



DEPARTMENT OF BIOLOGICAL AND  
ENVIRONMENTAL SCIENCES

# HAZARD ASSESSMENT OF 1 932 INDUSTRIAL CHEMICALS REGISTERED WITHIN REACH

**Andreas Hellohf**

---

Degree project for Master of Science (120 hec) with a major in Ecotoxicology

ES2520, Master Thesis in Ecotoxicology, 120 hec

Second cycle

Semester/year: Spring 2022

Supervisor: Thomas Backhaus and Mikael Gustavsson

Examiner: Bethanie Carney Almroth, Department of Biological & Environmental Sciences

## Abstract

This report was conducted to examine if industrial chemicals present a hazard to the aquatic environment in Europe. Industrial chemicals produced within or imported to the European Union are regulated according to the REACH Regulation and most of them must be registered in the ECHA database. The database contains information regarding the chemicals physical and chemical properties, production volumes, release categories, toxicological and ecotoxicological data. Industrial chemicals are produced in large volumes and some are not only toxic but also bioaccumulating and/or persistent.

In order to assess the hazard potential these chemicals might present to the environment, the dossiers for all registered chemicals were downloaded, organized and analyzed. Only substances not labeled as mixtures or intermediates and for which ecotoxicological data was available were included in this report. The toxicity of industrial chemicals was compared to the toxicity of groups of chemicals regulated or monitored by other authorities, to see how they ranked. An assessment factor is applied to the aquatic toxicity value to derive a PNEC value and are along with production volume crucial variables in chemical risk assessment. The use and correlation between these variables were analyzed to see if any patterns could be observed. Industrial chemicals with persistent and/or bioaccumulating and/or toxic properties (PBT) were sorted into different subgroups, consisting of substances possessing two or more PBT properties, and their total production volume was calculated. The results from acute and chronic exposure on algae, aquatic invertebrates and fish were compared to see how they correlated to each other, how frequently the different groups were used in toxicity testing and how often the result from one group was used to derive a  $PNEC_{\text{freshwater}}$ . The result shows that industrial chemicals as a group are less toxic on average than other groups of chemicals compared to. It did however also show that the most toxic industrial chemicals, the lower 5 % percentile, are as toxic on average as many of the other groups of known hazardous compounds. Some of these chemicals were labeled as persistent and/or bioaccumulating and are produced in considerable volumes and could be a threat to the environment. The comparison between different groups of species regarding acute and chronic toxicity showed some indications of correlation, but few definite conclusions could be reached. The use frequency of a species or a group of species in toxicity testing does not necessarily reflect how often the result is used to derive a  $PNEC_{\text{freshwater}}$ . The choice of species for toxicity testing is primarily dependent on availability and not it's assumed sensitivity to a chemical.

## Table of content

	ABSTRACT .....	1
1.	INTRODUCTION .....	3
1.1	Industrial chemicals and the European Chemical Agency .....	6
1.2	Information requirement for registrant .....	6
1.3	PBT and vPvB and toxicity screening for the aquatic environment .....	7
1.3.1	Persistence .....	10
1.3.2	Bioaccumulation .....	11
1.3.3	Toxicity .....	12
1.4	Assessment factor for derivation of PNEC <sub>freshwater</sub> .....	13
1.5	PNEC for other compartments .....	14
2.	METHOD .....	14
2.1	Establishment of list of substances from the ECHA database .....	14
2.1.1	Substances excluded and corrected from the initial list .....	15
2.1	Substances controlled by other regulations .....	16
2.1.1	Pharmaceuticals .....	16
2.1.2	OSPAR Priority Pollutants .....	16
2.1.3	Biocides .....	17
2.1.4	Pesticides .....	17
2.1.5	WFD Priority substances .....	18
2.2	Variables and data used in graphs .....	19
2.2.1	The octanol-water coefficient .....	19
2.2.2	The bioconcentration factor .....	20
2.2.3	Production volume .....	20
2.2.4	Scaling of y-axis in graphs .....	20
2.2.5	Geometric mean .....	20
2.2.6	Effect concentration 50 % and lethal concentration 50 % .....	20
2.2.7	No observed effect concentration .....	21
2.2.8	Predicted No Effect Concentration and Predicted Environmental Concentration .....	21
2.2.9	Settings for box plots .....	22
2.3	The next step; calculation of PEC and risk assessment .....	22
2.3.1	EUSES .....	22
3.	RESULTS AND DISCUSSION .....	23
3.1	Comparison of substances under different legislations .....	23
3.2	Persistent (P) Bioaccumulating (B) and Toxic (T) compounds .....	25
3.3	Toxicity and lipophilicity .....	28
3.4	Toxicity for different production volumes .....	30
3.5	The use of assessment factors .....	34
3.6	Species sensitivity and use frequency .....	36
3.7	Acute to chronic ratio .....	41
3.8	Comparison of EC <sub>50</sub> between species .....	44
3.9	Comparison of NOEC between species .....	46
3.10	PNEC .....	49
3.10.1	Comparison of EC <sub>50</sub> and NOEC for PNEC <sub>freshwater</sub> .....	49
3.10.2	Comparison of PNEC for different compartments .....	50
3.10.3	Comparison of NOEC and EC <sub>50</sub> between species .....	52
4.	CONCLUSIONS .....	54
5.	APPENDIX .....	57
6.	REFERENCES .....	58

# 1. Introduction

In 1998 representatives from Austria, Sweden, Denmark, Finland and The Netherlands met experts at an informal meeting to discuss problems with the current chemical legislation in Europe. The outcome from the meeting was a joint position for the five member states and the conclusion that a major problem was the absence of a general chemical policy for the whole European Union (EU). The initiative was the starting point for discussions and the subject was investigated by the European Council and European Commission (EC) along with other stakeholders. The result was the “White Paper on a Strategy for Future Chemicals Policy” and it was adopted by the EC in 2001. It presented a strategy for future chemical policy and the framework for how one system would regulate new and old substances in order to secure human and environmental health. The proposed system was called Registration, Evaluation and Authorization and Restriction of Chemicals (REACH) (Schörling and Lund 2004). The REACH Regulation came into force on the 1<sup>st</sup> of June 2007 after many years of discussions and evaluations. Today almost all chemicals and mixtures imported or manufactured within the EU in volumes of greater than one tonne falls under the REACH regulation. It also applies to products containing chemicals, such as paint, clothes, electrical products etc. Groups of substances that are exempted or partially exempted from the REACH Regulation includes pesticides, pharmaceuticals, vet. drugs etc. (Swedish Chemicals Agency 2014). There are also groups of substances of special concern that are being monitored by other authorities than REACH. These groups include chemicals that are very hazardous or possess certain physical and chemical properties that makes them a threat to a certain part of the environment or to human health.

Today tens of thousands chemicals are registered under the REACH Regulation. For substances regulated by the REACH Regulation, there is a requirement for the registrant to provide information about the substance they produce or import. Information regarding its hazardous properties, physical and chemical properties, handling instructions, labeling etc. is to be provided to the European Chemicals Agency (ECHA) before they can be put on the European market. The amount of data that is required from the company varies for different chemicals, initially depending on the production volume. In the next step in the screening process, chemicals are evaluated for persistence, bioaccumulation and toxicity (PBT) potential in order to determine its hazardous properties. The outcome from this assessment is used to decide whether further testing is required or if the chemical can be approved for the European market. The submitted data is not verified by ECHA before being published, it is solely the company’s obligation to assure that the provided data is accurate and up to date. Every registered substance has a dossier in the ECHA database that contains information concerning physical and chemical properties, usage, exposure and manufacturing of the substance (ECHA 2014b).

The traditional approach used in environmental chemical risk assessment has been single substance studies, where an organism is exposed to one chemical and the concentration-response relationship is observed. Most of the information about the chemicals used in

ecotoxicological studies derives from single substance exposures in laboratory or single compound studies in microcosms. From the experiments a No Observed Effect Concentration (NOEC) or an Effect Concentration ( $EC_x$ ) can be calculated. The effect concentration commonly used is  $EC_{50}$ , the concentration where 50 % effect can be observed in the exposed group. For the freshwater compartment, an assessment factor (AF) of 5-1000 is used to compensate for the uncertainty from extrapolating the result from the experiment to the field. The uncertainty is due to the fact that there might be more sensitive species in the environment and the species in the field can vary in group composition (age, gender, and genes etc.) when compared to the species used in the laboratory. Another factor is the conditions in a laboratory that do not only differ from the environment, but they do also differ from other laboratories as do the people performing the experiments in them. The AF is also compensating for the extrapolation from acute to chronic exposure (ECHA 2008). All these factors combined contributes to why a measured effect from a laboratory study does not necessarily coincide with the observed effect in the environment. The value derived after applying the AF to the NOEC or  $EC_x$  is called the Predicted No Effect Concentration (PNEC), a level where no effect is predicted to occur in the environment (Leeuwen and Vermeire 2007).

Within the REACH Regulation, the Predicted No Effect Concentration (PNEC) is used in the regulatory process. It is often used with the Predicted Environmental Concentration (PEC), which is the concentration of a substance that is expected to be found in a certain part of the environment. The PEC is usually calculated in a model that is based upon how much, where and how the chemical is released into the environment and the physical and chemical properties of the chemical. If the ratio between the PEC and the PNEC (referred to as the risk quotient) for a chemical is  $> 1$  it is considered a risk (Walker 2006). This risk quotient is fundamental in the risk assessment within REACH.

This report aims to provide a hazard assessment of the industrial chemicals registered under the REACH Regulation. The focus is on the inherent characteristics of the chemicals; how many are classified as persistent, bioaccumulative or toxic and in what volumes they are produced and imported. The toxicity from industrial chemicals is also compared to the toxicity from other groups of chemicals, regulated or monitored by other authorities, to compare the hazardous potential. The procedure of deriving  $PNEC_{\text{freshwater}}$  is analyzed with a focus on what group of species that is most frequently used and how the assessment factor is used for different production volumes.

10 533 chemicals were registered at the time the data was downloaded. Chemicals registered as mixtures are not included in this report and consequently 1 931 chemicals are included in this report. This applies to every part in this report where “industrial chemicals” are referred to.

**This report aims to examine:**

- Do industrial chemicals represent a hazard to the aquatic environment?
- How toxic are industrial chemicals compared to substances regulated or monitored by other authorities?
- What is the production volume of substances with PBT or vPvB properties?
- Is assessment factor used differently depending on production volumes?
- What species are used more frequently in toxicity testing, and what species are used most frequently to derive PNEC for freshwater?
- What species used in toxicity testing are most sensitive and how does the result from tests on different trophic levels correspond to each other?
- Do the results from acute and chronic exposure testing correspond to each other?
- Do the PNEC values differ for the different environmental compartments?

## 1.1 Industrial chemicals and the European Chemical Agency

The European Chemical Agency (ECHA) was founded in 2007 and is a regulatory authority of the EU that ensures that the EU chemical legislation is implemented. The stated goal is to improve the quality of human health and the environment. ECHA makes information on chemicals available to the public and to manufacturers and provide guidance documents for the chemical registration process, an important part in the REACH Regulation. They also provide information about safe handling of the chemicals to ensure that no adverse effect to human health or the environment occur. Replacing hazardous compounds with safer alternatives is a continuous part of the work of ECHA in order to reduce risk for humans' health and the environment.

In December 2014 there was approximately 11 000 chemicals registered in the ECHA database. By May 2018 another 70 000 substances in the tonnage band from 1-100 tonnes are expected to be registered (ECHA 2015). This means that a total production volume of somewhere between 70 000 and 7 000 000 tonnes a year is left out in this report. As a comparison the yearly production of the substances included in this report is minimum 177 300 000 tonnes. Industrial chemicals are not intentionally released into the environment in the way e.g. pesticides are. Industrial chemicals enter the environment in different ways through multiple routes, low levels of chemicals can be found in all parts of the ecosystem; in the tissue of organisms, water, air, soil etc.

The registration process strives to ensure that sufficient relevant information is available or else produced, to enable safe handling of the chemical. The aim is to provide a joint level for the actors on the European market, so that all manufacturers put in the same minimum effort to ensure environmental and human health standards (ECHA 2014b).

Companies are obliged to exchange information on known hazardous properties so they can be used by other companies. ECHA makes this information available and by doing so, the manufacturers expenses can be kept at a minimum and time can be saved by reusing existing data and it also keeps animal testing to a minimum (ECHA 2014b). A negative aspect with this approach is that experiments that has gone wrong or calculations that are incorrect might stay in the system for a long time and that modern and more effective methods might not be used when available.

## 1.2 Information requirement for registrant

The production volume of the chemical determines the initial amount of information required before a product can be sold on the European market. The larger the production volume, the greater hazard potential does a chemical constitute theoretically, which is reflected in the REACH Regulation. The registrant of a substance is obliged to collect all available relevant information and the minimum information required is established in the REACH Regulation

Annex VII-X. Table 1 shows how the information requirement depends upon the production volume (REACH 2006).

**Table 1.** Information requirement according to production volume (CNRS 2007).

	All available relevant data	Annex VII requirements	Annex VIII requirements	Annex IX requirements	Annex X requirements
1-10 t/y	•	• <sup>1</sup>			
10-100 t/y	•	•	•		
100-1 000 t/y	•	•	•	• <sup>2</sup>	
≥ 1 000 t/y	•	•	•	• <sup>2</sup>	• <sup>2</sup>

<sup>1</sup> For the lowest tonnage band (1 to 10 t per year per manufacturer or importer), the required minimum information is confined to the physic-chemical data:

- if the substance is a “phase-in substance”, i.e. a substance already manufactured or imported, under certain conditions, before the entry into force of REACH on 1 June 2007 and,
- if the substance is predicted not likely to meet the criteria for category 1 or 2 classification for carcinogenicity, mutagenicity or reproductive toxicity and the PBT or vPvB criteria (PBT: persistent, bioaccumulative and toxic - vPvB: very persistent and very bioaccumulative) and,
- the substance does not have a dispersive or diffuse use and it is predicted not likely to meet the criteria for classification for any human health or environmental hazard.

<sup>2</sup> At this level, the registrant must submit a proposal and a time schedule for fulfilling the information requirements of this annex.

### 1.3 PBT and vPvB and toxicity screening for the aquatic environment

The initial amount of data required from the manufacturer is dependent on the production volume. Depending on the outcome of the screening process in the risk assessment of the chemical, additional data can be required. The screening process for substances is based upon three properties of the chemical: its persistence (P), bioaccumulation (B) and toxicity (T). In the screening process all these properties are considered when estimating the PBT and the very persistence very bioaccumulating (vPvB) potential of a substance.

Substances imported or manufactured in volumes greater than 10 tonnes per year and regulated by the REACH Regulation are required to undergo a PBT/vPvB assessment. Substances excluded from the assessment are substances which are either isolated intermediates, substances that are parts of mixtures in concentrations < 0.1 % weight by weight or used in “Product and Process Oriented Research and Development”. The

substances are evaluated based upon the physicochemical data that should be provided in the technical dossier along with exposure information (ECHA 2012a).

The persistence of a substance is defined as the time it takes to biologically or chemically transform a substance or physically remove it from a certain environment. The time required for this process is called biodegradation time and takes place inside an organism or in the environment. Bioaccumulation is the concentration of a substance in an organism as a result of biomagnification and bioconcentration. Bioconcentration occurs in the organism if the uptake from the medium (e.g. water) through gills, lungs or skin exceeds the organism's capacity to excrete or metabolite the substance. Biomagnification is the process by which the concentration is increasing by each trophic level (meaning that the organism has higher concentration than its food). The concentration in the organism as a result of those two processes represents the bioaccumulation. The ratio between the concentration in the organism and the medium is called the bioconcentration factor (BCF). Toxicity is a substances capacity to harm an organism with reference to the quantity taken up by the organism (Duffus et al. 2007), the lower concentration needed to cause a certain effect, the more toxic is the substance.

In the PBT assessment, a substance is evaluated for persistence, bioaccumulation and toxicity potential before a conclusion can be reached about its hazardous properties. The long-term effects from PBT/vPvB substances are hard to predict and once released into the environment it can be difficult to reverse the process since persistent substances will remain in the nature for a long time after the emissions have stopped. The vPvB screening process differ from the PBT assessment since is does not rely on the toxicity of the substance. It recognizes that a substances fulfilling the vPvB criteria can be found at unexpected high levels due to bioaccumulation through the food chain and reach toxic levels. A substance can be categorized as PBT or vPvB through a definitive criteria or a screening criteria. If a substance fulfills a definitive criteria it is categorized as such, according to Table 2.

**Table 2.** Definitive criteria for PBT and vPvB (ECHA 2012a)

<b>Property</b>	<b>PBT-criteria</b>	<b>vPvB-criteria</b>
<p><b>Persistence</b> Based on available half-life data.</p>	<ul style="list-style-type: none"> <li>- T1/2 &gt; 60 days in marine water, or</li> <li>- T1/2 &gt; 40 days in fresh- or estuarine water, or</li> <li>- T1/2 &gt; 180 days in marine sediment, or</li> <li>- T1/2 &gt; 120 days in fresh- or estuarine sediment, or</li> <li>- T1/2 &gt; 120 days in soil.</li> </ul>	<ul style="list-style-type: none"> <li>- T1/2 &gt; 60 days in marine, fresh- or estuarine water, or</li> <li>- T1/2 &gt; 180 days in marine, fresh- or estuarine sediment, or</li> <li>- T1/2 &gt; 180 days in soil.</li> </ul>
<p><b>Bioaccumulation</b> Based on measured data on bioconcentration in aquatic species in fresh and marine water.</p>	BCF > 2 000 L/kg	BCF > 5 000 L/kg
<p><b>Toxicity</b></p>	<ul style="list-style-type: none"> <li>- NOEC/EC/10 (long-term) &lt; 0.01 mg/L for marine or freshwater organisms, or</li> <li>- substance meets the criteria for classification as carcinogenic, germ cell mutagenic, or toxic for reproduction or there is other evidence of chronic toxicity</li> </ul>	-

For substances that does not fulfill any definitive criteria but has the potential to fulfill the PBT or vPvB criteria, the screening criteria in Table 3 is used. A substance that fails one criteria, e.g. persistence, still needs to be assessed for toxicity and bioaccumulation before considered fully evaluated. A substance that fail one criteria marginally but exceeds two other criteria substantially, can be categorized as a PBT substance according to Annex XIII of the REACH Regulation (ECHA 2012a).

**Table 3.** Screening Criteria for PBT and vPvB substances (ECHA 2012a)

	Type of data	Criterion	Screening assignment
<b>Persistence</b>	Ready biodegradability test	readily biodegradable	Not P and not vP
	Enhanced ready biodegradability test	readily biodegradable	Not P and not vP
	Specified tests on inherent biodegradability Zahn-Wellens (OECD 302B)	$\geq 70\%$ mineralization (DOC removal) within 7 d; log phase no longer than 3d; removal before degradation occurs below 15 %; no pre-adapted inoculum	Not P
	MITI II test (OECD 302C)	$\geq 70\%$ mineralization (O <sub>2</sub> uptake) within 14 days; log phase no longer than 3d; no pre-adapted inoculum	Not P
	Biowin 2 (non-linear model prediction) and Biowin 3 (ultimate biodegradation time) or	Does not biodegrade fast (probability < 0.5) <sup>4</sup> and ultimate biodegradation timeframe prediction: $\geq$ months (value < 2.2) or	P
	Biowin 6 (MITI non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	Does not biodegrade fast (probability < 0.5) <sup>3</sup> and ultimate biodegradation timeframe prediction: $\geq$ months (value < 2.2)	P
<b>Bioaccumulation</b>	Convincing evidence that a substance can biomagnify in the food chain (e.g field data <sup>5</sup> )	e.g. BMF > 1	B or vB
	Octanol-water partitioning coefficient (experimentally determined or estimated by valid QSAR)	Log K <sub>ow</sub> $\leq$ 4.5	Not B and not vB
<b>Toxicity</b>	Short-term aquatic toxicity (algae, daphnia, fish)	EC <sub>50</sub> or LC <sub>50</sub> < 0.01 mg/L	T
	Short-term aquatic toxicity (algae, daphnia, fish)	EC <sub>50</sub> or LC <sub>50</sub> < 0.1 mg/L	T
	Avian toxicity (subchronic or chronic toxicity or toxic for reproduction)	NOEC < 30 mg/kg food	T

### 1.3.1 Persistence

Estimating the persistence of a chemical is a complex procedure involving many factors and the data can be contradictory, especially when estimating the biodegradability. Only assessing the primary biodegradation may be insufficient if the degradation product possesses PBT or vPvB-properties.

The biodegradation rate is a fundamental factor when predicting long-term effects and is used when calculating the PEC for a substance. The persistence of a substance is evaluated in a tiered approach and if there is no evidence from prior testing for or against persistence, an initial screening can be made using Quantitative Structure–Activity Relationship (QSAR) models, non-standard test, standard test etc. If the initial screening does not exclude persistence, further analyze of the substance can be required. The final testing for vP or P potential is assessed according to the OECDs guidelines that defines which test should be used, based upon likely exposure scenario of the substance. The chemical is then defined and labeled into a category according to Table 4, depending on the time it takes for the substance to degrade (OECD 2006).

**Table 4.** Categorization of biodegradability (ECHA 2012b)

*Readily biodegradable* is an arbitrary definition of a compound which after a screening process is assumed to mineralize rapidly and completely in aquatic environment under aerobic conditions.

*Inherently biodegradable* is a compound which in 28 days is unequivocal biodegradable somewhere between 20-70 %. The biodegradability is recognized through an approved test method. If the biodegradation rate is over 70 % the substance is considered as *inherent, ultimate biodegradability*.

*Inherently biodegradable but failing 10-day window* is a substance that is biodegradable, but the final criteria for degradation is not met within a 10 day window within the 28 days. This 10-day window starts when 10 % of degradation is reached (the *lag time*).

*Not biodegradable* is a substance that has a negligible degradation under the test conditions.

### 1.3.2 Bioaccumulation.

The bioaccumulation potential of a chemical is estimated using all relevant data available. If sufficient data is not available, the screening criteria in Table 3 should be used. A substance is considered bioaccumulative if the bioconcentration factor (BCF) is 2 000-5 000 and very bioaccumulative if  $BCF > 5\,000$ . Substances with a  $\log K_{ow} < 4.5$  are assumed to not be hydrophobic enough to exceed the next threshold in the screening process, a BCF value of  $> 2\,000$ . This does not take into consideration bioaccumulation through other mechanisms than passive diffusion. It is assumed that substances with a  $\log K_{ow} > 10$  have a  $BCF < 2000$  and are not considered to be bioaccumulative. Other factors such as molecular size, octanol solubility etc. also influence the bioaccumulation potential (ECHA 2012a).

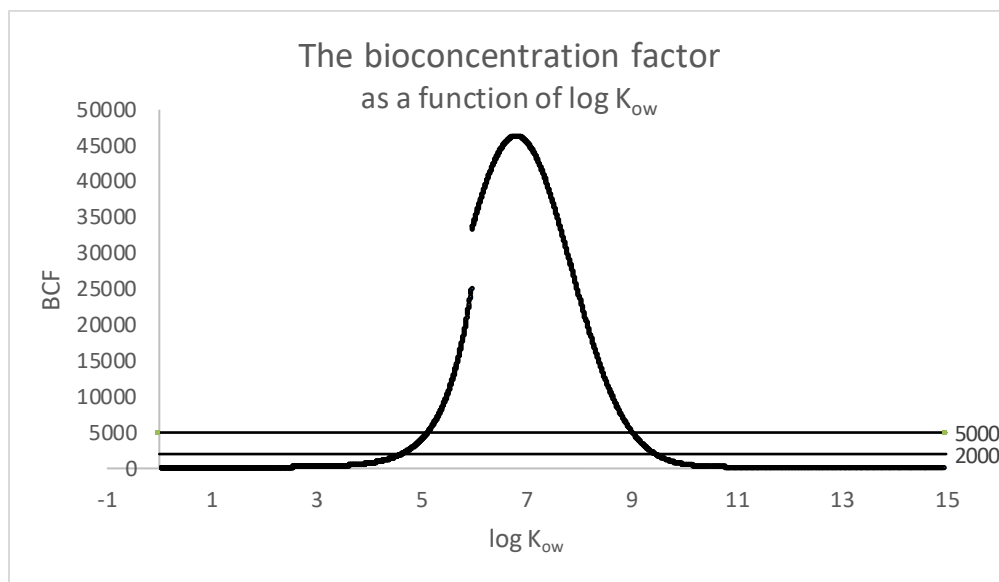
**Table 5.** Equations for calculating BCF (European Chemicals Bureau 2003)

$$1) \log BCF_{fish} = 0,85 \times \log K_{ow} - 0,70$$

$$2) \log BCF_{fish} = -0,20 \times \log K_{ow}^2 + 2,74 \times \log K_{ow} - 4,72$$

When assessing the bioaccumulation potential of a substance and its possible effect upon top predators, the predicted no-effect level is rarely based on internal concentration from wild

animals, instead it is based on their prey. By comparing the predicted concentration of the substance in the food with the predicted no-effect concentration of the predator an assessment can be based upon the quantity of food the top predator consumes. When calculating the bioaccumulation,  $BCF_{fish}$  is used. Equation 1 in Table 5 is used for substances with a  $\log K_{ow}$  2-6 and for substances with a  $\log K_{ow} > 6$  equation 2 is used (European Chemicals Bureau 2003). The equations are plotted in Figure 1. The gap in the curve is occurring since the two equations are not overlapping each other.



**Figure 1.** The bioconcentration factor (BCF) as a function of the lipophilicity. The line at 2000 represents the lower level when a substance is categorized as bioaccumulative and the line at 5000 represents when the substance is considered as very bioaccumulative. The gap in the graph is a consequence of the two different equations in Table 5 that do not overlap each other.

### 1.3.3 Toxicity

The definitive toxicity criteria for the aquatic environment is based upon the NOEC value. If a definitive criteria is not met, a screening criteria is used to determine the toxicity potential of the chemical.

The screening threshold values for toxicity is an acute  $E(L)C_{50}$  value of less than 0.1 mg/l from a standard toxicity test or valid QSARs. If this criteria is met, chronic toxicity testing is required, regardless of tonnage band. If the acute  $E(L)C_{50}$  value is less than 0.01 mg/l in a standard toxicity test it is labeled as a toxic substance. A substance with a value above 0.1 mg/l is not considered nontoxic, it can only be established that for a certain species there were no significant proof of toxicity.

For very lipophilic substances ( $\log K_{ow} > 5$ ) chronic toxicity ( $NOEC < 0.01 \text{ mg/l}$ ) cannot be excluded solely by the result from acute testing. Equilibrium partitioning may not have been reached in the limited time of an acute toxicity test and a chronic test could be necessary to rule out toxicity. For substances with a  $\log K_{ow} > 6$  species from the pelagic water phase may not be suitable for testing since the main part of the substance will likely partition out of the solution. For those substances bottom dwelling organisms may be more suited and provide more information since they are more likely exposed to the substance. Table 6 provides an overview of the screening criteria values for toxicity (ECHA 2012a).

**Table 6.** Screening criteria for toxicity (T) (ECHA 2012a).

Type of data	Criterion	Screening assignment***	Definitive assignment
Short-term aquatic toxicity*	$EC_{50}$ or $LC_{50} \geq 0.1 \text{ mg/L}$	presumably not T	-
Short-term aquatic toxicity*	$EC_{50}$ or $LC_{50} < 0.1 \text{ mg/L}$	potentially T	-
Short-term aquatic toxicity**	$EC_{50}$ or $LC_{50} < 0.01 \text{ mg/L}$	-	T

\* From acute tests or valid QSARs.

\*\* From acute tests.

\*\*\* The screening assignments should always be considered together for P, B and T to decide if the substance may be a potential PBT/vPvB candidate.

#### 1.4 Assessment factor for derivation of $PNEC_{\text{freshwater}}$

According to the REACH Regulation an assessment factor is applied to a  $L(E)C_{50}$  or  $NOEC$  value to ensure that no adverse effect will occur, this gives the  $PNEC$  value.  $PNEC$  values are available for different compartments, this section will however only cover  $PNEC_{\text{freshwater}}$  and does not include the marine environment.

Chemicals produced or imported in volumes larger than 10 tonnes/year with no previous test data available, requires testing before being allowed to be sold on the European market. Depending on the outcome from acute exposure testing, more tests on other species or other test methods might be required to pass the first tier in the risk assessment. An assessment factor is applied to the lowest endpoint value available from the toxicity testing to derive a  $PNEC_{\text{freshwater}}$  value. The magnitude of the assessment factor is depending on what tests that are available for the evaluated substance as shown in Table 7 (European Chemicals Bureau 2003).

**Table 7.** Assessment factor used to derive a PNEC for the freshwater compartment (European Chemicals Bureau 2003).

Available data	Assessment factor
At least one short-term L(E)C <sub>50</sub> from each of three trophic levels of the base-set (fish, Daphnia and algae)	1 000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1 (case by case)
Field data or model ecosystems	Case by case basis

## 1.5 PNEC for other compartments

The technical dossier for chemicals in the ECHA database includes PNEC values for other compartments e.g. marine water, marine sediments, soil etc. These PNEC values are derived by the same general procedure as described for PNEC<sub>freshwater</sub> in section 1.4. The dossier also holds PNEC values based upon toxicity through food; PNEC<sub>oral</sub>.

## 2. Method

### 2.1 Establishment of list of substances from the ECHA database

The ECHA database included 10 533 substances at the time the data was downloaded 5<sup>th</sup> November 2013. The ECHA database contains public information about all chemicals registered according to the REACH Regulation for production, import or sale in the EU. The data submitted to the database is not examined by ECHA, the manufacturer is liable for the information to be up to date and sufficient to avoid adverse effects to the environment and/or human health. Each substance has a dossier that contains data concerning PNEC and DNEL values, physical and chemical properties, production volumes and release categories for all substances in the database.

An overview of the content of the dossiers:

- Identity of the substance, CAS and EC, IUPAC and trade name and molecular formula
- The type of manufacture and the intended use of the substance
- Labelling and hazardous classification of the substance
- Information regarding safety when using or handling the substance
- A summary of the physical and chemical properties of the substance
- If needed, further testing proposals
- Exposure related information for substances produced in quantities of 1 tonne and higher (ECHA 2014b)

### 2.1.1 Substances excluded and corrected from the initial list

Chemicals that:

- were classified as intermediates were removed since they were assumed to not be found in the environment.
- had no CAS numbers were removed since their identities could not be determined.
- had confidential production volumes were removed.
- were labeled as UVCB (Unknown or Variable composition, Complex reaction products or Biological origin) or MSC (multi-constituent substances) were removed since they are compositions of different substances.
- had names indicating that they were mixtures were removed.
- had multiply entries and same or no  $PNEC_{\text{freshwater}}$  data were removed. When two entries contained the same set of data, the entry with the highest production volume was kept in accordance with worst case scenario. Substances with multiply entries but with different  $PNEC_{\text{freshwater}}$  values were kept as individual compounds.
- did not contain information about molecular weight were corrected using information from Chemspider (Chemspider 2014). Molecular weights with e.g.  $>/< 256.6 \text{ g/mol}$  were controlled against Chemspider and if a match were found, the symbol was removed and a note was made, if no match were found the substance was removed.

After removing all substances that did not fulfill the criteria above, 1 932 substances were left. Only those substances are included in this report.

## 2.1 Substances controlled by other regulations

Regulations for different groups of compounds differ from each other as they appear in the environment for different reasons. Pesticides and biocides have a designed mode of action and many of them are produced to be used in the environment e.g. rodent killers, algae growth inhibitors, insecticides etc. Pharmaceuticals and veterinary compounds also have a designed mode of action, but within the organism, and end up in the environment through leakage, production and excretion. Some substances are not produced themselves but occur in the environment from earlier use or as a result of leakage from production, unauthorized use, as result of persistence or as by-products from incineration. This creates a conflict when comparing the hazard potential, while some are produced with the intention of being used in the environment, some are not released deliberately into the environment at all.

### 2.1.1 Pharmaceuticals

Pharmaceuticals are evaluated by the European Medicines Agency (EMA) or through the national authorization procedure. EMA primarily handles innovative and high technology medicines. EMA does not create new regulations or laws, it only assures that they are implemented. They also provide guidelines on how a manufacturer of a product should proceed to be authorized for marketing in the Europe Economic Area (EEA). The EMA is evaluating applications for medicinal products intended for human and veterinary use from a scientific perspective. The European Commission (EC) is responsible for developing new laws or regulating existing laws within the pharmaceutical regulation. The EC is also responsible for the final approval of a product before it can be sold on the European market (European Medicines Agency 2014).

In the report “Human and Veterinary Pharmaceuticals, Narcotics, and Personal Care Products in the Environment” (Grung et al. 2007), the environmental situation in Norway with focus on Pharmaceuticals and Personal Care Products (PPCPs) is evaluated from an ecotoxicological perspective. The report contains ecotoxicological data for veterinary compounds, pharmaceuticals, personal care products and narcotics. The data from 109 substances were used in this report and sorted into respective sub-groups. Illegal substances were not included since they are regulated through jurisdiction not included in this report.

### 2.1.2 OSPAR Priority Pollutants

The list of Chemicals for Priority Action includes 42 substances, or groups of substances, of potentially problematic chemicals for the marine environment. The chemicals have been identified by the Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR). The convention was held in Oslo 1972 and in Paris in 1974 and was put into force in 1998. The conventions focus is on preventing and eliminating pollution in the marine area and protect it from adverse effects caused by human activity (EU 2006). The list of chemicals of high environmental concern has been implemented under the WFD and since

2004 OSPAR is relying on the REACH Regulation for evaluating new substances. The focus of OSPAR has since 2006 been to evaluate its list of substances of Possible Concern to see if it is adequately covered by the EC framework. The latter list is divided into four different lists, depending on the hazardous properties of the substance, how much information that is available and how well it is covered by EC initiatives or other forums. The lists are constantly updated as new information becomes available (OSPAR 2014). Substances with no  $PNEC_{\text{freshwater}}$  values were not included in this report.

### 2.1.3 Biocides

A biocide is defined as a biological or chemical pesticide used to regulate or prevent animals, micro-organisms (including viruses) and plants from causing damage on property or affect human health. Products intended for use as biocides are regulated by the Biocidal Product Regulation (Swedish Chemicals Agency 2014a).

The data from three reports were used. “Proposal for Environmental Quality Standards for certain pollutants” (Förslag till gränsvärden för särskilda förorenande ämnen) (Swedish Environmental Protection Agency) is a report made by the Swedish Chemical Agency on behalf of the Swedish Environmental Protection Agency. The purpose was to create Environmental Quality Standards (EQS) for substances that could pose a threat to the environment. EQS are available for different compartments e.g. surface water, living organisms, air etc. It is the concentration that should not be exceeded in order to protect human health and the environment. The substances that were included in the report constituted a risk to either aquatic animals or their predators or humans. Biocides (II) Refined aquatic environmental risk assessment of 28 priority biocides” (Mensink 2001) was published by the Dutch National Institute of Public Health and the Environment on behalf of the Directorate-General for Environmental Protection and the Directorate for Soil, Water and Rural Areas. The report is a follow up on “Preliminary environmental risk assessment of 93 biocides” (Mensink 1999) and the focus is on non-agricultural pesticides in a “rest-group”, named so as they did not fall under any of the biocide policies of the Dutch government and as a result were not included in the first report. The second report aims to fill the data gaps for these substances and to assess the hazard potential that these substances may present to the environment and or human health. “Prioritization of biocidal substances for an environmental monitoring” (Rüdel 2012) is a presentation of a project that assess the possibility of a new prioritizing order for environmental monitoring and the potential ecotoxicological effect they present. The  $PNEC_{\text{freshwater}}$  for substances in the list “Biocides (no current PPP) - relevant for monitoring in water” was used in this report.

### 2.1.4 Pesticides

Pesticides are substances created with the purpose of plant protection against fungi, pest and competing plants. The active substance in the product needs to be approved for use by the EU before being sold on the European market. The European Parliament is responsible for

the regulation of plant protection products. The regulation is binding in its entirety to all Member States of the EU but does not force the Member States to accept all products with approved substances, it gives them the option to do so (Swedish Chemical Agency 2014b). The report used “Compilation of protocol of Environmental Quality Standards for plant protection products in surface water” (Sammanställning av protokoll om riktvärden för växtskyddsmedel i ytvatten) (Swedish Chemical Agency 2008) was performed on behalf of the Swedish Environmental Protection Agency and it contains EQS for 100 active ingredients used in approved plant protection products. For some substances EQS for their metabolites were included in the analysis. Substances with no  $PNEC_{\text{freshwater}}$  were removed and substances with no unique CAS-number were removed. The original CAS-number for Fenoxaprop-p-ethyl was replaced with the correct number. The substances were divided into three subgroups depending on their intended use; insecticides (21), fungicides (28) and herbicides (45).

#### 2.1.5 WFD Priority substances

The Water Framework Directive (WFD) was created by the European parliament and council to identify chemical pollution in surface waters and how to minimize the damage in an economically and environmentally effective way. Relying on the precautionary principle and that preventive action should be taken, the directive states that pollution should be regulated at the source to avoid damage to the environment in a short- and long-term perspective. The directive includes a list of 33 priority substances and 8 certain other pollutants for which each state in the European Union must monitor the concentrations and inventory the emissions, discharges and losses of. The aim of the directive is to reach good ecological and chemical status for all water bodies identified by the member state. All substances listed in Annex I of the directive has an Environmental Quality Standards (EQS) assigned to them. The member states have to meet the EQS within a certain timeline, unless special conditions allow them to apply for exemptions. The concentration is either set by an annual average (AA) or by a maximum allowable concentration (MAC). The directive covers inland surface water, groundwater, transitional water and coastal water (one nautical mile from the shore) (EU 2010).

The current list of priority substances is found in Directive 2008/105/EC and are divided into three sub-groups: Priority substances (20), Priority hazardous substances (13) and Certain other pollutants (8). Priority substances are chemicals that according to Article 16 in the WFD are identified as presenting a significant risk for human or environmental health to or via the aquatic environment. Each state should take necessary action to gradually reduce pollution to keep those substances below the set threshold value. The substances regarded as the most dangerous are categorized as Priority hazardous substances and special measures/procedures should be taken with the aim of phasing out emissions, discharges and losses within 20 years after being labeled as such. Certain other pollutants is a list of substances that have an EQS but currently does not fulfill the criteria to be defined as a Priority substance. For other

substances of interest, not listed under the WFD, it is for the member state to lay down rules if found necessary to do so (Cleuvers 2004).

The substances used in this report are found in the Annex II of the proposed update of the current priority substance list. The proposed list contains 21 substances categorized as Priority hazardous substances and 27 Priority substances. The substances previously categorized as Other pollutants have now been included in the Priority substances list. Substances that did not have an EQS value were excluded from this report.

The focus of this thesis is on the effects upon the environment and not humans which is why the Quality Standard Freshwater ( $QS_{\text{freshwater}}$ ) and not the EQS was used. There are different QS depending on who or what it is aimed to protect e.g. drinking water, protection of aquatic life etc. The  $QS_{\text{freshwater}}$  is derived using toxicity data from three trophic levels (algae, aquatic invertebrates and fish). Substances classified as priority pollutants and priority hazardous pollutants in the report “Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy” was included. The  $QS_{\text{freshwater}}$  values were collected from the data sheets containing the background information for the different substances (CIRCAB 2014). Substances with no data sheet were removed since no  $QS_{\text{freshwater}}$  value could be retrieved.

There are also substances categorized as highly ranked substances, substances that are candidates to be defined as priority substances. The  $PNEC_{\text{freshwater}}$  for these substances was collected from Annex XV in “Prioritisation process: Final monitoring-based ranking” (European Commission 2009).

## 2.2 Variables and data used in graphs

### 2.2.1 The octanol-water coefficient

The octanol-water coefficient ( $K_{ow}$ ) is describing the balance between a substances water solubility and lipid solubility. A substance with a  $\log K_{ow}$  value much lower than 1 is very water soluble (hydrophilic) and a substance with a value much higher than 1 is very lipid soluble (lipophilic) (Walker 2006). As previously mentioned, the  $K_{ow}$  value can be used to estimate the bioaccumulation potential of a substance in PBT and vPvB assessment and in PEC calculation to predict how much of the substance that will end up in each compartment of the environment. The BCF is describing the bioaccumulation potential of a substance as described in section 1.3.2.

For substances with multiple  $K_{ow}$  values, the value with highest reliability was kept or the average of the ones with highest reliability if multiply values were available.

### 2.2.2 The bioconcentration factor

Bioconcentration is defined as an organism's net uptake of a substance in water. The net uptake is the concentration in the organism after taking uptake, elimination and distribution of the substance into account. The hazard of bioconcentration can be assessed using the bioconcentration factor (BCF). The BCF is defined as the ratio between the concentration in the organism and the concentration in the water surrounding it at steady state (Attias et al. 2005).

$$BCF_{fish} = \frac{C_{fish}}{C_{water}}$$

### 2.2.3 Production volume

Substances with confidential production volume were removed. Substances with no exact production volume, but only a minimum production volume were categorized in line with substances with the same minimum production volume. That means a maximum production volume of 10 times the minimum production volume.

### 2.2.4 Scaling of y-axis in graphs

For several figures a scale between 0 and 1 has been used to determine the percentage of compounds falling within a certain category. For this purpose the formula below was used to calculate the y-value.  $i$  is the sample number and  $n$  is the total amount of samples.

$$y = \frac{i - 0,5}{n}$$

### 2.2.5 Geometric mean

The geometric mean is a way to describe the average of a series of numbers and is calculated by using the formula below. The geometric mean was used since it is less sensitive to outliers than the arithmetic mean.

$$\text{Geometric mean} = \left( \prod_{i=1}^n x_i \right)^{\frac{1}{n}}$$

### 2.2.6 Effect concentration 50 % and lethal concentration 50 %

Effect concentration 50 % (EC<sub>50</sub>) and lethal concentration 50 % (LC<sub>50</sub>) are two endpoints in toxicity testing. The EC<sub>50</sub> is the concentration that causes a toxic response in 50 % of the individuals in an exposed group. The predetermined response can be inhibition, reproduction,

growth etc. LC<sub>50</sub> is the concentration that kills 50 % of the individuals in an exposed group (Walker 2006).

### 2.2.7 No observed effect concentration

The no observed effect concentration (NOEC) is the highest measured concentration of a chemical in a toxicity test where no significant effect was observed. The NOEC value is only valid for the specific test and the specific species that was used in the experiment. The NOEC is only the highest effect where no significant effect was observed and since the level of significance is highly dependent on the design of the experiment it cannot be assumed that there is no effect, only that no effect has been significantly proven (Kortenkamp et al. 2009).

### 2.2.8 Predicted No Effect Concentration and Predicted Environmental Concentration

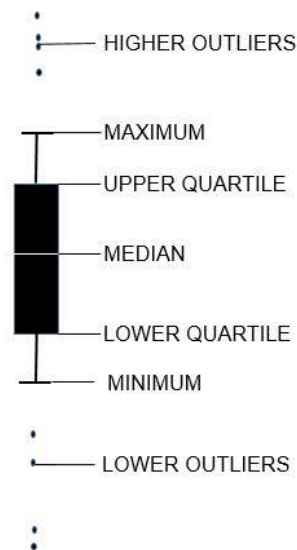
The predicted no effect concentration (PNEC) is a concentration where no effect is assumed to occur. The value is an estimation based upon a L(C)<sub>50</sub> value or a NOEC value from the most sensitive species that has been tested. This value is then divided with an assessment factor (1-1000) to decrease uncertainty. The assessment factor is depending on the amount of toxicity data available and is applied to compensate for the uncertainty that entails when extrapolating data. The predicted environmental concentration (PEC) is the amount of a substance that is predicted to be found in a specific compartment at a specific place under certain conditions and is calculated, for instance, using a model such as EUSES or MAMPEC. The PNEC value for a substance is often used with the predicted environmental concentration (PEC) in the equation below to estimate the risk.

$$risk\ quotient = \frac{PEC}{PNEC}$$

If the risk quotient is over 1 it is considered a risk and more testing is required for the substance. Through further testing and more knowledge about the substances characteristics, a lower assessment factor can be used as shown in Table 7, which will decrease the risk quotient (Walker 2006).

### 2.2.9 Settings for box plots

Figure 2 shows a boxplot and its definitions. The median value is the value in the middle of the data set, where there is as many values above as below. The box represent the 50 % of the values in the middle, the upper quartile is the highest value of the 25 % over the median and the lower quartile is the lowest value of the lower 25 %. The minimum and maximum represent the values that are highest and lowest without being labeled as outlier. An outlier is defined as a value that is 1.5 times higher than the length of the box;  $(\text{Upper quartile} - \text{lower quartile}) * 1.5$ . This gives a confidence interval of 99.3 % of the data within a normal distribution.



**Figure 2.** A boxplots structure and definitions.

## 2.3 The next step; calculation of PEC and risk assessment

In order to estimate the risk to the general environment as a result of exposure to industrial chemicals, the PEC value for all industrial chemicals, after exclusions, can be calculated using EUSES. By comparing the PEC and the PNEC value, a risk quotient can be derived as described in section 2.2.8.

### 2.3.1 EUSES

EUSES 2.0 (European Union System for the Evaluation of Substances) is a PC-program designed to be a decision-support system to be used for quantitative assessing of substances risk to humans and the environment. The program is constructed using the EU Technical Guidance Document for risk assessment of new and existing substances and biocides. EUSES was developed by EU Member States, the European Commission and the European Chemical Industry to be used for risk assessment, which is required for new substances by Directive 92/32/EC, for existing substances by EC Council Regulation (EC) 793/93 and for biocides by EC Directive 98/8/EC.

EUSES include micro-organisms in sewage treatment plants, populations of predators, terrestrial, aquatic and sediment ecosystem in both freshwater and marine environment. The model describes short- and long-term effects from a reasonable worst case scenario using input values or default values. The exposure assessment is made at three spatial scales: the

local scale, the regional scale and a personal scale. The personal scale is made for consumers and workers and do not include the environment. The local scale includes humans and the environment and describes the exposure near point sources. The regional scale also includes both humans and the environment and local point sources and the total regional emission is taken into account.

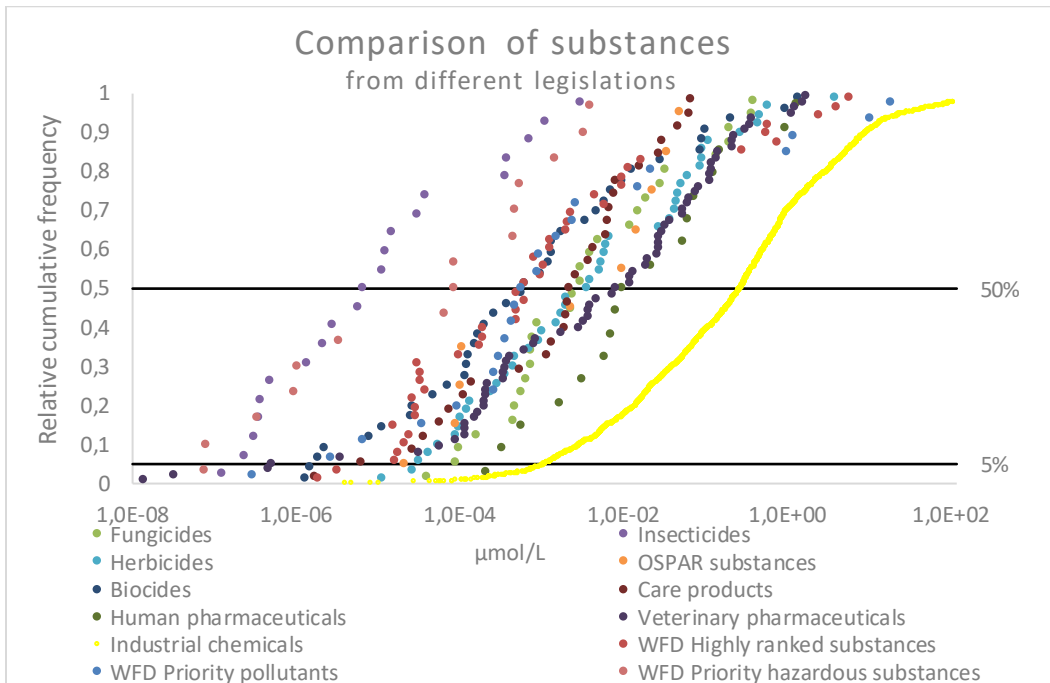
EUSES can be used in a tiered risk assessment, initially at a screening stage or later at a more refined stage. When handling large numbers of chemicals, it can be used to set priority where additional data search is needed or refined assessment of certain parameter values (Lijzen and Rikken 2004). EUSES could be used in the next step in the evaluation of the possible threat to the European aquatic environment from industrial chemicals, to carry out a risk assessment, preferably from a mixture toxicity perspective.

### **3. Results and discussion**

#### **3.1 Comparison of substances under different legislations**

Substances are assessed in different ways depending on their intended use. Substances categorized as industrial chemicals are regulated by other jurisdiction than e.g. pesticides. Industrial chemicals are not produced to interact with organisms while e.g. pesticides are designed to interact and harm its target organism through a specific mode of action. Industrial chemicals found in the environment is the result from leakage, while most pesticides intended use is in the environment. Those differences among others are the reasons for different approaches when assessing their hazardous potential. The Quality Standard Freshwater for WFD substances and the  $PNEC_{\text{freshwater}}$  for pharmaceuticals, pesticides, biocides, industrial chemicals and OSPAR hazardous substances were plotted in Figure 3. The graph shows that Industrial chemicals is the least toxic group by average and among the more toxic groups are insecticides, biocides, WFD Priority hazardous substances and WFD highly ranked substances. The result is expected since insecticides and biocides are designed to be toxic and the priority substances are known to be toxic.

Another factor that needs to be considered when assessing hazardous potential is the production volume. As previously mentioned, industrial chemicals are produced in large volumes compared to many of the other groups of compounds. In 2005 the total sale of insecticides in the EU (data only available for 22 countries) were 12 300 tonnes of active ingredient (European Commission 2012). As a comparison, the total production volume of the industrial chemicals included in this graph is somewhere between 177 300 000 (min) and 1 773 000 000 (max) tonnes/year.



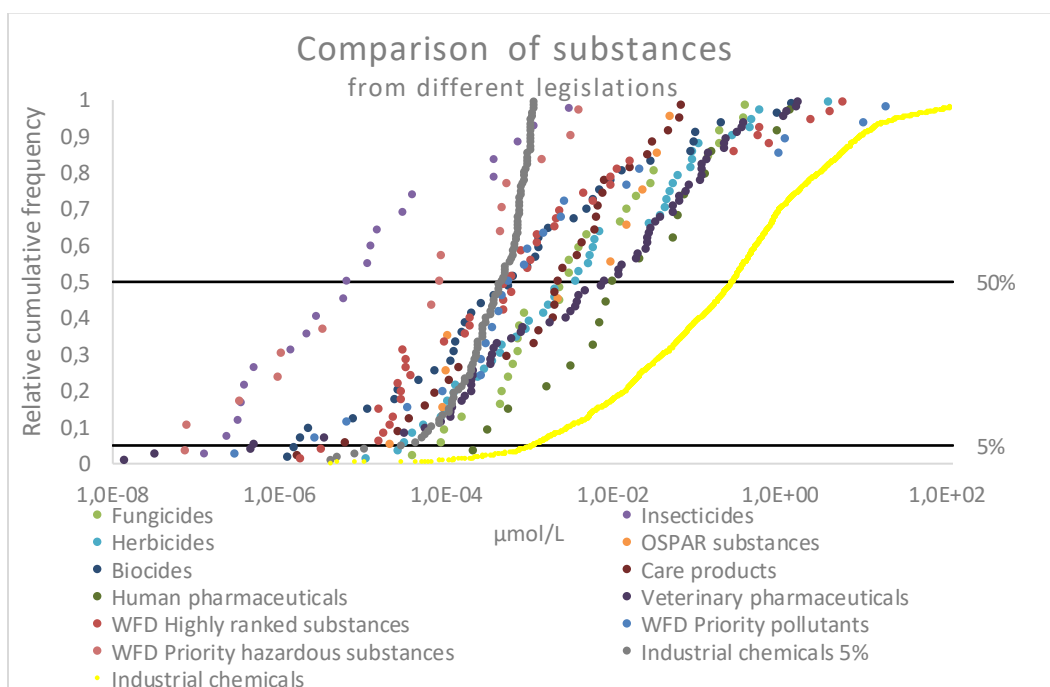
**Figure 3.** PNEC<sub>freshwater</sub> µmol/L for groups of compounds from different legislations. For WFD substances, the QS<sub>freshwater</sub> value is used and not PNEC<sub>freshwater</sub>. The 50 % line shows the median value and the 5 % line shows the lower 5 % percentile.

Table 8 shows that industrial chemicals found under the 5 % limit are at about the same concentration as many of the other substances mean values, the 50 % limit. The 97 industrial chemicals under the 5 % limit were used to create a new group of compounds: “industrial chemicals 5 %”.

**Table 8.** The value at the 5 % percentile and 50 % percentile in µmol/L in Figure 4.

Group	5 % percentile µmol/L	50 % percentile µmol/L
Industrial chemicals	1,14E-03	2,82E-01
Industrial chemicals 5 %	2,92E-05	4,84E-04
WFD Priority haz. substances	7,71E-08	8,68E-05
WFD Priority pollutants	1,97E-07	4,94E-04
Insecticides	2,38E-07	6,62E-06
Veterinary pharmaceuticals	5,11E-07	8,32E-03
Biocides	1,56E-06	6,51E-04
WFD High ranked substances	1,63E-05	5,00E-04
OSPAR haz. substances	2,21E-05	2,32E-03
Herbicides	3,34E-05	3,74E-03
Fungicides	9,13E-05	2,43E-03
Care products	6,40E-06	2,28E-03

The industrial chemicals 5 % were plotted with the other substances in Figure 4. It shows that industrial chemicals 5 % rank somewhere in the middle of the other groups and when comparing the median values in Table 8 the 50 % value for Industrial chemicals 5 % is the third most toxic. Considering that the total production volume of those chemicals is somewhere between 10 300 000 (min) and 103 000 000 (max) tonnes/year a greater hazard potential is revealed. A difference to keep in mind when comparing to e.g. insecticides, is that the whole production volume is not released into the environment, the part that ends up in the environment is a result of different kind of leakage. But even if just a small fraction, e.g. 1 % of the total produced and imported volume leaks into the environment in some way, a total volume of somewhere between 103 000 and 1 030 000 tonnes ends up in the environment every year compared to 12 300 tonnes of insecticides.



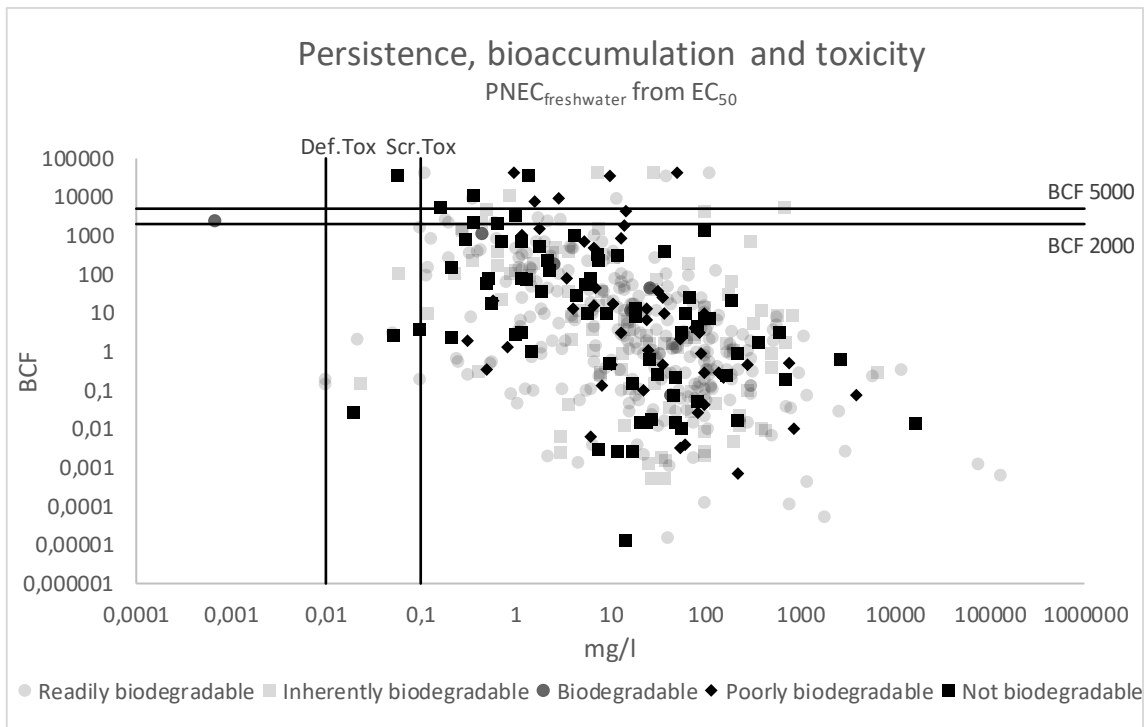
**Figure 4.**  $PNEC_{\text{freshwater}}$   $\mu\text{mol/L}$  for groups of compounds from different legislations and industrial chemicals 5 %. For WFD substances, the  $QS_{\text{freshwater}}$  value is used and not  $PNEC_{\text{freshwater}}$ . The 50 % line shows the median value and the 5 % line shows the lower 5 % percentile.

### 3.2 Persistent (P) Bioaccumulating (B) and Toxic (T) compounds

Screening for PBT and vPvB properties is a fundamental part in the risk assessment of new substances under the REACH Regulation, as described in section 1.3. The persistence, bioaccumulation and toxicity for each industrial chemical were plotted in the same graph. The substances were sorted into two groups, one with substances for which the  $PNEC_{\text{freshwater}}$  was derived from an  $EC_{50}$  value and one for which the  $PNEC_{\text{freshwater}}$  was derived from a

NOEC value. This separation was made since the endpoints are different and the definitive criteria are different for the two endpoints as described in section 1.3.3.

Figure 5 includes the substances for which an  $EC_{50}$  value was used to derive the  $PNEC_{\text{freshwater}}$  as described in section 2.2.8. The def.tox line is the limit for when a substance is categorized as definitely toxic. Substances to the left of this line are labeled as toxic regardless of other factors. The scr.tox line is the limit for the toxicity screening threshold. Substances to the left of this line needs to undergo further testing before being defined as toxic or non-toxic. The line labeled BCF 2 000 represent the lower limit for when a substance is categorized as bioaccumulative. The horizontal line labeled BCF 5 000 represent the lower limit for when a substance can be categorized as very bioaccumulative (vB). The substances persistence and very persistence potential are defined according to Appendix 1 and categorized into five groups, readily biodegradable, inherently biodegradable, biodegradable, poorly biodegradable and not biodegradable.



**Figure 5.** PBT and vPvB properties for substances with a  $PNEC_{\text{freshwater}}$  value derived from an  $EC_{50}$  value.

Few substances fulfill all three criteria and most substances are in the area where no criteria is fulfilled and no substance fulfill all the highest criteria, colored black and placed in the upper left corner of the graph in Figure 5. But considering that the substances fulfilling the toxicity screening criteria has a yearly production volume of somewhere between 14 701 and 147 010 tonnes a year and that some of the substances are labeled as either or both bioaccumulative and persistent, as shown in Table 9, there is a hazardous potential.

**Table 9.** Production volume in tonnes per year of substances fulfilling persistence and/or bioaccumulation and/or toxic criteria. Only substances for which the  $PNEC_{\text{freshwater}}$  was derived from an  $EC_{50}$  value were included.

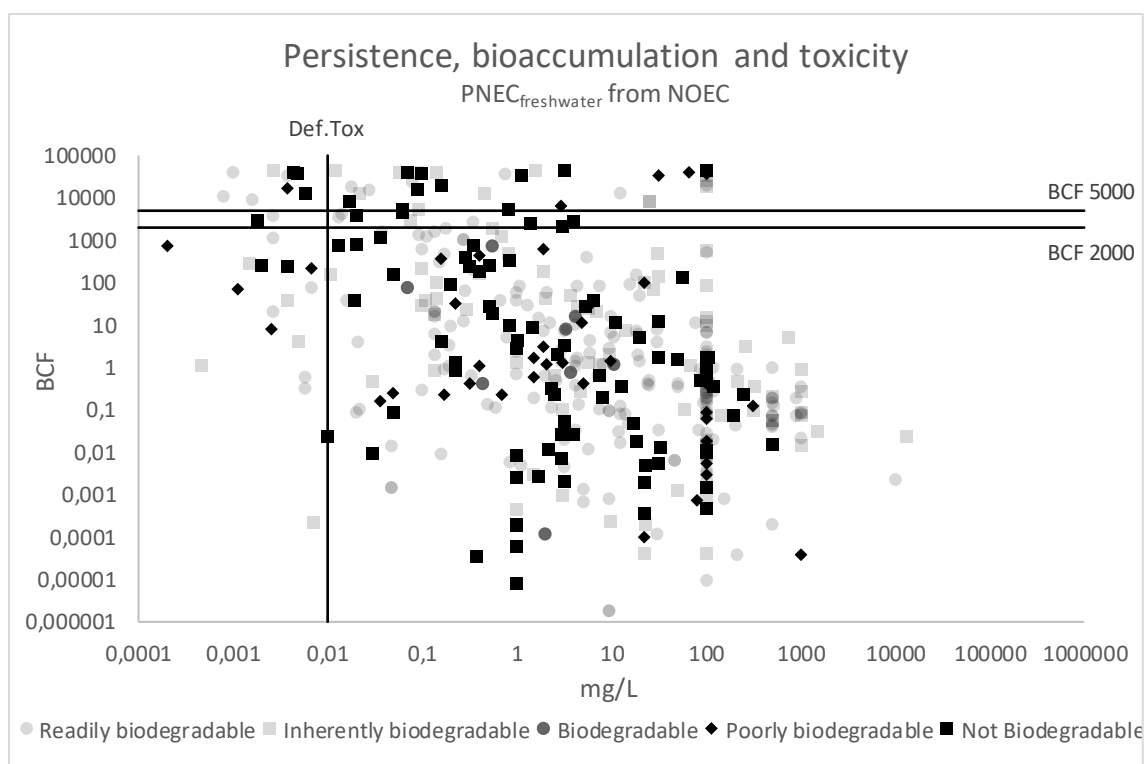
	Min production tonnes/year	Max production tonnes/year
<b>PB</b> *1	1 822	18 220
<b>BT</b> *2	1 100	11 000
<b>PT</b> *3	300	3 000

\*1 substances with  $BCF > 2000$  and defined as poorly or not biodegradable according to Appendix 1.

\*2 substances fulfilling toxicity screening criteria  $< 0.1$  mg/L and  $BCF > 2000$ .

\*3 substances fulfilling toxicity screening criteria  $< 0.1$  mg/L and defined as poorly biodegradable or not biodegradable according to Appendix 1.

Figure 6 has the same framework as Figure 5 except that it only has a definitive toxicity criteria instead of a screening and definitive criteria. The graph shows that many substances fulfill the PBT and vPvB criteria, colored black and placed in the top left corner of the graph. The total production volume of substances with PBT properties is somewhere between 102 010 and 1 020 100 tonnes a year.



**Figure 6.** PBT and vPvB properties for substances with a  $PNEC_{\text{freshwater}}$  value derived from an NOEC value.

The yearly production volume of substances defined as persistent and/or bioaccumulative and/or toxic is shown in Table 10. The reason why more hazardous substances end up in the

NOEC graph than the EC<sub>50</sub> graph might be that substances fulfilling the initial toxicity screening criteria, requiring long term exposure testing. Since the NOEC value usually is lower than the EC<sub>50</sub> value, it will be used to derive a PNEC.

**Table 10.** Production volume in tonnes per year of substances fulfilling persistence, bioaccumulation and toxic criteria. Only substances for which the PNEC<sub>freshwater</sub> was derived from a NOEC value were included.

	Min production tonnes/year	Max production tonnes/year
<b>PB</b> *1	219 410	2 194 100
<b>BT</b> *2	225 020	2 250 200
<b>PT</b> *3	113 420	1 134 200
<b>vPvB</b> *4	202 810	2 028 100
<b>PBT</b> *5	102 010	1 020 100

\*1 substances with BCF > 2 000 and defined as poorly or not biodegradable according to Appendix 1.

\*2 substances fulfilling definitive toxicity criteria < 0.01 mg/L and BCF > 2 000.

\*3 substances fulfilling definitive toxicity criteria < 0.01 mg/L and defined as poorly biodegradable or not biodegradable according to Appendix 1.

\*4 substances with BCF > 5 000 and defined as not biodegradable according to Appendix 1.

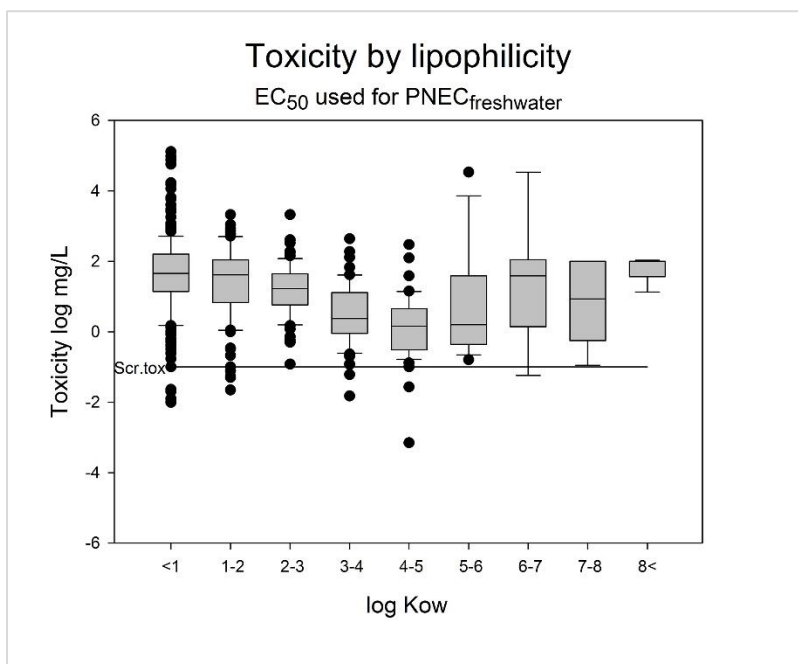
\*5 substances fulfilling definitive toxicity criteria < 0.01 mg/L and BCF > 2 000 and defined as not biodegradable according to Appendix 1.

### 3.3 Toxicity and lipophilicity

The lipophilicity is an important variable when assessing the bioaccumulation potential of a substance and since humans are top predators it is crucial when assessing the hazardous potential of a substance. This is considered in the REACH Regulation, lipophilicity is a variable used initially in the risk assessment of a substance, as shown in section 1.3.

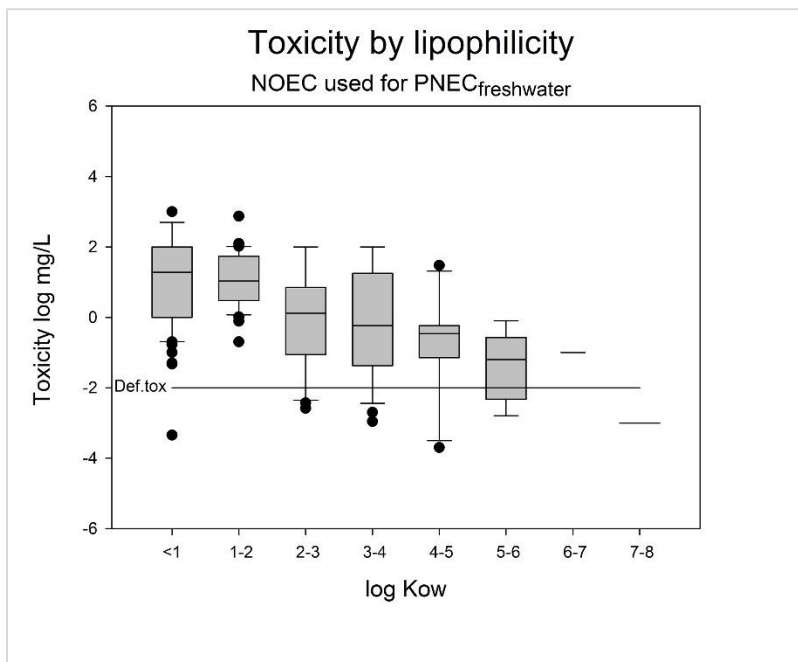
Substances produced in small volumes (1-10 tonnes/year), only have the physical and chemical properties of the chemical taken into account if there is no indication of hazard, as shown in Table 1. This means that a substance with a log K<sub>ow</sub> < 4.5 is assumed to present a low risk since it will not bioaccumulate and magnify through the food chain. As in the previous section, the EC<sub>50</sub> and NOEC values were separated into different graphs since the endpoints are different.

As shown in Figure 7 there are substances not defined as lipophilic below the screening limit for toxicity. This indicates a toxic mode of action not described by the narcotic effect. Narcotic effect, also known as baseline toxicity, is the toxic effect that a substance cause upon an organism solely from its lipophilic characteristics. If the toxicity is only a result of the lipophilicity, the toxicity would follow the pattern in Figure 1. Substances can diverge from this pattern if they have another mode of action or other physical and chemical properties that affect their toxicity.



**Figure 7.** Toxicity in log mg/L (EC<sub>50</sub>) for different spans of log K<sub>ow</sub>. The Scr.tox. line at -1 represents the 0.1 mg/L screening criteria for toxicity.

The average toxicity in Figure 7 increase with increasing level of lipophilicity and then decrease at high level of lipophilicity. One explanation for this pattern could be that substances that are very lipophilic are not very bioavailable since they do not dissolve completely in water. As a result many organisms living in the pelagic zone will never be exposed to those substances. The same pattern between toxicity and lipophilicity can be observed in Figure 8. Both graphs are following the predicted pattern from Figure 1. The average toxicity is higher in Figure 8 than in Figure 7, this can be explained by the use of different methods and endpoints. EC<sub>50</sub> is the concentration where 50 % of the test group is affected while NOEC is the concentration where no significant effect is observed. It could also be argued that substances that in the initial screening are labeled as possibly or definitely toxic are assigned to undergo chronic toxicity testing and as a result, they end up in Figure 8. The results from chronic toxicity tests are likely to be used to derive PNEC since the NOEC value usually is lower than the EC<sub>50</sub> value.

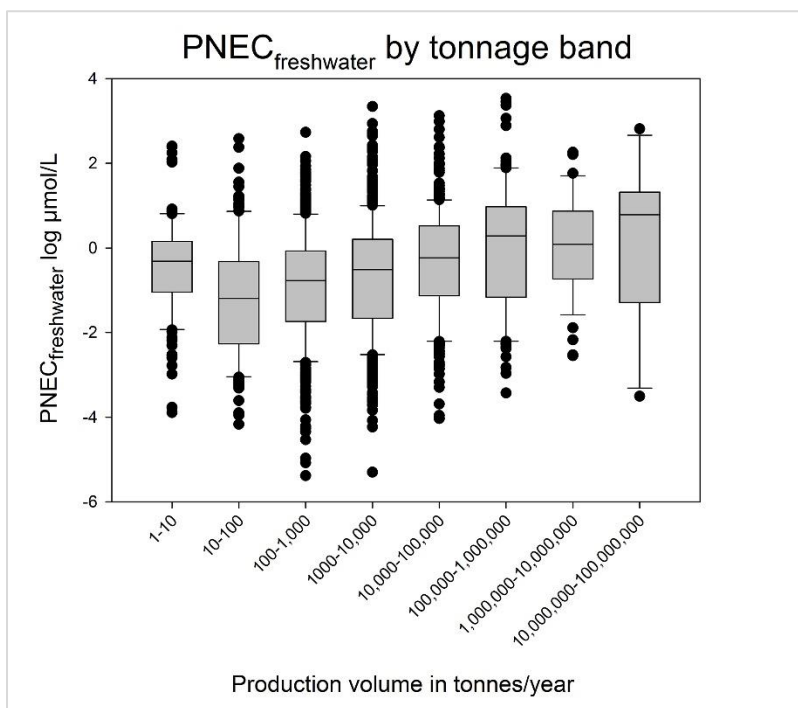


**Figure 8.** Toxicity in log mg/L (NOEC) for different spans of log  $K_{ow}$ . The Def.tox-line at -2 represents the 0.01 mg/L definitive criteria for toxicity. Log  $K_{ow} > 8$  is not included in this graph since there were no substances in that range for NOEC.

### 3.4 Toxicity for different production volumes

The risk potential of a substance is not only depending on its toxic properties but also on the production volume. The greater production volume, the greater is the risk potential since a larger amount of the substance potentially can end up in the environment.

For substances produced in volumes of less than 10 tonnes/year a PNEC value is not always required. The initial screening in the risk assessment is instead based upon the physical and chemical properties of the substance. If a criteria is not met as described in Table 1, no further toxicity testing is required and if no previous toxicity data is available, no PNEC value is included in the dossier. Substances below the def.tox-line in Figure 11 fulfill the definitive toxicity criteria in Table 2.



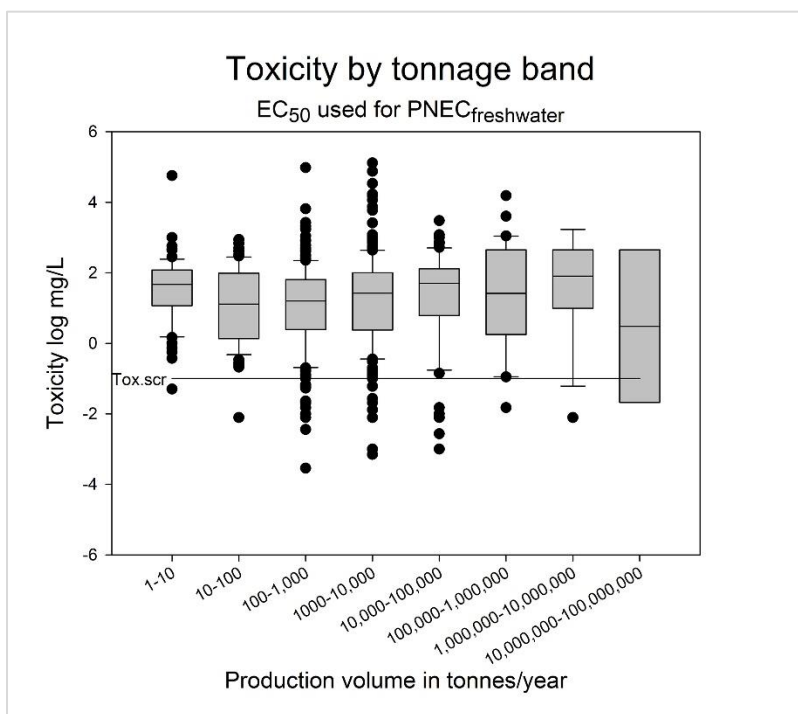
**Figure 9.** PNEC<sub>freshwater</sub> after production volume.

The trend for the average PNEC<sub>freshwater</sub> is an increasing value with increasing production volume from 10 - 100 tonnes onwards according to Table 11. This could be interpreted as that chemicals produced in smaller volumes are generally more hazardous than chemicals produced in large volumes. It could also be argued that substances produced in large volumes generally are predicted to occur in higher concentrations in the environment, which means that a lower PNEC is required in order to not exceed the risk quotient. This is achieved through additional toxicity testing so that a lower assessment factor can be used, as described in section 1.4.

**Table 11.** The average value for PNEC<sub>freshwater</sub> for different production volumes.

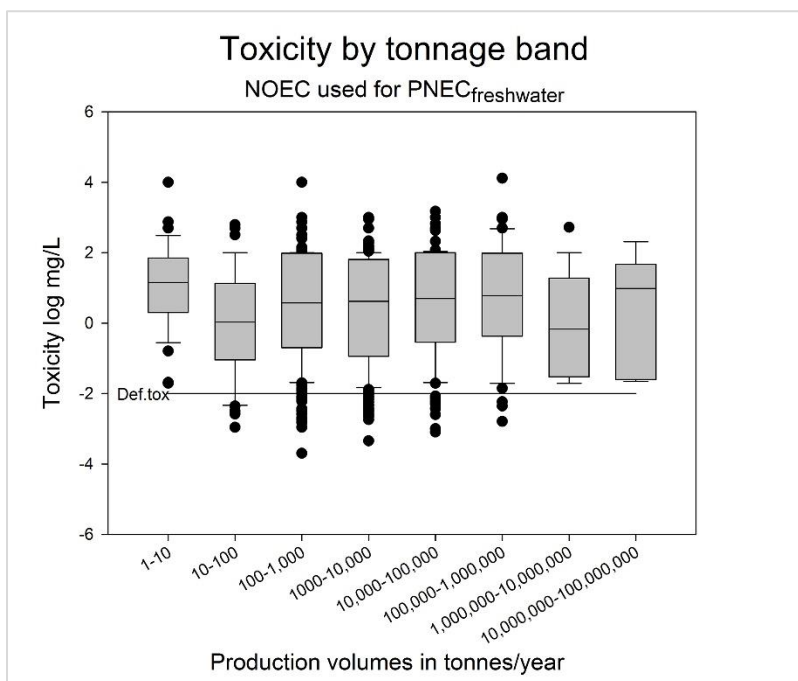
Min production volume tonnes/year	Average PNEC <sub>freshwater</sub> μmol/L	Median PNEC <sub>freshwater</sub> μmol/L
<b>1</b>	0.37	0.49
<b>10</b>	0.06	0.06
<b>100</b>	0.13	0.17
<b>1 000</b>	0.21	0.31
<b>10 000</b>	0.45	0.59
<b>100 000</b>	1.04	1.93
<b>1 000 000</b>	1.04	1.23
<b>10 000 000</b>	1.42	6.09

The  $PNEC_{\text{freshwater}}$  values in Figure 9 shows the predicted no hazard limit, where no adverse effect is assumed to occur. By removing the assessment factor from the  $PNEC_{\text{freshwater}}$  value, the  $EC_{50}$  and NOEC concentration from toxicity testing can be revealed.



**Figure 10.** The toxicity of compounds in log mg/L by production volume. The line at -1 represents the limit, 0.1 mg/L, the screening criteria for toxicity.

The result in Figure 10 is not confirming or rejecting the theory that the toxicity is decreasing with increasing production volume. The average toxicity is decreasing from 10-100 tonnes and then increasing at the largest production volume. It should be noticed that the sample size for the largest production volume is small, only two chemicals are represented. There are many outliers especially for the substances in the middle range and when comparing the median values the interpreted pattern is less visible.



**Figure 11.** The toxicity of compounds in log mg/L by production volume. The Def.tox line at -2 represents the limit, 0.01 mg/L; the definitive criteria for toxicity.

The bars in Figure 11 are not following the same pattern as in Figure 10. The average toxicity is varying irregularly with increasing volume. The highest average and median toxicity is found for the second largest production volume.

**Table 12.** The average value for  $PNEC_{\text{freshwater}}$  for different production volumes.

Min production volume tonnes/year	Average $EC_{50}$ mg/L	Average NOEC mg/L	Median $EC_{50}$ mg/L	Median NOEC mg/L
<b>1</b>	31.6	9.84	47.2	14.2
<b>10</b>	11.9	1.15	13.0	1.1
<b>100</b>	10.4	2.82	16.0	3.8
<b>1 000</b>	17.0	2.36	26.5	4.2
<b>10 000</b>	18.7	3.25	50.0	5.0
<b>100 000</b>	21.4	4.16	26.2	6.0
<b>1 000 000</b>	42.3	1.14	80.4	0.7
<b>10 000 000</b>	3.07	4.21	3.1	9.6

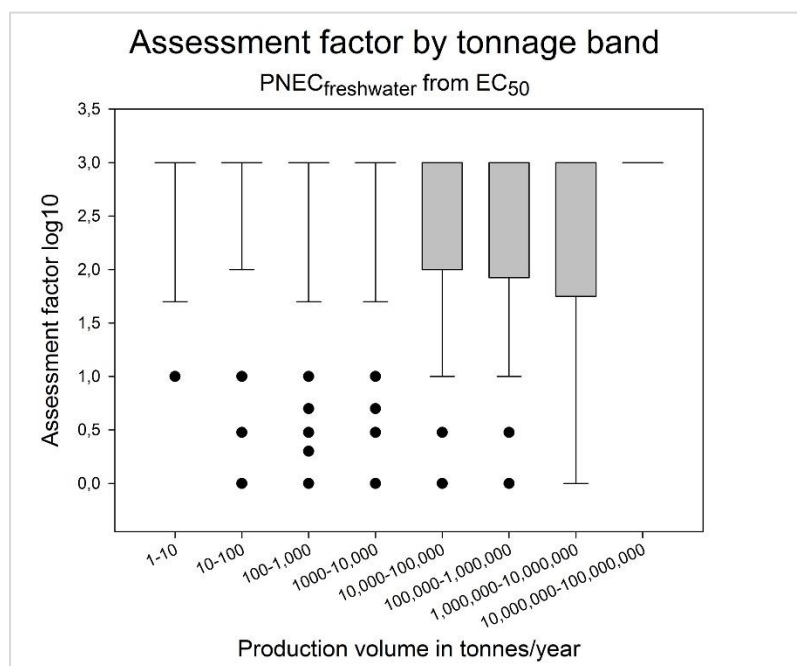
**Table 13.** The production volume of substances below the screening and definitive criteria for toxicity in Figure 10 and Figure 11.

	Min production tonnes/year	Max production tonnes/year
EC <sub>50</sub> < 0.1 mg/L	12 283 721	122 837 210
NOEC < 0.01 mg/L	418 470	4 184 700

The total minimum production volume of the substances below the toxicity screening limit in Figure 10 is 12 283 721 tonnes/year. The total minimum production volume of substances in Figure 11 below the definitive toxicity limit is 418 470 tonnes/year.

### 3.5 The use of assessment factors

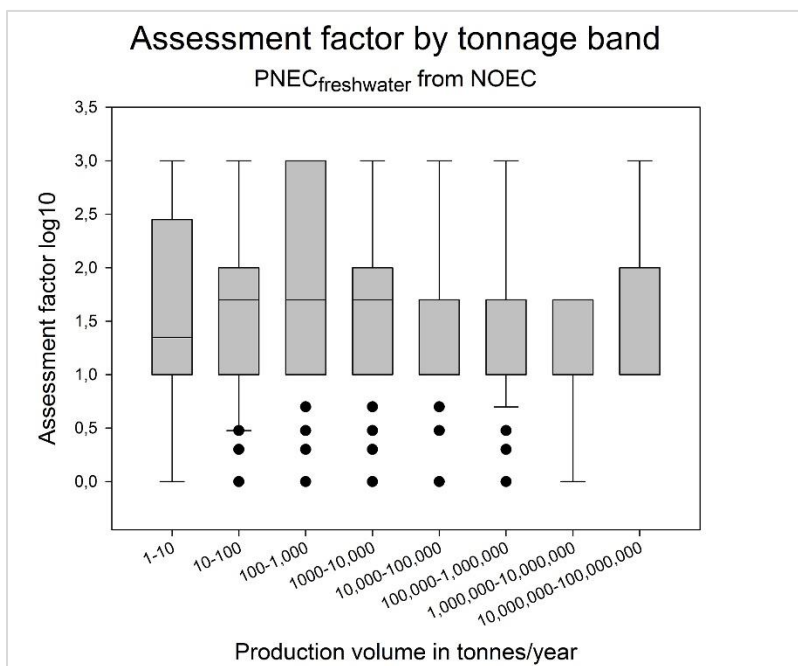
The assessment factor applied to the EC<sub>50</sub> and NOEC values to derive the PNEC<sub>freshwater</sub> values in Figure 9 was plotted with the production volume of the chemical.



**Figure 12.** The bar shows how the assessment factor varies for the different production volumes. The assessment factors have been used to derive PNEC<sub>freshwater</sub> from EC<sub>50</sub> values.

All median values in Figure 12 are at log<sub>10</sub> 3.0 which means that more than 50 % of the EC<sub>50</sub> values have had an assessment factor of 1 000 assigned to them. 1 000 is the highest AF that can be used for PNEC<sub>freshwater</sub> within the REACH Regulation and means that only the minimum amount of test data is available (or used); one acute test from algae, aquatic

invertebrates and fish respectively. The trend in the graph indicates that with increasing production volume a lower assessment factors have been used. This assumption is supported when comparing the average values, after 10 - 100 tonnes the average value is decreasing with increasing volume except for the largest production volume, whereas mentioned, the sample size is small. The same trend can be observed in Figure 13.



**Figure 13.** The bar shows how the assessment factor varies for the different production volumes. The assessment factors have been used to derive PNEC<sub>freshwater</sub> from NOEC values.

The median values in Figure 13 are by average lower than for the EC<sub>50</sub> values. The assessment factors have been applied to NOEC values which means that they are generally from chronic toxicity studies. One result from a chronic toxicity test do automatically allow the use of an assessment factor of 100 as shown in Table 7. The use of a lower assessment factors is possible since extrapolating from acute to chronic toxicity and 50 % effect to 0 % effect does not need to be considered when deriving PNEC<sub>freshwater</sub> from a NOEC value.

**Table 14.** Assessment factors used to derive  $PNEC_{\text{freshwater}}$  from  $EC_{50}$  and NOEC values. Outliers are not included.

<b>Min production volume tonnes/year</b>	<b>Average AF value used on <math>EC_{50}</math></b>	<b>Average AF value used on NOEC</b>
<b>1</b>	500	33
<b>10</b>	626	41
<b>100</b>	502	64
<b>1 000</b>	465	46
<b>10 000</b>	329	39
<b>100 000</b>	225	31
<b>1 000 000</b>	190	11
<b>10 000 000</b>	1000	30

### 3.6 Species sensitivity and use frequency

This section will examine which species and taxonomical groups that are most frequently used in toxicity assessment of industrial chemicals and if any group or species is more sensitive than the other. When assessing the toxicity of a substance, the aim is to expose the most sensitive organism so that the maximum effect from the exposure can be identified. By doing, the concentration from which no organism in the environment is expected to be harm can be established. The problem is that is never known, before or after, if the exposed species is the most sensitive one. In the environment, hundreds of species can be exposed to a single chemical and the examined species might not even be one of them. Many endpoints are not examined and collateral effects are hard to predict and take into account in experiments. To compensate for the uncertainty that this deficiency in data entails, an assessment factor is applied to the result from the toxicity test as described in section 1.4. The result is the  $PNEC$  value, in this section only the  $PNEC_{\text{freshwater}}$  will be included.

There were 1 616 chemicals for which a  $PNEC_{\text{freshwater}}$  could be linked to one or more species. For some substances more than one species could be linked to the final  $PNEC_{\text{freshwater}}$ , meaning that they had the same effect concentration in the toxicity test and the same assessment factor was used. As a result the sum is not 100 % when summarizing for the figures below.

The number of times the species were used in toxicity tests was divided with 1616 and the result is shown in percent in the “how often used” bar in Figure 14 and Figure 16. E.g. a species has been tested with 20 chemicals, then the “how often used” value would be  $20/1616 = 0.012$  this means that the probability for this species to be included in a toxicity test for an industrial chemical is 1.2 %. Species not defined as aquatic invertebrates, fish or algae were not included.

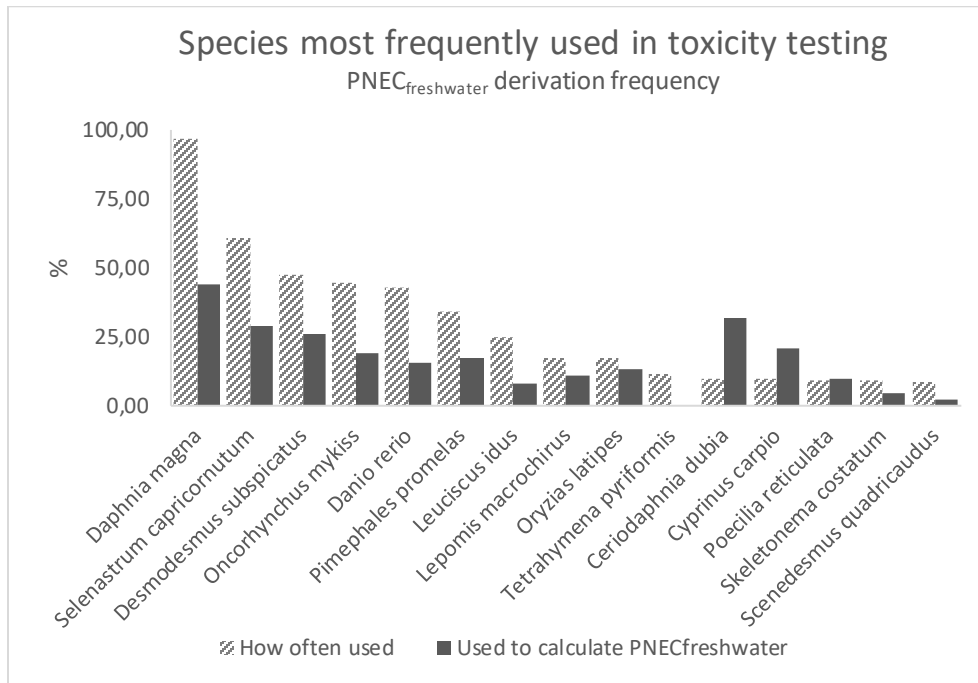
The “used for  $PNEC_{\text{freshwater}}$ ” shows the ratio between the numbers of times a species has been used for toxicity testing and how many times the result from the testing was used to

calculate a  $PNEC_{\text{freshwater}}$ . E.g. *Danio choprae* has been tested with 40 chemicals, for 20 chemicals the results from the experiments on *Danio choprae* were used to derive the  $PNEC_{\text{freshwater}}$  value. This means that the ratio for *Danio choprae* would be  $20/40 = 0.5$  which is 50 %. This means that when *Danio choprae* is used in an experiment the probability is 50 % the result is used for  $PNEC_{\text{freshwater}}$  derivation. Species tested with less than ten chemicals were not included since it would not be defensible to try to draw any conclusions from species used less. Species not defined as aquatic invertebrates, fish or algae were not included.

**Table 15.** The data that was used in Figure 14. The “used for  $PNEC_{\text{freshwater}}$ ” column shows how often a  $PNEC_{\text{freshwater}}$  could be linked to the result from an experiment on a species. The “used in experiments” column shows how many times the species has been used in experiments. There was a total of 1 616 chemicals for which a  $PNEC_{\text{freshwater}}$  was derived and at least one species could be linked. The “how often used” column shows the ratio between how often it is used and 1 616. The “used for  $PNEC_{\text{freshwater}}$  %” shows the ratio in percent between how often a species is used in an experiment and how often it is used to derive a  $PNEC_{\text{freshwater}}$ . The “tax” column gives the taxonomical group of the species; AI= Aquatic invertebrates, A = Algae, F = Fish.

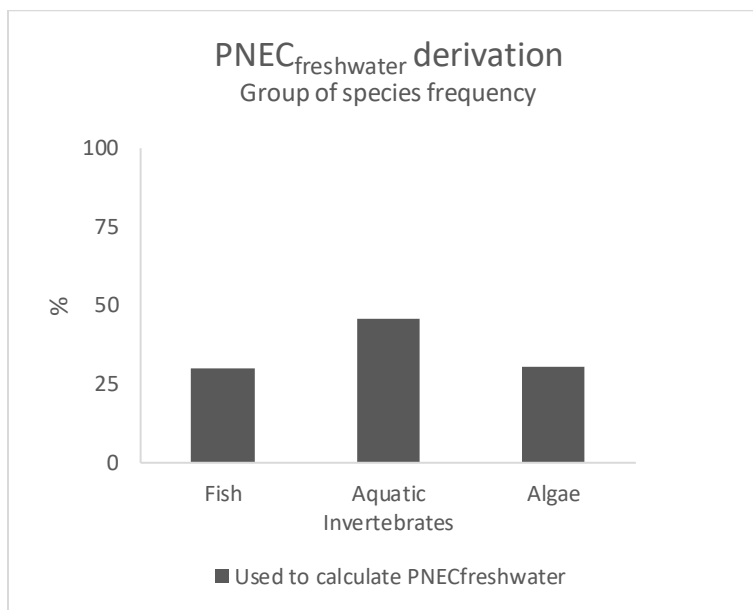
Species	Tax	Used for $PNEC_{\text{freshwater}}$	Used in experiment	How often used %	Used for $PNEC$ %
Selenastrum capricornutum	A	283	986	61.0	28.7
Desmodesmus subspicatus	A	202	770	47.6	26.2
Skeletonema costatum	A	7	147	9.10	4.76
Scenedesmus quadricaudus	A	3	141	8.73	2.13
					<b>61.8</b>
Daphnia magna	AI	693	1566	96.9	44.3
Ceriodaphnia dubia	AI	50	156	9.65	32.1
Tetrahymena pyriformis	AI	0	182	11.3	0.00
					<b>76.4</b>
Cyprinus carpio	F	32	156	9.65	20.5
Oncorhynchus mykiss	F	138	723	44.7	19.1
Pimephales promelas	F	96	550	34.0	17.5
Danio rerio	F	109	689	42.6	15.8
Oryzias latipes	F	36	277	17.1	13.0
Lepomis macrochirus	F	31	278	17.2	11.2
Poecilia reticulata	F	14	147	9.10	9.52
Leuciscus idus	F	32	399	24.7	8.02
					<b>114.6</b>

The species most frequently used in toxicity testing are presented in Figure 14. The graph shows that some species that are frequently used for toxicity testing are rarely used to derive  $PNEC_{\text{freshwater}}$ . This could be interpreted as that they are not very sensitive compared to other species used in toxicity testing. The reason for using them could instead be that they are well documented through earlier studies, inexpensive to use and/or easy to obtain. The data used in Figure 14 is presented in Table 15.



**Figure 14.** The figure shows the species most frequently used in toxicity testing based on the data in Table 15. The left bar shows how frequent a species is used for toxicity testing. The right bar shows how often the result from an experiment on a specific species is used to derive a PNEC<sub>freshwater</sub>.

Figure 15 shows which group was the critical one for deriving the PNEC<sub>freshwater</sub>. Although the result does not differ considerably for the different groups, the graph indicates that the results from toxicity testing on aquatic invertebrates is more frequently used for calculating PNEC<sub>freshwater</sub>. This could be interpreted as that they are generally more sensitive than fish and algae. It could also be other factors that affects the tolerance differently; uptake route, differences in size, feeding behavior etc and with a different design of the experiment, the outcome could be different. A fish may not be affected in a short-term experiment at concentrations that effects an aquatic invertebrate, but exposed over a year it could be affected at lower levels that do not affect the aquatic invertebrate. Another possibility could be that chronic toxicity on aquatic invertebrates are less expensive and easier to perform than on fish and a NOEC value is often lower than an EC<sub>50</sub> value from an acute test. As a result the value from aquatic invertebrates are used to derive the PNEC<sub>freshwater</sub>.



**Figure 15.** The figure shows the data from Table 15 when sorted taxonomically. The bar shows how frequently the result from an experiments on a group is used to derive a PNEC<sub>freshwater</sub>.

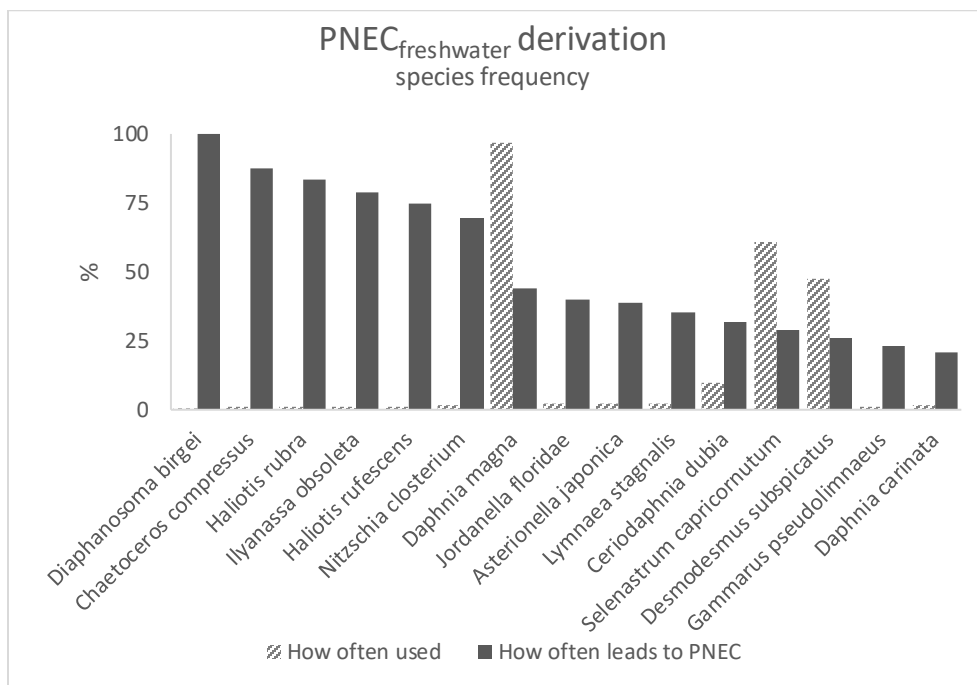
In Figure 16 the species most frequently used, in percent, to derive a PNEC<sub>freshwater</sub> are included. The graphs shows that the six species with the highest “used to calculate PNEC<sub>freshwater</sub>” value are all used in less than 2 % of all experiments. This could be interpreted as that they are more sensitive than other species and that they should be used more often. It should be noted that the sample size of these species is not great, by average they are used in 14 experiments. An explanation for the high use ratio could be that they have been used to derive PNEC<sub>freshwater</sub> for chemicals that is known to be toxic to a certain type of species.

The most frequently used species *Daphnia Magna* is also the seventh most used species for deriving PNEC<sub>freshwater</sub>. This could be interpreted as it is a sensitive species, but it could also be that it is used very often and aquatic invertebrates as a group is sensitive and consequently the used aquatic invertebrates are used to derive PNEC<sub>freshwater</sub>. As mentioned above there is also the possibility that chronic toxicity testing on aquatic invertebrates is cheaper and easier to perform and a NOEC value from a chronic toxicity test is often lower than the value from an acute test. As a result the value from the chronic toxicity is used to derive the PNEC<sub>freshwater</sub>.

**Table 16.** The data that was used in Figure 16. The “used for PNEC<sub>freshwater</sub>” column shows how often a PNEC<sub>freshwater</sub> could be linked to the result from an experiment on a species. The “used in experiments” column shows how many times the species has been used in experiments. There was a total of 1616 chemicals for which a PNEC<sub>freshwater</sub> was derived and at least one species could be linked. The “how often used” column shows the ratio between how often it is used and 1616. The “used for PNEC<sub>freshwater</sub> %” shows the ratio in % between how often a species is used in an experiment and how often it is used to derive a PNEC<sub>freshwater</sub>. The “tax” column gives the taxonomical group of the species; AI = Aquatic invertebrates, A = Algae, F = Fish.

Species	Tax	Used for PNEC <sub>freshwater</sub>	Used in experiment	How often used %	Used for PNEC %
<i>Selenastrum capricornutum</i>	A	283	986	61.0	28.7
<i>Desmodesmus subspicatus</i>	A	202	770	47.6	26.2
<i>Asterionella japonica</i>	A	14	36	2.22	38.9
<i>Nitzschia closterium</i>	A	16	23	1.42	69.6
<i>Chaetoceros compressus</i>	A	14	16	0.99	87.5
<i>Daphnia magna</i>	AI	693	1566	96.9	44.3
<i>Ceriodaphnia dubia</i>	AI	50	156	9.65	32.1
<i>Lymnaea stagnalis</i>	AI	12	34	2.10	35.3
<i>Daphnia carinata</i>	AI	6	29	1.79	20.7
<i>Haliotis rufescens</i>	AI	15	20	1.24	75.0
<i>Ilyanassa obsoleta</i>	AI	15	19	1.18	78.9
<i>Haliotis rubra</i>	AI	15	18	1.11	83.3
<i>Gammarus pseudolimnaeus</i>	AI	3	13	0.80	23.1
<i>Diaphanosoma birgei</i>	AI	12	12	0.74	100
<i>Jordanella floridae</i>	F	14	35	2.17	40.0

Only one species of fish is represented in Table 16 while being in the majority of species used for toxicity testing in Table 15. This indicates a discrepancy between the number of experiments on fish and the number of result from them that are used to derive a PNEC<sub>freshwater</sub>. An explanation for the many species of fish in Figure 14 could be that fish as a group is generally more difficult and expensive to keep in captivity and as a result fewer species are used and those that are used are used in high numbers.



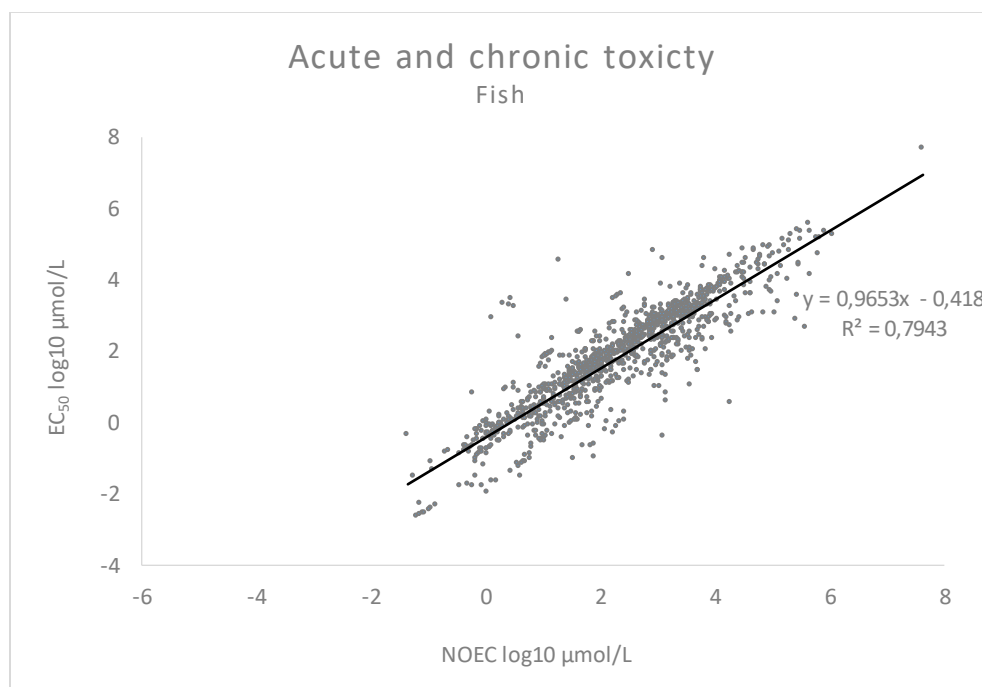
**Figure 16.** The figure shows the species that when used in experiments are most frequently used to derive a PNEC<sub>freshwater</sub>. The right bar shows how frequent a species is used for toxicity testing. The left bar shows how often the result from an experiment on a specific species is used to derive a PNEC<sub>freshwater</sub>.

Sensitivity cannot be the primarily factor when choosing species for toxicity testing since it is not known before the test. Species from which the results are very rarely used for PNEC<sub>freshwater</sub> calculation are continuously used to derive a PNEC<sub>freshwater</sub>. The reason could be that those results are available literature data that is used repeatedly. It could be argued that using this data is questionable since it is likely that data from more sensitive species within the same taxa is available. The result from this report leads to the recommendation that further analysis on whether all species within a taxa should be accepted or if a short list of species should be used.

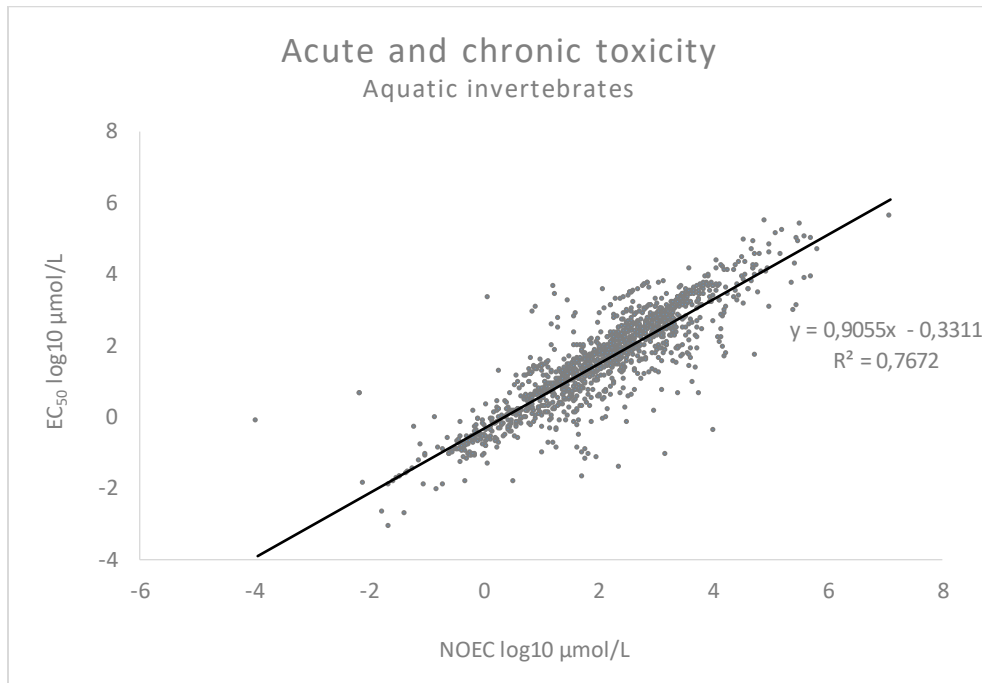
### 3.7 Acute to chronic ratio

A comparison between acute (EC<sub>50</sub>) and chronic toxicity (NOEC) was made to see how they correspond to each other. As mentioned before, the difference is not only in terms of exposure time, but also a comparison of different effect measurements. In the graph each dot represents all available EC<sub>50</sub> values for a substance compiled into one value, the geometric mean per species group. The same procedure is performed with the NOEC values. The geometric mean was calculated as described in section 2.2.5 and used in all graphs. For each substance the geometric mean for EC<sub>50</sub> (y-axis) and NOEC (x-axis) was plotted in the graph.

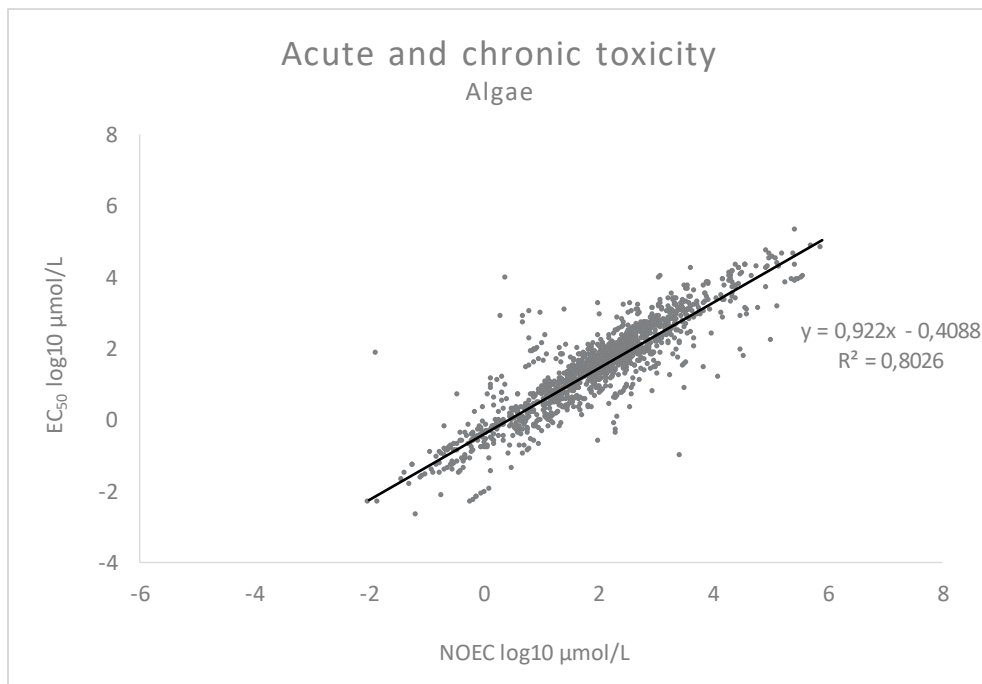
A strong correlation between the EC<sub>50</sub> value and the NOEC value could be valuable when extrapolating test data. A correlation between acute exposure and chronic toxicity could be used as an indication of the accuracy of the prediction. The higher R<sup>2</sup>-value, the stronger is the correlation. A low R<sup>2</sup>-value could be an indication of higher level of uncertainty when extrapolating the result from acute to chronic toxicity.



**Figure 17.** Comparison of data from acute and chronic toxicity tests on fish.



**Figure 18.** Comparison of data from acute and chronic toxicity tests on aquatic invertebrates.



**Figure 19.** Comparison of data from acute and chronic toxicity tests on algae.

The  $R^2$ -value for  $NOEC_{\text{algae}}$  and  $EC_{50\text{algae}}$  was the highest, 0.80, as shown in Figure 17. The  $R^2$ -value from the trendline is an indication of how well the  $EC_{50}$  value can be used to predict the NOEC value. The result shows that the chronic effect on algae can be predicted from acute toxicity testing and vice versa more accurately than for fish and aquatic invertebrates. An explanation for this could be the exposure scenario, the experiment time for chronic and acute toxicity testing on algae typically is the same; four days is often used in standard tests, and it is likely that removing the effect from time reduces the difference between the  $EC_{50}$  and NOEC test. The  $R^2$ -value for the other two groups, fish 0.79 and aquatic invertebrates 0.77, are not much lower and when the exposure scenario for algae is taken into account, possibly more relevant. The conclusion is that the relationship between acute and chronic toxicity does not vary considerably for the different groups of species.

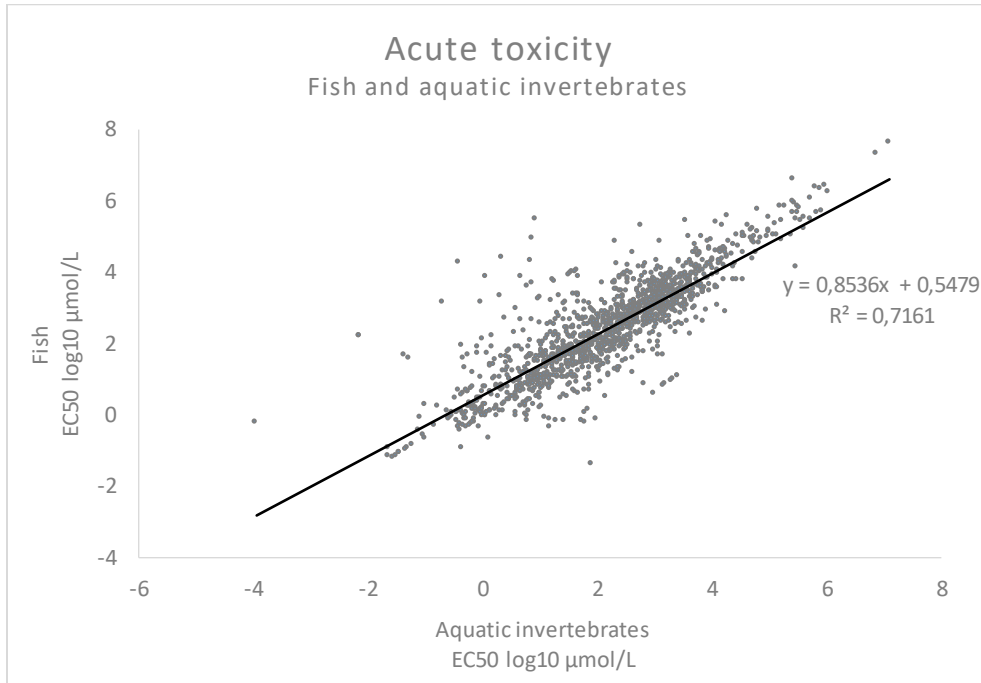
**Table 17.** The  $R^2$ -values from the graphs over acute and chronic toxicity.

Species	$R^2$
Fish	0.79
Aquatic Invertebrates	0.76
Algae	0.80

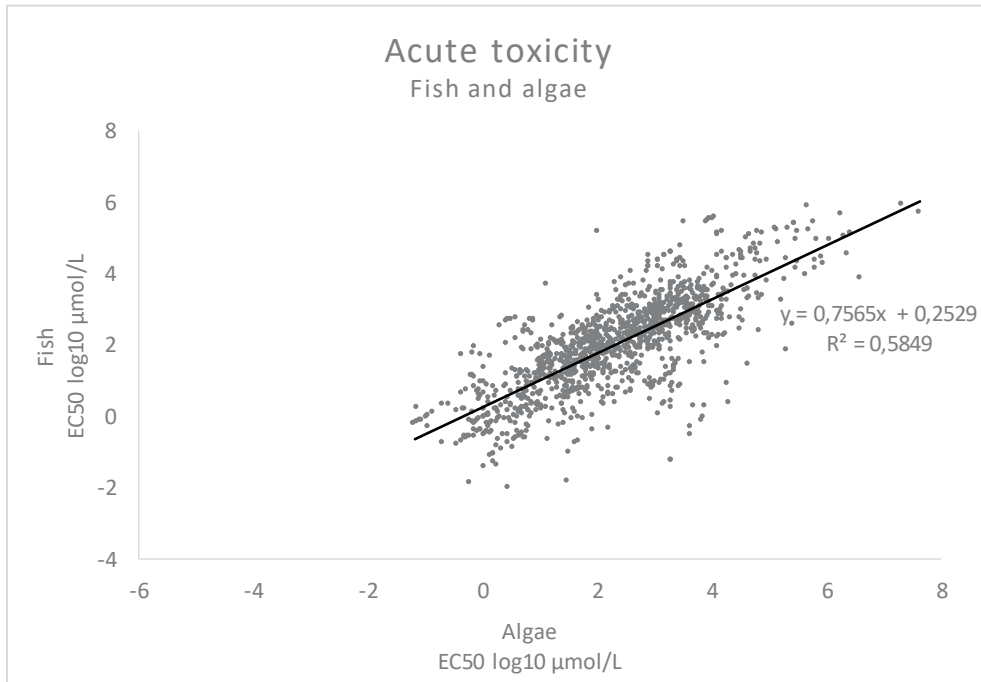
### 3.8 Comparison of $EC_{50}$ between species

A comparison of  $EC_{50}$  values from different groups of species was made to see if there was any correlation between them. The geometric mean was calculated and used in all graphs. The level of accuracy with which a prediction could be made, could be useful when extrapolating the result between species from different trophic levels. A strong correlation between two groups could be useful from both economic and ecotoxicological perspective. By choosing the less expensive species for the designated experiment, money could be saved by using e.g. *Daphnia magna* instead of *Oncorhynchus mykiss* since experiments on aquatic invertebrates generally are cheaper than experiments on fish. The result from the less expensive species could be used to predict the effect upon the latter one through extrapolation. It would also be a better alternative from an ethical perspective to use the species from a lower trophic level.

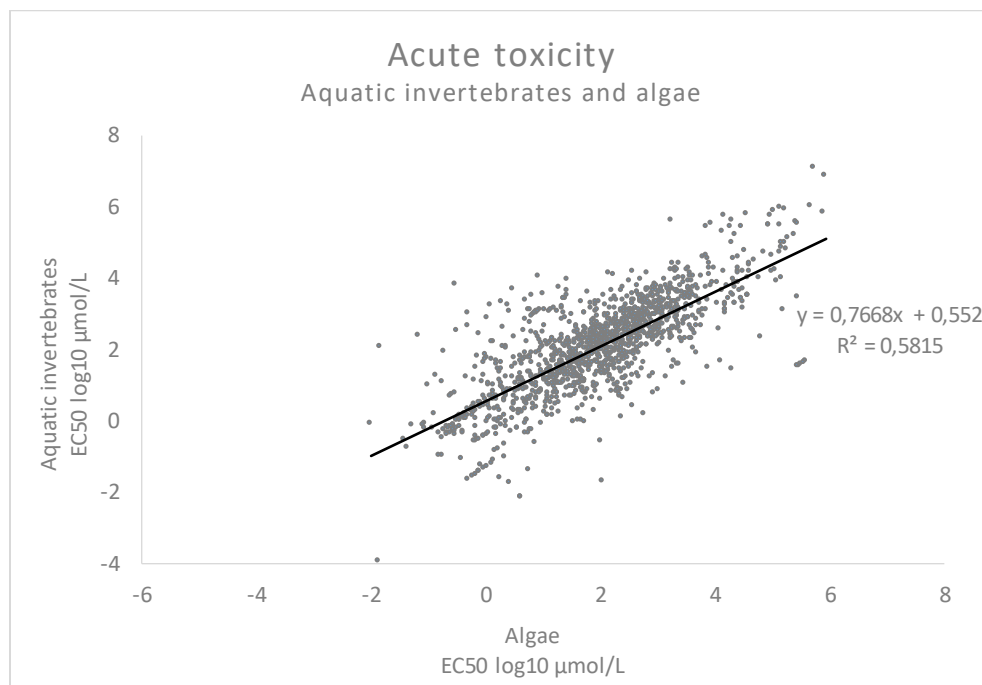
The strongest correlation was found when comparing the  $EC_{50\text{fish}}$  and  $EC_{50\text{aq.invertebrates}}$ , the  $R^2$ -value was 0.72 as shown in Figure 20. The other two comparisons both had an  $R^2$ -value of 0.58. A reason for the stronger correlation between fish and aquatic invertebrates than for any of them with algae could be their stronger likeness in anatomy and uptake routes.



**Figure 20.** Comparison of data from acute toxicity testing on fish and aquatic invertebrates.



**Figure 21.** Comparison of data from acute toxicity testing on fish and algae.



**Figure 22.** Comparison of data from acute toxicity testing on aquatic invertebrates and algae.

**Table 18.** The R<sup>2</sup>-values from the graphs of acute toxicity.

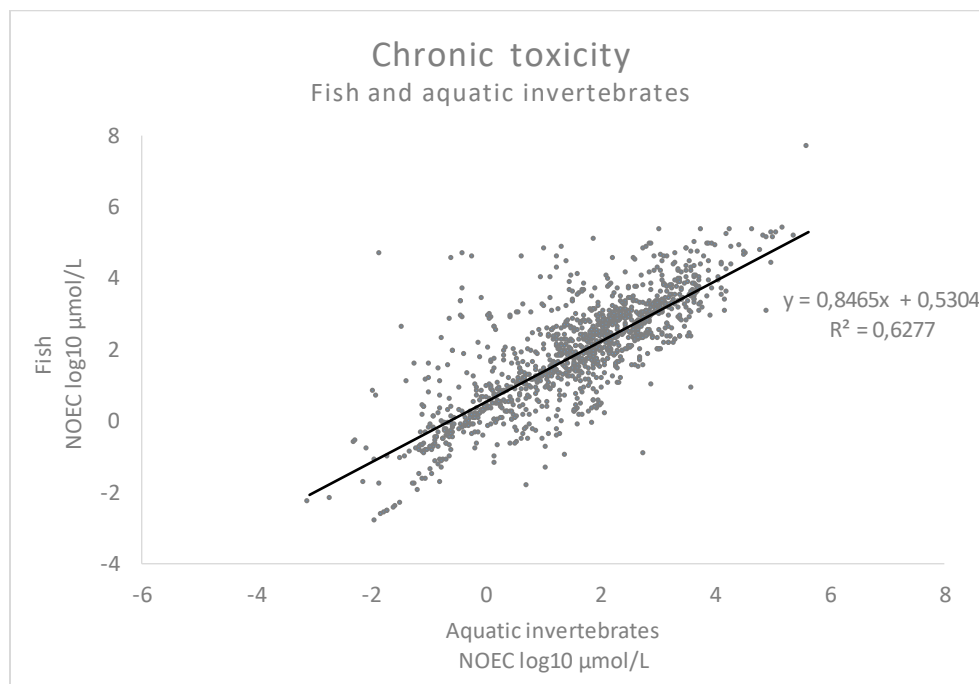
Species	R <sup>2</sup>
Fish – Aquatic invertebrates	0.72
Fish – Algae	0.58
Algae – Aquatic Invertebrates	0.58

### 3.9 Comparison of NOEC between species

A comparison of NOEC values from different group of species was made to see if there was any correlation between them. All graphs were plotted using the geometric mean for each substance. A strong correlation between two groups could be useful in the same way as described in section 3.8.

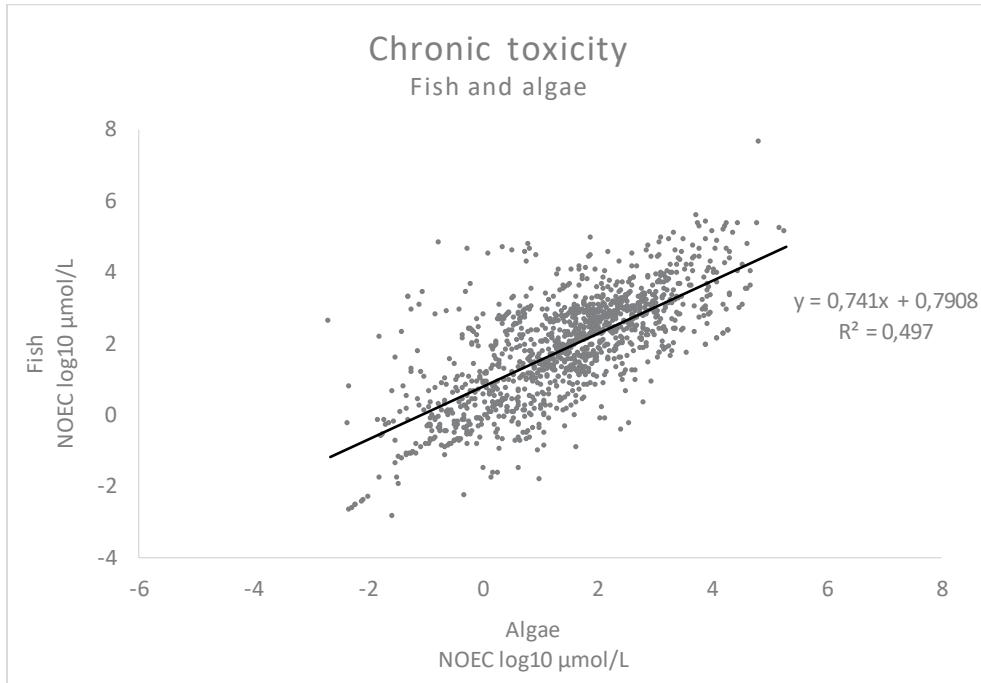
The correlation between  $NOEC_{aq.invertebrates}$  and  $NOEC_{fish}$  was not as strong as when comparing their  $EC_{50}$ ,  $R^2 = 0.63$  which could be interpreted as that their tolerance differ more when exposed to a substance long term. It should however be considered that an  $EC_{50}$  value is a value that has been tested and where a certain effect has been observed. The NOEC value is a value where something is *not* observed and is more dependent on the design of the experiment than the  $EC_{50}$ . Another factor to consider is that fishes usually are greater in size than aquatic invertebrates and that their tolerance thereby differ more over a longer time span,

as is the case in a chronic toxicity test. The same pattern could be observed when comparing the other groups;  $R^2 = 0.50$  for  $\text{NOEC}_{\text{algae}}$  and  $\text{NOEC}_{\text{fish}}$  and  $R^2 = 0.57$  for  $\text{NOEC}_{\text{aq.invertebrates}}$  and  $\text{NOEC}_{\text{algae}}$ .

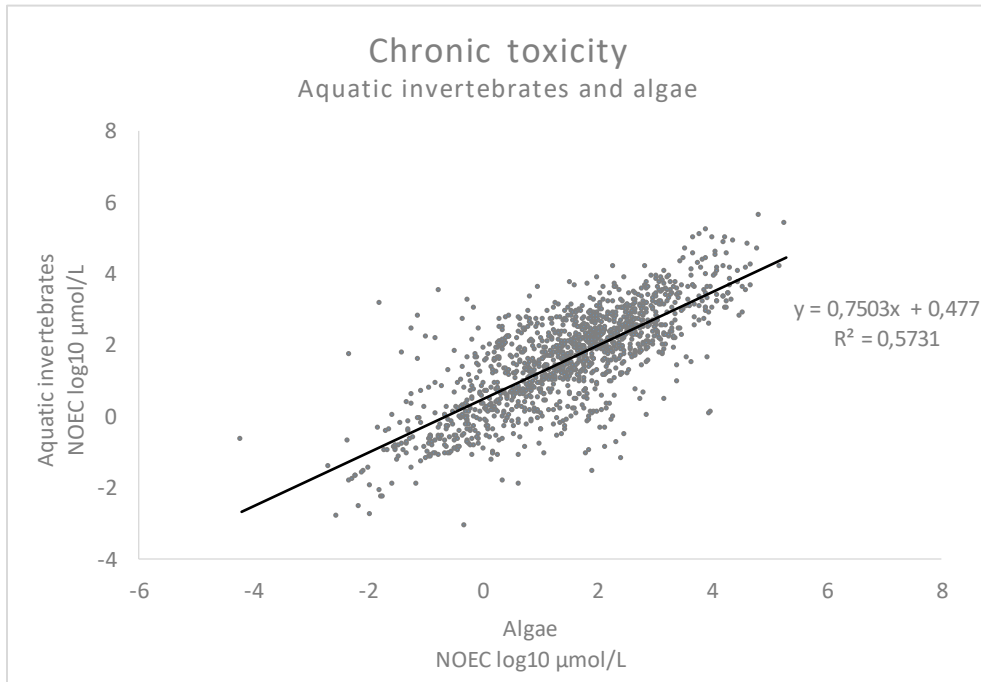


**Figure 23.** Comparison of data from chronic toxicity tests on fish and aquatic invertebrates.

The biggest difference was observed for both  $\text{EC}_{50}$  and  $\text{NOEC}$  when comparing the two groups that were two trophic level apart. Reason for this difference could be that the algae, being a primary producer have completely different anatomy and living behaviour than a secondary predator e.g. a rainbow trout. Their differences can make them vulnerable to exposure through different uptake routes and to different substances depending on the characteristic of the chemicals.



**Figure 24.** Comparison of data from chronic toxicity tests on fish and algae.



**Figure 25.** Comparison of data from chronic toxicity tests on aquatic invertebrates and algae.

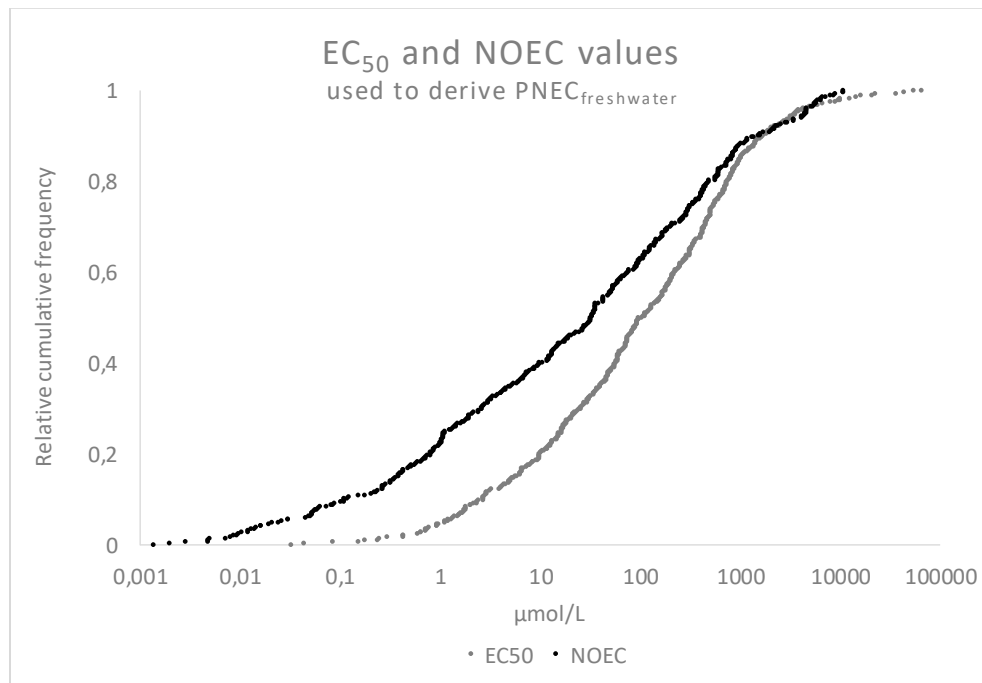
**Table 19.** The R<sup>2</sup>-values from the graphs of chronic toxicity.

Species	R <sup>2</sup>
Fish – Aquatic invertebrates	0.63
Fish – Algae	0.50
Algae – Aquatic Invertebrates	0.57

### 3.10 PNEC

#### 3.10.1 Comparison of EC<sub>50</sub> and NOEC for PNEC<sub>freshwater</sub>

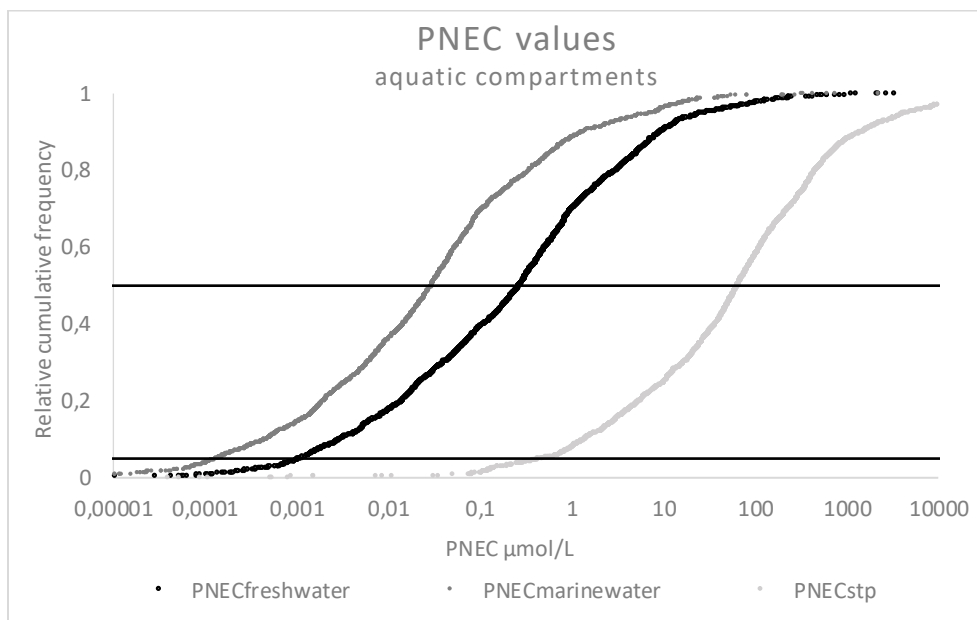
NOEC and EC<sub>50</sub> values were plotted in Figure 26. Only values that had been used to derive a PNEC<sub>freshwater</sub> were used. The average toxicity for EC<sub>50</sub> was 1174.8 µmol/L and the median value was 105.9 µmol/L. For NOEC the average toxicity was 634.0 µmol/L and the median value was 33.36 µmol/L. The difference was expected since the NOEC value usually is lower than the EC<sub>50</sub> value.



**Figure 26.** The EC<sub>50</sub> and NOEC values that were used for a PNEC<sub>freshwater</sub> were plotted against each other.

### 3.10.2 Comparison of PNEC for different compartments

A substances toxicity is assessed in different ways for different compartments. When toxicity data is not available for a compartment or toxicity testing is not possible because of the substances physical and chemical properties, extrapolating the result from another compartment can be the only solution. To compensate for the extrapolation, an assessment factor is applied as described in section 1.4. The assessment procedure for soil is the same as described for  $PNEC_{\text{freshwater}}$  except that terrestrial species are used instead of aquatic.  $PNEC_{\text{STP}}$  is calculated from  $PNEC_{\text{microorganisms}}$  since it is avoiding adverse effect on the microbial activity in the sewage treatment plant (STP) that is the main aim of the assessment.

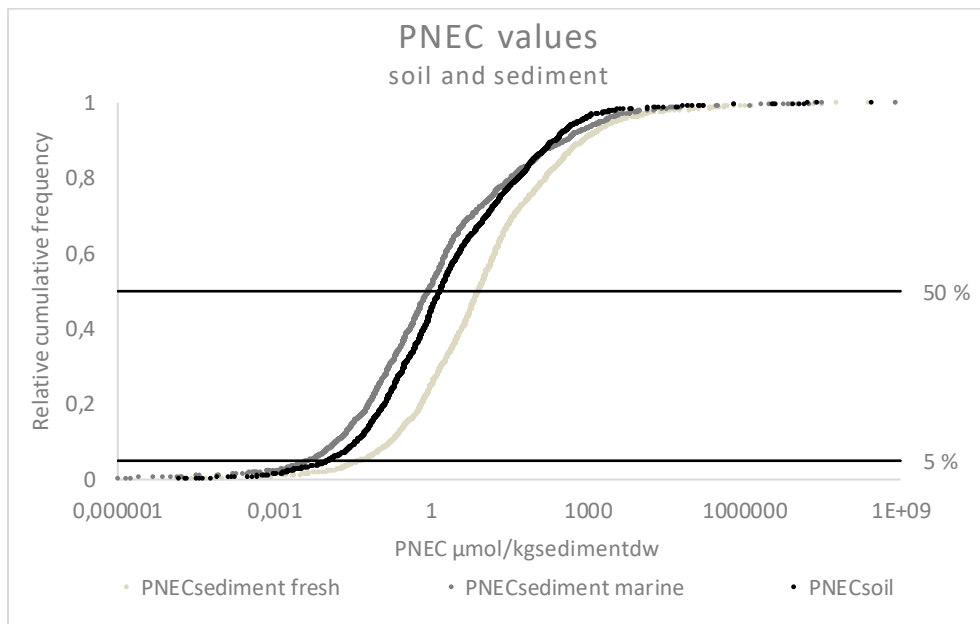


**Figure 27.** PNEC values for different water compartments. The 50 % line shows the median value and the 5 % line shows the lower 5 % percentile.

The 50 % percentile was 0.28  $\mu\text{mol/L}$  for  $PNEC_{\text{freshwater}}$  and 0.03  $\mu\text{mol/L}$  for  $PNEC_{\text{marinewater}}$  and 66.2  $\mu\text{mol/L}$  for  $PNEC_{\text{STP}}$ . This gives a ratio of 9.05 between the two aquatic compartments. The difference between the two compartments can be explained by the usage of a standard factor of 10 to extrapolate from fresh to marine water, which is often used since marine data is less often available. This could be looked upon as a weakness in risk calculation since it is not necessarily reflecting the environmental situation, that species in marine water are always 10 times as sensitive as species in fresh water. But the use of a higher assessment factor is compensating for the fact that marine ecosystem are more complex and contains more species, which increases the level of uncertainty. Compared to the freshwater compartment, there is lack of data for the marine compartment. This is partly for practical and economic reasons; marine species are more difficult and expensive to contain in captivity.

The same pattern appears when comparing the sediment for freshwater with sediment from marine water. The median value for  $PNEC_{sed.fresh}$  is 8.77 and 0.957 for  $PNEC_{sed.marine}$ . The ratio of 9.17 between the two compartments is very close to the ratio between the aquatic compartments, 9.05.

The 50 % percentile for  $PNEC_{STP}$  is 66.2. The lower toxicity for the STP could be a result of presence of particles in the samples which could lower the bioavailability of the tested chemical.



**Figure 28.** PNEC values for soil and sediments. The 50 % line shows the median value and the 5 % line shows the lower 5 % percentile.

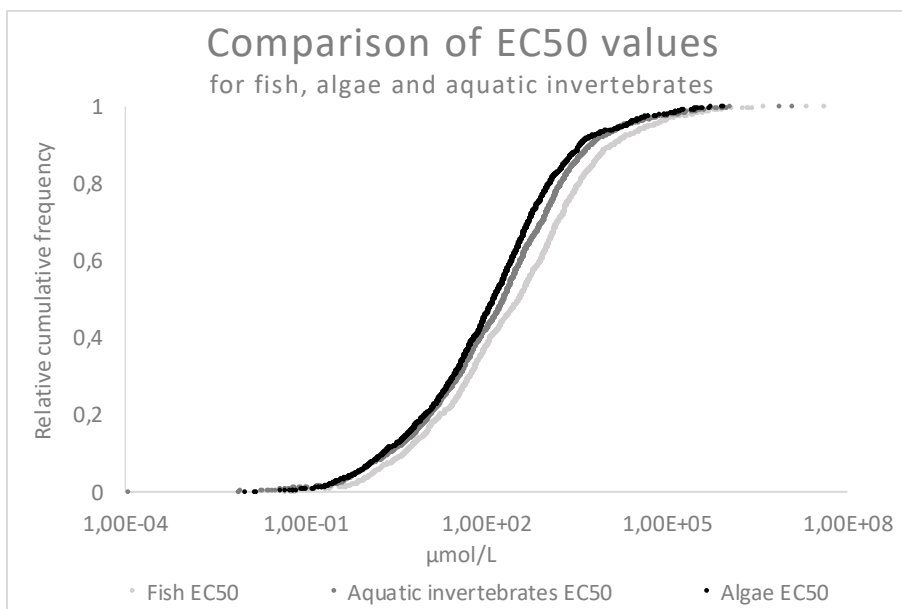
Figure 28 shows PNEC values in  $\mu\text{mol/kg sediment dw}$  from sediments and soil. The values for  $PNEC_{sed.fresh}$  and  $PNEC_{sed.marine}$  are very similar when comparing the 5 %-percentile and the average value. The median values follow the same patterns as in the comparison for the water compartment. The median for  $PNEC_{sed.fresh}$  is  $8.77 \mu\text{mol/kg sediment dw}$  and  $0.96 \mu\text{mol/kg sediment dw}$  for  $PNEC_{sed.marine}$ . This gives a ratio of 9.14, which is very similar to the results from Figure 27.

**Table 20.** The average, 5 % percentile and median value for the different compartments. When calculating the average value for the different compartments, outliers were removed.

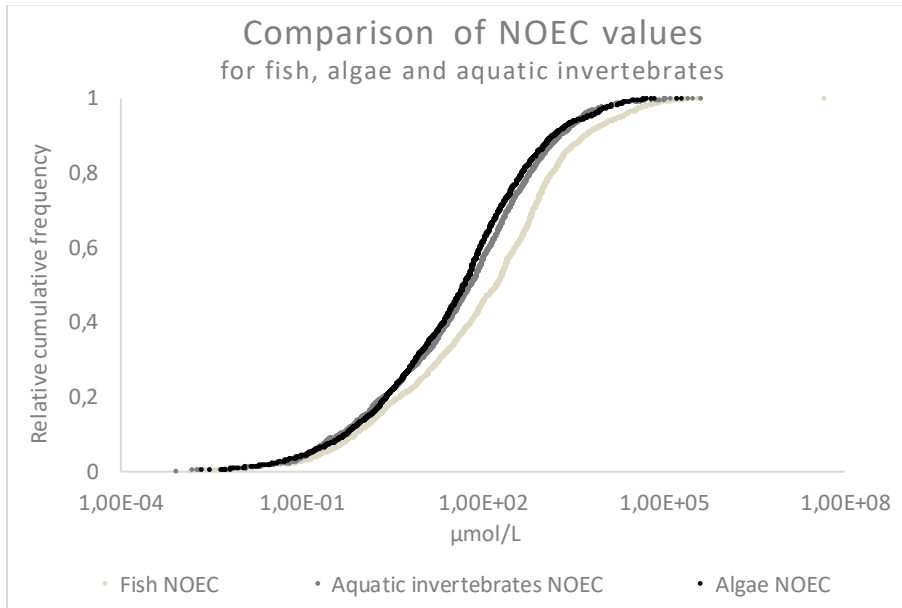
	Average $\mu\text{mol/l}$	5 %-percentile $\mu\text{mol/l}$	Median $\mu\text{mol/l}$
$\text{PNEC}_{\text{freshwater}}$	8.49	0.0011	0.28
$\text{PNEC}_{\text{marinewater}}$	1.23	0.00014	0.03
$\text{PNEC}_{\text{STP}}$	1669	0.44	66.2
	Average $\mu\text{mol/kg sediment dw}$	5 %-percentile $\mu\text{mol/kg sediment dw}$	Median $\mu\text{mol/kg sediment dw}$
$\text{PNEC}_{\text{sed.fresh}}$	18436	0.0049	8.77
$\text{PNEC}_{\text{sed.marine}}$	20235	0.0049	0.96
$\text{PNEC}_{\text{soil}}$	13837	0.011	1.57

### 3.10.3 Comparison of NOEC and EC<sub>50</sub> between species

A comparison of the results of acute and chronic toxicity was made to see if a group of species were more sensitive than others. Figure 29 shows that fish have higher tolerance by average, followed by aquatic invertebrates. Algae is the most sensitive group for both EC<sub>50</sub> and NOEC. The sensitivity distribution is very similar for the different groups of species. If one group was significantly more sensitive, it could be argued that it should be used more frequently since it is more likely to end up being used for PNEC calculation.



**Figure 29.** A comparison of EC<sub>50</sub> values for the freshwater compartment. Each color represents a groups of species.



**Figure 30.** A comparison of NOEC values for the freshwater compartment. Each color represents a groups of species.

Table 21 shows that algae as a group is more sensitive than the other two. The difference is however not great and previous mentioned differences in exposure scenarios could be a reason for the difference.

**Table 21.** Average concentration, lower 5 %-percentile and median value for EC<sub>50</sub> and NOEC from different groups of species.

<b>Average concentration</b>	<b>EC<sub>50</sub> µmol/l</b>	<b>NOEC µmol/l</b>
Algae	6100.8	1131.4
Aquatic invertebrates	9952.6	1362.3
Fish	19752.4	4299.9
<b>5 %-percentile in graph</b>	<b>EC<sub>50</sub> µmol/l</b>	<b>NOEC µmol/l</b>
Algae	0.703262	0.141156
Aquatic invertebrates	0.715094	0.144511
Fish	1.337695	0.241207
<b>Median</b>	<b>EC<sub>50</sub> µmol/l</b>	<b>NOEC µmol/l</b>
Algae	132.4	49.4
Aquatic invertebrates	194.8	62.4
Fish	351.8	174.3

## 4. Conclusions

The ambition of REACH is to ensure that no adverse effect occur to human health or the environment. This report analyzes the hazard of 1 932 industrial chemicals, a group of substances not considered as problematic for the environment as many other groups of substances, e.g. pharmaceuticals or biocides. The risk assessment within REACH does not fully consider the conditions in the environment, where an organism can be exposed to several chemicals at the same time. Every year millions of tons of industrial chemicals are produced and even if just a smaller fraction of these chemicals ends up in the environment, they undoubtedly pose a threat to the environment.

This report shows that several industrial chemicals are persistent and/or bioaccumulative and/or toxic. The result shows that the 5 % most toxic industrial chemicals are more or less as toxic as substances designed to have a toxic mode of action, such as pesticides, or substances identified as problematic, such as OSPAR Hazardous Substances. As shown, substances with these properties are produced in large volumes, meaning that even if a small percentage leaks into the environment the effect could be severe. Since the industrial chemical are usually not monitored and regulated as hard as these other groups of substances, there is a greater risk of them causing adverse effect in the environment undetected.

The result also shows that a lower assessment factor is generally used for substances produced in large volumes, which was expected since more data is required with increasing production volume. It could also be that substances produced in large volumes typically generates a higher PEC since they are used in large volumes and consequently need to derive a higher PNEC to get a risk quotient  $< 1$ . Since the PNEC is affected by the amount of data, one way of doing this is to generate more data.

A few species are used in a majority of the toxicity tests. However, the result shows that some species are not commonly used in toxicity tests, but when they are used the result is very often used to derive PNEC. That is an indication that the species is sensitive, since it was most effected by the substance. According to precautionary principle, it could be argued that these species should be used more often. The reason for this not happening could be that there are well established standards for commonly used species, such as *Daphnia Magna*, making it easy to use them. It could also be that they are cheaper, easier to breed or more easily available than other species. Another argument could be that if you conducted a certain test for many years, you do not want to change that and lose the possibility to compare to earlier results.

The level of exposure is fundamental in risk assessment and to ensure that no adverse effect occurs in the aquatic environment a more holistic approach would be desirable. That includes the use of a more realistic scenario where the effect from all exposure is considered in chemical legislation when conducting risk assessment of chemicals. As of today chemicals are usually assessed one by one in risk assessment. But since many chemicals can have the

same mode of action, and consequently have the same effect on an organism, the question whether this is the best way of doing it is valid. Toxicity from mixtures need to be further incorporated in legislation in order to protect the environment from hazardous chemicals.

## **Acknowledgement**

Thanks to prof. Thomas Backhaus and PhD Mikael Gustavsson for sharing their knowledge and giving their support and guidance. Thanks to Åsa Arrhenius, Bethanie Carney Almroth and Lennart Bornmalm for support, patience and time for supporting me with my thesis.

## 5. Appendix

### Appendix 1

Measurement	Bigger Than	Percent	Smaller Than	Percent	More than	Days	Interpretation
DOC Removal	>=	70	<=	Infinity	<=	10	Readily Biodegradable
BOD	>=	60	<=	Infinity	<=	10	Readily Biodegradable
O2 Consumption	>=	60	<=	Infinity	<=	10	Readily Biodegradable
CO2 Evolution	>=	60	<=	Infinity	<=	10	Readily Biodegradable
COD	>=	60	<=	Infinity	<=	10	Readily Biodegradable
DOC Removal	>=	20	<=	70	<=	84	Inherently Biodegradable
BOD	>=	20	<=	60	<=	29	Inherently Biodegradable
O2 Consumption	>=	20	<=	60	<=	29	Inherently Biodegradable
CO2 Evolution	>=	20	<=	60	<=	29	Inherently Biodegradable
COD	>=	20	<=	60	<=	29	Inherently Biodegradable
DOC Removal	>=	20	<=	Infinity	>=	30	Biodegradability 3
BOD	>=	20	<=	Infinity	>=	30	Biodegradability 3
O2 Consumption	>=	20	<=	Infinity	>=	30	Biodegradability 3
CO2 Evolution	>=	20	<=	Infinity	>=	30	Biodegradability 3
COD	>=	20	<=	Infinity	>=	30	Biodegradability 3
DOC Removal	>=	5	<	20	>=	10	Biodegradability 4
BOD	>=	5	<	20	>=	10	Biodegradability 4
O2 Consumption	>=	5	<	20	>=	10	Biodegradability 4
CO2 Evolution	>=	5	<	20	>=	10	Biodegradability 4
COD	>=	5	<	20	>=	10	Biodegradability 4
Any Measure	-	-	<=	5	>=	10	Biodegradability 5
Any Measure	-	-	<=	1	<=	1	Biodegradability 5

## 6. References

- Altenburger, R., M. Nendza and G. Schuurmann (2003). "Mixture toxicity and its modeling by quantitative structure-activity relationships." *Environ Toxicol Chem* **22**(8): 1900-1915.
- Attias, L., P. Boccardi, G. Boeije, D. Brooke, J. de Bruijn, M. Comber, B. Dolan, S. Fischer, G. Heinemeyer, V. Koch, J. Lijzen, B. Muller, R. Murray-Smith, M. Rikken, J. Tadeo and T. Vermeire (2005). "European union system for the evaluation of substances: the second version." *Chemosphere* **59**(4): 473-485.
- Backhaus, T., H. Blanck and M. Faust (2010). Hazard and Risk Assessment of Chemical Mixtures under REACH, Department of Plant and Environmental Sciences, University of Gothenburg Sweden and F&B Environmental Consulting, Germany.
- Chemspider. (2014). "Chemspider." from <http://www.chemspider.com/>.
- CIRCAB. (2014). "Background sheet for priority substances." from <https://circabc.europa.eu>.
- Cleuvers, M. (2004). "Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid." *Ecotoxicology and Environmental Safety* **59**(3): 309-315.
- CNRS. (2007). "Information requirements for the technical dossier for registration." Retrieved 2014-08-21, from [http://www.prc.cnrs-gif.fr/reach/en/data\\_requirements.html](http://www.prc.cnrs-gif.fr/reach/en/data_requirements.html).
- Duffus, J. H., M. Nordberg and D. M. Templeton (2007). "Glossary of terms used in toxicology, (IUPAC recommendations 2007)." *Pure and Applied Chemistry* **79**(7): 1153-1344.
- ECHA (2008). "Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment."
- ECHA (2012a). Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment.
- ECHA (2012b). Guidance on information requirements and chemical safety assessment: Chapter R.7b: Endpoint specific guidance.
- ECHA (2014a). Guidance on information requirements and Chemical Safety Assessment; Chapter R.7a: Endpoint specific guidance.
- ECHA. (2014b). "ECHA." from [www.echa.europa.eu](http://www.echa.europa.eu).
- ECHA (2015). ECHA's REACH 2018 Roadmap.
- EU. (2006). "OSPAR Convention." from [http://europa.eu/legislation\\_summaries/environment/water\\_protection\\_management/l28061\\_en.htm](http://europa.eu/legislation_summaries/environment/water_protection_management/l28061_en.htm).
- EU. (2010). "Water protection and management (Water Framework Directive)." Retrieved 2014-08-25, from [http://europa.eu/legislation\\_summaries/agriculture/environment/l28002b\\_en.htm#key](http://europa.eu/legislation_summaries/agriculture/environment/l28002b_en.htm#key).
- European Chemicals Bureau, I. f. H. a. C. P. (2003). Technical Guidance Document on Risk Assessment part II.
- European Commission (2009). Prioritisation process: monitoring-based ranking, Annex XV.
- European Commission. (2012). "Agri-environmental indicator - consumption of pesticides." from [http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental\\_indicator\\_-\\_consumption\\_of\\_pesticides](http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_consumption_of_pesticides).

European Medicines Agency. (2014). "What we do." from [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Brochure/2014/08/WC500171674.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Brochure/2014/08/WC500171674.pdf).

Grung, M., E. Heimstad, M. Moe, M. Schlabach, A. Svenson, K. Thomas and A. Woldegiorgis (2007). "Human and veterinary pharmaceuticals, narcotics, and personal care products in the environment. Current state of knowledge and monitoring requirements." Norwegian Pollution Control Authority, Oslo.

Kortenkamp, A., T. Backhaus and M. Faust (2009). State of the Art Report on Mixture Toxicity. Report for Directorate General for the Environment of the European Commission.

Leeuwen, C. J. v. and T. Vermeire (2007). Risk assessment of chemicals : an introduction. Dordrecht, The Netherlands, Springer.

Lijzen, J. P. A. and M. G. J. Rikken (2004). EC (2004) European Union System for the Evaluation of Substances 2.0 (EUSES 2.0), National Institute of Public Health and the Environment.

Mensink, B. (1999). "Biocides (I) Preliminary environmental risk assessment of 93 biocides."

Mensink, B. (2001). "Biocides (II) Refined aquatic environmental risk assessment of 28 priority biocides."

Newman, M. C. (2010). Fundamentals of ecotoxicology. Boca Raton, FL, CRC Press.

OECD (2006). Principles and strategies related to the testing of degradation of organic chemicals. OECD Guidelines for the testing of chemicals, section 3 Introduction. OECD/OCDE.

OSPAR. (2014). Retrieved 2014-10-13, from <http://www.ospar.org>.

REACH (2006). Registration of substances; Chapter 1; Article 12.

Rüdel, H. (2012). Prioritization of biocidal substances for an environmental monitoring.

SCHER, S., SCENIHR (2012). Opinion on the Toxicity and Assessment of Chemical Mixtures.

Schörling, I. and G. Lund (2004). "REACH—The Only Planet Guide to the Secrets of Chemicals Policy in the EU. What Happened and Why." Brussels: Inger Schörling, Greens/European Free Alliance in the European Parliament, available at [http://www. mp. se](http://www.mp.se).

Silva, E., N. Rajapakse and A. Kortenkamp (2002). "Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects." Environmental science & technology **36**(8): 1751-1756.

Swedish Chemical Agency (2008). "Sammanställning av protokoll om riktvärden för växtskyddsmedel i ytvatten."

Swedish Chemical Agency (2014b). "Plant Protection Products."

Swedish Chemicals Agency. (2014). REACH Retrieved 2014-10-23, from <http://www.kemi.se/sv/Start/Reach-forordningen/>.

Swedish Chemicals Agency. (2014a). "Biocidal Products." from <https://www.kemi.se/en/Content/Pesticides/Biocidal-products/>.

Swedish Environmental Protection Agency, R. (2008). 5799, Förslag till gränsvärden för särskilt förorenande ämnen—stöd till vattenmyndigheternas statusklassificering och fastställande av MKN (2008), ISSN.

Walker, C. H. (2006). Principles of ecotoxicology. Boca Raton ; London, CRC.