# Venezia2021

# Programma di ricerca scientifica per una laguna “regolata”

**Linea 2.3**

***Contaminanti emergenti in laguna esposizione ed effetti***

D2.3.4.2

*Report sulla derivazione dei valori di PNEC*

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# Indice

## Sommario

La caratterizzazione dello stato di contaminazione dell'ambiente lagunare non può prescindere dal considerare, oltre alle sostanze prioritarie monitorate ai sensi della Direttiva 2013/39/CE, anche i contaminanti emergenti. Tra i contaminanti emergenti ritroviamo alcuni erbicidi e pesticidi (ad esempio, glifosato e neonicotinoidi), prodotti farmaceutici (come ormoni e antibiotici) e per la cosmesi, sostanze perfluoroalchiliche (PFAS), composti industriali (ad esempio, bisfenolo A) e microplastiche. Nonostante i contaminanti emergenti siano stati oggetto di interesse e di approfondimenti scientifici in tempi relativamente recenti, per la laguna di Venezia non è ancora disponibile un censimento delle loro concentrazioni e, tantomeno, una caratterizzazione del rischio ecologico ad essi potenzialmente associato.

All'interno del progetto di ricerca Venezia2021, la Linea 2.3 si prefigge di colmare questa lacuna tramite un approfondimento dello stato di contaminazione delle acque e dei sedimenti a opera degli inquinanti emergenti. Con il fine ultimo di creare una Watch List per la laguna di Venezia utile a definire futuri programmi di monitoraggio della qualità dell'ambiente lagunare, nella Linea 2.3 l'attività del WP2.3.4 "Analisi di rischio" prevede una valutazione del rischio ecologico tramite un'analisi di rischio di *screening* per i contaminanti emergenti identificati e quantificati nei WP2.3.1 "Valutazione della rilevanza del problema e sviluppo del modello concettuale" e 2.3.2 "Caratterizzazione dell'esposizione".

Nello specifico, il WP2.3.4 prevede una prima fase di caratterizzazione dell'esposizione con la stima, attraverso l'integrazione di approcci sperimentali e modellistici, dei valori di concentrazione a cui sono esposti gli organismi acquatici (valori di Predicted Environmental Concentration, PEC). Nella fase di valutazione del rischio, tali valori verranno poi confrontati con la soglia di concentrazione ambientale al di sotto della quale non sono attesi effetti avversi sull'ecosistema (Predicted No Effect Concentration, PNEC).

Il presente lavoro si inserisce tra gli obiettivi del WP2.3.4, andando a stimare i valori di PNEC per i contaminante emergenti selezionati durante la prima fase progettuale. Nella presente deliverable si è proceduto alla determinazione dei valori di PNEC seguendo linee guida internazionali. A tal fine, i dati ecotossicologici disponibili in letteratura e in database internazionali raccolti nel database corrispondente alla Milestone M2.3.1.1 "Completamento della raccolta e revisione dei dati ecotossicologici", compilato nel 2020 e recentemente aggiornato, sono stati integrati con i risultati dei test ecotossicologici condotti nell'ambito del WP2.3.3 "Caratterizzazione degli effetti dei contaminanti emergenti" su singoli contaminanti in condizioni controllare (che saranno presentati nella deliverable D2.3.3.1 "Test di tossicità su matrici ambientali contaminate con concentrazioni note di contaminanti emergenti").

La derivazione dei valori di PNEC è stata effettuata seguendo le linee guida per l'implementazione del regolamento REACH No 1907/2006 (ECHA, 2008), che prevedono due possibili approcci: un approccio deterministico attraverso l'applicazione di appropriati "Assessment Factors" (Fattori di valutazione) al dato ecotossicologico più stringente oppure un approccio probabilistico tramite la costruzione di Curve di Sensibilità delle Specie (Species Sensitivity Distribution, SSD) nel caso in cui sia disponibile un numero adeguato di dati per più gruppi trofici. Inoltre sono state prese in considerazione le linee guida promosse dalla Commissione Europea per la derivazione degli Standard di Qualità Ambientale (Environmental Quality Standard, EQS) nell'ambito della Direttiva Quadro Acque, e più in generale gli approcci recenti all'analisi di rischio ecologico per ambienti acquatici disponibili nel contesto internazionale.

La derivazione dei valori di PNEC servirà successivamente nel WP2.3.4 alla stima del rischio ecologico per l'ambiente lagunare che verrà effettuata attraverso il calcolo di un Hazard Quotient dato dal rapporto PEC/PNEC.

# 1. Introduction

Understanding the occurrence and behaviour of emerging contaminants in the Venice lagoon is of crucial importance to complements the knowledge about priority substances and achieve a comprehensive evaluation of environmental quality status. Emerging contaminants include herbicides and pesticides (e.g., glyphosate, pyrethroids, neonicotinoids), pharmaceuticals and cosmetics, perfluorinated alkylated substances (PFAS), bisphenol A, and microplastics. Environmental risks associated to these substances can be mainly linked to their ability of generating long-term effects on aquatic species, individually and in mixture. To date, an inventory of the occurrence of emerging contaminants in the Venice Lagoon has never been conducted. A risk characterization for these substances appears then fundamental.

The specific objectives of Line 2.3 are to deepen the knowledge regarding the contamination status of water and sediments in the Venice lagoon due to the presence of emerging contaminants, and to investigate the distribution and fate of these contaminants in the water environment of the lagoon, by integrating experimental and modelling approaches. The ultimate goal is the formulation of a "Watch List" for the Venice lagoon, consisting of emerging substances that will be identified through the assessment of their ecotoxicological effects and associated environmental risks. This result can support the development of future environmental monitoring plans for the Venice Lagoon.

Contaminants to be included in the Watch List for the Venice lagoon will be selected via an ecological screening risk analysis, where the predicted environmental concentrations (PEC) of such contaminants in the environmental matrices will be compared with the predicted no effect concentrations (PNECs). Within Line 2.3, WP2.3.4 works in this direction by estimating: i) the concentrations of emerging contaminants to which lagoon aquatic organisms are exposed (i.e. PEC values), through the integration of modelling approaches and experimental data, ii) the concentration of such contaminants at which there are no negative effects for the aquatic organisms (i.e. PNEC values), by using both the experimental results of WP2.3.3 and data available in the literature, and finally iii) the ecological risks posed by emerging contaminants by integrating PEC and PNEC values.

The second objective of WP2.3.4, that is the derivation of PNEC values for the contaminants of interest, is the focus of this deliverable. To this end, ecotoxicological data previously collected and described in Milestone 2.3.3.1 were updated to incorporate the most recent findings on acute and chronic results for marine and estuarine organisms. Where possible, ecotoxicity data available in literature and public databases were integrated with experimental results of bioassays on copepods and bivalves presented in Deliverable 2.3.3.1.

In compliance with the REACH Regulation No 1907/2006, depending on the adequacy and completeness of the ecotoxicity databases, the appropriate Assessment Factor (AF) was selected to be applied to the most sensitive ecotoxicity data in order to deterministically derive PNEC values. When data were particularly abundant, it was possible to probabilistically calculate PNECs with a Species Sensitivity Distribution analysis (SSD). Two guidance documents (ECHA, 2008; 2011) assisted in the correct interpretation of the REACH requirements while the guideline on the derivation of Environmental Quality Standards (EQS) under the Water Framework Directive (Guidance Document No. 27, 2018) and other international guidelines supported the evaluation of ecotoxicological data. Pragmatic choices based on expert judgement were made for unconventional situations.

The PNEC value calculated in this work for each emerging contaminant, when compared with the corresponding PEC value derived in Task 2.3.4.B, would serve to fulfil the third objective of WP2.3.4, that is the determination of the risk posed by these chemicals to the aquatic ecosystem of the Venice Lagoon.

# 2. Methodology

## 2.1 Data sources and criteria for data search and selection

Water ecotoxicity data for marine and estuarine organisms were collected from different existing databases and peer-reviewed literature as described in Milestone M2.3.1.1 (delivered in April 2020) and hereafter briefly summarised. The search started in early 2019 and was regularly updated until February 2022.

Database searches were carried out on:

- the OPP Pesticide Ecotoxicity Database (https://ecotox.ipmcenters.org/), developed by the US EPA Office of Pesticide Programs, which includes pesticides registered or previously registered in the U.S. It contains mainly unpublished data, reviewed by EPA, drawn from toxicological studies conducted by commercial laboratories and submitted by pesticide companies, and from published studies conducted by U.S. Environmental Protection Agency (US EPA), U.S. Department of Agriculture (USDA), and U.S. Fish & Wildlife Service (US FWS) laboratories.
- the US EPA ECOTOX (https://cfpub.epa.gov/ecotox/), a well-known and extensive database that contains ecotoxicity data for a large number of substances, derived mainly from the peer-reviewed literature and also from US EPA sources.
- the WFD CIRCA library (https://circabc.europa.eu/), including a section dedicated to the implementation of the Water Framework Directive (WFD), where the EC Dossiers on the derivation of EU Environmental Quality Standards (EQS) can be accessed. Here both approved and draft Dossiers (still under discussion) are publicly available. Draft Dossiers cover, among others, also some neonicotinoid insecticides, perfluoroalkyl substances and pharmaceuticals and report review of ecotoxicological data from literature and other sources.

Data published in peer-reviewed literature were collated firstly from the source papers found through the search in the ECOTOX database. Additional or most recent data were retrieved through a supplementary literature search on Scopus and Google Scholar, using as relevant search terms the names of each compound or group of compounds and other keywords (as singular words or in combination), such as ecotoxicity, toxicity, assay, test, organism, marine, estuarine, saltwater, emerging, contaminants, watch list, and others.

Searches were carried out both by chemical name and by CAS (Chemical Abstract Service) number. Data for pesticides tested as formulations were not included in the database, since they may show a different ecotoxicity compared to the active ingredient tested alone, as shown for example in studies on glyphosate (Elandaloussi et al., 2008, Tsui and Chou, 2003). For Diclofenac, also data for Diclofenac sodium were included and then merged together into a single dataset.

Data for which insufficient information was reported (e.g., lack of details on testing methods and toxicity values calculation) were discarded, as well as data showing a poor statistical significance (e.g., data obtained from low quality dose-response curves).

In general, data search included endpoints associated to effects of contaminants on growth, mortality, and reproduction while biochemical and genetic responses, not usable for PNEC derivation, were not taken into account.

Test organisms are reported with their scientific name, together with additional information, such as the taxonomic group (algae, mollusca, crustacea, fish, etc.), and the trophic level (primary producer, primary consumer, etc.). The life stage of the organisms considered in the test is also reported, in some cases together with the organism's age and/or size.

Ecotoxicity tests are briefly described, and different columns report the test type (acute, sub-chronic, chronic), the exposure type (static, static renewal, flow-through), the exposure time, the endpoint (e.g., ECx, LCx, NOEC/L, LOEC/L), and the measured effect (e.g., mortality, growth, hatching). When used, standard test protocols are reported and detailed. All the toxicity values are given in μg/L.

This research supported the creation of an Excel database structured into different sheets for 24 chemicals, grouped according to the categories used in D2.3.1 (delivered in 2019), as listed below:

- Industrial chemicals;
- Pharmaceuticals;
- Neonicotinoid insecticides;
- Plant protection products;
- Perfluoroalkyl substances.

Given the variety of factors influencing toxicity of microplastics, such as type of plastic, dimensions and shape, potential absorbed chemicals, for the purpose of this deliverable this class is not taken into account.

Table 1 provides a summary of the dataset, reporting the selected chemicals along with the species groups for which toxicity data are available.

For some chemicals, it was possible to integrate literature data with experimental values from ecotoxicity tests with copepod *Acartia Tonsa* and bivalve *Mytilus galloprovincialis* performed by the Ecotoxicological group of UNIVE-DAIS (to be included in D2.3.3.1). These bioassays were performed on marine water samples artificially spiked with single chemicals, with the goal of providing new insights into the effects of emerging contaminants on marine/estuarine species (additional details about the selection of tested organisms and substances will be available in D2.3.3.1).

In some detail, a 48 hours test on larval development of *Mytilus galloprovincialis* was carried out under progressively increasing concentrations (from 10 ng $L^{-1}$ up to 10 mg $L^{-1}$) of each of the selected chemicals, i.e. neonicotinoids (imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam), antibiotics (amoxicillin, ciprofloxacin, clarithromycin, erythromycin), diclofenac, and plant protection products (methiocarb, triallate, oxadiazone).

Among the substances of interest for this deliverable, experiments on copepods *A. Tonsa* provided data from exposure to neonicotinoids. The objective of the experimental design was to investigate the effect of contaminants exposure on: i) the larval development and survival of generation $F_0$, ii) the production of eggs for the first generation and iii) the survival and development of $F_1$ larvae obtained from the parental generation $F_0$. The entire duration of the tests was about 21 days, with observation between the 21$^{st}$ and the 26$^{th}$ day. More information will be provided in D2.3.3.1.

Table 1. Summary of the species groups for which toxicity data are available. Red "x" indicates data generated from ecotoxicity tests with copepod *Acartia Tonsa* and bivalve *Mytilus galloprovincialis* by the UNIVE Ecotoxicological group. Chemicals belonging to one of the five categories are presented with the same colour.

| Chemical | Species groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Algae | Crustacea | Fish | Mollusca | Echinoida | Bacteria | Additional groups |
| EHMC | x | x | | x | x | x | |
| Bisphenol A | x | x | x | x | x | | x (Ascidiacea) |
| 17-beta-estradiol | | x | x | | x | | |
| 17-alpha-ethynylestradiol | | x | x | x | x | | |
| Estrone | | x | | | x | | |
| Amoxicillin | x | | | x | x | | |
| Ciprofloxacin | x | | | x | | x | |
| Azithromycin | | x | x | | | | x (Diatomea) |
| Clarithromycin | | x | | x | | x | x (Diatomea) |
| Erythromycin | x | x | | x | | x | |
| Diclofenac | x | x | x | x, x | x | x | x (Asteroidea, Polychaeta) |
| Imidacloprid | | x, x | x | x, x | | | x (Insecta) |
| Thiacloprid | | x, x | x | x, x | | x | |
| Clothianidin | x | x, x | x | x, x | | | |
| Thiamethoxan | x | x, x | x | x, x | | | |
| Acetamiprod | x | x, x | x | x, x | | | |
| Methiocarb | x | x | x | x, x | | x | |
| Oxadiazon | x | x | x | x, x | | | |
| Triallate | x | x | x | x | | | |
| Metaflumizone | | x | x | x | | | |
| Glyphosate | x | x | x | x | x | x | |
| AMPA | | | | x | x | | x (Coelenterata, Protozoa, Spermatophyta) |
| PFOA | x | x | x | x | x | x | |
| PFOS | x | x | x | x | x | x | |

## 2.2 Reference guidance for PNECs derivation

The derivation of PNEC values described in this work complies with the REACH (Registration, Evaluation and Authorisation of Chemicals) Regulation No 1907/2006 of the European Parliament and of the Council, by following the guidance documents of the European Chemicals Agency released in May 2008 (ECHA, 2008; 2011). The guidance documents help with the fulfilling of the REACH requirements for the Chemical Safety Assessment by giving detailed recommendations for the assessment of the effect of a chemical substance on the environment.

Information contained in the guidance document was additionally integrated with the European Commission's Guidance Document No. 27 for deriving Environmental Quality Standards (EC, 2018) since it covers many of the key technical issues involved in the interpretation and classification of ecotoxicity data. Also the Technical Rationale for the derivation of Australian and New Zealand water quality guidelines (Batley et al., 2014) was used, because it provides detailed recommendations on how to discriminate acute and chronic toxicity tests based on test duration, endpoint and tested life cycle stage. Within the assessment framework provided by REACH regulation, technical indications reported in the considered

guidelines helped outlining the criteria used in this deliverable for the assessment and categorization of ecotoxicological data.

The completeness and adequacy of ecotoxicity datasets are crucial in the determination of PNEC in water compartment, which is the concentration below which no adverse effects are likely to occur in organisms exposed to chemicals for long or short lengths of time. Given the variety of species populating an ecosystem, such evaluation cannot rely on tests performed on few individual organisms. For this reason, PNEC values used in risk assessments are extrapolated from the results of tests with two methods: the deterministic assessment factor methods and the probabilistic sensitivity distribution methods.

According to REACH guidelines, PNECs can be deterministically estimated by dividing the lowest value generated by toxicity tests with the relevant Assessment Factor (AF). Criteria to derive the AFs depend on the number of chronic tests and the number of different taxonomic groups represented in the dataset. The quantity of data available for deriving a PNEC in the aquatic compartment can vary, and in case of saltwater environments it is greatly affected by the larger species diversity. To assess the potential impact of a substance on marine environment, the presence of a number of taxa that occur only in that environment implies a broader distribution of sensitivities on organisms and, thus, a higher uncertainty in extrapolation of a no-effect concentration.

Uncertainty and species variety is accounted for by higher AFs than those applied for the derivation of freshwater PNECs. For this reason, the procedure follows the freshwater guidelines by looking at data covering the three main trophic levels (algae, invertebrates-crustaceans, and fish), but it also accounts for data available across additional marine taxonomic groups, for example rotifers, echinoderms or molluscs (Figure 1). Should data on additional marine taxonomic groups be available, then the uncertainties are reduced and the magnitude of the assessment factor applied to a dataset can be lowered.

The assessment factors depend also on the quality of dataset, with higher AFs for short-term acute tests, and gradually reduced factors when long-term chronic data become available. Table 2, which is based on and expands Table R.10-5 of the ECHA document, describes the assessment factors that would be applied to the dataset available. Since it cannot cover all possible scenarios, expert judgment should pragmatically guide the derivation of the most appropriate AF as explained in the next section.

Alongside Table 2, Figure 1 illustrates the organization of ecotoxicity data as required in the deterministic derivation of the most appropriate factor. To summarize, the most complete dataset consists of short and long-term test results covering the three main trophic levels with additional long-term data from marine taxonomic groups with different feeding strategies or life form. In this case the minimum AF applies, i.e. AF = 10. By reducing the availability of ecotoxicity data across different marine species, the AF increases up to a maximum of 10000 (when only acute results are generated) in order to be protective towards the most sensitive group.

Where a large dataset from long-term tests for different taxonomic groups is available (at least 10 long-term test results, preferably more than 15, for different species covering at least 8 taxonomic groups), PNECs can be statistically obtained with a species sensitivity distribution (SSD). In this situation the AF ranges between 5 and 1, depending on an evaluation of the uncertainties around the derivation of the $5^{th}$ percentile.

Table 2. Assessment factors proposed for deriving PNEC for saltwater for different data sets, after ECHA document (2008).

| Case ID | Dataset | AF | Apply to: | Additional notes from ECHA (2008) |
|---|---|---|---|---|
| 1 | **Short-term L(E)C50** from freshwater or saltwater representatives **of three taxonomic groups** (algae, crustaceans and fish) of three trophic levels | 10,000 | lowest L(E)C50 | Under no circumstances should a factor lower than 1000 be used in deriving a PNEC for saltwater from short-term toxicity data. |
| 2 | **Short-term L(E)C50** from freshwater or saltwater representatives of **three taxonomic groups** (algae, crustaceans and fish) of three trophic levels, **plus two additional marine** taxonomic groups (e.g. echinoderms, molluscs) | 1000 | lowest L(E)C50 | **Short term** values on three taxonomic groups of **three trophic** levels **plus two additional marine** taxonomic groups. |
| | | 1000 | lowest EC10/NOEC | 1000 applies to a **single long-term** result (e.g. EC10 or NOEC) (freshwater or saltwater crustacean or fish) if this result was **generated for the taxonomic group showing the lowest L(E)C50** in the short-term algal, crustacean or fish tests. |
| | | 10,000 | lowest L(E)C50 | **If not**, the hazard assessment is based on the **short-term data with an assessment factor of 10,000**. However, normally the lowest PNEC should prevail. |
| | | 1000 | lowest EC10/NOEC | 1000 applies also to the lowest of the **two long-term results** (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results (e.g. EC10 or NOEC) have **not been generated for the species showing the lowest L(E)C50** of the short-term tests. |
| | | 1000 | lowest L(E)C50 | This not apply in cases where the **acutely most sensitive species has an L(E)C50-value lower than the lowest long term value**. In such cases the PNEC might be derived by applying an assessment factor of 1000 to the lowest L(E)C50 of the short-term tests. |
| 3 | **One long-term** result (e.g. EC10 or NOEC) (from freshwater or saltwater **crustacean reproduction** or **fish growth** studies) | 1000 | | See notes for case 2 |
| 4 | **Two long-term** results (e.g. EC10 or NOEC) from freshwater or saltwater species representing **two** | 500 | lowest EC10/NOEC | Two long-term results of **two trophic levels** |

| Case ID | Dataset | AF | Apply to: | Additional notes from ECHA (2008) |
|---|---|---|---|---|
| | **trophic levels** (algae and/or crustaceans and/or fish) | 500 | lowest EC10/NOEC | 500 applies to the lowest of **two long term results** (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results have been **generated covering those trophic levels showing the lowest L(E)C50** in the short-term tests with these species. |
| | | 500 | lowest EC10/NOEC | 500 applies to the lowest of **three long term results** (e.g. EC10 or NOEC) covering three trophic levels, when such results have **not been generated from the taxonomic group showing the lowest L(E)C50** in short-term tests |
| | | 1000 | lowest L(E)C50 | In the case where the **acutely most sensitive species has an L(E)C50 value lower than the lowest long term result** (e.g. EC10 or NOEC) value |
| | | 100 | lowest EC10/NOEC | High probability that the most sensitive species covering fish, crustacea and algae has been examined, that is that a **further longer-term result** (e.g. EC10 or NOEC) **from a third taxonomic group would not be lower than available data** |
| | | 100 | lowest EC10/NOEC | **Short-term tests for one additional species** representing marine taxonomic groups (for example echinoderms or molluscs) have been carried out and **indicate that these are not the most sensitive group** and high probability that long-term results (e.g. EC10 or NOEC) generated for these marine groups would not be lower |
| | | 50 | lowest EC10/NOEC | Short-term tests for **two additional species representing marine taxonomic groups** (for example echinoderms or molluscs) have been carried out and indicate that these are **not the most sensitive group** and high probability that long-term results (e.g. EC10 or NOEC) generated for these marine groups would not be lower |
| 5 | **Long-term** results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing **three trophic** | 100 | lowest EC10/NOEC | **Longer-term toxicity results** (e.g. EC10 or NOEC) are available from three freshwater or saltwater species (algae, crustaceans and fish) **across three trophic levels** |

| Case ID | Dataset | AF | Apply to: | Additional notes from ECHA (2008) |
|---|---|---|---|---|
| | **levels** | 10 | lowest EC10/NOEC | **Short-term tests for additional species representing marine taxonomic groups** (for example echinoderms or molluscs) have been carried out and indicate that these are **not the most sensitive group**, and it has been determined with a high probability that long-term results (e.g. EC10 or NOEC) generated for these species would not be lower than that already obtained |
| | | 10 | lowest EC10/NOEC | **Short-term tests for additional taxonomic groups** (for example echinoderms or molluscs) have indicated that one of these **is the most sensitive group acutely and a long-term test has been carried out for that species**. Longer term results (e.g. EC10 or NOEC) generated from other taxa will not be lower than the long term results already available |
| 6 | **Two long-term** results (e.g. EC10 or NOEC) from freshwater or saltwater species representing **two trophic levels** (algae and/or crustaceans and/or fish) + **one long-term result from an additional marine** taxonomic group (e.g. echinoderms, molluscs) | 50 | lowest EC10/NOEC | |
| 7 | **Long-term** results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing **three trophic levels** + **two long-term results from additional marine** taxonomic groups (e.g. echinoderms, molluscs) | 10 | lowest EC10/NOEC | |

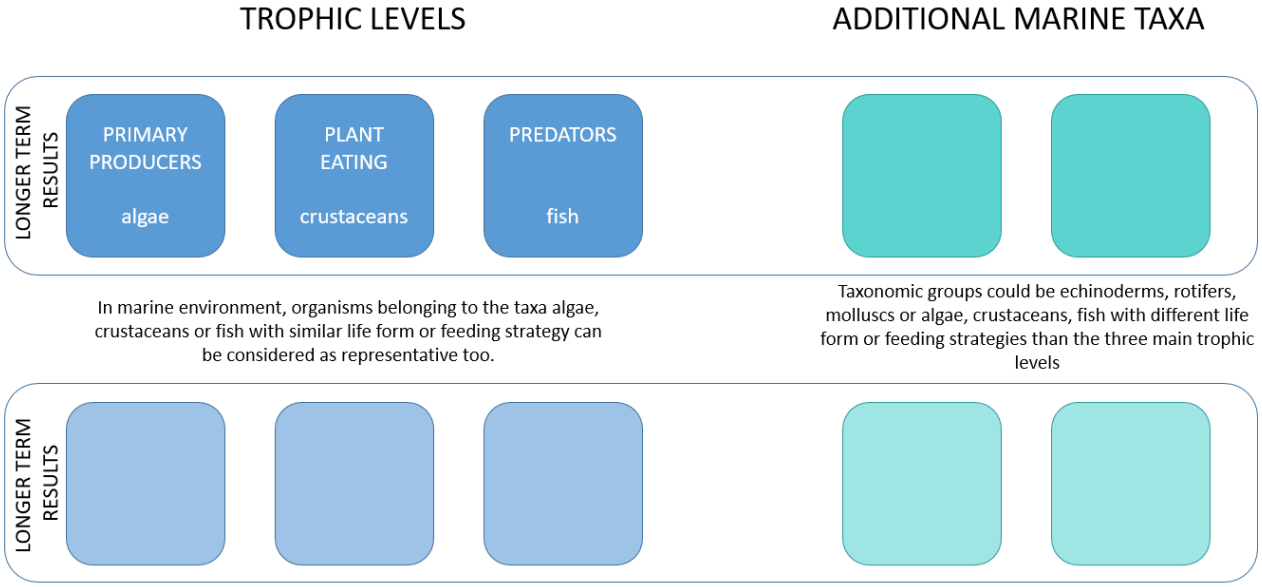


Figure 1. Graphical interpretation of the REACH requirements (ECHA, 2008; 2011) for the assessment of saltwater AF and corresponding PNEC. Long and short-term results are organized into three main trophic levels (generally algae, crustaceans and fish) plus at least two additional marine taxonomic groups.

## 2.3 Interpretation of ecotoxicological datasets

In the evaluation of ecotoxicological data, the PNEC derivation requires the assessor to discriminate between short-term and long-term studies.

Short-term studies are described as studies in which the organisms are exposed to the toxicant for a duration that is relatively short in comparison to the extent of the life-cycle of the organisms (ECHA, 2011; Batley et al., 2014). These studies provide acute toxicity values, that is concentrations at which 50% mortality or inhibition of a function (e.g. growth) was observed and are expressed as the lethal concentration (LC50) or the effect concentration (EC50).

Conversely, a long-term toxicity study is defined as a study that is in general relatively long in relation to the duration of the organisms' life-cycles (ECHA, 2011). Guidance 27 adds specifications of what should be intended as a "long" study: a test in which (i) the species is exposed to the toxicant for at least one complete life cycle, or (ii) the species is exposed to the toxicant during one or more sensitive life stages (EC, 2018). The Technical Rationale for the derivation of Australian and New Zealand water quality guidelines (Batley et al., 2014) further specifies that chronic toxicity is the result of exposure to chemicals for a substantial proportion of the organism's life span or a study of adverse sub-lethal effects on a sensitive early life stage. These studies provides chronic toxicity data most frequently reported as L/EC10 or as NOEC (No Observed Effect Concentration), which corresponds to the highest tested concentration for which there are no statistical significant difference of effect when compared to the control group. In this work, when both data were available, EC10 values were preferred to NOEC as measurement of toxicity since they are not affected by the inappropriate application of hypothesis testing (Batley et al., 2014).

The perception of what is chronic and what is acute is dependent on the species type, the study endpoint and experimental design and, often, on the individual decision of the assessors. Definition of acute and chronic can greatly affect the AF, with a PNEC variation up to three orders of magnitude as shown in a study by Hahn et al. (2013). In the voidance of transparent shared directives, decision-making process is here based on available guidance documents, acknowledging that they are not worldwide accepted and

that regional jurisdiction and personal judgment may vary the assumptions based on which the AF and relative PNEC are derived.

In this work, the following assumptions guided the determination of acute or chronic values.

For fish, where multigeneration tests are essentially impossible, but also for other species such as amphibians, tests on early life-stage tests (ELS), in which eggs or larvae are exposed and the effects on hatching, malformation and growth are considered, were here accepted as chronic toxicity tests, in line with the Guidance Document No. 27 if the duration of the exposure is at least 7 days (Batley et al., 2014).

According to the Technical Rationale of Australian and New Zealand Guidelines (Batley et al., 2014), ELS tests on sensitive endpoints for invertebrates, are judged in this work as more indicative of longer term exposure (see Table 3 after Batley et al, 2014). Acknowledging that ELS tests rather provide indications of adverse sub-chronic effects, that is these tests study endpoints on a more sensitive life stage of the organism than acute tests but their duration is more similar to an acute exposure, tests on the larval development of micro and macroinvertebrates for exposures of at least 48 hours are here tabulated as chronic studies. Under this assumption and in the necessity of labelling ecotoxicity data as either chronic or acute, the sub-chronic experimental results on larval development of *M. Galloprovincialis* after a 48 hours exposure are considered as indicative of a chronic effect. Likewise, experiments on *A. Tonsa* generated chronic data from both F0 and F1 larval development studies.

Differently, in algae studies, which are multigeneration studies over 72 hours, the EC50 of growth rate or biomass tests is considered as acute, while the NOEC or EC10 of the same test is regarded as a chronic value. For this reason, in this study chronic data on algae were accounted for only if supported by long-term tests on other trophic levels. According to REACH guidelines (ECHA, 2008), studies with bacteria (e.g. growth tests) can be used and are accepted as short-term tests, although EC50 from bacterial tests cannot substitute any of the three main trophic levels (algae, invertebrate, and fish). Likewise, studies on gametes are considered as acute and, when available, only L(E)C50 values were tabulated for PNEC derivation.

Given the broad variety of species in marine environments, marine organisms that belong to the taxa algae, crustaceans or fish with similar life form or feeding strategy are here considered representatives of the three main trophic levels. If life form or feeding strategy differ, then these organisms can be accepted as additional marine taxonomic groups and will allow a reduction in the AF.

This work makes use of ecotoxicity studies aimed at assessing the direct toxicity of chemical substances, while possible effects associated with bioaccumulation processes and, therefore, on secondary poisoning along the food chain were not considered. When for the same species there was more than one set of data, endpoint, test duration, life stage and testing condition, the greatest importance was given to the most reliable and relevant one. To do so, it was often necessary to look into more detail at the study reported in the original source and to pragmatically select the most sensible assessment factor based on professional judgment.

Table 3. Classification of acute and chronic toxicity tests for invertebrates, based on species, test duration, life stage and endpoint. After Batley et al., 2014).

| Species | Life stage | Relevant endpoint | Test duration |
|---|---|---|---|
| **Acute** | | | |
| Macroinvertebrates (i.e. decapods, echinoderms, molluscs, annelids, corals, amphipods) | Adults/Juveniles | All | < 14d |
| | Embryos/larvae | All except fertilisation, larval development/ metamorphosis | < 7d |
| | Embryos/larvae | Larval development/ metamorphosis | < 48 h |
| Microinvertebrates (i.e. ladocerans, copepods, conchostracans, and hydra) | Adults/juveniles/larvae | All | < 7 d |
| **Chronic** | | | |
| Macroinvertebrates (i.e. decapods, echinoderms, molluscs, annelids, corals, amphipods) | Adults/Juveniles | All | ≥ 14d |
| | Larvae | Lethality, immobilisation, growth | ≥ 7 d |
| | Larvae | Larval development/ metamorphosis | ≥ 48 d |
| | Embryos | Fertilisation | ≥ 1 h |
| Microinvertebrates (i.e. ladocerans, copepods, conchostracans, and hydra) | Adults/juveniles/larvae | All (except lethality) | ≥ 7 d (or 3 reproductive broods for cladocerans) |
| | | Lethality | ≥ 21 d |
| | Larvae | Development | ≥ 48 d |
| | | Fertilisation | ≥ 1 h |

# 3. PNECs derivation

Hereafter ecotoxicity data are tabulated for each chemical according to the three main trophic levels (primary producers represented by algae, plant eating animals represented by invertebrates and predators represented by fish) with the inclusion of additional marine taxonomic groups when at disposal.

First, a summary of the ecotoxicological dataset for each chemical is presented, including the number of trophic levels, species groups and species covered by the data.

Then, tables report short-term and long-term test results divided into acute and chronic data respectively, along with information on the species names, the duration of tests, the tested endpoint and the bibliographic reference. Where available, acute and chronic data for the most sensitive species are presented for each trophic levels. If a result from a sub-chronic study is selected as the lowest value, this is tabulated as chronic value but with an explicit indication.

With regard to the methodological approach for PNEC derivation described in Paragraph 2.3, for every dataset expert considerations guided the derivation of the most adequate AF to be applied to the lowest ecotoxicity data for the derivation of deterministic PNECs. Only one dataset was sufficiently populated to calculate a probabilistic PNEC via a SSD analysis, i.e. bisphenol A. If additional data from Deliverable 2.3.3.1 were available, the determination of the correct AF was carried out including those results on copepods and/or bivalves.

### 3.1 EHMC

Trophic levels: 4

Species groups: 5

Species: 5

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Isochrysis galbana*, 72 h, growth EC10 = 51.506 | Paredes et al., 2014. Chemosphere 104, 44–50. |
| | Acute | *Isochrysis galbana*, 72 h, growth EC50 = 74.72 | |
| **Invertebrates** (µg/l) | Chronic | | Paredes et al., 2014. Chemosphere 104, 44–50. |
| | Acute | *Siriella armata*, 96 h ELS, mortality EC50 = 199.43 | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional marine taxonomic groups (*Echinoida*)** (µg/l) | Chronic | | Paredes et al., 2014. Chemosphere 104, 44–50. |
| | Acute | *Paracentrotus lividus*, 48 h ELS, growth EC50 = 283.69 | |
| **Additional marine taxonomic groups (*Mollusca*)** (µg/l) | Chronic | *Mytilus galloprovincialis*, 48 h ELS, development (subchronic) EC10 = 430.648 | Paredes et al., 2014. Chemosphere 104, 44–50. |
| | Acute | | |

Since the only chronic result for the main three trophic levels was generated for algae, the maximum AF of 10000 should apply to the lowest acute data, i.e. $EC50_{algae}$ = 74.72 µg/l. Such PNEC is protective towards the other species for which data are available, however, the scarcity of the database highlights the uncertainty associated to this value. Only additional investigations of toxicity posed by EHMC on marine species could help in retrieving a more sensible PNEC value.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (algae) | 74.72 | 10000 | 0.0075 |

## 3.2 Bisphenol A

Trophic levels: 4

Species groups: 6

Species: 15

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 96h, biomass<br>EC10 = 400 | Alexander et al., 1988. Environ. Toxicol. Chem. 7, 19–26. |
| | Acute | *Skeletonema costatum*, 96h, growth<br>EC50 = 1000 | |
| **Invertebrates** (µg/l) | Chronic | *Tigriopus japonicas*, 21d, reproduction<br>NOEC = 10 | Marcial et al., 2003. Environ. Toxicol. Chem. 22, 3025–3030<br><br>Shaukat et al., 2014. J. Ocean Univ. China 13, 141–145 |
| | Acute | *Artemia sinica*, 72h, mortality<br>LC50 = 17.3 | |
| **Fish** (µg/l) | Chronic | *Cyprinodon variegatus*, 115d ELS, reproduction<br>NOEC = 66 | Mihaich et al., 2018. Env. Tox. and Chem., 37, Number 2, pp. 398–410<br><br>Alexander et al., 1988. Environ. Toxicol. Chem. 7, 19–26. |
| | Acute | *Menidia menidia*, 72h, mortality<br>LC50 = 9400 | |
| **Additional marine taxonomic groups (*Echinoida*)** (µg/l) | Chronic | *Paracentrotus lividus*, 72h ELS, development (subchronic)<br>NOEC = 420 | Roepke et al., 2005. Aquat. Toxicol. 71, 155–173 |
| | Acute | | |
| **Additional marine taxonomic groups (*Mollusca*)** (µg/l) | Chronic* | *Mytilus galloprovincialis*, 48h ELS, development (subchronic)<br>NOEC = 0.01 | Fabbri et al., 2014. Mar. Environ. Res. 99, 1–8 |
| | Acute | | |
| **Additional marine taxonomic groups (*Ascidiacea*)** (µg/l) | Chronic | | |
| | Acute | *Ciona intestinalis*, 22h ELS, mortality<br>NOEC = 1240 | |

*Alternative value: *Hhaliotis diversicolor supertexta*, 12h ELS development, EC10 = 16 µg/l (Liu et al., 2011).

Short and long-term results are available for three saltwater species (algae, crustaceans and fish) representing three trophic levels, with two long-term results from additional marine taxonomic groups (echinoderms and molluscs). According to case 7 of the REACH guidelines, an AF of 10 should be applied to the lowest chronic result, being it NOEC = 0.01 µg/l provided by Fabbri et al. (2014). Since this toxicity data is much lower than data provided for other species by other studies, it has been considered not enough robust and an alternative value is here considered more adequate, that is a EC10 = 16 µg/l from a 12 hours ELS development test of *Hhaliotis diversicolor supertexta* (Liu et al., 2011). The lowest long-term test result is then invertebrates' NOEC =10 µg/l, generating a PNEC of 1 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (invertebrates) | 10 | 10 | 1 |

Following the REACH guidelines a probabilistic value of PNEC can be derive when at least 10 chronic results for different species covering at least 8 taxonomic groups are available. Given the abundance of toxicity test results for Bisphenol A, a probabilistic approach is applied to the 12 available long-term results, i.e. NOEC or EC10, summarized in Table 4. The test data applicable to the most sensitive endpoint was taken as representative for the species and when multiple data on the same end-point and species were available the geometric mean was used as input.

Table 4. Species groups and names with relative long-term toxicity value used for the construction of the SSD.

| Species group | Species scientific name | Value µg/L |
|---|---|---|
| Crustacea | *Tigriopus japonicus* | 10 |
| Mollusca | *Hhaliotis diversicolor supertexta* | 27 |
| Fish | *Cyprinodon variegatus* | 66 |
| Crustacea | *Acartia tonsa* | 100 |
| Crustacea | *Americamysis bahia* | 170 |
| Echinoida | *Paracentrotus lividus* | 420 |
| Algae | *Skeletonema costatum* | 545 |
| Echinoida | *Strongylocentrotus nudus* | 710 |
| Echinoida | *Hemicentrotus pulcherrimus* | 710 |
| Algae | *Cyclotella caspia* | 4720 |
| Algae | *Stephanodiscus hantzschii* | 1000 |
| Algae | *Cochlodinium polykrikoides* | 3470 |

The Species Sensibility Distribution curve (SSD), obtained with the USEPA SSD Generator software (2005) where laboratory data are fitted with a linearized log-normal distribution, provides a 5% cumulative value, $HC_5$, equal to 13.17 µg/l. Such value is comparable with $HC_5$ obtained in other studies, e.g., $EC_5$ = 18 µg/l (Mihaich et al., 2018), $EC_5$ = 9.8 µg/l (Guan et al., 2018), $EC_5$ = 10.5 (CIRCABC EQS draft dossier, 2021). An assessment factor of 5 is then applied to this value to make sure that the most sensitive species, whose long-term result is lower than $HC_5$ (crustacean *Tigriopus japonicus*), is protected. The probabilistic PNEC equal to 2.634 µg/l is consistent with the deterministic value of 1 µg/l.

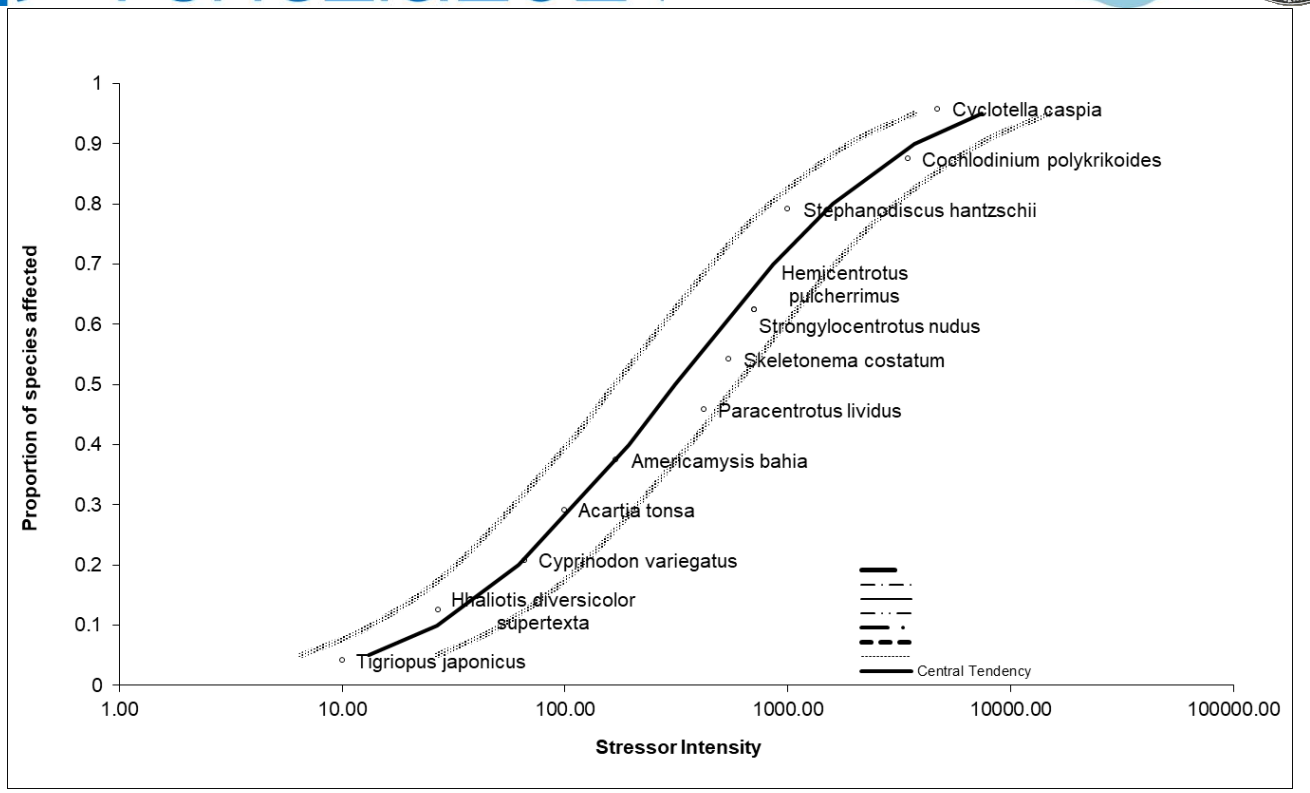


Figure 2. Species sensibility distribution curve where laboratory toxicity data for several species are fitted by a linearized log-normal distribution with confidence intervals. The fitting is denoted by $R^2 = 0.98$. Concentrations are expressed in µg/l.

### 3.3 17-beta-estradiol

Trophic levels: 4

Species groups: 3

Species: 9

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | *Elminius modestus*, 12 months, reproduction<br>NOEC = 10 | Billinghurst et al., 2001. J. Exp. Mar. Bio. Ecol. 257, 255–268<br><br>Andersen et al., 2001. Environ. Toxicol. Chem. 20, 2821–2829 |
| | Acute | *Acartia tonsa*, 5d, development<br>EC50 = 720 | |
| **Fish** **(µg/l)** | Chronic | | |
| | Acute | *Fundulus heteroclitus*, 96h ELS, mortality<br>LC50 = 5094 | Kelly and Di Giulio, 2000. Environ. Toxicol. Chem. 19, 2564–2570 |
| **Additional marine taxonomic groups** **(µg/l)** | Chronic | | |
| | Acute | | |

Having a single long-term test over the three main trophic levels, that is the crustacean reproduction test with NOEC = 10 µg/l, it would fall within the case 3 of the REACH guidelines providing an AF of 1000 to that value since this result was generated for the species showing the lowest L(E)C50 available.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (invertebrates) | 10 | 1000 | 0.01 |

### 3.4 17-alpha-ethynylestradiol

Trophic levels: 4

Species groups: 4

Species: 12

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | *Acartia tonsa*, 5d ELS, development<br>EC10 = 46 | Andersen et al., 2001. Environ. Toxicol. Chem. 20, 2821–2829 |
| | Acute | *Nitocra spinipes*, 96h, mortality<br>EC50 = 510 | Breitholtz and Bengtsson, 2001. Mar. Pollut. Bull. 42, 879–886 |
| **Fish** **(µg/l)** | Chronic | *Fundulus heteroclitus*, 25d ELS, development<br>NOEC = 0.01 | Boudreau et al., 2004. Environ. Toxicol. Chem. 23, 415–25 |
| | Acute | | |
| **Additional marine taxonomic groups (Echinoida)** **(µg/l)** | Chronic* | *Paracentrotus lividus*, 48h ELS, development (subchronic)<br>NOEC = 0.005 | Capolupo et al., 2018. Environ. Sci. Pollut. Res. 25, 32196–32209 |
| | Acute | | |
| **Additional marine taxonomic groups (Mollusca)** **(µg/l)** | Chronic | | |
| | Acute | | |

*additional data: *Hemicentrotus pulcherrimus*, 48h ELS, NOEC = 92 µg/l (EQS draft dossier)

Long-term development tests are available covering two main trophic levels (crustacean and fish). An assessment factor of 500 would then apply to the lowest of the two long term results. For this reason, an AF of 500 applied to the lowest long-term test on fishes was considered as protective for also other taxonomic groups. The resulting PNEC is 0.00002 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (fish) | 0.01 | 500 | 0.00002 |

## 3.5 Estrone

Trophic levels: 2

Species groups: 2

Species: 3

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | | |
| | Acute | | |
| **Invertebrates** (µg/l) | Chronic | *Acartia tonsa*, 5d ELS, development (subchronic) EC10 = 250 | Andersen et al., 2001. Environ. Toxicol. Chem. 20, 2821–2829 |
| | Acute | | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional marine taxonomic groups (Echinoida)** (µg/l) | Chronic | | |
| | Acute | | |

Because of the scarcity of data, a precautionary assessment factor of 10000 applies to a single long-term result covering crustaceans development test since this result was generated for the taxonomic group showing the lowest L(E)C50 in the short-term tests. The resulting PNEC is 0.025 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC10 (invertebrates) | 250 | 10000 | 0.025 |

### 3.6 Amoxicillin

Trophic levels: 2

Species groups: 2

Species: 5

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Phaeodactylum trocornutum*, 96h, growth<br>NOEC = 250000 | De Orte et al., 2013. Chem. Ecol. 29, 554–563.<br><br>Lützhøft et al., 1999. Arch. Environ. Contam. Toxicol. 36, 1–6 |
| | Acute | *Rhodomonas salina*, 72h, growth<br>EC50 > 500000 | |
| **Invertebrates** (µg/l) | Chronic | | |
| | Acute | | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional marine taxonomic groups (Echinoida)** (µg/l) | Chronic | *Arbacia lixula*, 72h ELS, development (subchronic)<br>LOEC = 100000 | Carballeira et al., 2012. Arch. Environ. Contam. Toxicol. 63, 249–261 |
| | Acute | | |
| **Additional marine taxonomic groups (Mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 1 | Deliverable D2.3.3.1 |
| | Acute | | |

A single long-term test is available covering the three main trophic levels and was generated for aquatic plants. If the algae tests were the only data available, in general they should not be considered. Additionally, chronic tests for two marine taxonomic groups (echinoderms and molluscs) show that algae are not the most sensitive species, hence an AF of 10000 was applied to the lowest short-term test, that in this case would be the EC50 of algae, generating a PNEC of 50 µg/l. This value is although not protective of the most sensitive species (molluscs). As a consequence, a pragmatic but conservative PNEC of 0.001 µg/l was obtained by dividing the lowest long-term data by a precautionary AF of 1000. A revision of this proposed value is desirable as soon as additional long-term data become available to reduce the uncertainty on the assessment.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (mollusca) | 1 | 1000 | 0.001 |

### 3.7 Ciprofloxacin

Trophic levels: 2

Species groups: 2

Species: 3

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | | Hagenbuch and Pinckney, 2012. Water Res. 46, 5028–5036 |
| | Acute | *Cylindrotheca closterium*, 4-5d, growth IC50 = 55430 | |
| **Invertebrates** (µg/l) | Chronic | | |
| | Acute | | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional species (bacteria)** (µg/l) | Chronic | | Hernando et al., 2009. Chemosphere 68, 724–730 |
| | Acute | *Vibrio fischeri*, 5 min, Bioluminescence EC50 > 5900 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development NOEC = 1 | Deliverable D2.3.3.1 |
| | Acute | | |

No long-term tests are available for the main trophic levels or for additional marine species. By not considering the tests on bacteria, the highest AF is applied to the IC50 of short-term test with algae, so that the PNEC value results equal to 5.54 µg/l. Again, as in the case of amoxicillin, this is not protective for the most sensitive species. Similarly to the amoxicillin context, a precautionary AF of 1000 was applied to the most sensitive endpoint, i.e. NOEC of molluscs. The resulting PNEC is 0.001 µg/l. A revision of this proposed value is desirable as soon as additional long-term data become available to reduce the uncertainty on the assessment.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (mollusca) | 10000 | 10000 | 1 |

## 3.8 Azithromycin

Trophic levels: 3

Species groups: 3

Species: 3

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** | Chronic | | Minguez et al., 2016. Environ Sci Pollut Res (2016) 23:4992–5001 |
| **(µg/l)** | Acute | *Skeletonema marinoi*, 72 h, growth<br>EC50 = 214 | |
| **Invertebrates** | Chronic | | Minguez et al., 2016. Environ Sci Pollut Res (2016) 23:4992–5001 |
| **(µg/l)** | Acute | *Artemia salina*, 48 h ELS, immobilization<br>EC50 > 100000 | |
| **Fish** | Chronic | | Mhadhbi et al., 2020. Drug Chem Toxicol. 22:1-7. |
| **(µg/l)** | Acute | *Dicentrarchus labrax*, 96h ELS, mortality<br>LC50 = 30800 | |
| **Additional marine taxonomic groups** | Chronic | | |
| **(µg/l)** | Acute | | |

Results generated for fishes are affected by uncertainty in their derivation from the dose-effect curve. Uncertainty is also presented in the CIRCABC draft dossier for the acute results covering plants and invertebrates. Based on this, a precautionary AF of 10000 was applied to the lowest short-term result (EC50 algae). The PNEC value is then equal to 0.0214 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (algae) | 214 | 10000 | 0.0214 |

### 3.9 Clarithromycin

Trophic levels: 3

Species groups: 3

Species: 5

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Phaeodactylum tricornutum*, 72 h, growth<br>NOEC = 100 | CIRCABC - DRAFT EQS Dossier, Polleichtner 2020 |
| | Acute | *Phaeodactylum tricornutum*, 72 h, growth<br>EC50 = 2679 | |
| **Invertebrates** (µg/l) | Chronic | | Minguez et al., 2016. Environ Sci Pollut Res |
| | Acute | *Artemia salina*, 48h ELS, immobilization<br>EC50 > 100000 | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional species (bacteria)** (µg/l) | Chronic | | Yamashita et al., 2006. Water Sci. Technol. 53, 65-76 |
| | Acute | *Vibrio fischeri*, 15 min, Bioluminescence<br>EC50 > 8200 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 1000 | Deliverable D2.3.3.1 |
| | Acute | | |

Since long-term results are available only for algae and results for invertebrates are presented in the CIRCABC draft dossier as uncertain, an assessment factor of 10000 was applied to the lowest EC50 available. The scarcity of data forced to consider test results that were presented with a degree of uncertainty, for this reason the highest AF seemed to be a sensible choice. PNEC results equal to 0.27 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (algae) | 2679 | 10000 | 0.27 |

## 3.10 Erythromycin

Trophic levels: 3

Species groups: 3

Species: 8

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Dunaliella tertiolecta*, 96h, growth<br>EC10 = 1880 | Machado and Soares, 2019. J. Appl. Phycol. 31, 399–408. |
| | Acute | *Dunaliella tertiolecta*, 96h, growth<br>EC50 = 5750 | |
| **Invertebrates** (µg/l) | Chronic | *Penaeus vannamei*, 48 h ELS, total toxicity (subchronic)<br>NOEC = 16900 (replicate test 4900) | Williams et al., 1992. J. Aquat. Anim. Health 4, 262–270 |
| | Acute | *Penaeus vannamei*, 24 h ELS, total toxicity<br>EC50 = 29200 | |
| **Fish** (µg/l) | Chronic | | |
| | Acute | | |
| **Additional species (bacteria)** (µg/l) | Chronic | | |
| | Acute | *Vibrio fischeri*, 5 min, Bioluminescence<br>EC50 > 100000 | Hernando et al., 2007. Chemosphere 68, 724–730 |
| **Additional marine taxonomic groups** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC > 10000 | Deliverable D2.3.3.1 |
| | Acute | | |

Two out of three main trophic levels present long-term studies, i.e. algae and crustaceans. The uncertainty of the subchronic data on *Penaeus vannamei* in terms of considered endpoint with reference to the guidelines considered in this work (see Table 3), leaves us with only long-term data for algae and molluscs. An AF of 10000 was then sensibly applied to the lowest of the acute tests available, that is $EC50_{algae}$, resulting in a PNEC = 0.58 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (algae) | 5750 | 10000 | 0.58 |

### 3.11 Diclofenac (acid and sodium)

Trophic levels: 7

Species groups: 9

Species: 13

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** | Chronic | | Schnmidt et al., 2011. Mar. Pollut. Bull. 62, 1389–1395 |
| **(µg/l)** | Acute | *Skeletonema costatum*, 72 h, growth<br>IC50 = 5000 | |
| **Invertebrates** | Chronic | *Palaemon longirostris*, until reaching the first juvenile stage, development<br>NOEC = 750 | González-Ortegón et al., 2016. Sci. Tot. Env. 540, 260-266. |
| **(µg/l)** | Acute | *Siriella armata*, 96 h, mortality<br>EC50 = 2919 | Pérez et al., 2015. Ecotoxicology 24, 1229–1238 |
| **Fish** | Chronic | | Nassef et al., 2009. J. fac. Agr., Kyushu Univ., 54:. 407-411 |
| **(µg/l)** | Acute | *Oryzias latipes*, 96 h, mortality<br>EC50 = 10100 | |
| **Additional species (bacteria)** | Chronic | | Schnmidt et al., 2011. Mar. Pollut. Bull. 62, 1389–1395 |
| **(µg/l)** | Acute | *Vibrio fischeri*, 30 min, Bioluminescence<br>EC50 = 27800 | |
| **Additional marine taxonomic groups (Mollusca)** | Chronic | *M. galloprovincialis*, 48 h ELS, development (subchronic)<br>NOEC = 0.01 | Deliverable D2.3.3.1 |
| **(µg/l)** | Acute | | |
| **Additional marine taxonomic groups (Echinoida)** | Chronic | | Mohd Zanuri et al., 2007. Environ. Res. 127, 126–136 |
| **(µg/l)** | Acute | *Psammechinus miliaris*, gametes 1 h, sperm mobility<br>EC50 = 247 - 429 | |
| **Additional marine taxonomic groups (Polychaeta) (µg/l)** | Chronic | | Mohd Zanuri et al., 2007. Environ. Res. 127, 126–136 |
| | Acute | *Arenicola marina*, 2 h gametes, sperm mobility & fertilization<br>EC50 = 106 – 565 | |
| **Additional marine taxonomic groups (Asteroidea) (µg/l)** | Chronic | | Mohd Zanuri et al., 2007. Environ. Res. 127, 126–136 |
| | Acute | *Asterias rubens*, 1 h gametes, sperm mobility & fertilization<br>EC50 = 616 – 2610 | |

The three main trophic levels present a single long-term result for invertebrates with endpoint development, although this value is higher than the toxicity results on molluscs ELS development tests and on tests with gametes for other species. In this case, where the acutely most sensitive species has an

L(E)C50-value lower than the lowest long term value, the PNEC might be derived by applying an assessment factor of 10000 to the lowest L(E)C50 of the short-term tests. Considering gametes tests for polychaeta, an averaged EC50 was calculated, being 334 µg/l. If an AF of 10000 is applied to this value, the resulting PNEC is 0.0334 µg/l. This PNEC is although not protective towards the most sensitive species (molluscs). For this reason, the lowest EC50 value for polychaeta (106 µg/l) was preferred instead of the average. In this way, the new PNEC is 0.011 µg/l, which is comparable (but not lower) to the lowest chronic NOEC (for molluscs).

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (Polychaeta) | 106 | 10000 | 0.011 |

## 3.12 Imidacloprid

Trophic levels: 6

Species groups: 4

Species: 12

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | *Acartia Tonsa, F0 larval develpment* NOEC = 0.02 | Deliverable 2.3.3.1 |
| | Acute | | |
| **Fish** **(µg/l)** | Chronic | | EPA-OPP Pesticide Ecotoxicity Database, 1990. Toxikon Environmental Sciences, Jupiter, Florida |
| | Acute | *Cyprinodon variegatus*, 96h, mortality LC50 = 163000 | |
| **Additional species (Insecta)** **(µg/l)** | Chronic | | Song et al., 1997. Environ. Toxicol. Chem. 16:2494-2502 |
| | Acute | *Aedes taeniorhynchus*, 48 h, mortality LC50 = 13 | |
| **Additional marine taxonomic groups (Mollusca)** **(µg/l)** | Chronic | *M. galloprovincialis, 48 h ELS, larval development* NOEC = 1000 | Deliverable 2.3.3.1 |
| | Acute | | |

A single long-term result on a crustacean study is available, but the endpoint is larval development (instead of reproduction) thus, we cannot apply the case 3 of REACH guidelines with an AF of 1000 applied to the lowest chronic value. Being Imidacloprid an insecticide, tests on insects should be evaluated. In this case, the acute test for insect generated a LC50 values of 13 µg/l and the application of a conservative AF (10000) results in a PNEC of 0.0013 µg/l, which looks still protective of other taxonomic groups.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEL (invertebrates) | 13 | 10000 | 0.0013 |

## 3.13 Thiacloprid

Trophic levels: 4

Species groups: 4

Species: 4

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | *Acartia Tonsa,* F1 larval development<br>NOEC < 0.01 | Deliverable D2.3.3.1 |
| | Acute | | |
| **Fish** **(µg/l)** | Chronic | | EPA-OPP Pesticide Toxicity Database, 1998. Bayer Co., Agricultural Division, U.S. |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 = 19700 | |
| **Additional species (bacteria)** **(µg/l)** | Chronic | | Escher et al., 2017. Environ Sci Process Impacts.19:414-428. |
| | Acute | *Aliivibrio fischeri*, 30min, Bioluminescence inhibition<br>EC50 = 159460 | |
| **Additional marine taxonomic groups (Mollusca)** **(µg/l)** | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 100 | Deliverable 2.3.3.1 |
| | Acute | | |

Chronic data are available only for one of the main three trophic levels, i.e. from larval development study with crustaceans. Given that invertebrates represent the most sensitive species, the AF of 1000 is applied to the lowest long-term test result for copepods. The PNEC so generated is equal to 0.0001 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC(invertebrates) | 0.01 | 1000 | 0.00001 |

## 3.14 Clothianidin

Trophic levels: 5

Species groups: 4

Species: 5

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 96 h, growth NOEL = 6350 | EPA-OPP Pesticide Toxicity Database, 2012. Bayer Co., Agricultural Division, U.S. |
| | Acute | *Skeletonema costatum*, 96 h, growth EC50 = 17600 | |
| **Invertebrates** (µg/l) | Chronic | *Acartia Tonsa,* F1 larval development NOEC = 0.01 | Deliverable D2.3.3.1 |
| | Acute | | |
| **Fish** (µg/l) | Chronic | | EPA-OPP Pesticide Toxicity Database, 1999. Dr. V. Noack Lab for Applied Biology, Sarstedt, Germany |
| | Acute | *Cyprinodon variegatus*, 96h, mortality EC50 > 93600 | |
| **Additional marine taxonomic groups (Mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h, ELS larval development NOEC = 100 | Deliverable 2.3.3.1 |
| | Acute | | |

Two trophic levels are covered by chronic tests showing that *Acartia Tonsa* is the most sensitive species. For this reason, an AF of 500 can be applied to the lowest long-term result for invertebrates. An additional chronic result is presented for marine taxonomic group showing that molluscs is not the most sensitive species and allowing so to lower the AF to 100 according to case 4.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC(copepods) | 0.01 | 100 | 0.0001 |

### 3.15 Thiamethoxan

Trophic levels: 4

Species groups: 4

Species: 4

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 72 h, growth<br>EC10 = 8700 | Finnegan et al., 2017. Environ. Toxicol.Chem.36: 2838-2848 |
| | Acute | *Skeletonema costatum*, 72 h, growth<br>EC50 > 99000 | |
| **Invertebrates** (µg/l) | Chronic | *Arcatia tonsa*, F1 larval development<br>NOEC > 0.1 | Deliverable D2.3.3.1 |
| | Acute | | |
| **Fish** (µg/l) | Chronic | *Cyprinodon variegatus*, 33 d, growth<br>NOEC = 1700 | Finnegan et al., 2017. Environ. Toxicol.Chem.36: 2838-2848<br><br>Moeris et al., 2021. Env. Tox. Chem. 40, 1343-1367 |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>EC50 > 111000 | |
| **Additional marine taxonomic groups (Mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 1000 | Deliverable 2.3.3.1 |
| | Acute | | |

Long-term results covering the three main trophic levels are provided. In this case, an AF of 100, as specified in the scenario 5 under the REACH guidelines, should be applied. Although, the AF of 100 can be further reduced to 10 as long-term tests for additional species representing a marine taxonomic group (molluscs) have been carried out and they indicate that these are not the most sensitive group. The AF of 10 is then applied to the lowest chronic test available, NOEC of copepods, resulting in a PNEC of 0.01 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC(copepods) | 0.1 | 10 | 0.01 |

### 3.16 Acetamiprid

Trophic levels: 4

Species groups: 4

Species: 4

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | *Skeletonema costatum*, 120 h, growth<br>NOEL = 1000 | EPA-OPP Pesticide Toxicity Database, 1997. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Skeletonema costatum*, 120 h, growth<br>EC50 > 1000 | |
| **Invertebrates** **(µg/l)** | Chronic | *Acartia tonsa*, F1 larval development<br>NOEC = 0.01 | Deliverable D2.3.3.1 |
| | Acute | | |
| **Fish** **(µg/l)** | Chronic | | EPA-OPP Pesticide Toxicity Database, 1998. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 = 100000 | |
| **Additional marine taxonomic groups (Mollusca)** **(µg/l)** | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC > 10000 | Deliverable 2.3.3.1 |
| | Acute | | |

Two long-term tests covering the main trophic levels of algae and invertebrates are available. According to case 6 of the REACH guidelines, an AF of 50 should be applied since a further long-term result from an additional marine taxonomic group (molluscs) is provided and it shows that this is not the most sensitive species. The PNEC value resulting from the application of an AF = 50 to the lowest chronic test (NOEL invertebrates) is 0.0002 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEL (invertebrates) | 0.01 | 50 | 0.0002 |

### 3.17 Methiocarb

Trophic levels: 7

Species groups: 5

Species: 9

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | *Skeletonema costatum*, 96 h, growth<br>NOEL = 180 | EPA-OPP Pesticide Ecotoxicity Database, 2014. Wildlife International Inc. |
| | Acute | *Skeletonema costatum*, 96 h, growth<br>EC50 = 630 | |
| **Invertebrates** **(µg/l)** | Chronic | *Americamysis bahia*, 28 d, growth<br>NOEL = 1.65 | EPA-OPP Pesticide Ecotoxicity Database, 2014. Wildlife International Inc. |
| | Acute | *Americamysis bahia*, 96 h, mortality<br>LC50 = 12.4 | |
| **Fish** **(µg/l)** | Chronic | *Cyprinodon variegatus*, 33 d, growth<br>NOEL = 5.9 | EPA-OPP Pesticide Ecotoxicity Database, 2014. Wildlife International Inc.<br><br>EPA Ecotox, 1985. Brunson, M.W. et al, - Louisiana Agricultural Experiment Station (USA) |
| | Acute | *Menidia menidia*, 4 d, mortality<br>LC50 = 51 | |
| **Additional marine taxonomic groups (Mollusca)** **(µg/l)** | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 1000 | Deliverable 2.3.3.1 |
| | Acute | | |
| **Additional species (bacteria)** **(µg/l)** | Chronic | | Escher et al., 2017. Environ Sci Process Impacts.19::414-428. |
| | Acute | *Aliivibrio fischeri*, 30 min, Bioluminescence<br>EC50 = 10300 | |

Long-term and short-term data are available for three saltwater species (algae, crustaceans and fish) representing three trophic levels, thus belonging to the case 5 of the REACH guidelines. In addition, long-term tests for species representing marine taxonomic group molluscs have been carried out and indicate that these are not the most sensitive group. Based on this consideration, the AF of 100 can be further reduced to 10 and applied to the lowest chronic test result (NOEL invertebrates). The resulting PNEC is 0.165 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEL (invertebrates) | 1.65 | 10 | 0.165 |

## 3.18 Oxadiazon

Trophic levels: 4

Species groups: 4

Species: 4

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 120 h, growth<br>NOEL = 1.4 | EPA-OPP Pesticide Ecotoxicity Database, 1990. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Skeletonema costatum*, 120 h, growth<br>EC50 = 5.2 | |
| **Invertebrates** (µg/l) | Chronic | *Americamysis bahia*, 28 d, growth<br>NOEL = 44 | EPA-OPP Pesticide Ecotoxicity Database, 2004. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Americamysis bahia*, 96 h mortality<br>LC50 = 270 | EPA-OPP Pesticide Ecotoxicity Database, 1992. Springborn Laboratory Inc., Wareham, MA |
| **Fish** (µg/l) | Chronic | | EPA-OPP Pesticide Ecotoxicity Database, 1992. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 = 1500 | |
| **Additional marine taxonomic groups (Mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 1000 | Deliverable 2.3.3.1 |
| | Acute | | |

According to the case 4 from REACH guidelines, the AF should be 500 as long-term tests representing two of the main trophic levels are available. The AF of 500 can be lowered to 100 since long-term tests for one additional species representing marine taxonomic groups (molluscs) have been carried out and indicate that these are not the most sensitive group. Applying the AF 100 to the lowest chronic result (NOEL of algae), generates a PNEC of 0.014 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEL (algae) | 1.4 | 100 | 0.014 |

## 3.19 Triallate

Trophic levels: 4

Species groups: 3

Species: 8

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 96 h, growth<br>NOEL = 34 | EPA-OPP Pesticide Ecotoxicity Database, 2002. Wildlife International Inc. |
| | Acute | *Skeletonema costatum*, 96 h, growth<br>EC50 = 330 | |
| **Invertebrates** (µg/l) | Chronic | *Americamysis bahia*, 28 d, growth<br>NOEL = 2.3 | EPA-OPP Pesticide Ecotoxicity Database, 2017. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Americamysis bahia*, 96 h, mortality<br>LC50 = 210 | |
| **Fish** (µg/l) | Chronic | *Cyprinodon variegatus*, 34 d ELS, growth<br>NOEL = 99 | EPA-OPP Pesticide Ecotoxicity Database, 2017. Springborn Laboratory Inc., Wareham, MA |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 = 900 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic | *M. galloprovincialis*, 48 h ELS, larval development<br>NOEC = 0.01 | Deliverable D2.3.3.1 |
| | Acute | | |

Long-term and short-term data are available for three saltwater species (algae, crustaceans and fish) representing three trophic levels, thus belonging to the case 5 of the REACH guidelines. However, these results were not generated for the species showing the most sensitive endpoints. An AF of 100 should be applied to the lowest chronic result (NOEC molluscs), resulting in a PNEC equal to 0.0001 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEL (invertebrates) | 0.01 | 100 | 0.0001 |

## 3.20 Metaflumizone

Trophic levels: 3

Species groups: 3

Species: 3

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | | EPA-OPP Pesticide Ecotoxicity Database, 2001. Analytical Biochemical Laboratory, USA |
| | Acute | *Americamysis bahia*, 96 h, mortality<br>LC50 > 289 | |
| **Fish** **(µg/l)** | Chronic | | EPA-OPP Pesticide Ecotoxicity Database, 2001. BASF Corporation, Germany |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 > 257 | |
| **Additional marine taxonomic groups (mollusca)** **(µg/l)** | Chronic | | |
| | Acute | | |

The scarcity of tests for this chemical and the uncertainty related to the endpoint of test on molluscs have forced to arbitrarily impose a precautionary AF of 10000. The value considered is the unbounded short-term result on fish studies, providing a PNEC = 0.0257 which is protective for the most sensitive species (i.e. molluscs).

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| LC50 (fish) | 257 | 10000 | 0.0257 |

## 3.21 Glyphosate

Trophic levels: 8

Species groups: 9

Species: 16

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 7 d, growth<br>NOEL < 240 | EPA-OPP Pesticide Ecotoxicity Database, 1987. Malcolm Pernie Inc., US |
| | Acute | *Skeletonema costatum*, 7 d, growth<br>EC50 = 870 | |
| **Invertebrates** (µg/l) | Chronic | *Neohelice granulate*, 90 d, growth<br>LOEC = 24.5 | Avigliano et al., 2018. Water Air Soil Pollut. 229: 44.<br><br>Vurm et al., 2021. Toxics 9, 275 |
| | Acute | *Artemia salina*, 24 h, mortality<br>LC50 = 811 | |
| **Fish** (µg/l) | Chronic | *Gasterosteus aculeatus*, 42 d, growth<br>NOEC = 100 | Le Mer et al., 2013. Ecotoxicol. Environ. Saf.89(0): 174-181<br><br>EPA-OPP Pesticide Ecotoxicity Database, 1996. Brixham Lab., UK |
| | Acute | *Cyprinodon variegatus*, 96 h, mortality<br>LC50 = 240000 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic | | Bringer et al., 2021. Sci. Tot. Env. 779, 144363 |
| | Acute | *Crassostrea gigas*, 24 h ELS, development arrest<br>EC50 = 241 | |
| **Additional marine taxonomic groups (Echinoida)** (µg/l) | Chronic | | Xu et al., 2011. Hum. Exp. Toxicol.30(8): 1009-1021 |
| | Acute | *Glyptocidaris crenularis*, 50 h, development<br>EC50 = 1074 | |
| **Additional marine taxonomic groups (Coelenterata)** (µg/l) | Chronic | | Demetrio et al., 2012. Bull. Environ. Contam. Toxicol.88: 15-19 |
| | Acute | *Hydra attenuate*, 96 h, mortality<br>LC50 = 15700 | |
| **Additional species (bacteria)** (µg/l) | Chronic | | Vurm et al., 2021. Toxics 9, 275 |
| | Acute | *Aliivibrio fisheri*, 30 min, Bioluminescence inhibition<br>IC50 = 2928 | |
| **Additional species (protozoa)** (µg/l) | Chronic | | Elandalloussi et al., 2008. Bull Environ Contam Toxicol 80, 512–515 |
| | Acute | *Perkinsus olseni*, 72 h, Viability<br>IC50 = 574800 | |

Long and short-term results are provided across the main three trophic levels (algae, invertebrates and fish). Based on this, case 5 of the REACH guidelines recommends an assessment factor of 100. This value can be decreased to 10 since additional short term tests were generated for species that are not the most sensitive ones, i.e. echinoida, mollusca, coelenterata, bacteria and protozoa. The lowest long-term result

matches with the LOEC for invertebrates *Neohelice granulate* on growth tests. When only LOEC values are available, a NOEC can be estimated as NOEC = LOEC/2 if the LOEC data corresponds to effects from 10% up to 20 (ECHA, 2008). In the study by Avigliano et al. (2018), LOEC for invertebrates corresponds to a 40% of effect, thus it has to be discarded. The second available long-term data is represented by fish, i.e. NOEC =100 µg/l. The application of an AF of 10 to this value would generated a PNEC of 10 µg/l, which is protective towards all marine groups tested.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (fish) | 100 | 10 | 10 |

## 3.22 AMPA

Trophic levels: 2

Species groups: 2

Species: 2

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Invertebrates** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Fish** **(µg/l)** | Chronic | | |
| | Acute | | |
| **Additional marine taxonomic groups (mollusca)** **(µg/l)** | Chronic | *Crassostrea gigas*, 48 h, development (subchronic) NOEC = 10000 | Mottier et al., 2013. Aquat. Toxicol. 128–129, 67–78 |
| | Acute | *Crassostrea gigas*, 36 h, development EC50 = 50780 | Di Poi et al., 2018. Environ. Sci. Pollut. Res. 25, 6122-6134 |
| **Additional marine taxonomic groups (echinoida)** **(µg/l)** | Chronic | | Asnicar et al., 2020. J. Mar. Sci. Eng. 8, 661 |
| | Acute | | |

Having tests covering only additional marine species (molluscs and echinoderms) forced to consider the most protective AF, i.e. 10000 to apply to the lowest short-term result.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 | 50780 | 10000 | 5.078 |

### 3.23 PFOA

Trophic levels: 5

Species groups: 6

Species: 11

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Isochrysis galbana*, 72 h, growth NOEC = 25000 | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| | Acute | *Isochrysis galbana*, 72 h, growth EC50 = 163600 | |
| **Invertebrates** (µg/l) | Chronic | | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| | Acute | *Siriella armata*, 96 h, mortality EC50 = 15500 | |
| **Fish** (µg/l) | Chronic | | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| | Acute | *Psetta maxima*, 144 h ELS, mortality (subchronic) EC50 = 11900 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic* | *Mytilus galloprovincialis*, 48 h ELS, development (subchronic) NOEC = 0.01 | Fabbri et al., 2014. Mar. Environ. Res. 99, 1–8 |
| | Acute | | |
| **Additional marine taxonomic groups (echinoida)** (µg/l) | Chronic | *Strongylocentrotus purpuratus*, 96 h, normal development (subchronic) NOEC = 4700 | Hayman et al., 2021, Chemosphere 273, 129699 |
| | Acute | | |
| **Additional species (bacteria)** (µg/l) | Chronic | | Hayman et al., 2021, Chemosphere 273, 129699 |
| | Acute | *Pyrocystis lunula*, 24 h, Light output EC50 = 18000 | |

*alternative value: NOEC = 390 µg/l, test of 96 h ELS, normal development (Hayman et al., 2021)

Considering Fabbri et al.'s values ambiguous, a more recent test on 96 hours normal development of molluscs presenting a NOEC = 390 µg/l is here considered more indicative of chronic toxicity for this species (Hayman et al., 2021). We have now short-term results covering the three main trophic levels with also results from two additional marine taxonomic groups (mollusca and echinoida). Case 2 of REACH guidelines should be followed by application of an AF = 1000 to the lowest EC50, which is $EC50_{fish}$ = 11900 µg/l, and resulting PNEC equal to 11.9 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| EC50 (fish) | 11900 | 1000 | 11.9 |

### 3.24 PFOS

Trophic levels: 5

Species groups: 6

Species: 12

| | | | Master reference |
|---|---|---|---|
| **Algae and aquatic plants** (µg/l) | Chronic | *Skeletonema costatum*, 96 h, growth<br>NOEC = 3200 | EQS draft dossier, tab 7.1, 2021. |
| | Acute | *Isochrysis galbana*, 72 h, growth<br>EC50 = 37500 | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| **Invertebrates** (µg/l) | Chronic | *Americamysis bahia*, 35 d, reproduction and growth<br>NOEC = 250 | EQS draft dossier, tab 7.7 from updated EQS dossier on PFOS (2017) |
| | Acute | *Americamysis bahia*, 96 h, mortality<br>EC50 = 3600 | |
| **Fish** (µg/l) | Chronic | | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| | Acute | *Psetta maxima*, 144 h ELS, mortality<br>EC50 = 110 | |
| **Additional marine taxonomic groups (mollusca)** (µg/l) | Chronic* | *Mytilus galloprovincialis*, 48 h ELS, development<br>NOEC = 0.01 | Fabbri et al., 2014. Mar. Environ. Res. 99, 1–8 |
| | Acute | | |
| **Additional marine taxonomic groups (echinoida)** (µg/l) | Chronic | *Strongylocentrotus purpuratus*, 96 h ELS, normal development<br>NOEC = 1100 | Hayman et al., 2021. Chemosphere 273 (2021) 129699 |
| | Acute | *Paracentrotus lividus*, 48h ELS, growth<br>EC50 = 20000 | Mhadhbi et al., 2012. J. Environ. Monit. 14, 1375-1382 |
| **Additional species (bacteria)** (µg/l) | Chronic | | Hayman et al., 2021. Chemosphere 273 (2021) 129699 |
| | Acute | *Pyrocystis lunula*, 24 h, light output<br>EC50 = 4900 | |

*alternative value: NOEC = 880 µg/l, test of 96 h ELS, normal development (Hayman et al., 2021)

Considering Fabbri et al.'s values ambiguous, a more recent test on 96 hours normal development of molluscs presenting a NOEC = 880 µg/l is here considered more indicative of chronic toxicity for this species (Hayman et al., 2021). According to case 2, and AF of 1000 should be applied to the lowest of two long term results covering two trophic levels (algae and invertebrates) since such results have not been generated for the species showing the lowest acute data of the short-term tests, which is for fish EC50 = 110 µg/l. The resulting PNEC is then equal to 0.25 µg/l.

| End point | End point value (µg/l) | AF | $PNEC_{sw}$ value (µg/l) |
|---|---|---|---|
| NOEC (invertebrates) | 250 | 1000 | 0.25 |

# 4. Conclusions

In this work the Predicted No Effect Concentration (PNEC) was calculated for 24 substances classified as emerging chemicals that can pose a risk to the aquatic ecosystem of the Venice lagoon. The procedure complied with the REACH regulation for chemicals risk assessment and was supported by two guidance documents which cover many of the key technical issues involved in the interpretation and classification of marine-water ecotoxicity data. Data consisted of previously collected results available from different existing databases and peer-reviewed literature as described in Milestone M2.3.1, together with experimental bioassays results on copepods and bivalves presented in Deliverable 2.3.3.1.

The rationale for assessing the adequacy and completeness of ecotoxicity data assisted the determination of the PNEC values. Acknowledging the difficulty of classifying data as indicative of either chronic or acute toxicity, guidelines were constantly accompanied by professional judgment and pragmatic choices. For 23 substances PNECs were deterministically calculated by application of the appropriate Assessment Factor to the most sensitive ecotoxicity data available. Only for bisphenol A, whose dataset was particularly abundant, it was possible to derive a probabilistic PNEC with a Species Sensitivity Distribution analysis. While experimental data obtained in WP 2.3.3 on copepods and bivalves contributed to the completeness of some datasets for the derivation of the AF, this work highlighted emerging chemicals for which ecotoxicity information on saltwater marine species is still scarce and the need for future experimental tests may seem desirable (e.g. EHMC, hormones and plant protection product metaflumizone).

The next task within Line 2.3 of Venezia2021 will make use of the PNEC values here derived and will compare them with the Predicted Environmental Concentrations (PECs) obtained from experimental and modelling activities in order to characterize the environmental risks associated to emerging contaminants and contribute to the identification of a “watch list” for the Venice lagoon.

## Bibliography


Alexander, H.C., Dill, D.C., Smith, L.W., Guiney, P.D., Dorn, P., 1988. Bisphenol A: Acute aquatic toxicity. Environ. Toxicol. Chem. 7, 19–26. doi:10.1002/etc.5620070104.

Andersen, H.R., Wollenberger, L., Halling-Sorensen, B., Kusk, K.O., 2001. Development of copepod nauplii to copepodites - A parameter for chronic toxicity including endocrine disruption. Environ. Toxicol. Chem. 20, 2821–2829. doi:10.1002/etc.5620201222.

Avigliano, L., Canosa, I.S., Medesani, D.A., Rodriguez, E.M., 2018. Effects of Glyphosate on Somatic and Ovarian Growth in the Estuarine Crab Neohelice granulata, During the Pre-Reproductive Period. Water Air Soil Pollut 229, 44 (2018). doi; 10.1007/s11270-018-3698-0.

Batley, G.E., van Dam, R.A., Warne, M.St.J., Chapman, J.C., Fox, D.R., Hickey, C.W. and Stauber, J.L., 2014. Technical Rationale for Changes to the Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants. CSIRO.

Billinghurst, Z., Clare, A., Depledge, M., 2001. Effects of 4-n-nonylphenol and 17β-oestradiol on early development of the barnacle Elminius modestus. J. Exp. Mar. Bio. Ecol. 257, 255–268. doi:10.1016/S0022-0981(00)00338-5.

Boudreau, M., Courtenay, S.C., MacLatchy, D.L., Bérubé, C.H., Parrott, J.L., Van der Kraak, G.J., 2004. Utility of morphological abnormalities during early-life development of the estuarine mummichog, Fundulus heteroclitus, as an indicator of estrogenic and antiestrogenic endocrine disruption. Environ. Toxicol. Chem. 23, 415–25. doi: 10.1897/03-50

Breitholtz, M., Bengtsson, B.E., 2001. Oestrogens have no hormonal effect on the development and reproduction of the harpacticoid copepod Nitocra spinipes. Mar. Pollut. Bull. 42, 879–886. doi:10.1016/S0025-326X(01)00046-7.

Capolupo, M., Díaz-Garduño, B., Martín-Díaz, M.L., 2018. The impact of propranolol, 17α-ethinylestradiol, and gemfibrozil on early life stages of marine organisms: effects and risk assessment. Environ. Sci. Pollut. Res. 25, 32196–32209. doi:10.1007/s11356-018-3185-6.

Carballeira, C., De Orte, M.R., Viana, I.G., DelValls, T.A., Carballeira, A., 2012. Assessing the Toxicity of Chemical Compounds Associated With Land-Based Marine Fish Farms: The Sea Urchin Embryo Bioassay With Paracentrotus lividus and Arbacia lixula. Arch. Environ. Contam. Toxicol. 63, 249–261. doi:10.1007/s00244-012-9769-0.

Demetrio, P.M., Rossini, G.D.B., Bonetto, C.A., Ronco, A.E., 2012. Effects of Pesticide Formulations and Active Ingredients on the Coelenterate Hydra attenuata (Pallas, 1766). Bull. Environ. Contam. Toxicol.88: 15-19 doi:10.1007/s00128-011-0463-0.

De Orte, M.R., Carballeira, C., Viana, I.G., Carballeira, A., 2013. Assessing the toxicity of chemical compounds associated with marine land-based fish farms: The use of mini-scale microalgal toxicity tests. Chem. Ecol. 29, 554–563. doi:10.1080/02757540.2013.790381.

European Commission, 2011. Technical guidance for deriving environmental quality standards. Guidance Document No 27**,** Common Implementation Strategy for the Water Framework Directive, European Commission, Brussels, 204 pp.

[ECHA] European Chemicals Agency, 2011. Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. Version 2.1, December 2011. ECHA-11-G-16-EN, European Chemicals Agency.

[ECHA] European Chemicals Agency, 2008. Guidance for implementation of REACH: Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterization of dose [concentration] - response for environment. Helsinki: European Chemicals Agency.

Elandalloussi, L. M., Leite, R. B., Rodrigues, P. M., Afonso, R., Cancela, M. L., 2008. Effect of the Herbicide Roundup® on Perkinsus olseni in vitro Proliferation and in vivo Survival when Infecting a Permissive Host, the Clam Ruditapes decussatus. Bull Environ Contam Toxicol 80: 512–515 doi: 10.1007/s00128-008-9412-y.

Escher, B., Baumer, A., Bittermann, K., Henneberger, L., König, M., Kühnert, C., Klüver, N., 2017. General baseline toxicity QSAR for nonpolar, polar and ionisable chemicals and their mixtures in the bioluminescence inhibition assay with Aliivibrio fischeri. Environ Sci Process Impacts 19:414-428, doi: 10.1039/C6EM00692B.

Fabbri, R., Montagna, M., Balbi, T., Raffo, E., Palumbo, F., Canesi, L., 2014. Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in Mytilus galloprovincialis. Mar. Environ. Res. 99, 1–8. doi:10.1016/J.MARENVRES.2014.05.007.

Finnegan, M.S., Baxter, L.R., Maul, J., Hanson, M.L., Hoekstra, P.F., 2017. Comprehensive characterization of the acute and chronic toxicity of the neonicotinoid insecticide thiamethoxam to a suite of primary producers, invertebrates, and fish. Environ. Toxicol. Chem.36: 2838-2849. doi:10.1002/etc.3846.

Fontes, M.K., Kachel Gusso-Choueri, P., Maranho, A., Moledo De Souza Abessa, D., Almeida Mazur, W., Galv, B., Ao De Campos, Lopes, L., Aes, G., Sergio De Toledo, M., Lebre, D., Rodrigues Marques, J., Felicio, A.A., Cesar, A., Alves Almeida, E., Dias, C., Pereira, S., 2018. A tiered approach to assess effects of diclofenac on the brown mussel Perna perna: A contribution to characterize the hazard. Water Res. 132: 361–370.

González-Ortegón, E., Blasco, J., Nieto, E., Hampel, M., Le Vay, L., & Giménez, L., 2016. Individual and mixture effects of selected pharmaceuticals on larval development of the estuarine shrimp Palaemon longirostris. Sci. Tot. Env. 540, 260-266. doi:10.1016/j.scitotenv.2015.06.081.

Hagenbuch, I.M., Pinckney, J.L., 2012. Toxic effect of the combined antibiotics ciprofloxacin, lincomycin, and tylosin on two species of marine diatoms. Water Res. 46, 5028–5036. doi:10.1016/J.WATRES.2012.06.040.

Hahn, T., Diamond, J., Dobson, S., Howe, P., Kielhorn, J., Koennecker, G., Lee-Steere, C., Mangelsdorf, I., Schneider, U., Sugaya, Y., Taylor, K., Van Dam R. and Stauber, J.L., 2013. Predicted no effect concentration derivation as a significant source of variability in environmental hazard assessments of chemicals in aquatic systems: An international analysis. Integr. Environ. Assess. Manag. , 10, 30-36.

Hayman, T. N., Rosen, G., Colvin, M.A., Conder, J., Arblaster, J.A., 2021. Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. Chemosphere 273 (2021) 129699.

Hernando, M.D., De Vettori, S., Martínez Bueno, M.J., Fernández-Alba, A.R., 2007. Toxicity evaluation with Vibrio fischeri test of organic chemicals used in aquaculture. Chemosphere 68, 724–730. doi:10.1016/J.CHEMOSPHERE.2006.12.097

Kelly, S.A., Di Giulio, R.T., 2000. Developmental toxicity of estrogenic alkylphenols in killifish (Fundulus heteroclitus ). Environ. Toxicol. Chem. 19, 2564–2570. doi:10.1002/etc.5620191024.

Le Mer, C., Roy, R.L., Pellerin, J., Couillard, C.M., Maltais, D., 2013. Effects of Chronic Exposures to the Herbicides Atrazine and Glyphosate to Larvae of the Threespine Stickleback (Gasterosteus aculeatus). Ecotoxicol. Environ. Saf.89(0): 174-181. doi: 10.1016/j.ecoenv.2012.11.027.

Lützhøft, H., Halling-Sørensen, B., Jørgensen, S., 1999. Algal Toxicity of Antibacterial Agents Applied in Danish Fish Farming. Arch. Environ. Contam. Toxicol. 36, 1–6. doi: 10.1007/s002449900435.

Machado, M.D., Soares, E. V., 2019. Sensitivity of freshwater and marine green algae to three compounds of emerging concern. J. Appl. Phycol. 31, 399–408. doi:10.1007/s10811-018-1511-5.

Marcial, H.S., Hagiwara, A., Snell, T.W., 2003. Estrogenic compounds affect development of harpacticoid copepod Tigriopus japonicus. Environ. Toxicol. Chem. 22, 3025–3030. doi:10.1897/02-622.

Mhadhbi, L., El Ayari, T., Tir, M., Kadri, D., 2020. Azithromycin effects on the European sea bass (Dicentrarchus labrax) early life stages following acute and chronic exposure: Laboratory bioassays. Drug Chem Toxicol. 22:1-7. doi: 10.1080/01480545.2020.1822388.

Mhadhbi, L., Rial, D., Pérez, S., Beiras, R., 2012. Ecological risk assessment of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in marine environment using Isochrysis galbana, Paracentrotus lividus, Siriella armata and Psetta maxima. J. Environ. Monit. 14, 1375-1382 doi:10.1039/c2em30037k.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., Halm-Lemeille, M. P., 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environ Sci Pollut Res (2016) 23:4992–5001.

Moeris, S., Vanryckeghem, F., Demeestere, K., De Schamphelaere, K. A., 2021. Neonicotinoid insecticides from a marine perspective: acute and chronic copepod testing and derivation of environmental quality standards. Environ. Toxicol. Chem. 40, 1353-1367. doi:10.1002/etc.4986.

Mohd Zanuri, N.B., Bentley, M.G., Caldwell, G.S., 2017. Assessing the impact of diclofenac, ibuprofen and sildenafil citrate (Viagra®) on the fertilisation biology of broadcast spawning marine invertebrates. Environ. Res. 127, 126–136. doi:10.1016/j.marenvres.2017.04.005.

Mottier, A., Kientz-Bouchart, V., Serpentini, A., Lebel, J.M., Jha, A.N., Costil, K., 2013. Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas. Aquat. Toxicol. 128–129, 67–78 doi:10.1016/J.AQUATOX.2012.12.002.

Nassef, M., S. Matsumoto, M., Seki, I.K., Kang, J., Moroishi, Shimasaki, Y., Oshima, Y., 2009. Pharmaceuticals and personal care products toxicity to Japanese medaka fish (Oryzias latipes). J. fac. Agr., Kyushu Univ., 54, 407-411.

Paredes, E., Perez, S., Rodil, R., Quintana, J.B., Beiras, R., 2014. Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. Chemosphere 104, 44–50. doi:10.1016/J.CHEMOSPHERE.2013.10.053.

Pérez, S., Rial, D., Beiras, R., 2015. Acute toxicity of selected organic pollutants to saltwater (mysid Siriella armata) and freshwater (cladoceran Daphnia magna) ecotoxicological models. Ecotoxicology 24, 1229–1238. doi: 10.1007/s10646-015-1489-6.

Roepke, T.A., Snyder, M.J., Cherr, G.N., 2005. Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquat. Toxicol. 71, 155–173. doi:10.1016/j.aquatox.2004.11.003.

Shaukat, A., Liu, G., Li, Z., Xu, D., Huang, Y., Chen, H., 2014. Toxicity of five phenolic compounds to brine shrimp Artemia sinica (Crustacea: Artemiidae). J. Ocean Univ. China 13, 141–145. doi:10.1007/s11802-014-1980-3.

Schmidt, W., O'Rourke, K., Hernan, R., Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar. Pollut. Bull. 62, 1389–1395. doi:10.1016/J.MARPOLBUL.2011.04.043.

Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environ. Toxicol. Chem. 16, 2494–2500 doi:10.1002/etc.5620161209.

Williams, R.R., Bell, T.A., Lightner, D., 1992. Shrimp Antimicrobial Testing. II. Toxicity Testing and Safety Determination for Twelve Antimicrobials with Penaeid Shrimp Larvae. J. Aquat. Anim. Health 4, 262–270.

Xu, X., Wang, X., Li, Y., Wang, Y., Wang, Y., 2011. Acute Toxicity and Synergism of Binary Mixtures of Antifouling Biocides with Heavy Metals to Embryos of Sea Urchin Glyptocidaris crenularis. Hum. Exp. Toxicol.30(8): 1009-1021.

Yamashita, N., Yasojima, M., Nakada, N., Miyajima, K., Komori, K., Suzuki, Y., Tanaka, H., 2006. Effects of antibacterial agents, levofloxacin and clarithromycin, on aquatic organisms. Water Sci. Technol. 53, 65. doi:10.2166/wst.2006.338 .