atmosphere at a shipyard environment remains possible. However, there is no specific related study for coatings containing cybutryne and the possible adverse effects they elicit in regards to air quality.

In general the user spraying paints containing antifouling biocides runs a highly increased probability of exposure. Spray mists are containing air-borne particles that can be inhaled and may be hazardous due to the biocides that give the paint its antifouling properties especially for a shipyard environment.

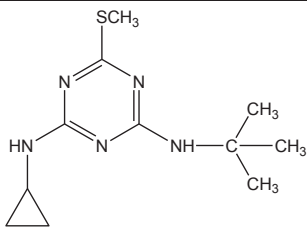
The hazards will vary from one antifouling to another. However the safety measures that need to be applied do not differ for biocides containing cybutryne. The user should wear all the necessary protective equipment (including respiratory equipment) when spraying an antifouling coating according to national and international occupational health regulations. For a shipyard environment the banning of cybutryne can contribute to the overall reduction of waste materials that need to be managed. In table 1.8-1, some of the major activities in shipyards are summarized, and the contribution of cybutryne to the adverse environmental effects highlighted.

<b>Shipbuilding work</b>	<b>Adverse effects</b>	<b>Cybutryne ban will reduce adverse effects</b>
<b>Painting and coating</b>	Inhalation of toxic biocides and other vapours generated from the painting process affecting human health	Yes
	Painting particles contamination of surrounding water during the coating process	Yes
	VOC's emission during the spray painting	No
<b>Welding operation</b>	N/A	
<b>Surface treatment cleaning and de-greasing preparation for painting</b>	Particle emission to the atmosphere and increased exposure to micro sized particles that could have adverse effects to human health	It may have an impact
	Water contamination from paint parts combined with other cleaning solvents	Yes
<b>Blasting</b>	The antifouling removal and formation of air borne compounds containing antifouling substances	Yes
	Water contamination with antifouling substances	Yes
<b>Maintenance work and Repair</b>	N/A	

Table 1.8-1: Shipbuilding activities and how will the cybutryne contribute to the overall environmental effects.

## 2. Physical and Chemical properties of cybutryne (PART II)

2.1. Physical and Chemical properties of cybutryne. (Including melting point, boiling point, density (relative density), vapour pressure, water solubility / pH / dissociation constant (pKa), oxidation/reduction potential, molecular mass, molecular structure; and other physical and chemical properties identified in the initial proposal)

CAS-No.	28159-98-0
EINECS-No.	248-872-3
US EPA chemical code	128996
Other No. (CIPAC, ELINCS)	No CIPAC No. not assigned.
Chemical name	<i>N</i> -cyclopropyl- <i>N'</i> -(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
Common name, synonyms	cybutryne (ISO), Cybutryne, TK 13079, Irgarol® 1051, Irgarol® 1071, Irgaguard® D 1071
Molecular formula	C <sub>11</sub> H <sub>19</sub> N <sub>5</sub> S
Structural formula	
Molecular weight (g/mol)	257.37

As a pure active substance, it is a white, cloudy, solid substance with an odour resembling that of leek or garlic. It melts at 128.4 °C, and its boiling point lies between 347.3 and 375 °C. It has low solubility in tap water (7-9 mg/L at pH 7 and 20°C) and a low vapour pressure (3.4\*10<sup>-5</sup> Pa at 25°C). Higher salinity of water results in lower water solubility. Its density accounts for 1.11 g/cm<sup>3</sup> at 20°C. Due to its surface tension below 60mN/m for solutions of 90 % saturation, it is regarded as a surface-active substance. The substance is not highly flammable nor explosive; it does not have oxidising potential. Its log P<sub>OW</sub> (3.1 - 3.2) indicates a potential for bioaccumulation. Cybutryne is regarded as a surface-active substance, since its surface tension is below 60 mN/m.

### 3. Further data requested in paragraph 1(b) of Annex 3 of the AFS Convention (PART III)

#### 3.1. Data on the environmental fate and effect modes of degradation/dissipation

##### Persistence in the relevant media

The main route of entry into the environment is *via* leaching of cybutryne during service life and *via* discharge from docks or marine lifts as a result of application and removal of antifouling. The dominant receiving environmental compartment is seawater. (ECHA, 2015)

Cybutryne was found not to be ready biodegradable. Additionally, higher tier tests in freshwater and marine water are available. No significant degradation of cybutryne was found (DT50 > study duration of 12 month). However, the dissipation from seawater was investigated in an outdoor microcosm under natural climatic conditions. Cybutryne dissipated from the microcosm water under the actual test conditions with a DT<sub>50</sub> of approximately 22.5 days. GS 26575 (also called M1) was the only metabolite found in the test system peaking at a maximum concentration of 150 ng active substance /L after one month, then declining with approximately the same half-life as cybutryne (22.7 days).

In a freshwater microcosm cybutryne dissipated from the microcosm water under the actual test conditions with a half-life of approximately 35 days. Again M1 (GS 26575) was the only metabolite found in the test system. No DT50-values for M1 (GS 26575) in the water phase and cybutryne in the sediment could be calculated since the dataset was too small. Due to the data in the microcosm studies cybutryne is regarded as persistent.

Cybutryne does not biodegrade in marine sediment. On basis of the available studies, however, it is not possible to establish half-lives and thus to properly address the P-criterion. Therefore, cybutryne and its major metabolite M1 (GS 26575) should be considered as potentially persistent or very persistent

The principal transformation product of cybutryne, M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine)(GS 26575), is produced through n-dealkylation via biodegradation (Liu et al. 1997), photodegradation (Okamura et al. 1999) or (Hg<sup>2+</sup> – catalysed) hydrolysis (Liu et al. 1999) (Figure 3.1.1-1).

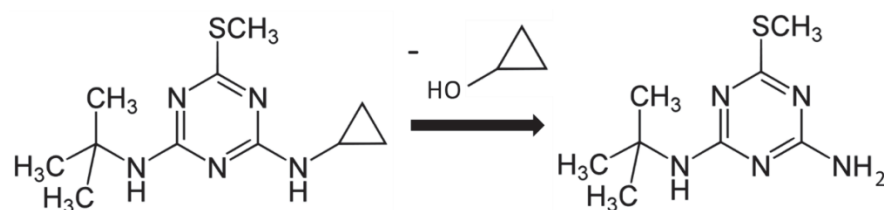


Figure 3.1.1-1: Main degradation pathway of Cybutryne to M1 (GS 26575).

Other degradation products include M2 (3-[4-tertbutylamino-6-methylthiol-s-triazin-2-ylamino]-propionaldehyde (the N-propionaldehyde derivative of M1) (Lam et al. 2005) and M3, N0-di-tert-butyl-6-methylthiol-s-triazine-2,4-diamine. All metabolites can be detected in the environment ([Lam et al., 2005](#)).

M1 (GS 26575) is relatively stable in water (half-life > 200 days) and sediment (half-life > 260 days) (Okamura 2002; Thomas et al. 2002), well above the thresholds to be considered very persistent. Reported levels of M1, are up to 1.9 µg/L ([Okamura et al., 2000](#)) in seawater and 0.023 µg/g ([Gatidou et al., 2007](#)) in marine sediment, respectively. Those numbers are generally lower than those of Cybutryne, indicating slow degradation rates of the parent compound (Zou, 2008).

Findings by Lam et al (2005) indicate that M3 is thermally stable and stable towards photo- and biodegradation.

### **Biotic degradation (aerobic and anaerobic)**

Cybutryne is not readily biodegradable. Results from an OECD 301B test (EU-CAR, 2014) showed that in the course of a 41 day test period, negligible amounts of cybutryne were degraded by activated sludge. A significant increase in degradability was observed in the presence of an emulsifying agent, which shows that bioavailability can be a limiting factor for microbial degradation. These findings were confirmed by results from a modified OECD 302 test (Meinecke et al., 2006) that showed that after 28 days, less than 10% of the substance had been degraded. The authors derived half-lives for primary degradation between 36 and 109 days (valid until day 120 after dosing), depending on the initial concentration.

### **Freshwater compartment**

Cybutryne and its major metabolite M1 (GS 26575) are recalcitrant to biodegradation in both freshwater and the respective sediment. Cybutryne does not biodegrade readily and exhibits aqueous half-life between 100 and 350 days (Thomas and Brooks 2010). Substances with aqueous half-life > 180 days are classified very persistent (vP).

There is only minor aerobic degradation of cybutryne in a freshwater water/sediment system in the dark: The initial concentration decreased by ~25% within 30 days and the water/sediment distribution was 45:48. Non-extractable residues (NER) were at <7% of initial measured dose at the final sampling date. However, the study is not considered reliable (RI 3) because the incubation period was too short to calculate reliable degradation rates. Figure 3.1.1-2 shows the proposed degradation pathway of cybutryne in water/sediment and mesocosm systems. (EU-CAR, 2014)

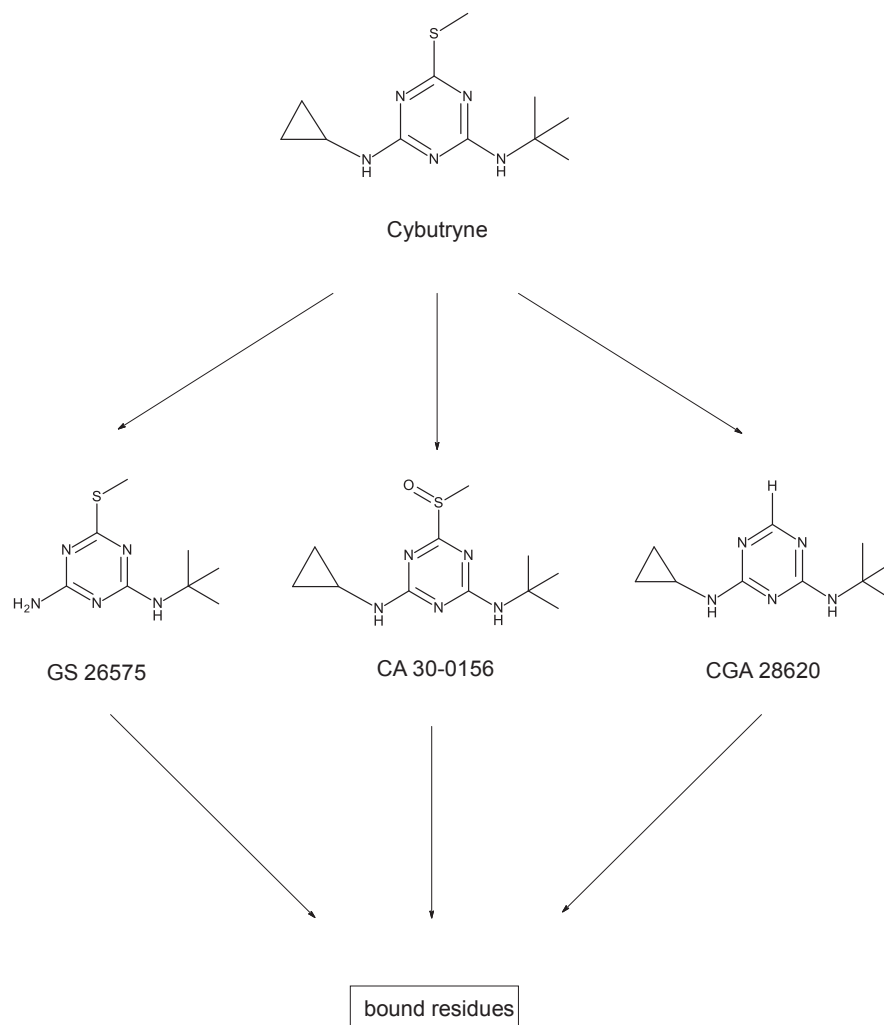


Figure 3.1.1-2. Proposed degradation pathway of cybutryne in water/sediment and mesocosm systems. (Schmidt and Head, 1991)

Under anaerobic aquatic conditions, no significant degradation of cybutryne was found during a 1-year period in the dark (Schmidt, 1992, unpublished report).

A total dissipation half-life of 118 days ( $DT_{50\text{water}} + DT_{50\text{sediment}}$ ) for cybutryne in freshwater outdoor mesocosms under static conditions is reported (EU-CAR, 2014). The aqueous dissipation half-life was reported 35 days for the parent and 246 days for the metabolite M1, the latter being extrapolated data. For sediment, the extrapolated half-life for cybutryne was 90 days. Since biofilms, macrophytes and bound residues were not analysed, no conclusion can be drawn on the overall fate in in the total system (RI 3). However, the study shows that cybutryne and the major metabolite M1 (GS 26575) are recalcitrant to biodegradation in both water and sediment.



## Marine compartment

Cybutryne degrades slowly in seawater with reported  $DT_{50}$  of 7d – 6.5y. Paint flakes represent a long-term environmental release source for both the substance itself and its metabolite M1 (GS 26575). M1 (GS 26575) shows a higher degree of persistence vis-à-vis environmental degradation processes compared to the parent compound.

Concentrations of cybutryne in seawater worldwide vary between non-detectable and low  $\mu\text{g/L}$  (Sargent et al., 2000; Biselli et al., 2000; Sakkas et al., 2002; Okamura et al., 2003). Concentrations up to 4.2  $\mu\text{g/L}$  have been detected in coastal areas (Basheer et al., 2002), whereas in the UK the highest concentration observed was 1.4  $\mu\text{g/L}$  (Thomas et al., 2001). In sediment sample concentrations are typically in the ng/g range (Albanis et al., 2002; Gatidou et al., 2007) but levels as high as 1  $\mu\text{g/g}$  have been detected in marinas (Boxall et al., 2000). The highest concentrations of M1 (GS 26575) found are 1.9  $\mu\text{g/L}$  (Okamura et al., 2000) and 23 ng/g (Gatidou et al., 2007) for seawater and marine sediment, respectively. Metabolite concentrations are thus generally lower than those of cybutryne, indicating slow degradation rates of the parent compound.

In seawater, biodegradation occurs via aerobic metabolism. In sediment, both aerobic and anaerobic degradation is slow, resulting in persistent occurrence. In general, the major metabolite M1 (GS 26575) seems to be even more persistent than cybutryne.

Findings by Okamura et al (2002) suggest that cybutryne is gradually released from antifouling paint into the surrounding water and reacts with sunlight in the upper water column to decompose itself rapidly into M1 (GS 26575), which is heat resistant and stable against hydrolysis (Okamura, 2002).

Kaonga et al (2016) reported a biodegradation half-life in seawater of 6.5 years (21 °C). The authors explained their finding with a suppressed microbial activity in the marine environment due to high salt content, as compared to freshwater environments. ([Kaonga et al., 2016](#))

Hall et al. (1999) and Thomas et al. (2002) report half-life values in a range of 100 to 350 days, indicating that cybutryne is a fairly stable substance in seawater, where the main mode of biotic cybutryne degradation appears to be aerobic microbial metabolism to form M1 (GS 26575) ([Hall et al., 1999](#)).

In a seawater/sediment fate study on artificially prepared paint particles, cybutryne did not show any significant signs of degradation during 42 days and the authors concluded that the substance was particularly persistent under the anaerobic conditions in sediments ([Thomas et al., 2003](#)). However, the reported data does not allow for a sound conclusion on  $DT_{50}$  (degradation curve  $R^2=0.14$ ).

Degradation in marine sediments is slow under both aerobic and anaerobic conditions, resulting in persistent occurrence in sediments. As a result, marine sediments may serve as both storage and secondary sources of the substance (Thomas et al., 2003, Zhou, 2008).



Sapozhnikova et al (2009) conducted a study in a modular estuarine mesocosm and reported an average half-life of Cybutryne in water of 7 days ( $\pm 3$  days). The aqueous concentration of the metabolite M1 (GS 26575) increased over the course of the experiment to up to about 3% as compared to the parent compound. In addition, M1 (GS 26575) significantly accumulated in the mesocosm sediments (average  $16.7 \pm 2.5\%$  of total cybutryne dose).

Zou (2008) sampled British coastal waters and investigated the occurrence of cybutryne in paint residues. The authors reports that the concentrations of the cybutryne were higher in paint residues from vessels than in sediments nearby. In addition, remobilisation of cybutryne from paint residues was found to be very slow with a half-life of approximately 1 y. Interestingly, M1 (GS 26575) was only detected in 20% paint samples at very low levels. Even though cybutryne has been banned since 2003 for application in vessels of any sizes, it was still present in relatively higher concentrations in paints than in sediments. These finding point towards a high degree of persistence of Cybutryne in paint matrix.

M1 (GS 26575) has been detected in coastal waters and sediments as a result of natural transformation processes such as photodegradation and biodegradation (Thomas et al., 2000, 2002; Martinez and Barceló, 2001; Ferrer and Barceló, 2001; Lam et al., 2005; Gatidou et al., 2007).

The ratio of M1 (GS 26575) to cybutryne in seawater was found to increase with time since the banning of cybutryne in small vessels. The ratio increased from 1:5 in the year 2000 to an average of 1:3 in the years 2004 and 2005, indicating a greater environmental persistence of M1 (GS 26575) as compared to the parent compound. (Zou, 2008)

Findings by Thomas and Brooks (2010), however, seem to show that the principal metabolite M1 (GS 26575) degrades more quickly in water than the parent, with a aqueous half-life of approximately 80 days. The half-life in sediment was reported 260 days, indicating persistence in marine sediments.

### **Soil compartment**

For the aerobic degradation of Cybutryne in sandy loam (<1% organic carbon content) a half-life of 101 days (at 25 °C) is reported, which converts to a half-life of 286 days at 12 °C, when applying the Arrhenius equation. The  $DT_{50}$  soil threshold for classification of a substance as very persistent is 180 days (at 12 °C) (EU-CAR, 2014).

### **Abiotic degradation**

#### **Hydrolysis**

Cybutryne and its major metabolite M1 (GS 26575) are hydrolytically stable in the natural environment. Significant hydrolysis of cybutryne occurs in the presence of  $Hg^{2+}$  ions only, independent on the pH (Liu et al. 1999).

Hydrolysis of cybutryne was investigated and reported for a duration over 30 days (25°C, pH8, US EPA FIFRA N-161-1), the authors found no evidence of hydrolysis (EU-CAR, 2014).

Findings by Okamura et al. (1999) confirm that cybutryne does not significantly degrade by hydrolysis in sea water, river water or buffered solutions (pH 5, 7, 9; 1 week; 50°C).

Okamura (2002) reported 11-25% hydrolysis of cybutryne over 10 month and observed that the major metabolite M1 is more stable to hydrolysis as compared to the parent.

### **Photolysis**

Photolysis can be an important degradation route in natural waters in the presence of dissolved and particulate organic matter.

Photodegradation of both cybutryne and M1 (GS 26575) occurs at acidic pH rather than at neutral or basic pH (Okamura et al., 1999). The Swedish Chemicals Agency investigated photolysis in natural waters (pH 5-9 (25°C) and concluded that it is of minor importance (KEMI, 1998). Photodegradation half-life in seawater was shown to be 57 d (Kaonga et al., 2016). Doyle (1991a) reported photodegradation rates for Cybutryne of 4.1% - 7.8% over 1 month in sterile buffer and sterile artificial seawater, respectively, in the absence of organic matter.

Most importantly, the photodegradation rate for Cybutryne significantly accelerates in the presence of humic and fulvic acids, as reported by Okamura et al. (1999) and Sakkas et al. (2002). These findings can be explained with a photosensitizing effect of naturally occurring organic matter (Okamura, 2004). Sakkas et al. (2002) reported half-life values in the range 52-60 d for natural water (52 d in lake water pH 7.7, 60 d in river water, pH 7.9 and 56 d in sea water pH 7.7). Lam et al. (2009) reported a half-life of 72 d for coastal seawater.

During the life cycle as marine antifouling substance cybutryne is not considered to enter the terrestrial compartment. The calculated half-life of the photochemical degradation of cybutryne in the atmosphere is 159.6 h (24-h day,  $0.5 \times 10^6$  OH/cm<sup>3</sup> (AOPWIN v. 1.92a). However, given the low vapour pressure, cybutryne is not considered to be prone to evaporation from natural waters. (NL, 2014)

### **Sediments / water partitioning**

Cybutryne may partition into suspended solids, which precipitates onto sediment. The distribution of cybutryne between sediments and water is strongly correlated with the organic carbon content of sediment and the level of disturbance of the sediment. Interestingly, cybutryne in paint particles is very persistent in sediment.

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 and sediment.

KEMI (1998) reported that 60-80 % of cybutryne partitions to the sediment (KEMI, 1998).

Thomas et al. (2000) suggest a low affinity of M1 (GS 26575) for particulate matter and marine sediments. They justify their conclusion with the absence of M1 (GS 26575) in samples from a number of coastal and marine sediments while the same substance was present in ambient surface waters in the range 13-99 ppt. The authors explained their findings with a lower lipophilicity of the degradation product as compared to the parent due to dealkylation, resulting in a relatively low affinity for particulate matter and marine sediments.

Zou (2008), on the other hand, report findings that indicate a strong correlation of the occurrence of cybutryne and M1 (GS 26575) in sediment with elevated organic carbon content, indicating organic rich sediments may become a sink for Cybutryne.

A study by Kaonga et al. (2016) on the distribution of cybutryne revealed that 74 % of the substance partitions from seawater into sediments and accumulates to a large extent. The same study reports that the substance biodegrades very slowly, resulting in persisting for at least several months.

Comber et al. (2002) investigated the sediment-water partitioning of cybutryne. They derived organic carbon/water partition coefficients (K<sub>oc</sub>) for in the range logK<sub>oc</sub> 2.41 - 4.89. The partitioning of the compound to suspended solids increased with increasing sediment concentration. However, much of this variation was eliminated when the calculated partition coefficients were normalised to the organic carbon content (log K<sub>oc</sub>).

The authors conclude that in the natural environment where suspended sediment concentrations are likely to be lower than those investigated in this study, cybutryne will be predominantly in the dissolved form.

The findings by Comber et al. (2002) are supported by field monitoring data where the majority of sediment samples contained less than 10 ppt of cybutryne, compared with dissolved concentrations in the range of 10–100 ppt (Boxall et al., 2000). Their experimental and field data suggest that in a marina environment, where high boat density leads to high levels of antifoulants in the water and where suspended solid concentrations are generally low (<20 mg/l), the booster biocides examined here will be transported out of the marinas in the dissolved phase rather than adsorb to and settle out on particulate matter. Accumulation in the sediment, producing a potentially toxic 'pool' of contaminants is therefore unlikely, although their presence in the water column means that the biocides will be transported further afield, and may be more bioavailable.

In an estuarine mesocosm study, after 35 days of exposure, 7 per cent of cybutryne remained unchanged in the water while 75 percent accumulated in sediments (Shapoznikova et al, 2009). Studies conducted by Tolhurst et al. (2007) and Voulvoulis et al. (2002) showed on the other hand, that disturbance of sediments contaminated with cybutryne can cause desorption of cybutryne with the rate of 1.9–2.4% per 24 h, resulting in a re-release into seawater (Saleh, 2016). Zou (2008) reports desorption of cybutryne with a half-life of 63 days. The half-life of desorption was 346 days for cybutryne bound to paint particles, showing a high degree of persistence in sediments under realistic post-application conditions.

## Leaching rates of biocides or active ingredients

The emission of biocides in antifouling products from ship hulls during service life is determined by the leaching rate and the total underwater area covered with antifouling paint. The leaching mechanism of antifouling paints like the one presented by the manufacturer during the EU assessment, a self-polishing paint, involves the slow dissolution of the coating polymer matrix, which releases the active substance into water. The leaching rate is therefore a critical parameter in the environmental exposure assessment. A laboratory study was conducted to measure the leaching rate for cybutryne from antifouling paint, yielding a leaching rate of  $6.45 \mu\text{g}/\text{cm}^2/\text{day}$  for the reference product. However some laboratory methods are generally considered overly conservative and are not believed to assess the actual environmental loading of the biocide into the environment (OECD, 2004). As an alternative to laboratory methods, the leaching rate may be estimated by mass balance, using the fact that total release of biocide can never exceed the amount incorporated into the coating.

The European Paint Industry (CEPE) has developed a calculation method for the determination of leaching rates based on the assumption that the total release of biocide can never exceed the amount incorporated into the coating. Data generated by this method has been accepted as an interim solution by some countries' authorities (a.o. The Netherlands) as the method used for submission of release rate data with a product application. This mass balance method was used to estimate the release rate for cybutryne from the reference product.

The method is a simplified generic model of biocide release, which is based on the assumption that the majority of biocide in the paint that is applied is released at a constant rate during the specified lifetime. The calculated release rate derives from the volume of dry paint film applied, the loading of biocide in the paint, and the specified lifetime of the product.

The model assumes that:

- the biocide release rate falls linearly for the first 14 days following immersion;
- the biocide release rate is thereafter constant from day 14 until the last day of the coating's specified life-time;
- the ratio of the cumulative amount of biocide released during the first 14 days following immersion to the average release rate during the remainder of the coating's specified lifetime is 30;
- a fraction of biocide is retained in the paint film at the end of its specified lifetime. According to the workshop on "Harmonisation of leaching rate determination for antifouling products under the Biocidal Products Directive" in 2006, the amount of biocide retained in the paint film at the end of its specified lifetime is set at 10%.

Based on these assumptions, and from knowledge of the biocide content of the paint, specified dry film thickness and its specified lifetime, it is possible to calculate both the amount of biocide released during first 14 days ( $\mu\text{g}/\text{cm}^2$ ) and the average leaching rate during the rest of the lifetime ( $\mu\text{g}/\text{cm}^2/\text{day}$ ). For the purpose of this work, no other data on

cybutryne containing products was available, therefore the leaching rate calculated for the EU evaluation has been used as reference.

Based on this mass balance method average leaching rates for service periods ranging from 12 to 60 months of 1.9 µg/cm<sup>2</sup>/day and 1.6 µg/cm<sup>2</sup>/day were calculated for 'new building' and 'maintenance & repair' applications respectively. Average leaching rates for vessels with different service periods, and over the lifetime of the paint, are considered appropriate for the exposure calculations due to the number of vessels specified in the scenarios (e.g. ~27 for the OECD commercial harbour scenario), the length of time required to reach steady-state concentrations and the realistic worst-case estimations of the default OECD scenarios (hydrology, shipping characteristics and dimensions of the receiving compartment). However, as a conservative measure, the average leaching rate for new building, i.e. 1.9 µg/cm<sup>2</sup>/day, was used in the PEC calculations rather than the more typical value of 1.6 µg/cm<sup>2</sup>/day calculated for maintenance and repair.

The leaching rate of cybutryne from paint was also determined to be 2.2 µg/cm<sup>2</sup> per day (Thomas et al., 2002). The Swedish Chemicals Agency (KEMI, 2006) has estimated cybutryne emissions to be 1.89 µg/cm<sup>2</sup>/day when the substance is applied to boats.

### **Mass balance of cybutryne**

Mass balance is not relevant for this compound. The behaviour and partition into different environmental compartments has been described in previous sections (i.e. section 3.1.1, 3.1.2). The leaching behaviour and partitioning to water phase has been described in section 3.1.3.

### **Bioaccumulation, partition coefficient octanol/water coefficient**

Cybutryne has a log P<sub>ow</sub> (3.1 - 3.2) which indicates a potential for bioaccumulation. Cybutryne is regarded as a surface-active substance, since its surface tension is below 60 mN/m. for further details on bioaccumulation potential see section 3.2.6

### **Novel reactions on release or known interactive effects**

There are no known interactive effects with other substances.

### **3.2. Data on unintended effects in aquatic plants, invertebrates, fish, seabirds, marine mammals, endangered species, other biota, water quality the seabed, or habitat of non-target organisms, including sensitive and representative organisms:**

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<sub>2</sub> in organic molecules is inhibited, ultimately leading to an inhibition in growth. In standard laboratory tests the lowest 72-h NOEC for cybutryne was observed with the freshwater diatom *Navicula*

*pelliculosa* (NOEC 20 ng active substance /L), while marine diatoms *Skeletonema costatum* were slightly less susceptible: NOEC 22 ng active substance /L.

It is 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-h LC<sub>50</sub> 11 and 1.50 mg/L, respectively), and highly but slightly less toxic to marine algae (120-h NOEC is 180 ng/L). Freshwater algae were much less susceptible: 120-h NOEC was 77,000 ng/L.

In a microcosm study, in which natural marine algae, zooplankton, 3 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 active substance /L. The results of an indoor freshwater mesocosm study will be used to discuss the decision on pooling or not pooling data on freshwater and marine organisms. Furthermore as no thorough statistical analysis was included in the report and it was not possible to carry out such an analysis on basis of the limited raw data submitted additionally. Therefore this study could not be used to derive a PNEC freshwater during the EU assessment.

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 needed to clarify this finding.

Short term dietary toxicity tests showed low acute toxicity of cybutryne (5 days LD<sub>50</sub> of >5620 and >2000 mg active substance /kg food) towards birds and mammals. Sub-chronic exposure of rats gave a 90 days NOEC of 150 mg active substance /kg food.

### Mode of action

Cybutryne as a triazine algicide is known to inhibit photosynthesis. This is in line with the observation that primary producers (algae and aquatic macrophytes), were the most sensitive aquatic species. The active substance, however, appeared to be also highly toxic to fish and invertebrates. The mode of action in these organisms is, nevertheless, unknown. There is no reasoning available to assume that the working mechanism in freshwater and marine should be considered as different.

### Differences in sensitivity between fresh and marine water species

A summary of the endpoints for the most susceptible standard species tested in aquatic toxicity tests with the active substance cybutryne is presented.

Test type	Freshwater	Exposure	Endpoint	Marine	Exposure	Endpoint
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	Test species	Design	Duration	Type	µg active substance /L	Test species	Design	Duration	Type	µg active substance /L
Fish acute	<i>Oncorhynchus mykiss</i>	F (n)	96 h	LC <sub>50</sub>	860	<i>Menidia beryllina</i>	S (mm)	96 h	LC <sub>50</sub>	1760
Aquatic invertebrates acute	<i>Daphnia pulex</i>	S (n)	24 h	EC <sub>50</sub>	5700	<i>Mysidopsis bahia</i>	S (n)	96 h	EC <sub>50</sub>	480
Aquatic invertebrates chronic	<i>Daphnia magna</i>	F (mm)	21 d	NOEC	510	<i>Mysidopsis bahia</i>	F (mm)	28 d	NOEC	110
Aquatic inverte. chronic	Number embryos <i>Potamopyrgus antipodarum</i>	R (n)	56 d	NOEC	<0.05	-	-	-	-	-
	Number eggs <i>Lymnaea stagnalis</i>	R (TWA)	56 d	NOEC	>117	<i>Ilyanassa obsoleta</i>	-	45d	NOEC	1500
Algae growth inhibition	<i>Navicula pelliculosa</i>	S (TWA)	72 h	EC <sub>50</sub>	1.47	<i>Skeletonema costatum</i>	-	96 h	EC <sub>50</sub>	0.17
			72 h	EC <sub>10</sub>	0.020				NOEC	0.022
Aquatic macrophytes	<i>Lemna gibba</i>	S (m)	14 d	EC <sub>50</sub>	1.65	<i>Ruppia maritima</i>	-	28 d	EC <sub>50</sub>	0.843
				NOEC	0.671					
Fish chronic	Growth / ELS <i>Oncorhynchus mykiss</i>	F (mm)	95 d	NOEC	4	<i>Cyprinodon variegatus</i>	F (mm)	33 d	NOEC	170
Sediment dwelling organisms	<i>Chironomus riparius</i>	spiked water – S	28 d	NOEC	≥30.3 µg/L (>1.2 [mg/kg dw])	<i>Ampelisca abdita</i>	spiked sediment – SS (mm)	10 d	NOEC	44 [mg/kg dw]
Additional marine taxonomic groups										
Coral - Cnidaria						<i>Seriatophora hystrix</i>		10 h	EC <sub>50</sub>	0.7
Coral - Cnidaria						<i>Acropora formosa</i>		10 h	EC <sub>50</sub>	0.9
Ascidia - urochordata (sea squirt)						<i>Ciona intestinalis</i>		24 h	EC <sub>50</sub>	2.11
Echinodermata						<i>Paracentrotus lividus</i>		48 h	EC <sub>50</sub>	6.03

n = nominal; mm = mean measured  
F = flow-through; R = renewal; S = static

Table 3.2-1 Freshwater and seawater aquatic toxicity data cybutryne

Regarding laboratory data, it can be seen from Table 3.2-1 that the sensitivity difference between freshwater and marine species is > 10 for aquatic invertebrates (both acute and chronic), in the chronic fish studies and chronic snail studies. However, given the mode of action of cybutryne and the fact that freshwater and marine primary producers are the most sensitive species and have a similar sensitivity, it is proposed to pool the freshwater and marine ecotoxicity data.

### Acute toxicity

## Toxicity to fish

Acute and chronic effects of cybutryne on fish were investigated both in marine and freshwater species. For the marine species, inland silverside (*Menidia beryllina*), the 96-h LC<sub>50</sub>-value was 1.76 mg active substance /L. And for the freshwater species, rainbow trout (*Oncorhynchus mykiss*), the 96-h LC<sub>50</sub>-value was 0.86 mg active substance /L. This indicates that there is no difference in the sensitivity of fish from the different environments (Table 3.2.1-1). This conclusion is also supported by the additional data from the EU Environmental Quality Standards (EQS) report which are added to the table without further evaluation. The effect of the metabolite GS 26575 was tested with the sheepshead minnow, *Cyprinodon variegatus*. The observed 96-h LC<sub>50</sub>-value of is 11 mg active substance /L

Guideline / test method	Species	Endpoint type of test	Exposure		Results [mg active substance /L]			Remarks	Reference
			Design	Duration	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>		
cybutryne									
<b>Freshwater</b>									
OECD 203	Rainbow trout, <i>Oncorhynchus mykiss</i>	Mortality / acute	Flow-through	96-h	0.58	0.86	1.8	non-GLP freshwater (n)* RI=2	Rufli, 1985 EU CAR
		<i>Oncorhynchus mykiss</i>		Mortality	7-d		25.0		
<b>Marine</b>									
FIFRA 72-3	Inland silverside, <i>Menidia beryllina</i>	Mortality / acute	Static	96-h	1.16	1.76	3.28	GLP saltwater (mm) RI=2	Chandler, 1989 EU CAR
		<i>Fundulus heteroclitus</i>		mortality	96-h		3.22		
GS 26575									
<b>Marine</b>									
FIFRA 72-3	Sheepshead minnow, <i>Cyprinodon variegatus</i>	Mortality / acute	Static	96-h	5.2	11	21	GLP saltwater (mm) RI=1	Cafarella, 1999a EU CAR

n = nominal; mm = mean measured

Table 3.2.1-1 Acute toxicity of cybutryne and its major metabolite GS 26575 to fish

## Toxicity to invertebrates

The 96-h LC<sub>50</sub>-value of cybutryne towards the marine mysid shrimp *Mysidopsis bahia* was 0.48 mg active substance /L (Table 3.2.1-2) which is in the same order of magnitude as the acute toxicity observed for fish. Most other acute LC<sub>50</sub> 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 are available for 3 more marine taxonomic groups mollusks, echinoderms and cnidarians. Acute toxicity L(E)C<sub>50</sub>'s to echinoderms and mollusks 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 0.7 and 0.9 µg/L after 10 hours exposure. In the EQS report the following text is included on cnidaria: "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 (Owen *et al.*, 2002). Effects on isolated zooxanthellae have also been shown by Owen *et al.* (2003). Zooxanthellae isolated from the coral species *M. mirabilis*, *Diploria strigosa* and *Favia framum* were affected after exposure to 2 µg/L cybutryne. No toxicity values related to the cnidarian hosts relevant for the EQS derivation is 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) (Sheikh *et al.*, 2009), and for *M. mirabilis*, Downs and Downs (2007) showed changes in expressions of proteins related to the cnidarian after exposure to 10 µg/L."

The 96-h LC<sub>50</sub>-value of 1.5 mg active substance /L of the metabolite GS 26575 (Table 3.2.1-2) to *M. bahia* was, as for fish, higher than the reported solubility of < 1 mg/L.

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [mg active substance /L]			Remarks	Reference
			Design	Duration	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>		
<b>cybutryne</b>									
<b>Freshwater</b>									
	Crustacea <i>Daphnia magna</i>	Mortality / acute	Static	48-h		8.3		Freshwater (n) RI=2	Okamura et al., 2000b EU CAR
	Crustacea <i>Daphnia magna</i>	mortality		48-h		2.4		RI=1	Vial (1990)
	Crustacea <i>Daphnia pulex</i>	Mortality / acute	Static	24-h		5.7		Freshwater (n) RI=2	Okamura et al., 2000b EU CAR
	Crustacea <i>Daphnia magna</i>	immobilisation		48-h		7.3		RI=2	Fernandez-Alba et al. (2002)
	Crustacea <i>Thamnocepharus platyurus</i>	Mortality / acute	Static	24-h		12.0		Freshwater (n) RI=2	Okamura et al. (2000b)
<b>Marine</b>									
FIFRA 72-3	Mysid shrimp, <i>Mysidopsis bahia</i>	Mortality / acute	Static	96-h	0.22	0.48*	1.0	NCA, GLP saltwater (n) RI=2	Hoberg, 1986 EU CAR
FIFRA 72-3	Eastern oyster	larval development / acute	Static	48-h	0.76	3.2	>6.0	GLP, no saltwater	Surprenant, 1986 (not evaluated)
	Ascidia <i>Ciona intestinalis</i>	embryogenesis		24h		2.11		RI=2	Bellas (2006)
	Cnidaria <i>Acropora formosa</i>	photosynthesis of symbiotic dinoflagellates		10h		0.0009		RI=2	Jones and Kerswell (2003)

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [mg active substance /L]			Remarks	Reference
			Design	Duration	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>		
	Cnidaria <i>Seriatophora hystrix</i>	photosynthesis of symbiotic dinoflagellates		10h		0.0007		RI=2	Jones and Kerswell (2003)
	Crustacea <i>Nitocra spinipes</i>	mortality		96h		4.5		RI=2	Karlsson et al. (2006)
	Crustacea <i>Palaemonetes pugio</i>	larval mortality		96h		1.52		RI=2	Key et al. (2008)
	Crustacea <i>Palaemonetes pugio</i>	adult mortality		96h		2.46		RI=2	Key et al. (2008)
	Crustacea <i>Balanus albicostatus</i>	mortality		48h		0.556		RI=2	Khandeparker et al. (2005)
	Crustacea <i>Artemia salina</i>	mortality		24h		1.62		RI=2	Bakoulia et al. (2002)
	Echinodermata <i>Paracentrotus lividus</i>	embryogenesis		48h		4.02		RI=2	Bellas (2006)
	Echinodermata <i>Paracentrotus lividus</i>	growth		48h		6.03		RI=2	Bellas (2006)
	Mollusca <i>Mytilus edulis</i>	embryogenesis		48h		1.54		RI=2	Bellas (2006)
	Mollusca <i>Ilyanassa obsoleta</i>	adult mortality		96h		3.73		RI=2	Finnegan et al. (2009)
	Mollusca <i>Ilyanassa obsoleta</i>	larval mortality		96h		3.16		RI=2	Finnegan et al. (2009)
	<b>GS 26575</b>								
	<i>Daphnia magna</i>	Mortality acute	/ Static	48-h		11		Freshwater (n) RI=2	Okamura et al., 2000b EU CAR
	<i>Daphnia pulex</i>	Mortality acute	/ Static	24-h		27		Freshwater (n) RI=2	Okamura et al., 2000b EU CAR
	Crustacea <i>Thamnocepharus platyurus</i>	Mortality acute	/ Static	24-h		12		Freshwater (n) RI=2	Okamura et al. (2000b) EU CAR
FIFRA 72-3	Mysid shrimp, <i>Mysidopsis bahia</i>	Mortality acute	/ Static	96-h	0.88	1.5	3.2	GLP, saltwater (mm) RI=1	Cafarella, 1999b EU CAR
	Crustacea <i>Artemia salina</i>	Mortality acute	/ Static	24-h		>40		Saltwater (n) RI=2	Okamura et al. (2000b) EU CAR

n = nominal; mm = mean measured; NCA = not chemically analysed; \* = recalculated value;

Table 3.2.1-2 Acute toxicity of cybutryne and its metabolite to invertebrates

## Growth inhibition of algae and macrophytes

The effects of cybutryne and its metabolite GS 26575 to growth inhibition of algae (Table 4.2.1-5) were investigated in a marine (*Skeletonema costatum*) and a freshwater (*Navicula pelliculosa*) diatom species. Additionally in the EQS report a great number of algal tests were reported, which were added to the dossier without further evaluation. The effects on aquatic macrophytes were tested in two related freshwater species (*Lemna gibba* and *Lemna minor*) (Table 3.2.1-3). The toxicity in algae ranged between EC<sub>50</sub>-values of 0.12 to 12 µg active substance /L (and one outlier LC<sub>50</sub> of 5000 µg/L) as compared to the macrophyte EC<sub>50</sub>-values ranging between 1.65 and 8.1 µg active substance /L. The algae *Navicula pelliculosa* appears to be the most sensitive species. At the lowest test concentration of 0.0756 µg active substance /L 40% effect on growth rate was observed. Further statistical analysis of the mean data indicated a 3 days E<sub>r</sub>C<sub>10</sub> of 0.02 µg active substance /L. Due to the high variability within the control group and also within the solvent control group, it was not possible to derive a NOEC on basis of the raw data. The data suggest that not all replicates started with the same cell count. The cause of the observed variability remains unclear because cell counts were not performed at t=0h, t=24h and t=48h. This causes that the period between 0-72 hours contains a certain lag phase, which cannot be excluded from the data. Thus it is also not possible to conclude that during the 72-96 hours period the growth rate is still exponential. Thus the derived 72 h EC<sub>10</sub> of 0.020 µg/l could still be an underestimation. However, as this value is the lowest, and in the same range as the valid *Skeletonema* study (96 h EC<sub>10</sub> = 0.022 µg/L) it was considered acceptable to use the 72 h EC<sub>10</sub> of 0.020 µg/L for the risk assessment.

Late review of the Buma *et al.* (2009) study made clear that next to the reported E<sub>r</sub>C<sub>50</sub> values in the EQS report also E<sub>r</sub>C<sub>10</sub> values are included, and next to growth rate also photosystem II (PSII) efficiency was tested as effect parameter. The extra E<sub>r</sub>C<sub>10</sub> values for *Thalassiosira weissflogii* (diatom), *Emiliania huxleii* (cocolithophore), *Tetraselmis sp.* (green alga), *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 EC<sub>10</sub> = 0.017 µg/L). At present, however, there is not enough knowledge concerning this effect parameter. For consistency reasons and the late discovery of this observation in literature it was decided to leave this issue for future revision.

The metabolite GS 26575 was less toxic to algae than cybutryne, the marine species (120h-EC<sub>50</sub>-value of 16 µg active substance /L) was considerably more sensitive than the freshwater species (120h-EC<sub>50</sub>-value of 190 µg active substance /L). Please notice that these values are indicative only, as exponential growth could not be determined (see the discussion above).

Guideline / Species test method	Endpoint / type of test	Exposure		Results [µg active substance /L]		Remarks	Reference
		Design	Duration	NOEC	EC <sub>50</sub>		
<b>cybutryne</b>							
<b>Freshwater</b>							

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference
			Design	Duration	NOEC	EC <sub>50</sub>		
	<i>Chlamydomonas intermediata</i>	growth		6 d		0.5	RI=2	Berard et al.(2003)
	freshwater algae <i>Chlorella vulgaris</i>	growth		4 d		1.5	RI=2	Berard et al. (2003)
	<i>Chlorella vulgaris</i>	growth		4 d		1.45	RI=2	Nyström et al. (2002)
	<i>Closterium ehrenbergii</i>	growth		5 d		2.5	RI=2	Okamura et al. (2000b)
	<i>Closterium ehrenbergii</i>	embryogenesis		5 d		3.6	RI=2	Okamura et al. (2000b)
FIFRA 123-2	<i>Navicula pelliculosa</i>	Growth inhibition	Static	120-h 72-h	<0.0756 <b>0.02*</b>	0.0957 1.47	GLP TWA	RI=2 Hughes & Alexander, 1993b EU CAR
	<i>Navicula accomoda</i>	growth		4 d		0.5	RI=2	Berard et al. (2003)
	<i>Navicula accomoda</i>	growth		4 d		0.45	RI=2	Nyström et al. (2002)
	<i>Nitzschia sp.</i>	growth		4 d		0.8	RI=2	Berard et al. (2003)
	<i>Nitzschia sp.</i>	growth		4 d	0.1	0.75	RI=2	Nyström et al. (2002)
	<i>Pseudokirchneriella subcapitata</i>	growth		4 d		3.3	RI=2	Berard et al. (2003)
	<i>Scenedesmus acutus</i>	growth		4 d		5.1	RI=2	Berard et al. (2003)
	<i>Scenedesmus vacuolatus</i>	reproduction		24 h	0.507	5.57	RI=2	Arrhenius et al. (2006)
	<i>Scenedesmus vacuolatus</i>	reproduction		24 h		12.903	RI=2	Neuwoehner et al. (2008)
	<i>Scenedesmus vacuolatus</i>	photosynthesis		24 h		6.072	RI=2	Neuwoehner et al. (2008)
	freshwater algae <i>Selenastrum capricornutum</i>	growth		3 d		10.8	RI=2	Fernandez-Alba et al. (2002)
	<i>Selenastrum capricornutum</i>	growth		3 d		1.6	RI=2	Okamura et al. (2003)
	<i>Selenastrum capricornutum</i>	cell number-area		72 h		1.6	RI=2	Okamura et al. (2000a)
	<i>Selenastrum capricornutum</i>	cell number-growth rate		72 h		2.3	RI=2	Okamura et al. (2000a)
	<i>Staurastrum sebalidii</i>	growth		6 d		2.5	RI=2	Berard et al. (2003)
<b>Marine species</b>								
	<i>Ceramium</i>	growth		7 d		0.96	RI=2	Karlsson et

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference
			Design	Duration	NOEC	EC <sub>50</sub>		
	<i>tenuicorne</i>							al. (2006)
	<i>Chaetocerus gracilis</i>	growth		3 d		1.1	RI=2	Koutsaftis et al. (2006)
	<i>Dunaliella tertiolecta</i>	growth		4 d	0.09	0.73	RI=2	DeLorenzo and Serano (2006)
	<i>Dunaliella tertiolecta</i>	growth		3 d		1.1	RI=2	Gatidou and Thomaidis (2007).
	<i>Eisena bicyclis</i>	growth		4 d	3.2	5.9	RI=2	Okamura et al. (2000b)
	<i>Eisena bicyclis</i>	cell division		7 d	0.32	2.2	RI=2	Okamura et al. (2000b)
	<i>Eisena bicyclis</i>	growth		7 d	1	2	RI=2	Okamura et al. (2000b)
	<i>Eisena bicyclis</i>	growth		7 d	0.32	2.1	RI=2	Okamura et al. (2000b)
	<i>Emiliana huxleyi</i>	Growth rate PSII efficiency		3 d	0.168 0.047	0.406 0.596	RI=2 measured	Buma et al. (2009)
	<i>Emiliana huxleyi</i>	growth		3 d		0.25	RI=2	Devilla et al. (2005)
	<i>Enteromorpha intestinalis</i>	growth		6 d	0.05	0.33	RI=2	Scarlett et al. (1997)
	<i>Enteromorpha intestinalis</i>	photosynthesis		72 h		2.5	RI=2	Scarlett et al. (1997)
	<i>Fibrocapsa japonica</i>	Growth rate PSII efficiency		3 d	0.029 0.018	0.618 0.121	RI=2, Measured	Buma et al. (2009)
	<i>Fucus serratus</i>	zygote germination (area)		72h	8		RI=2	Braithwaite and Fletcher (2005)
	<i>Fucus vesiculosus</i>	fertilization		3 d		0.325	RI=2	Andersson (1995)
	<i>Hormosira banksii</i>	photosynthesis		2 h		0.17	RI=2	Seery et al. (2006)
	<i>Navicula forcipata</i>	Growth		3 d		1.1	RI=2	Gatidou and Thomaidis (2007)
	<i>Porphyra yezoensis</i>	growth		4 d		0.6	RI=2	Okamura et al. (2000b)
	<i>Porphyra yezoensis</i>	lethality		4 d	1500	5000	RI=2	Okamura et al. (2000b)
	<i>Porphyra yezoensis</i>	germination		4 d	1.2	4.1	RI=2	Okamura et al. (2000b)
	<i>Skeletonema costatum</i>	growth		96 h	<b>0.022</b>	0.17	RI=2	Zhang et al. (2008)**

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference
			Design	Duration	NOEC	EC <sub>50</sub>		
FIFRA 123-2	<i>Skeletonema costatum</i>	Growth inhibition	Static	120-h	0.146	0.452	GLP Initial Measured Concentrations	RI=3 Hughes & Alexander, 1993a A7.4.1.3/02
	<i>Tetraselmis sp.</i>	Growth rate PSII efficiency		3 d	0.023 0.017	0.116 0.229	RI=2 measured	Buma et al. (2009)
	<i>Thalassiosira pseudonana</i>	Growth		4 d	0.047	0.27	RI=2	Zhang et al. (2008)**
	<i>Thalassiosira weissflogii</i>	Growth rate PSII efficiency		3 d	0.056 0.032	0.303 0.302	RI=2 measured	Buma et al. (2009)
	cyanobacteria <i>Synechococcus sp.</i>	growth		72 h		0.16	RI=2	Devilla et al. (2005)
<b>GS 26575</b>								
FIFRA 123-2	Freshwater diatom, <i>Navicula pelliculosa</i>	Growth inhibition	Static	120-h 72 h	<77 n.d.	190	GLP (mm)	RI=3 Hoberg, 1999b A7.4.1.3/05
FIFRA 123-2	Marine diatom, <i>Skeletonema costatum</i>	Growth inhibition	Static	120-h 72 h	0.18 n.d.	16	GLP (mm)	RI=3 Hoberg, 1998a A7.4.1.3/04
*: E <sub>10</sub> , C <sub>10</sub> calculated applying probit analysis using Toxrat								
**The values are derived based on results for the exposure concentrations up to 1 $\mu\text{g/l}$ . mm = mean measured								

Table 3.2.1-3 Growth inhibition of algae by cybutryne and its metabolite GS 26575

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference
			Design	Duration	NOEC	EC <sub>50</sub>		
<b>Freshwater</b>								
FIFRA 123-2	Inflated duckweed, <i>Lemna gibba</i>	Growth inhibition	Static	14-d	0.671	1.65	GLP freshwater Lowest measured concentration	RI=1 Hughes & Alexander, 1993e EU CAR
	<i>Lemna gibba</i>	growth		7 d		11	RI=2	Okamura et al. (2000)
	<i>Lemna minor</i>	growth		7 d		8.1	RI=2	Okamura et al. (2000)
<b>Marine</b>								
	<i>Potamogeton pectinatus</i>	dry weight		28 d		6.115	RI=2	Hall et al. (1999a)
	<i>Ruppia maritima</i>	growth		28 d		0.843	RI=2	Hall et al. (1999a)
	<i>Zostera marina</i>	Photosynthetic efficiency	Semi-static	10-d		1.1	Saltwater RI=2 (n)	Chesworth et al., 2004 EU CAR

Guideline / Species test method	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference	
		Design	Duration	NOEC	EC <sub>50</sub>			
	<i>Zostera marina</i>	Photosynthesis and growth		10 d	0.5	2.5	RI=2	Scarlett et al. (1999)

n = nominal

Table 3.2.1-4 Cybutryne: growth inhibition of aquatic macrophytes

## Chronic toxicity

### Toxicity to fish

In chronic testing, hatch and survival of fish larvae were not significantly affected in an Early Life Stage (ELS) test with sheepshead minnow (*C. variegatus*; up to treatment level 330  $\mu\text{g}$  active substance /L) and rainbow trout (*O. mykiss*; up to treatment level 9.1  $\mu\text{g}$  active substance /L). Growth, however, was affected by cybutryne (*C. variegatus*; 33-d NOEC 170  $\mu\text{g}$  active substance /L and *O. mykiss*; 95-d NOEC 4.0  $\mu\text{g}$  active substance /L).

Guideline / Species test method	Endpoint / type of test	Exposure		Results [ $\mu\text{g}$ active substance /L]		Remarks	Reference	
		Design	Duration	NOEC	LOEC			
<b>Freshwater</b>								
FIFRA 72-4	Rainbow trout, <i>Oncorhynchus mykiss</i>	Growth / ELS	Flow-through	95-d	4.0	9.1	GLP freshwater (mm) RI=1	Cohle & Veltri, 1994 EU CAR
<b>Marine</b>								
FIFRA 72-4	Sheepshead minnow, <i>Cyprinodon variegatus</i>	Growth / ELS	Flow-through	33-d	170	330	GLP saltwater (mm) RI=1	Sousa, 2001 EU CAR

ELS = early life stage test; mm = mean measured

Table 3.2.2-1 Chronic toxicity of cybutryne to fish

The potential long-term risk to fish resulting from the exposure to cybutryne residues can be evaluated on basis of the ELS studies. In ELS studies usually more sensitive developmental stages of fish are exposed for longer periods to the test substance, and therefore a prolonged toxicity study with fish is not considered necessary.

### Toxicity to invertebrates

The 28-d NOEC based on growth/reproduction of cybutryne on *Mysidopsis bahia* was 110  $\mu\text{g}$  active substance /L (Table 3.2.2-2), which is of the same magnitude as the chronic toxicity observed for sheepshead minnow (Table 3.2.2-1). Freshwater daphnids are less susceptible within a factor of 5: NOEC<sub>mortality, 30d</sub> was 510  $\mu\text{g}$  active substance /L (Table 3.2.2-2).

Guideline / Species test method	Endpoint / type of test	Exposure	Results [ $\mu\text{g}$ active substance /L]	Remarks	Reference
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			Design	Duration	NOEC	LOEC		
<b>Freshwater</b>								
FIFRA 72-4	<i>D. magna</i>	survival, growth, reproduction	Flow-through	21-d	510	560	GLP (mm) RI=1	Putt, 1999a EU CAR
	<i>Lymnaea peregra</i>	mortality	Semi-static	30-d	≥10		Freshwater (n) RI=2	Morley et al., 2004 EU CAR
	<i>Physa fontinalis</i>	mortality	Semi-static	30-d	≥10		Freshwater (n) RI=2	Morley et al., 2004 EU CAR
OECD 2010	<i>Lymnaea stagnalis</i> *	reproduction	Semi static	56-d	≥117		Freshwater (TWA) RI=1	Habekost, 2010 EU CAR
OECD 2010	<i>Potamopyrgus antipodarum</i> *	reproduction	Semi static	56-d	<0.05		Freshwater (n) verified conc. RI=1	Oehlmann & Ziebart, 2011 EU CAR
<b>Marine</b>								
FIFRA 72-4	Mysid shrimp, <i>Mysidopsis bahia</i>	Growth / reproduction	Flow-through	28-d	110	260	GLP Saltwater (mm) RI=1	Boeri & Ward, 1991 EU CAR
	mollusc <i>Ilyanassa obsoleta</i>	mortality		45 d	1500		RI=2	Finnegan et al. (2009)

n = nominal; mm = mean measured tw = time weighted average; \* = Discussed further in the section on endocrine disruption

Table 3.2.2-2 Chronic toxicity of cybutryne to invertebrates

### Effects on birds

The acute oral toxicity of cybutryne to bobwhite quail (*Colinus virginianus*) was determined in a laboratory test. Birds received a single dose via gavage and were monitored until 14-d post-exposure. No mortalities were observed in the control and dosages up to and including 810 mg active substance /kg bw, whereas 20% of the birds died at the two highest dosages (1350 and 2250 mg active substance /kg bw). The results are summarised in Table 3.2.2-3.

Guideline / test method	Species	Endpoint / type of test	Exposure duration	Results [mg active substance /kg bw]		Remarks	Reference
				LD <sub>0</sub>	LD <sub>50</sub>		
FIFRA 71-1	Bobwhite quail, <i>Colinus virginianus</i>	Mortality / acute oral	14-d post dosing	810	>2250	GLP RI=1	Beavers, 1985a EU CAR

Table 3.2.2-3 Acute effects on birds

In an avian dietary toxicity tests with mallard duck (*Anas platyrhynchos*), birds were fed a diet containing cybutryne for 5 days followed by a 3-day period fed untreated food. Mortality occurred only in the highest treatment group (5620 mg active substance /kg food; 10% mortality) (Table 3.2.2-4). Body weight gain was reduced at concentrations ≥ 1780 mg active

substance /kg food, and food consumption in the first 5 days were reduced at the two highest concentrations (3160 and 5620 mg active substance /kg food).

Guideline / test method	Species	Endpoint / type of test	Exposure duration	Results [mg active substance /kg food]		Remarks	Reference
				LC <sub>0</sub>	LC <sub>50</sub>		
FIFRA 71-2	Mallard duck, <i>Anas platyrhynchos</i>	Mortality / short-term dietary	5-d dosing and 3-d post dosing	1000	>5620	GLP RI=1	Beavers 1985b EU CAR

Table 3.2.2-4 Short-term effects on birds

### Effects on mammals

Cybutryne has a low acute toxic potential. Acute and long-term endpoints from studies of mammalian toxicity were presented and evaluated during the EU assessment. The most sensitive acute study was performed with rats (Kobel, 1984a), which gave an LD50 of >2000 mg/kg bw. The lowest short term studies were performed with rats. Dietary exposure during 28 and 90 days resulted in NOEC values of 100 and 150 mg/kg food, respectively. Effects observed were decreased body gain and food consumption in males and increased haemosiderosis in the spleen at concentrations of 600 and 1000 mg/kg food, respectively.

### Developmental and reproductive toxicity

See section above

### Endocrine Disruption

A higher tier freshwater study was made available at the time of the EU evaluation. 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 00, 0.006, 0.031\*, 0.211 and 1.425\* µg/L (Time weighted average). The endocrine effects of Cybutryne on the freshwater snail *Radix balthica* sampled from the indoor freshwater mesocosms were described in a separate study in more detail (Oehlmann J, B. Watermann (2005)).

Endocrine disrupting effects in *Radix balthica* between controls and cybutryne-treated mesocosms were evaluated using appropriate univariate and multivariate statistical methods. The authors calculated EC<sub>10</sub> –values of the different endpoints. Applying regression analysis a lowest EC<sub>10,nominal</sub> of 0.014 µg/L (TWA) was derived for the parameter spermatogenesis and a EC<sub>10,nominal</sub> of 0.025 µg/L (TWA) at day 60 for the parameter albumen gland hypertrophy.

The authors indicated that it is clear that cybutryne exhibits a strong reproductive toxicity in *Radix* and the specificity of these effects on male reproductive organs, spermatogenesis – in both cases an inhibitory effect – and the stimulating effects on female reproductive organs point to a potential endocrine-mediated effect of cybutryne in *R.balthica*. The authors

therefore concluded that cybutryne (and eventually its main metabolite GS 26575) may induce endocrine effects in snails and other invertebrates

According to the manufacturer of cybutryne the reliability of the studies is considered to be low, and it cannot be concluded that the observed effects were directly caused by cybutryne, let alone that they were the result of endocrine disruption. This does not necessarily imply that cybutryne is incapable of causing a reduction in the reproductive success of these organisms through an endocrine disrupting mode of action, although this seems unlikely on the available evidence, but further work would be needed to demonstrate such effects in a reliable manner.

As a follow up, an additional study was evaluated on chronic reproduction with the freshwater pond snail *Lymnaea stagnalis* which demonstrated that pulmonate molluscs such as *L. stagnalis* are not particularly sensitive to cybutryne.

The pond snail is one of the recommended test species in a detailed review paper on mollusc (partial) life-cycle toxicity testing (OECD, 2010). No effect on growth or on reproduction was observed during the exposure period, demonstrating that pulmonate molluscs such as *L. stagnalis* are not particularly sensitive to Cybutryne.

Furthermore, a chronic reproduction study with the freshwater mudsnail *Potamopyrgus antipodarum* was provided. The mud snail is also a candidate species in a detailed review paper on mollusc (partial) life-cycle toxicity testing (OECD, 2010).

As endpoints mortality and the number of embryos in the brood pouch of females were recorded, distinguishing shelled and unshelled embryos.

From the study it was concluded that cybutryne caused a significant increase of total embryo numbers in all exposure groups after 4 and 8 weeks, including the lowest concentration (0.05 µg/L). Also the number of embryos without shell increased under cybutryne exposure, although this effect was only significant in a concentration window between 0.13 and 0.8 µg/L after 4 weeks and between 0.05 and 0.8 µg/L after 8 weeks. This increase attained factor 4 at 0.13 µg/L after 8 weeks for the total embryo number and was therefore comparable in intensity to the increase observed under exposure to the known xeno-estrogens Bisphenol A and Ethinylestradiol (EE2).

The concentration-response relationship for cybutryne resembled an inverted U as described in several other studies with xeno-estrogens.

However, it must be noted that the test addresses the assessment of hormonally active substances but is not exclusively sensitive to EDCs and is equally suitable for the detection of adverse effects on reproduction mediated via other modes of action. This is in line with the following information given in the discussion of the study report: "there is currently no convincing explanation for the molecular mechanism by which cybutryne may act as a xeno-estrogen in *P. antipodarum*". Furthermore, a no effect concentration (EC<sub>10</sub> or NOEC) was not derived for the endpoints studied, only a LOEC of 0.05 µg/L.

In general it can be concluded that no distinct reproductive effects of cybutryne were observed in the chronic laboratory studies with snail species and in the higher tier mesocosm studies that firmly can be related to an endocrine mode of action of cybutryne. The xeno-estrogenic effects of cybutryne observed, however, are similar to known endocrine disrupters

such as Bisphenol A and Ethinylestra-diol, the molecular mode of action is, however, unknown. There is insufficient evidence to identify cybutryne as an endocrine disrupter, but the information available is considered sufficient to identify cybutryne as 'potential' endocrine disrupter.

It is at present not fully clear what the ecological relevance is of observed effects in snails. It is suggested that an increasing reproduction as induced in the current investigation in the freshwater mudsnail by cybutryne is not beneficial for the population. Estrogenic chemical exposure of females out of the breeding season leads to a stimulation of reproduction, which ultimately may cause a rupture of the oviduct. Furthermore, this stimulation is likely to cause energy shortages in growth, maintenance and reserves. When exposure occurs out of season, offspring will encounter unfavourable circumstances in the outside world (e.g. sub-optimal temperatures, lack of food and hiding places). Estrogenic chemical exposure of females in the breeding season could lead to a reduced reproductive performance, which ultimately reduces the number of offspring during the most favourable time for juvenile growth and survival in the environment. Whether these adverse effects indeed occur under field conditions is unknown.

It should also be kept in mind that invertebrate endocrine systems are different from those in vertebrates. In a 2 generation reproduction study with rats and two development toxicity studies with rats and rabbits no endocrine disruptive effects were observed. It was agreed at the EU evaluation of the substance that more research is needed to be able to conclude on the endocrine disruption properties of the compound.

## Sediment toxicity

### Toxicity to sediment-dwelling organisms

Acute toxicity of cybutryne to sediment dwelling organisms was tested in a spiked-sediment test with a marine amphipod (*Ampelisca abdita*). The NOEC-value was 44 mg active substance /kg dry weight sediment and based on measured concentration in sediment. An EC50 of 0.04 mg active substance /kg dw was determined for a brackish-freshwater amphipod (*Monoporeia affinis*) which showed a reduced burial in sediment when exposed to cybutryne (Table 3.2.5-1).

Guideline / test method	Species	Endpoint / type of test	Exposure		Results [mg active substance /kg Measured concentration]			Remarks	Reference
			Design	Duration	NOEC	LOEC	EC50		
OPPts 850.1735	Marine amphipod, <i>Ampelisca abdita</i>	Mortality / acute	Spiked sediment - semi static	10-d	44	140	*	2 test conc., GLP RI=2 Mean Measured concentration at day 9	Putt, 1999b EU CAR
	Brackish-freshwater amphipod, <i>Monoporeia</i>	Avoidance response (reduced burial in		24h			<b>0.04</b>	RI=2	Eriksson Wiklund et al. (2009)

\* The highest response in the test showed a 40% effect.

Table 3.2.5-1 cybutryne: acute toxicity to sediment dwelling organisms

In a chronic spiked-water test with the freshwater midge *Chironomus riparius*, cybutryne had no effect on mortality, emergence success and development rate. Both concentrations in the water phase and in the sediment were analysed. In the course of the test period, the concentrations in the water decreased from 18 to 3 µg active substance /L and from 100 µg to 12 µg active substance /L, in two of the test concentrations. The concentration in sediment increased to 50 and 200 µg active substance /kg wet sediment by day 7, and to 50. The concentration in sediment increased to 50 µg and 20 µg active substance /kg wet sediment by day 7, and to 50 µg and 240 µg active substance /kg wet sediment by day 28. The number of midges emerging in the cybutryne treatments were not statistically different from the controls. NOEC therefore is  $\geq 30.3$  µg active substance /L ( $\geq 0.240$  µg active substance /kg wet sediment by day 28). Results are given in Table 3.2.5-2.

Guideline / test method	Species	Endpoint / type of test	Exposure		Results		Remarks	Reference
			Design	Duration	NOEC	LOEC		
OECD 219	Midge, <i>Chironomus riparius</i>	Development, Emergence / chronic sediment-water test	Spiked water - static	28-d	$\geq 30.3$ [µg active substance /L] $\geq 0.24$ [mg/kg ww] $\geq 1.2$ [mg/kg dw]	$> 30.3$ [µg active substance /L] $> 0.24$ [mg/kg ww] $> 1.2$ [mg/kg dw]	GLP RI=2 geomean measured concentrations	Luit, 2000 EU CAR
	Midge, <i>Chironomus riparius</i>	Development / emergence		10-d	100 µg active substance /L		RI=3	Desmares-Koopmans (1997)* evaluated by KEMI (1998)

\* study was included in the EQS dossier but considered invalid for PNEC derivation

Table 3.2.5-2 Chronic toxicity of cybutryne to sediment dwelling organisms

### Bioavailability/biomagnification/bioconcentration

Cybutryne did not bioconcentrate in fish (BCF = 250 L/kg) in a OECD 305E test, but the BCF in green macro algae is  $> 5000$  L/kg, indicating that Cybutryne is very bioaccumulative in plants. It is important to notice that only tests with fish (OECD 305) and mussels (ASTM E1022-94 (if available) directly can be used for comparison with the B and vB criteria (ECHA guidance document r11 page 25). BCF values determined in other invertebrates (e.g. algae) should not be used, since they are prone to high uncertainty due to adsorption (ECHA guidance document R 7C). On the other hand also substances adsorbed on e.g. algae may result in bioaccumulation and biomagnification in higher trophic levels. At present no guidance exists on how to test studies on bioaccumulation in invertebrates and plants to the B criterion.

A microcosm study however, showed highest BCF<sub>SS</sub> values of 110 L/kg ww for oyster (*Crassostrea virginica*; suspension feeder) and 307 L/kg ww 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 trigger (max is 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 EPI Suite QSAR estimation the logKow is 2.73 indicating that there is not a potential for bioaccumulation. Cybutryne and metabolite GS 26575 are therefore not regarded as bioaccumulative compounds.

## Food web/ population effects

### Higher-tier tests

In regard of the laboratory test results, higher-tier tests were conducted exposing natural phyto- and zooplankton communities under more realistic conditions for several weeks.

In an extensive outdoor marine microcosm study, fauna and flora naturally present in intertidal sediment and unfiltered, undiluted seawater were treated with cybutryne at a nominal concentration of 100, 200, 400 and 800 ng active substance /L. Three times each week, 30% of the water was replaced with newly collected seawater to simulate a portion of the natural tidal flushing, and to provide an immigration source for phyto- and zooplankton. Cybutryne was reapplied at each water replacement to maintain the cybutryne concentration close to the target concentration. Periphyton and phyto- and zooplankton were regularly sampled during the 10-week test period. Additionally, macrophyte biomass and abundance of macro invertebrates were determined once or twice. Differences in algal functional parameters, community structure, and taxonomic abundance of the major plant and invertebrate taxa between controls and cybutryne-treated microcosms were evaluated using appropriate univariate and multivariate statistical methods. NOEC-values (mean-measured concentrations) of the different groups of organisms are presented in Table 3.2.7-1.

Guideline / test method	Taxa	NOEC [ng active substance /L; Mean measured concentration]	Reference
Marine microcosms (three-four replicates); semi-static; 70 d; 3-times a week, 30% of the microcosm volume was exchanged with fresh medium containing an appropriate amount of cybutryne. Four concentrations (100, 200, 400, and 800 ng active substance /L; nominal values)	Phytoplankton		Hoberg, 2004 EU CAR
	Pigments (chlorophyll)	572	
	Pigments (phaeophytin)	572	RI=2
	Photosynthesis/respiration	288	
	Taxonomic abundance	288	
	Periphyton		
	Pigments (chlorophyll)	572	
	Pigments (phaeophytin)	572	
	Photosynthesis/respiration	572	
	Taxonomic abundance	572	
	Zooplankton	572	
	Eelgrass, <i>Zostera marina</i>	288 <sup>A</sup>	
Marsh grass, <i>Spartina alterniflora</i>	572 <sup>A</sup>		
Macro invertebrates	572 <sup>A</sup>		
Marine, 12 weeks; Biomass and abundance; Pilot study. Only one exposure concentration and two replicates	Plankton, macrophytes and macro-invertebrates	TWA 186,000	Giddings (2002)
Marine, photosynthesis; 21 days	Periphyton	16	Dahl and Blanck (1996)



<sup>A</sup> NOEC based on single event data (biomass at termination of the study).

Table 3.2.7-1 Effects of cybutryne in higher-tier tests

Exposure to cybutryne at concentrations up to nominal 400 ng active substance /L (288 ng active substance /L as mean measured concentration) 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 (single sampling date) and 800 ng active substance /L. Therefore NOEC<sub>microcosm study</sub> is nominal 400 ng active substance /L (288 ng active substance /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 70d test).

The EQS report showed 2 more chronic micro/mesocosm studies with NOECs of 16 ng/L and 186,000 ng/L, respectively.

A higher tier freshwater study was provided for the EU assessment. 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 active substance /L.

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 EC<sub>10</sub> and EC<sub>50</sub>-values, that are based upon nominal and TWA-based concentrations, of the different endpoints are presented in Table 3.2.7-2.

Taxa	Nominal concentration				TWA concentration				Reference
	EC <sub>10</sub> [ng substance (C.I.)	active /L]	EC <sub>50</sub> [ng substance (C.I.)	active /L]	EC <sub>10</sub> [ng substance (C.I.)	active /L]	EC <sub>50</sub> [ng substance (C.I.)	active /L]	
Periphyton									Schmidt et al., 2007 EU CAR
Total biomass (day 9)	60 (2-2490)		310 (60-1640)		40 (1-1620)		190 (30-1110)		
Chlorophytes (day 135)	<b>10 (3.1E-3-18.3E3)</b>		340 (20-1210)		<b>0.5 (4.5E-6-5.74E4)</b>		50 (0.61-4480)		
Zooplankton (day 78)									
Cyclopoid copepods	<b>10 (1-140)</b>		<b>90 (30-240)</b>		<b>2 (0.1 – 40)</b>		<b>20 (5-60)</b>		
Macrophyte biomass (day 150)									
<i>Myriophyllum verticillatum</i>	60 (3-1240)		210 (100-420)		10 (0.00-180)		30 (20-70)		
Filamentous algae	340 (4-2.67E4)		2130 (370-1.2E4)		50 (0.00-9840)		500 (60-4220)		
<i>Potamogeton nodosus</i>	760 (n.a.)		920 (n.a.)		60 (0.00-2.2E4)		140 (20-1060)		
Phytoplankton; 24 d Bray-curtis index	NOEC: 4								Nyström et al. 2002

C.I. = 95% confidence interval  
n.a. = not available

Table 3.2.7-2 Nominal and TWA-based effect concentrations as observed in an indoor freshwater mesocosm study



Both periphyton, zooplankton and macrophytes communities were directly affected by a single application of cybutryne. The periphyton chlorophytes were most susceptible, the lowest EC<sub>10</sub> (nominal) of 0.01 µg/L occurred after 135 days for the chlorophytes (EC<sub>10</sub> TWA 0.0005 µg/L). The EQS reported one more chronic freshwater micro/mesocosm study with phytoplankton deriving a 24 d NOEC of 4 ng/L. Examination of the response of periphyton, phytoplankton, macrophytes, as well as secondary effects to zooplankton, to cybutryne exposure reveals a consistent pattern of effects in the 5 µg/L group and to a lesser extent in the 1 µg/L group. Responses in the two lowest treatment groups (0.04 and 0.2 µg/L) are not generally distinguishable from the control response. Calculated EC<sub>10</sub> and EC<sub>50</sub> values lack statistical precision.

### **Observations of adverse effects in the field/fish kills/strandings/tissue**

No information was found in regards to this section

### **Residues in seafood**

No information was found in this regard and consumption of contaminated food by humans is of low relevance (see section 3.2.9).

### **3.3. Data on the potential for human health effects (including, but not limited to, consumption of affected seafood).**

Cybutryne has a low systemic availability in rats (oral absorption is estimated to be 50%). It is distributed mainly into blood and highly perfused organs, metabolised in seven metabolites, predominately excreted via faeces. In the 7-day repeated dosing oral study no plateau was reached, indicating possible accumulation at (semi-) chronic exposure.

Cybutryne has low acute toxicity profile, is not a skin or eye irritant; it is a skin sensitiser. Cybutryne is considered to be non-genotoxic and non-carcinogenic.

The critical NOAEL was set at 15 mg/kg bw/day based on effects in dams and foetues in rabbit teratogenicity study of Becker, Pöss-neckerand Flade, 2006 (effects at the LOAEL 45 mg/kg bw/d: dams, reduced food consumption and body weight loss; foetuses, reduced body weight and increased post implantation loss). A safety factor of 100 for inter- and intra-species variation was considered sufficient for the calculation of the Acceptable Exposure Level (AEL) of cybutryne during the EU assessment as a biocide, adjusted for the oral absorption. AEL for short, medium and long term was estimated to be 0.08 mg/kg bw/d.

An Acceptable Daily Intake (ADI) was derived using the critical NOAEL of 15 mg/kg bw/d and an assessment factor of 100, resulting in an ADI of 0.15 mg/kg bw/day. Since no acute toxicity effects were observed, the acute reference dose (ARfD) was not required for the risk assessment of cybutryne used in biocidal products.

Exposure to cybutryne can occur during production and use of the antifouling paint by professionals only. The main routes of exposure are by inhalation and by dermal exposure.

The internal dose after dermal and inhalation exposure was calculated taking into the dermal absorption of 5% in human skin and the default value for inhalation absorption of 100%.

According to the exposure and risk assessment carried out during the EU evaluation, professional user's exposure to antifouling paint containing 2.3 % cybutryne may lead to adverse health effects when no PPE is used. However, when using gloves and double coverall for spray painting or gloves and impermeable coverall for brushing and rolling, paint stripping and for pot men/ancillary worker, no adverse health effects are expected. For the grit filler, a risk index of 1.03 was identified for the exposure calculations despite the application of gloves and impermeable coverall. It is to be considered that the exposure estimations were based on worst-case approaches and assumptions, hence a slight exceedance of the risk should be attributed to the worst-case exposure calculations. Thus, it can be concluded that no adverse health effects are expected for the protected (gloves and impermeable coverall) grit filler.

Access of unauthorised personal to professional shipyards was considered to be unlikely, hence specific bystander exposure scenario was not included in the biocides assessment during the EU evaluation. To keep unauthorised persons from entering the treatment area, the product label should carry the phrase "Unprotected persons should be kept out of treatment areas".

Based on the environmental exposure assessment, consumer exposure via food/drinking water was not considered relevant. Relevant residues are not expected in matrices for human consumption. Nonetheless, a reverse reference scenario was performed to calculate the amount of fresh fish eaten by a person every day of his life before filling up the ADI.

Using a ADI of 0,15 mg/kg bw/d, a default body weight of 60 kg for a person, a person can be exposed to 9 mg cybutryne a day over a lifetime without presenting an appreciable risk to health. According to the Predicted Environmental Concentration for predators as specified in the EU CAR of 52.6 µg active substance /kg<sub>wet fish</sub> and using a reverse reference scenario, one could eat  $9000/52.6 = 171$  kg wet fish a day for a lifetime without appreciable risk to health.

This value is considered to be worst-case for shellfish as the value for wet fish is considered for a fish containing 5% fat (in which cybutryne could potentially accumulate).

## Annex I. Full data set on monitoring information

Country	Port	Port Area/	Port Type	Concentration	Concentration	GS26575	Author	Journal
Singapore	Sembawang Park	Sy, P		3800			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Punggol	P, J, M		3300			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Pasir Ris	Sy		2800			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Changi	M, J		4000			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Off Pulau Tekong	SL		below detectio			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	East Coast	–		3600			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Off East Coast	–		below detectio			<a href="#">Basheer et al</a>	Marine Pollution
Singapore	Off Marina	P		below detectio			<a href="#">Basheer et al</a>	Marine Pollution
Iran	Jalali marina		Small boats	nd			<a href="#">Saleh et al</a>	Marine Pollution

Iran	Jalali marina		Dhows moorin	63.4 ± 16		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Open seawater 1 (Persian Gulf)		Nearshore	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Open seawater 1 (Persian Gulf)		Offshore	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Bandargah marina		Small boats	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Bandargah marina		Dhows moorin	11.9 ± 1.5		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Open seawater 2 (Persian Gulf)		Nearshore	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Open seawater 2 (Persian Gulf)		Offshore	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Jofreh marina		Small boats	13.1 ± 2.3		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Jofreh marina		Dhows moorin	17.2 ± 1.9		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Jabri marina		Small boats	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Bushehr port internal canal		Anchor age 2	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Sadra Ship building factory		Ship buildin	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Sadra Ship building factory		Repairing yard	nd		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Solhabad marina		Dhows moorin	10.9 ± 0.4		<a href="#">Saleh et al</a>	Marine Pollution
Iran	Bushehr port internal canal		Middle of the	nd		<a href="#">Saleh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Bawe Island	near Touris	1.54		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Bawe Island 2	near Touris	1.95		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Bwawani area 1	near Shippin	3.64		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Bwawani area 2	near Shippin	6.71		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Bwawani area 3	near Shippin	15.44		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Chapwani Island	near Touris	1.35		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Chapwani Island	near Touris	1.68		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Chumbe Island 1	near Touris	2.33		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Chumbe Island 2	near Touris	1.66		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Malindi harbour	near Cargo	5.14		<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Malindi harbour	Passenger	5.8		<a href="#">Sheikh et al</a>	Marine Pollution

United Republic	Zanzibar coral	Malindi harbour	near Shippin	5.55			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Kizimkazi	near Touris	6.46			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Kwale	near Touris	3.87			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Mnemba 1	near Touris	5.24			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Mnemba 2	near Touris	2.06			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Mtoni port 1	near Local	3.5			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Mtoni port 2	near Local	7.22			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Mtoni port 3	near Local	5.39			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Muroga Reef 1	near Touris	2.4			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Muroga Reef 2	near Touris	4.12			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Nyage 1	near Touris	3.08			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Nyage 2	near Touris	3.04			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Pange	near Touris	1.77			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Sheni 1	near Touris	2.76			<a href="#">Sheikh et al</a>	Marine Pollution
United Republic	Zanzibar coral	Sheni 2	near Touris	3.08			<a href="#">Sheikh et al</a>	Marine Pollution
United States,	Oahu	Ala Wai Marina 1		n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 2		29.3			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 3		31.1			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 4		29.7			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 5		58.6			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 6		55.9			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 7		n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 8		57.1			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 9		n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Ala Wai Marina 10		n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 1		42.7			<a href="#">Knutson et al</a>	Ecotoxicology

United States,	Oahu	Kaneohe Bay Yacht Club 2	34.8			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 3	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 4	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 5	67.4			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 6	25.4			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 7	57			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 8	116			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 9	52			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Yacht Club 10	45			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	17.8			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	46			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	202			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	131			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	157			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	283			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	152			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kaneohe Bay Makani Kai Marina	32			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 1	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 2	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 3	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 4	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 5	19			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 6	18.2			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 7	94			<a href="#">Knutson et al</a>	Ecotoxicology

United States,	Oahu	Sand Island Keehei Marina 8	23			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 9	25			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Sand Island Keehei Marina 10	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 1	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 2	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 3	38			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 4	114			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 5	146			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 6	66			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 7	80			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 8	59			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 9	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Waikiki Yacht Club 10	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 1	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 2	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 3	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 4	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 5	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 6	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 7	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 8	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 9	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Kewalo Marina 10	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 1	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 2	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 3	n/d			<a href="#">Knutson et al</a>	Ecotoxicology



United States,	Oahu	Heeia Kea Marina 4	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 5	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 6	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 7	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 8	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 9	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Heeia Kea Marina 10	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Coral reef reference site near	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Coral reef reference site near	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States,	Oahu	Coral reef reference site near	n/d			<a href="#">Knutson et al</a>	Ecotoxicology
United States	Pier 39 Marina 1		3.02		4.06	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Pier 39 Marina 2		3.05		1.43	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Pier 39 Marina 3		3.11		1.35	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Pier 39 Marina 4 (reference)		0.93		0.97	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Ballena Isle Marina 1		156		46.9	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Ballena Isle Marina 2		15.2		5.81	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Ballena Isle Marina 3		10.2		5.29	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Ballena Isle Marina 4 (reference)		3.26		1.55	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alameda Marina 1		40.1		12.4	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alameda Marina 2		29.4		8.74	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alameda Marina 3		33.4		11.1	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alameda Marina 4 (reference)		17.7		8.38	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Berkeley Marina 1		84.3		27.5	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Berkeley Marina 2		28.7		9.17	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Berkeley Marina 3		25.2		7.73	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Berkeley Marina 4 (reference)		1.7		1.11	<a href="#">Hall Jr. et al</a>	Marine Pollution

United States	Sausalito Yacht Harbor 1		12.5		3.45	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Sausalito Yacht Harbor 2		44.4		10.4	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Sausalito Yacht Harbor 3		49.4		13.1	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Sausalito Yacht Harbor 4 (reference)		2.02		0.83	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 1		90.6		38.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 2		83		33.6	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 3		73.3		28.6	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 4		77.1		30	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 5		59.6		24.1	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 6		49.8		23.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marina Del Rey 7 (reference)		2.82		1.58	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Kings Harbor 1		214		52.7	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Kings Harbor 2		273		64.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Kings Harbor 3		339		74.4	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Kings Harbor 4 (reference)		1.45		0.78	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Watchorn Basin 1		23.8		16.8	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Watchorn Basin 2		28.6		10.6	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Watchorn Basin 3		34		13.3	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Queensway Bay 2		65.4		22.3	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Queensway Bay 3		40.8		14.1	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Queensway Bay 4 (reference)		1.71		0.84	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alamitos Harbor Marina 1		4.92		1.6	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alamitos Harbor Marina 2		4.55		1.26	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alamitos Harbor Marina 3		1.45		ND	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Alamitos Harbor Marina 4 (reference)		8.35		1.92	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 1		58.8		17	<a href="#">Hall Jr. et al</a>	Marine Pollution

United States	Shelter Island 2			42.3		10.9	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 3			75.8		18.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 4			33.3		10.4	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 5			26		7.86	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 6			22.5		6.21	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Shelter Island 7 (reference)			0.62		ND	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Harbor Island Marina 1			35.9		11	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Harbor Island Marina 2			48.1		13.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Harbor Island Marina 3			10.3		3.7	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Harbor Island Marina 4 (reference)			6.44		1.9	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marriott San Diego 1			31.9		9.76	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marriott San Diego 2			39		10.7	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marriott San Diego 3			39.8		14.2	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Marriott San Diego 4 (reference)			7.14		2.25	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Chula Vista Harbor 1			50.6		18.5	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Chula Vista Harbor 2			42.7		15.5	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Chula Vista Harbor 3			26.4		13.1	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Chula Vista Harbor 4 (reference)			8.08		5.22	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Coronado Cay Marina 1			25.1		11.5	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Coronado Cay Marina 2			43.4		14.7	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Coronado Cay Marina 3			29.4		12.7	<a href="#">Hall Jr. et al</a>	Marine Pollution
United States	Coronado Cay Marina 4 (reference)			7.99		5.77	<a href="#">Hall Jr. et al</a>	Marine Pollution
Malaysia	Kemaman	KM1	Fishing and	391			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Kemaman	KM2	Fishing and	624			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Kemaman	KM3	Fishing and	622			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Kemaman	KM4	Fishing and	686			<a href="#">Ali et al</a>	Marine Pollution

Malaysia	Kemaman	KM5	Commercial/p	846			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KNP1	Oil tanks	932			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KNP2	Cargo area	515			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KNP3	Cargo area	835			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KNP4	Cargo area	802			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KNP5	Cargo area	6			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KSP1	Passengers	845			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KSP2	Flour contain	668			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KSP3	Flour contain	690			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KSP4	Flour contain	752			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KSP5	Tourists and	858			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KWP1	Commercial	783			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KWP2	Commercial	1115			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KWP3	Commercial	44			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KWP4	Commercial	1277			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Klang Port	KWP5	Commercial,	2021			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG1	Local fishing	15			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG2	Coast guard	1397			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG3	Commercial	641			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG4	Commercial,	827			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG5	Pilot parking	563			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG6	Main port/lon	856			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG7	Oil tanks	554			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Pasir Gudang	PSG8	Agriculture and	788			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Bidong Island	PB1	Open sea	8			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Bidong Island	PB2	UMT marine	55			<a href="#">Ali et al</a>	Marine Pollution

Malaysia	Bidong Island	PB4	Fishing	29			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Bidong Island	PB5	Residential	71			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Redang Island	PR1	Marine park,	351			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Redang Island	PR2	Jetty, leisure	321			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Redang Island	PR3	Turtle bay	471			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Redang Island	PR4	Residential	597			<a href="#">Ali et al</a>	Marine Pollution
Malaysia	Redang Island	PR5	Leisure and	1370			<a href="#">Ali et al</a>	Marine Pollution
United States	Dana Point	D1	Marina	138			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Dana Point	D2	Marina	244			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Dana Point	D3	Marina	304			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Dana Point	D4	Marina	151			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Dana Point	D5	Marina	254			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Oceanside Harbor	O1	Marina	23			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Oceanside Harbor	O2	Marina	64			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Mission Bay	M1	one of the	8			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Mission Bay	M2	one of the	3			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	Mission Bay	M3	one of the	7			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S1	Deep water	15			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S2	Deep water	18			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S3	Deep water	8			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S4	Deep water	61			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S5	Deep water	28			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S6	Deep water	15			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S7	Deep water	27			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S8	Deep water	10			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S9	Deep water	17			<a href="#">Shapoznikova et al</a>	Marine Pollution

United States	San Diego	S10	Deep water	34			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S11	Deep water	23			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S12	Deep water	40			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S13	Deep water	29			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S14	Deep water	71			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S15	Deep water	36			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S16	Deep water	23			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S17	Deep water	42			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S18	Deep water	25			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S19	Deep water	1			<a href="#">Shapoznikova et al</a>	Marine Pollution
United States	San Diego	S20	Deep water	30			<a href="#">Shapoznikova et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Puerto Del	9.33			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Puerto Del	22			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Puerto Del	9.67			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Villa Marina	26.33			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Villa Marina	25.33			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Fajardo, Puerto	Puerto Rico—	Villa Marina	32.67			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	San Juan	<1			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	San Juan	5			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	San Juan	2.5			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	Club Nautico	2.67			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	Club Nautico	17.67			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	Cangrejos	1			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	San Juan Metro,	Puerto Rico—	Cangrejos	<1			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Lajas, Puerto	Puerto Rico—	Club Nautico	3.33			<a href="#">Carbery et al</a>	Marine Pollution
<u>United States</u>	Lajas, Puerto	Puerto Rico—	Varadero	<1			<a href="#">Carbery et al</a>	Marine Pollution

<a href="#">United States</a>	Ponce, Puerto	Puerto Rico—	Ponce Fishing	<1			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	Guayama, Puerto	Puerto Rico—	Club Nautico	3.67			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	Boqeron, Puerto	Puerto Rico—	Club Nautico	20			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	American	13			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	American	61			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	Benner Bay	281.67			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	Charlotte	3.67			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	Charlotte	5			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	Benner Bay	736.33			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. Thomas,	USVI—	Benner Bay	825			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Coral Bay	19			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Coral Bay	7			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Coral Bay	6			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Coral Bay	2			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Cruz Bay	9			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	St. John, US Virgin	USVI—	Cruz Bay	3.5			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	Shelf Edgs,	Oceanic Referen	Shelf Edge	<1			<a href="#">Carbery et al</a>	Marine Pollution
<a href="#">United States</a>	CATS, Referenc	Oceanic Referen	Caribbean	<1			<a href="#">Carbery et al</a>	Marine Pollution
United States	Florida Keys	Key Largo,	coastal	12.2 (1.5)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Key Largo –	waterway	144.2 (16.2)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Tavernier Key,	marina	10.6 (0.2)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Marathon Key,	marina	99.7 (8.6)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Ohio Key,	marina	29.1 (3.9)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Key West,	marina	94.4 (5.9)			<a href="#">Owen et al</a>	Marine Pollution
United States	Florida Keys	Key West,	marina	60.0 (0.7)			<a href="#">Owen et al</a>	Marine Pollution
United States	St. Croix	Gallow's Bay,	harbour	90.3			<a href="#">Owen et al</a>	Marine Pollution



United States	Bermuda	Inner Hamilton	harbour	234.7 (11.3)			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Inner Hamilton	harbour	37.4 (1.4)			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Inner Hamilton	harbour	206.1 (4.9)			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Mill's Creek,	harbour	77			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Mill's Creek,	harbour	75.6 (0.7)			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Ely's Harbour,	harbour	294			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	HogFish Beacon,	coastal	3.1			<a href="#">Owen et al</a>	Marine Pollution
United States	Bermuda	Hogfish Beacon,	coastal	17.5			<a href="#">Owen et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok1	Fishery harbour	14			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok2	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok3	Fishery harbour	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok4	Fishery harbour	34			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok5	Marina	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok6	Fishery harbour	143			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok7	Fishery harbour	20			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok8	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok9	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok10	Marina	19			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok11	Fishery harbour	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Okayama prefectur	Ok12	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H1	Port	13			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H2	Fishery harbour	13			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H3	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H4	Fishery harbour	63			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H5	Marina	59			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H6	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution

Japan	Hiroshima	H7	Fishery harbour	41			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H8	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H9	Fishery harbour	68			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H10	Marina	27			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H11	Fishery harbour	15			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H12	Fishery harbour	10			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H13	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H14	Fishery harbour	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H15	Fishery harbour	39			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H16	Port	19			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H17	Fishery harbour	148			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H18	Fishery harbour	16			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H19	Fishery harbour	42			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H20	Fishery harbour	22			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H21	Marina	18			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H22	Port	118			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H23	Fishery harbour	17			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H24	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H25	Fishery harbour	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H26	Marina	32			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H26'	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H27	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H28	Marina	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H29	Marina	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H30	Marina	29			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H31	Marina	22			<a href="#">Okamura et al</a>	Marine Pollution

Japan	Hiroshima	H32	Marina	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H33	Fishery harbour	10			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H34	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H35	Fishery harbour	93			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H36	Fishery harbour	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H37	Fishery harbour	72			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hiroshima	H38	Port	45			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y1	Marina	117			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y2	Fishery harbour	157			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y3	Fishery harbour	107			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y4	Marina	144			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y5	Fishery harbour	33			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y6	Fishery harbour	25			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y7	Fishery harbour	54			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y8	Fishery harbour	34			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y9	Fishery harbour	38			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y10	Fishery harbour	55			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y11	Port	20			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y12	Fishery harbour	14			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Yamaguchi	Y13	Fishery harbour	38			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Fukuoka prefecture	F1	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Fukuoka prefecture	F2	Marina	26			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Fukuoka prefecture	F3	Marina	37			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ooita prefecture	Ot1	Marina	18			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ooita prefecture	Ot2	Fishery harbour	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ooita prefecture	Ot3	Fishery harbour	10			<a href="#">Okamura et al</a>	Marine Pollution

Japan	Ooita prefectur	Ot4	Port	14			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ooita prefectur	Ot5	Fishery harbou	41			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ooita prefectur	Ot6	Fishery harbou	111			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E1	Fishery harbou	12			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E2	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E3	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E4	Fishery harbou	18			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E5	Fishery harbou	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E6	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E7	Fishery harbou	75			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E8	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E9	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E10	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E11	Port	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E12	Fishery harbou	11			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Ehime prefectur	E13	Fishery harbou	10			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K1	Fishery harbou	41			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K2	Port	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K3	Port	10			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K4	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K5	Marina	48			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Kagawa prefectur	K6	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Tokushim a	T1	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Tokushim a	T2	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Tokushim a	T3	Fishery harbou	21			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy1	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution

Japan	Hyogo prefectur	Hy2	Fishery harbou	97			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy3	Fishery harbou	13			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy4	Fishery harbou	29			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy5	Fishery harbou	105			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy6	Fishery harbou	14			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy7	Fishery harbou	262			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy8	Marina	27			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Hyogo prefectur	Hy9	Marina	16			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Osaka prefectur	Os1	Fishery harbou	10			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Osaka prefectur	Os2	Marina	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Osaka prefectur	Os3	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Osaka prefectur	Os4	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Osaka prefectur	Os5	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W1	Port	21			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W2	Fishery harbou	16			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W3	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W4	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W5	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W6	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W7	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W8	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W9	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W10	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W11	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Wakaya ma	W12	Port	136			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi1	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution

Japan	Mie prefectur	Mi2	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi3	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi4	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi5	Fishery harbou	78			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi6	Fishery harbou	34			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi7	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi8	Fishery harbou	ND			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi9	Port	16			<a href="#">Okamura et al</a>	Marine Pollution
Japan	Mie prefectur	Mi10	Fishery harbou	18			<a href="#">Okamura et al</a>	Marine Pollution
ROK	Masan	M1	Major (Industrial Bay	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Masan	M2	Major (Industrial Bay	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Masan	M3	Major (Industrial Bay	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Masan	M4	Major (Industrial Bay	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Masan	M5	Major (Industrial Bay	2.1			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Haengam	H1	Major Bay (Small scale shipyards	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Haengam	H2	Major Bay (Small scale shipyards	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Haengam	H3	Major Bay (Small scale shipyards	1			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Haengam	H4	Major Bay (Small scale shipyards	1.3			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Haengam	H5	Major Bay (Small scale shipyards	2.7			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G1	Major Bay (A large scale shipyard)	1.2			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G2	Major Bay (A large scale shipyard)	1.5			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G3	Major Bay (A large scale shipyard)	7.4			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G4	Major Bay (A large scale shipyard)	6.2			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G5	Major Bay (A large scale shipyard)	2.5			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gohyun	G6	Major Bay (A large scale shipyard)	3.1			<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Jinhae Bay	J1	Fishing Port (Inside of ports for small	ND			<a href="#">N.S. Kim et al.</a>	Marine Pollution

ROK	Jinhae Bay	J2	Fishing Port (Inside of ports for small	11.5		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Jinhae Bay	J3	Fishing Port (Inside of ports for small	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Jinhae Bay	J4	Fishing Port (Inside of ports for small	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Jinhae Bay	J5	Fishing Port (Inside of ports for small	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Jinhae Bay	J6	Fishing Port (Inside of ports for small	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Jinhae Bay	J7	Fishing Port (Inside of ports for small	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Incheon	IC1	Harbor and ferry boats	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Kunsan	KS2	Harbor and ferry boats	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Mokpo	MP3	Harbor and ferry boats	2.9		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Yeosu	YS4	Harbor			ND	<a href="#">N.S. Kim et al.</a>	Marine Pollution
ROK	Gwangyang	GY5	Container port	ND		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Tongyeong	TY6	Harbor and shipyards	1.1		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Busan	BS7	Harbor and shipyards	3.5		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Ulsan	US8	Harbor and shipyards and	1.9		<a href="#">N.S. Kim et al.</a>	Marine Pollution	
ROK	Sokcho	SC9	Harbor			ND	<a href="#">N.S. Kim et al.</a>	Marine Pollution
Japan	Otsuchi Bay	A 1	shipyard			2.3 µg kg <sup>-1</sup>	<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	A 2	small fishing port	0.13 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 3	small fishing port	0.05 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 4	small fishing port	0.08 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 5	small fishing port	0.16 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 6	small fishing port	0.08 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 7	small fishing port	0.09 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 8	small fishing port	0.06 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 9	small fishing port	0.08 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 10	small fishing port	<0.05 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	
Japan	Otsuchi Bay	A 11	small fishing port	0.13µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ	



Japan	Otsuchi Bay	A 12	small fishing port	0.08 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	A 13	small fishing port	0.11 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	A 14	small fishing port	0.87 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	A 15	small fishing port	21 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 1	close to shipyard	0.15 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 2	close to shipyard	0.24 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 3	close to shipyard	0.45 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 4	close to shipyard	1.9 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 5	close to shipyard	5.5 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 6	close to shipyard	0.43 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 7	close to shipyard	0.52 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 8	close to shipyard	2.6 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 9	next to shipyard	100 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 10	close to shipyard	0.55 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 11	close to shipyard	5.8 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 12	close to shipyard	0.18 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 13	close to shipyard	4.2 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 14	close to shipyard	0.31 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 15	close to shipyard	2.8 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 16	close to shipyard	0.21 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ
Japan	Otsuchi Bay	B 17	close to shipyard	0.41 µg kg <sup>-1</sup>		<a href="#">Harino et al</a>	Arch Environ