Risk assessment of chemical mixtures in the aquatic environment

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It is better to be approximately right than precisely wrong

- *Warren Buffett*

ABSTRACT

The total chemical production within the European Union amounts to more than 300 million tonnes per year, of which 140 million tonnes are considered as hazardous to the environment. In order to ensure that these chemicals do not have harmful effects under normal use, likely environmental concentrations are compared to assumed environmentally safe concentration, and only if the resulting risk is deemed acceptable is the product or chemical allowed for use. Depending on the use category of the chemical or product, market approval might require authorization from competent authorities, or a registration by the producer/importer. However, neither of the approval systems considers the combined risk from the coincidental mixtures which will be formed when several individually approved substances co-occur in the environment.

This thesis first analyses the hazard from chemicals from different regulatory classes. Thereafter the combined risks from coincidental mixtures detected in the Swedish aquatic environment from three different monitoring programs are estimated.

When using Swedish water quality objectives and a Kaplan-Meier adjustment to account for non-detects, 73% of 1308 samples taken in Swedish agricultural streams were estimated to be at risk. In contrast, using hazard-values calculated specifically per trophic level (algae, aquatic invertebrate and fish) according to the current pesticide guidelines, only 4% of the samples are estimated to be at risk. This demonstrates the risk estimates dependence on how hazardous concentrations are determined, a single substance issue which gets incorporated into the mixture risk assessment. The thesis also demonstrates that despite the Swedish national goal (adopted 1999) of a non-toxic environment, there is no perceivable trend in the estimated risk in the agricultural streams between 2002 and 2013.

Similarly, samples taken along the Swedish west coast and analyzed for 172 organic chemicals show exceedances of estimated safe concentration at five out of five locations. Also concentrations measured in fish tissue exceed concentrations thresholds set for human consumption, both for individual compounds and for their mixtures.

The thesis adds to a growing body of literature which demonstrates that chemical concentrations detected in the environment frequently exceeds concentrations perceived as safe. This indicates that the current chemical load in the environment should, at least, be considered during market approval.

Populärvetenskaplig sammanfattning

Inom EU produceras varje år över 300 miljoner ton kemikalier varav 140 miljoner ton antas vara skadliga för miljön. För att undvika skador krävs därför både ansvarsfull hantering och användning av sådana kemikalier. Inom EU finns en rad olika regelverk som styr vilka ämnen som får produceras och säljas, samt under vilka omständigheter de får användas.

De studier som presenteras i denna avhandling har två olika fokus som båda relaterar till risker associerade med kemikalier i vattenmiljöer. Dels studeras koncentrationerna som antas vara säkera för enskilda kemikalier med avseende på vilket regelverk som kemikalien faller under. Dels har risken från blandningar av kemikalier bestämts i en rad prover tagna inom ramen för olika miljö-övervakningsprogram i Sverige.

Övervakningsdatan som studerats har haft tre olika inriktningar. Ett övervakningsprogram mäter veckovis förekomsten av över 100 växtskyddsmedel på sex olika platser i södra Sverige. Studien inkluderar prover tagna 2002-2013 och risken från de blandningar som detekterats har bestämts. Särskilt anmärkningsvärt är att trots att Sveriges riksdag 1999 antog ett övergripande miljömål om en giftfri miljö kan ingen trend i risk ses mellan 2002 och 2013. Vidare visare studien att i 73% av all prover överstiger den sammantagna risken de svenska miljökvalitetsnormer som satts inom ramen för miljö-målet. Detta trots att risken från ett flertal ämnen med specifik påverkan på insekter kontinuerligt underskattas eftersom dessa ämnen inte är detekterbara vid de koncentrationer som satts som miljökvalitetsnormer.

De två övriga set av övervakningsdata som analyserats har bestämt förekomsten av kemikalier i marin miljö, dels i vatten och dels i vävnadsprover. Av fem vattenprov tagna längst Sveriges västkust 2012 visar samtliga prover att koncentrationen av enskilda ämnen eller blandningar av kemikalier överstiger de koncentrationer som antas vara säkra för organismer som lever i marin miljö.

Slutligen visar även vävnadsprover tagna i fisk på halter av framförallt poly-bromerade flamskyddsmedel som överstiger de värden som anses säkra för human konsumtion. De flamskyddsmedel som främst bidrar till risken är sedan 2009 förbjuda för användning och produktion inom EU, men finns kvar i miljö på grund av tidigare bruk. Den art som provtagits, tånglake, är inte en typisk matfisk och de halter som uppmäts är i linje med koncentrationer som tidigare rapporterats och på vilka nuvarande kost-råd är baserade.

Sammantaget visar studierna på en kemikalieanvändning som inte kan anses hållbar. De uppmätta halterna överskrider regelbundet de gränser som ställts upp inom

nuvarande regelverk. Studierna visar även på en klar brist inom dessa regelverk då ingen hänsyn tas de koncentrationer som redan uppmätts i miljön då nya ämnen registreras eller tillåts för försäljning på den europeiska marknaden. Om målet med en giftfri miljö skall uppnås krävs en övergripande strategi för att sänka utsläppen av skadliga kemikalier. Både substitution av de mest problematiska ämnena samt en minskad totalanvändning måste anses vara hörnstenar i en sådan strategi.

List of papers

The thesis is based on the following articles and manuscripts. The papers will be referred to by their roman numerals.

I. **Evaluating the environmental hazard of industrial chemicals from data collected during the REACH registration process.**
Mikael B. Gustavsson, Andreas Hellohf, Thomas Backhaus. (2017).
Science of the Total Environment. Vol 586, pp. 658-665
Doi:10.1016/j.scitotenv.2017.02.039
Also available as a pre-print via PeerJ.
Doi: 10.7287/peerj.preprints.2785v2

II. **Pesticide mixtures in the Swedish streams: environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation.**
Mikael B. Gustavsson, Jenny Kreuger, Mirco Bundschuh, Thomas Backhaus. (2017).
Accepted for publication in *Science of the Total Environment.*
Also available as a pre-print via PeerJ.
Doi: 10.7287/peerj.preprints.2779v1

III. **Chemical monitoring of Swedish coastal waters indicates common exceedances of environmental thresholds, both for individual substances as well as their mixtures.**
Mikael B. Gustavsson, Jörgen Magnér, Bethanie Carney-Almroth, K. Martin Eriksson, Joachim Sturve, Thomas Backhaus. (2017).
Submitted to Marine Pollution Bulletin.
Also available as a pre-print via PeerJ.
Doi: 10.7287/peerj.preprints.2894v1

IV. **Toward a Conceptual Approach for Assessing Risks from Chemical Mixtures and Other Stressors to Coastal Ecosystem Services.**
Kristian Syberg, Gary Banta, Peter Bruce, Jonas S Gunnarsson, Mikael Gustavsson, Wayne R Munns Jr, Robert Rämö, Henriette Selck, Thomas Backhaus. (2016).
Integrated Environmental Assessment and Management. Vol 13, pp. 376-386
Doi:10.1002/ieam.1849

Papers and reports not included in the thesis

1. **A quick and robust method for quantification of the hypersensitive response in plants.**
   Oscar N. Johansson, Anders K. Nilsson, Mikael B. Gustavsson, Thomas Backhaus, Mikael B. Gustavsson, Mats X. Andersson, Mats Ellerström. (2015).
   PeerJ 3:e1469
   Doi:10.7717/peerj.1469
2. **Formation of oxidized phosphatidylinositol and 12-oxo-phytodienoic acid containing acylated phosphatidylglycerol during the hypersensitive response in Arabidopsis.**
   Anders K. Nilsson, Oscar N. Johansson, Per Fahlberg, Feray Steinhart, Mikael B. Gustavsson, Mats Ellerström, Mats X. Andersson. (2014).
   Phytochemistry, Vol 101, pp. 65-75
   Doi: 10.106/j.phytochem.2014.01.020
3. **Mixture toxicity contribution of emerging and legacy contaminants in environmental monitoring data from Norway.**
   Thomas Backhaus, Mikael Gustavsson, Daniel Yngsell. (2015)
   Report M-464|2015 to the Norwegian Environmental Agency.
   Available: www.miljodirektoratet.no/Documents/publikasjoner/*M464/M464.pdf*

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Abbreviations

BAC = Background Assessment Criteria

CA = Concentration Addition

EAC = Environmental Assessment Criteria

EC50 = Effect Concentration of 50%, as derived from dose-response relationship

ECHA = European Chemicals Agency

EFSA = European Food Safety Authority

EQS = Environmental Quality Standard, as derived within the WFD

HC5 = Hazardous Concentration for 5% of species, as derived from a SSD

IA = Independent Action

LC50 = Effect Concentration of 50%, as derived from dose-response relationship, specifically measuring lethality

LOD = Level of Detection

MCR = Maximum Cumulative Ratio

MEC = Measured Environmental Concentration

MSFD = Marine Strategy Framework Directive

NOEC = No Observable Effect Concentration, lowest tested value with insignificant effect

PEC = Predicted Environmental Concentration

PNEC = Predicted No Effect Concentration

POP = Persistent Organic Pollutant

QS = Quality Standard, as derived within the WFD

RAC = Regulatory Acceptable Concentration, as derived according to (EFSA, 2013)

RQ = Risk Quotient

SSD = Species Sensitivity Distribution

TU = Toxic Unit, as derived from CA

WFD = Water Framework Directive

1 Introduction

In 2009 Rockström et al. suggested that living conditions on earth are kept stable only if humanity does not transgress nine different planetary boundaries, one of which was a boundary on chemical pollution (figure 1). In the original paper no threshold was given for that boundary, but an effect from chemical pollution, ozone depletion, was given an individual boundary. It has been suggested that the following criteria will likely be met by a chemical that is closing in on its planetary boundary; i) The chemical or mixture of chemicals has a disruptive effect on a vital earth system process. ii) The disruptive effect is not discovered until it is, or inevitably will become, a problem at a planetary scale. iii) The effects of the pollutant in the environment cannot be reversed (Persson et al., 2013). The interpretation is thus that the effects of chemicals are specific, and that a chemical boundary should be broken down into sub-boundaries for individual effects once they are discovered. A similar view is taken by Diamond et al. 2015 who argues that the Montreal protocol and the Stockholm convention shows that humanity has already acknowledged that there are chemical boundaries for certain groups of chemicals.

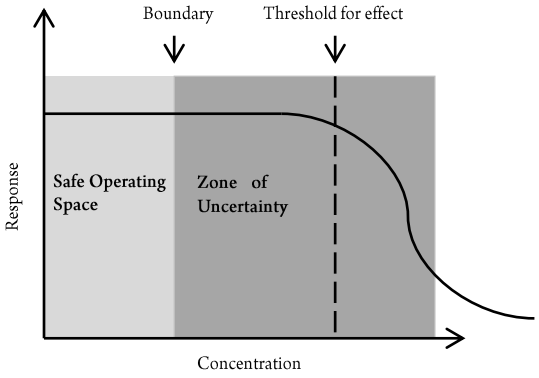

Figure 1: Conceptual overview of the planetary boundary concept. The chemical boundary is transgressed when the concentrations of 'chemicals' exceed levels that we assume are safe. After that transgression there is zone of uncertainty, of unknown width, before effects will be seen.

This thesis will explore the risk from chemical contamination, and the hazard of chemicals produced within the European Union using current regulatory frameworks as a base. Although the thesis does not make claims on a planetary, or even continental, scale it is the author's hope that by presenting this conceptual idea early on, the reader will be able to put the environmental risk assessment of chemical mixtures into a larger context. The thesis will start of discussing single substance (or

product) risk assessment and then focus the discussion on the risk from chemical mixtures.

1.1 Chemical production in the European Union

In 2014 the total production of chemicals within the EU-28 was 324 million tonnes, 205 million of those tonnes were classified as hazardous to human health and 140 million tonnes were classified as hazardous to the aquatic environment (Eurostat, 2016). To put these numbers into perspective, the EU-28 has an approximate land area of 4.5 million km^2, which means that in 2014 the production of compounds classified as hazardous to the aquatic environment was 31 g/m^2. Since 2005 the production of compounds classified as hazardous to the aquatic environment has fluctuated between 160 (2007) and 130 (2009) million tonnes per year (figure 2, Eurostat, 2016). Production volumes are themselves not problematic as they do not directly translate into environmental exposure, but they give an indication of the prevalence of hazardous chemicals.

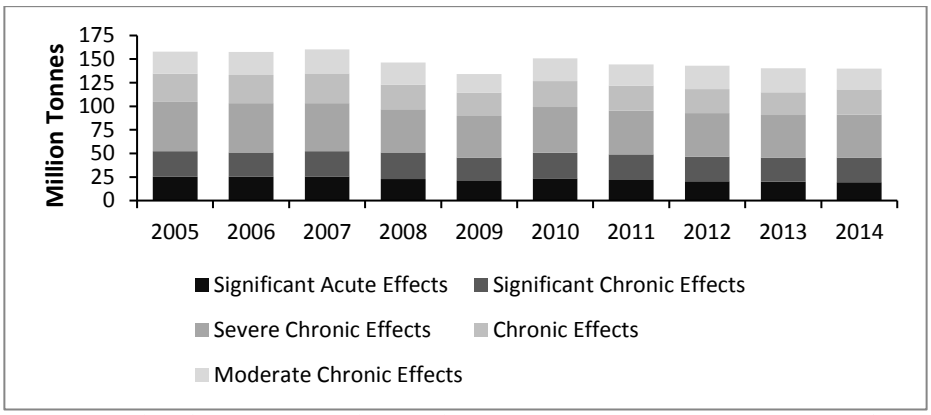

Figure 2: Total production of chemicals harmful to the aquatic environment in the EU28. The data is stacked from the most harmful (Significant Acute Effect), to the least harmful (Moderate Chronic Effects). The total peaks in 2007 at 160.3 million tonnes and is at its lowest 2009 at 134 million tonnes (Eurostat, 2016).

In order to control the risk from chemical exposures the EU has introduced a number of legal frameworks which limit acceptable concentrations in the environment. The acceptable concentrations are in turned determined by performing a hazard assessment of the compound (or product) itself which is then compared to a predicted or measured environmental concentrations (PEC or MEC). As all work within this thesis is based on MECs a discussion on exposure assessment is beyond its scope and the topic is only briefly covered after the discussion on hazard assessment.

1.2 Environmental hazard assessment in EU chemical regulation

The EU regulates chemicals through a number of different legal frameworks which are typically either substance, ecosystem or actor-oriented. The actor oriented legislations are beyond the scope of this thesis but include for instance the Industrial Emissions Directive (2010/75/EU) and the Directive 2001/80/EC dealing specifically with large combustion plants.

Table 1 provides an overview of 6 different legal frameworks with either a substance or ecosystem focus and which together covers the majority of chemical substances, as well as a majority of the aquatic environment. The substance oriented regulations aims to ensure a safe use of individual chemicals or products under normal circumstances. However, when determining if a certain use poses a risk or not, the substance-oriented regulations do not include chemicals already present in the environment. In contrast, the ecosystem-oriented frameworks aims to achieve a 'good ecological status' in the EU freshwater and coastal waters (water framework directive, WFD), or a 'good environmental status' in the coastal and marine waters (marine strategy framework directive, MSFD).

The substance oriented frameworks either deal with the authorization of individual chemicals or chemical products (plant protection products, biocides and pharmaceuticals), or the registration of individual chemicals and chemical products not covered by other regulations (referred to as industrial chemicals). Compounds or products which require authorization is allowed for sale only after a decision by the competent authorities, while industrial chemical are allowed for sale after the producer or importer has provided a specified set of information to the European Chemicals Agency (ECHA).

Table 1: Listing 6 of the legal frameworks which deal with chemical hazard and risk assessment in the EU.

	REACH 1907/2006/EC	**Plant Protection Products** 1107/2009/EC	**Biocides** 528/2012/EU	**Pharmaceuticals** 2001/83/EC and 2001/82/EC	**Water Framework Directive** 2000/60/EC	**Marine Strategy Framework Directive** 2008/56/EC
Pre-market requirement	Registration	Authorization	Authorization	Authorization	-	-
Focus	Industrial Chemicals	Pesticides	Biocides	Pharmaceuticals	Ecosystem	Ecosystem
Entered into force	2007	2009	2013	2004	2000	2008
Type	Regulation	Regulation	Regulation	Regulation	Directive	Directive
Environmental threshold	PNEC	RAC	PNEC	PNEC	EQS	EQS/EAC/BAC
Guidance Document	(ECHA, 2008)	(EFSA, 2013)	(ECHA, 2014)	(EMEA, 2006) and (EMEA, 2016))	(European Commission, 2011)	(Law et al., 2010)

Within the WFD 'good ecological status' is defined as "the values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions" (WFD, Annex V). The biological quality elements are in turn specified using more narrow biological, hydro-morphological and physio-chemical sub-goals. Within the MSFD 'good environmental status' is defined as "[where] marine waters provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations" (MSFD, article 3.5). As the combined effects from all compounds in the particular ecosystem is considered in the ecosystem oriented regulations they have a more holistic view than the substance oriented regulations.

Each of the frameworks listed in table 1 has one or more specific protection goal stated in the respective legislative document. However those goals are often too vague to be useful for risk assessment schemes and they are turned into operational protection goals in technical guidance documents (Rudén et al., 2016). For chemicals in the aquatic environment these operational protection goals often take the form of specific concentrations which should not be exceeded, herein referred to as environmental thresholds.

1.2.1 Industrial chemicals

Regulation 1907/2006/EC 'on the registration, evaluation, authorization and restriction of chemicals' (REACH) aims for a chemical safety assessment to be performed for all chemicals not specifically covered by other regulations. REACH therefore requires a registration of all chemicals produced within, or imported to, the European Union at more than 1 tonne per year (for a single manufacturer/importer). Data on such compounds are compiled by the registrant of the compound and submitted in individual compound dossiers to the European Chemicals Agency (ECHA).

The amount of ecotoxicological data required within the registration process increases with increasing production or import tonnages. For example, only data from a short term test with aquatic invertebrates and from an algae growth inhibition test is required for chemicals produced or imported at 1-10 tonnes per year (1907/2006/EC, Annex VII), while compounds produced or imported at 100-1 000 tonnes per year require the addition of information from acute and chronic tests with fish and aquatic invertebrates (1907/2006/EC, Annex IX).

For compounds produced or imported at more than 10 tonnes per year, as well as for compounds classified as PBT (persistent, bioaccumulative or toxic) or vPvB (very persistent or very bioaccumulative) REACH also requires the derivation of an environmental threshold, termed a Predicted No Effect Concentration (PNEC).

The PNEC is defined as "the concentration of the substance below which adverse effects in the environmental sphere of concern are not expected to occur." (1907/2006/EC, Annex I) and is determined individually for freshwater, marine water, sewage treatment plants, freshwater sediment, marine sediment and soil. The actual determination is performed by dividing an effect concentration (i.e. a concentration at which a specific effect has been seen, typically a 50% or 'no observed' effect, abbreviated EC50 and NOEC respectively) with an Assessment Factor (AF).

$$PNEC = \frac{\min(Effect\ Concentration)}{Assessment\ Factor} \qquad \text{eq:1}$$

The effect concentration used to derive the PNEC is typically the lowest effect concentration from a test on algae, aquatic invertebrates and fish, and the AF is determined by the availability of data. As an example, the specific requirements for freshwater AFs can be found in table 2.

The AF is applied to cover sensitivity differences between species, sensitivity differences within species due to gender, age, sub-population and life-stages, differences between the lab and field, differences between short term and life-long exposure, and differences within and between laboratories (ECHA, 2008).

It should be noted that studies registered under REACH are given a reliability score between 1 and 4. This scoring method was developed by Klimisch et al. 1997 and is based on the documentation and test-type. The system is interpreted as follows: 1 is 'reliable without restrictions', 2 is 'reliable with restriction', 3 is 'not reliable' and 4 is 'not assignable'. In practice the reliability score of the test with the lowest effect concentration may sometimes be high, leading to use of other effect concentrations. Typically the PNEC will be derived using the lowest value (per species group) from the reliability group with the lowest score.

Table 2: Assessment factors used to derive the $PNEC_{freshwater}$ (ECHA, 2008).

Avilable Data	**Assessment Factor**
At least one short-term L(E)C50 from each of the three trophic levels (fish, invertebrates (preferred *Daphnia*) and algae)	1000
One long-term EC10 or NOEC (either fish or *Daphnia*)	1000
Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Long term results (e.g. EC10 or NOECs) from at least three species (normally fish, *Daphnia* and algae) representing three trophic levels	10
Species sensitivity distribution method	5-1
Field data or model ecosystem	Review on case by case basis

After the PNEC has been estimated by the registrant it is compared to the Predicted Environmental Concentration (PEC). The PEC is determined by modelling the compounds release and distribution between different environmental compartments, as determined by its release categories and its physio-chemical properties. (Specific predictions are also made for occupational and consumer exposure (ECHA, 2016a, 2015) but these are beyond the scope of this thesis). Only if the PNEC is higher than the estimated environmental exposure is the compound eligible for registration (ECHA, 2009). Finally the conditions for safe use are communicated to users via safety data sheets.

1.2.2 Biocides

Regulation 528/2012/EU 'concerning the making available on the market and use of biocidal products' requires pre-sale authorization for biocidal products, covering 21 markedly different use categories, including for example 'embalming and taxidermist fluids', 'material preservatives' and 'anti-fouling paints' (boat hull paints). In order to be authorized for sale it must be demonstrated that "the biocidal product has no unacceptable effects itself, or as a result of its residues, on the environment" (528/2012/EU, Article 19).

Therefore, in order to be authorized for sale the biocidal product must be subjected to a full risk assessment and it must be shown that the product will not be released at concentrations which lead to unacceptable effects under normal use. This requires the risk assessment to include not only the active substance, but also any substances

of concern present in the biocidal product. An active substance is in turn defined as a "substance or a micro-organism that has an action on or against harmful organisms" (528/2012/EU, article 3c). Substance of concern has a wider definition and includes substances which have an inherent capacity to cause adverse effects, now or in the future (exemplified for instance as compounds which are PBT, vPvB or a POP) (528/2012/EU, article 3f). This means that although a specific active substance is authorized for use, all products containing the substance must also be authorized for sale individually.

A dossier on a biocidal product is prepared by the applicant and then evaluated by the competent authorities from the EU member states. Authorization of active substances is done EU-wide while authorization of individual biocidal products is performed on a national level. Also, although an applicant may apply for mutual recognition, i.e. that the biocidal product is allowed in one member state and therefore should be allowed in another, each individual member state is allowed to deny such recognition. Currently EU-wide applications for biocidal products are being phased in and the process is supposed to be finished by 2020 (ECHA, 2016b).

For the aquatic compartment the same method as employed within REACH is used to derive the environmental thresholds for the individual active substances (ECHA, 2015). For combined products all relevant ingredients are included in a four tiered system. On the first tier a PEC/PNEC summation (see section on risk assessment of chemical mixtures) of all substances of concern is performed. If that approach fails to demonstrate safe use, the assessment enters tier two and three, where the summation is based on data from individual throphic levels instead. In tier four full product testing is performed in order to (try to) demonstrate that the product is safe to use (ECHA, 2014).

1.2.3 Plant protection products

Regulation 1107/2009/EC on 'the placing of plant protection products (PPP) on the EU market' requires authorization of all PPPs before they enter the market. The classification is, similarly to the biocide regulation, based on the use of the product (1107/2009/EC, Article 1). PPPs basically includes all products designed to prevent harmful effects on crops, either by pests or competing plants, as well as those products that influence the growth of crops (with the exception of nutrients). The specific protection goal of the legislation is that "substances or products produced or placed on the market do not have any harmful effect on human or animal health or any unacceptable effects on the environment" (EC/1107/2009, Article 8).

In order to be authorized for sale it must therefore be demonstrated that the PPP under prescribed use does not have an unacceptable effect on the environment. The

regulation also stipulates that "interaction between the active substance, safeners, synergists and co-formulants shall be taken into account." (1107/2009/EC, Article 29). Similarly to biocides a dossier on the PPP is prepared by an applicant and then evaluated by the competent authorities from the EU member states.

Also similar to biocides, pesticide active substances must be authorized on EU-level, while authorization of products is performed on a member state level. Also similarly to biocides an applicant may apply for mutual recognition of a product, but only under comparable agricultural conditions. These comparable conditions are in turn defined as the member states belonging to the same agricultural zone (north, central or south).

The specific protection goal is operationalized through the so called regulatory acceptable concentrations (RACs). The RAC are defined in the latest guidelines on edge of field effects according to the following "The Annex VI of Directive 91/414/EEC stipulates that an authorization may be granted if e.g. the predicted short-term exposure does not exceed the concentration of the lowest LC or EC50 divided by 100 i.e., such concentration would be considered acceptable under the regulatory criteria of Annex VI, hence this term" (EFSA, 2013 (footnote on page 45)).

The RAC may be set either according to an ecological threshold option (ETO) where only negligible population effects are allowed, or as an ecological recovery option (ERO) where some population-level effects are accepted, if recovery takes place within an acceptable time-period. The RAC is, in both cases determined in accordance to:

$$RAC = \frac{Effect\ Concentration}{Assessment\ Factor} \qquad \text{eq:2}$$

The RAC is calculated independently for algae, aquatic invertebrates, macrophytes, sediment dwellers (*Chironomus* sp.) and fish and uses a different set of assessment factors than the PNEC derivation: 100 for EC50 data from aquatic invertebrates and fish, 10 for NOEC data from aquatic invertebrates, *Chironomus* sp. and fish, as well as for EC50-data from algae and macrophytes. NOEC data from algae and macrophytes are disregarded. The data requirement for all PPPs includes tests with an algae, *Daphnia* sp. and rainbow trout. For insecticides an additional test with an arthropod and the sediment dweller *Chironomus* sp. is required and for herbicides additional non-green algae and macrophyte testing is required.

The pesticide guidelines (just as the REACH guidelines) allow the use of species sensitivity distributions (see section 1.3) to derive the RAC. The guidelines also suggest a method for dealing with a situation where data from several different

species within an organism group is present, but too few data are present to use a species sensitivity distribution. In such instances the RAC may be determined using the geometric mean of the effect data of all species within the specific organism group (EFSA, 2013).

The ERO-RAC can only be derived from micro-/mesocosm tests using an AF of 3-4. It can for example be used to set RACs based on pulsed-exposure experiments, where the short pulsed exposures have led to effects, but where the species composition of the micro-/mesocosm return to pre-exposure composition within an acceptable timeframe (days to weeks depending on the affected species group).

1.2.4 Pharmaceuticals

Both Directive 2001/83/EC (concerning human pharmaceuticals) and Directive 2001/82/EC (concerning veterinary pharmaceuticals) requires that the risk of "any undesirable effects on the environment" (article 28 and 19 respectively) is determined before a new pharmaceutical product is put on the market (products approved before 2006 are excluded from the environmental risk assessment (Ågerstrand et al., 2015)). For veterinary pharmaceuticals the risks posed by the pharmaceutical compounds is then weighted against the positive therapeutic effects in a risk benefit analysis within the authorization process (EMEA, 2016; Directive 201/82/EC, article 27, paragraph 3) while for human pharmaceuticals "this [environmental] impact should not constitute a criterion for refusal of a marketing authorisation" (EMEA, 2006).

For human pharmaceuticals the guideline follows the REACH approach, with the exception that long-term test are immediately required for the base set of organisms (fish, invertebrates and algae. For anti-microbial substances test with microorganisms are also required) since continuous exposure of the aquatic environment via sewage treatment plant effluents is assumed. (EMEA, 2006; ECHA, 2008). The AFs used to determine the PNEC from the required NOEC data is in turn 10. For veterinary pharmaceuticals bio-assays are provided with individual assessment factors joined to each test and determined endpoint (EMEA, 2016; VICH, 2004). The process is similar to that of the PPPs as different AFs are used for different assays (100 for algae and 1000 for *Daphnia magna* and fish EC50; 10 for NOEC tests from algae, *Daphnia magna* and fish). It should be noted that as of 2014 no veterinary pharmaceutical had been refused authorization based on a negative environmental risk benefit analysis (Kuster and Adler, 2014).

1.2.5 Water framework directive

Directive 2000/60/EC, the water framework directive (WFD), aims for a progression towards, and achievement of, 'good ecological' and 'good chemical'

status in EU freshwater and coastal waters. One of the requirements for achieving a 'good ecological status' is that the water also has a 'good chemical status'. A 'good chemical status' is achieved when the concentrations of so called priority pollutants and certain river basin specific substances is below a "concentration of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment" (Directive 2000/60/EC, Art 35). That environmental threshold is in turn termed an environmental quality standard (EQS).

Which compounds that are priority pollutants and their respective EQSs are formally determined by the European Commission and the list and EQSs are periodically reviewed. Priority substances and priority hazardous substances are given EQSs which apply across Europe while the authorities responsible for each river basin district shall develop EQSs for "pollution by other substances identified as being discharged in significant quantities into the body of water" (WFD, Annex V).

The EQS is determined by first deriving eleven individual Quality Standards (QS) where each QS covers an individual aspect of ecosystems or human-health (e.g. specific QS exist for sediment dwelling organisms, for pelagic organisms and for fish tissue to ensure that human consumption is safe). In order to derive a QS for freshwater and marine water the same method is adopted as for deriving PNECs within REACH (European Commission, 2011 (p37 & 45); ECHA, 2008). Finally, the EQS is determined by the lowest of the derived QSs. This means that an EQS in contrast to a PNEC or RAC also includes impacts on human health.

The specific compounds for which an EQS must be derived are not determined within the WFD and the original list (2008/105/EC), comprising 33 compounds, were determined through the use of a combined monitoring-based and modelling-based priority setting scheme (COMMPS). The first candidate list contained 658 chemicals and the system scored compounds based on exposure and effect scores to yield a final list of 33 compounds (Klein et al., 1999). That list has since been amended with an additional 12 compounds and compound-groups (Directive 2013/39/EU, Annex X).

The practical work within the WFD is performed in six years long management cycles. The first of these cycles started in 2009, and management plans for all river basins were required to be in place at the start of that cycle. Initially the goal was that all waterbodies, except heavily modified ones, were to reach good ecological status by 2015, with a potential prolongation of the deadline until 2027 if needed. Unfortunately it is not likely that the goal will be reached even within the extended timeframe (Hering et al., 2010). However, the implementation of the WFD has led to substantial knowledge gains in water management (Hering et al., 2010).

1.2.6 Marine strategy framework directive

Similarly to the WFD the marine strategy framework directive (MSFD, Directive 2008/56/EC) aims to achieve, or progress towards, a 'good environmental status' in all EU marine waters. In order to operationalize that goal eleven different quality descriptors have been established (Directive 2008/56/EC, Annex I). Chemical pollution is primarily covered by descriptor eight which requires that "concentrations of contaminants are at levels not giving rise to pollution effects" (Law et al., 2010). This relates to both the WFD priority pollutants, defined in Directive 2008/105/EC and Directive 2013/39/EU, as well as to chemicals which "may entail significant risks to the marine environment from past and present pollution in the marine region" (2010/477/EU).

Environmental thresholds within the MSFD context have been suggested to be equal to background assessment criteria (BAC) and environmental assessment criteria (EAC). These criteria have previously been specified by the Baltic Marine Environment Protection Commission (HELCOM) and the Oslo-Paris convention (OSPAR) which aims to protect the marine environment of the North-East Atlantic (see for instance Nyberg et al., 2013 for an example of EAC for poly-aromatic hydrocarbons). The EAC cover a range of different environmental matrices (biota, sediment, water etc.) and more recent attempts to derive EACs have followed the same approach as the one used to derive EQSs (Law et al., 2010). In contrast, the BAC has previously been derived as the upper 90% confidence interval of concentrations detected at supposedly pristine sites (OSPAR, 2008). A three graded scale has been proposed where the environmental status is to be considered as unacceptable only if the EAC (which is by definition higher than the BAC) is exceeded (Vethaak et al., 2017). Furthermore it has been suggested that as 'good environmental status' will be assessed using a range of EACs, covering multiple biological matrices and comprising both chemical and biological endpoints, a certain amount (currently suggested as 5-10%) of exceedances of EACs should be allowed before an area or region is considered to not have a 'good environmental status' (Lyons et al., 2017; Vethaak et al., 2017).

Similarly to the WFD the MSFD is structured around management cycles with the first cycle started in 2012. During the first management cycle initial assessments, monitoring programs and implementation of the measures needed in order to reach good environmental status is required. It remains to be seen whether the ambitious goals of the MSFD will be achieved by 2020, which is the current time-frame.

1.3 Using species sensitivity distributions for environmental thresholds

Within most of the presented legal frameworks a species sensitivity distribution (SSD) can be used to derive environmental thresholds. The SSD concept is based on the assumption that different species have different sensitivities towards the same compound, thereby allowing for a distribution of sensitivities to be determined (Posthuma et al., 2002). Once determined, it is possible to derive environmental thresholds which are assumed to have a comparatively low uncertainty. An AF of 1-5 is suggested for chemicals registered under REACH and 3-6 for PPPs (ECHA, 2008; EFSA, 2013). As both the environmental thresholds for biocides and the WFD EQSs for freshwater and marine water uses the REACH approach an SSD may be used to determine also those thresholds ((ECHA, 2015; European Commission, 2011 (p. 37 & p. 45); ECHA, 2008). SSDs are not mentioned in the current guidelines for human or veterinary pharmaceuticals (EMEA, 2016, 2006; VICH, 2004).

If individual species groups are identified as being particularly sensitive to specific modes of action, only species from those groups are allowed to be included in the SSD when determining the environmental threshold (ECHA, 2008). This approach is also adopted for the Biocides and WFD EQS derivation (ECHA, 2014; European Commission, 2011). For PPPs each species groups is treated independently, and the lowest RAC will consequently be based only on the most sensitive species group (EFSA, 2013).

More specifically the environmental threshold is within both the PPP guidance (EFSA, 2013), biocide guidance (ECHA, 2015) and the REACH guidance defined (ECHA, 2008) as:

$$PNEC = \frac{HC05\ (50\%c.i.)}{Assessment\ Factor} \qquad \text{eq:3}$$

Where the HC05 is the concentration corresponding to the 5% percentile (see figure 3), and the 50%c.i. is the lower boundary for the 50% confidence interval for this estimate.

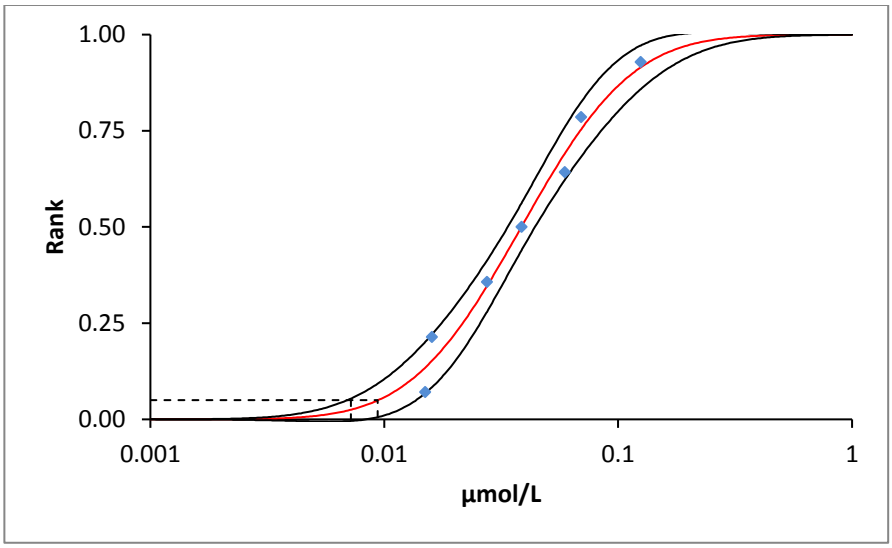

Figure 3: The figure shows an example of a species sensitivity distribution and the associated 50%-confidence interval. The points are effect concentrations (for instance EC50 or NOEC) from a single compound tested with seven different species. The dashed line shows the 5% level on the y-axis and the corresponding HC05 on the x axis (0.009 µmol/L), the lower limit HC05(50%ci) is 0.007 µmol/L.

1.4 Exposure assessment

Environmental concentrations can either be estimated by modelling (Predicted Environmental Concentration, PEC) or determined by direct measurements (Measured Environmental Concentration, MEC).

Modelling environmental concentrations of chemicals carries uncertainties as to whether the specific situation modelled will fit into the generic framework, or if there are special circumstances that is not accounted for. On the other hand modelling allows for prospective risk assessments as environmental concentrations from new or altered emission sources may be estimated. In contrast, measurements are specific and the representativeness of the sample can be questioned.

For samples taken within the Swedish monitoring program for pesticides in agricultural streams (discussed in paper II), it has been shown that the concentrations differed depending on whether the sample was taken in a time-averaged or flow-averaged manner (Bundschuh et al., 2014). Comparing the two sampling strategies also demonstrated that the sampling strategy that yielded the highest concentration was dependent on the flow pattern during the time of sampling.

For highly lipophilic compounds the concentration in water typically is low and bioaccumulation makes it easier to detect the compound in tissue samples (Quevauviller, 2011). However, both concentration-response relationships and environmental thresholds are typically set for water rather than tissue concentrations. Thus it is most often necessary to recalculate the tissue concentrations into the corresponding water concentrations (Dyer et al., 2011) for an estimation of risk to be possible.

In chemical risk assessment the risk from an individual substance or product may be determined by comparing the PEC or MEC (determined in the exposure assessment) to the environmental threshold (determined in the hazard assessment).

1.5 Environmental risk assessment for the aquatic environment

Within the frameworks listed in table 1 the risk towards the aquatic environment is estimated by comparing the environmental threshold, with a MEC or a PEC. The comparison takes the form

$$Risk\ \text{Quotient} = \frac{Environmental\ Concentration}{Environmental\ Threshold} \qquad \text{eq:4}$$

If the Risk Quotient (RQ) is equal to or above one, the situation is perceived as at risk and safe use cannot be ensured. This results in no market approval for the biocides, pesticides and industrial chemicals and calls for risk mitigation measures within the WFD and MSFD. For human pharmaceuticals the environmental risk does not influence the market approval, while for veterinary pharmaceuticals the environmental risk is considered in a risk/benefit analysis before a decision on authorisation is taken.

1.6 Environmental risk assessments of chemical mixtures

Compounds seldom appear alone in the environment. Chemical monitoring campaigns have shown that mixtures of chemicals exist in lakes (Chèvre et al., 2008), streams (Boström et al., 2015; Ccanccapa et al., 2016; Kolpin et al., 2002; Moschet et al., 2014; Schreiner et al., 2016; Stone et al., 2014, paper II), wetlands (Allinson et al., 2015), marine water (Ghekiere et al., 2013; paper III) and groundwater (Gilliom, 2001). The combined results of these monitoring campaigns clearly demonstrate the occurrence of chemical mixtures in the environment.

Furthermore, studies have shown that combined effects of chemical mixtures are larger than can be explained by the largest individual contributor (Kortenkamp et al., 2009). It has also been shown that mixture effects occur even if all components of the mixture is present below their individual NOECs (Altenburger and Greco, 2009; Backhaus, 2008; Faust et al., 2003, 2001; Hass et al., 2007; Kortenkamp, 2008; Rajapakse et al., 2002; Silva et al., 2002) or even their individual EQS (Carvalho et al., 2014).

The effect from chemical mixtures can be determined either directly, by performing tests with the actual mixture of interest (for instance during whole product testing), or by using a component-based approach. In the component-based approach the effect from the mixture is estimated from the effect of each of the individual mixture components.

1.6.1 Concentration addition and independent action

The combined effects of chemical mixtures are often predicted using either concentration addition (CA) or independent action (IA), (Kortenkamp et al., 2009).

The risk quotient of a mixture can, according to CA, be expressed as:

$$RQ_{CA} = \frac{c_{mix}}{ECx_{mix}} = \sum_{i=1}^{n} \frac{c_i}{ECx_i} = \sum_{i=1}^{n} TU_i \qquad \text{eq: 5}$$

were c_{mix} is the total concentration of the mixture, ECx_{Mix} is the mixture concentration causing an effect x, while c_i and ECx_i denote the corresponding concentrations of substance i. The ratio c_i/ECx_i provides a dimensionless measure of the toxicity contribution of compound i, usually termed a Toxic Unit (TU). Conceptually CA assumes that all compounds of a mixture share the same mode of action (Kortenkamp et al., 2009). According to CA, all components of a mixture contribute to the total risk, no matter what concentration they are present at.

Eq. 5 can also be re-arranged to predict the expected toxicity of a mixture in accordance to:

$$ECx_{Mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1} \qquad \text{eq:6}$$

were p_i is the proportion of compound i in the mixture. This shows that any mixture ECx can be determined if the ECx and proportion of each mixture component is

known. For instance, the commonly reported EC50 of individual components can be used to determine the EC50 of the mixture.

Independent Action (IA) can be expressed as:

$$E(c_{mix}) = 1 - \prod_{i=1}^{n}(1 - E(c_i)) \qquad \text{eq:7}$$

were $E(c_{mix})$ is the combined effect of the mixture provoked by a total concentration of c_{mix}. $E(c_i)$ is the effect of compound i when present alone at a concentration c. Conceptually IA assumes that all components of the mixture have independent modes of action (Kortenkamp et al., 2009). Eq:7 also shows that in order to determine the effect of a mixture the effect of each component at the concentrations they are present in the mixture must be known. This translates into IA having a higher data demand than CA. Also, only mixture components which individually cause effects contribute to the combined effect. This may be interpreted as compounds present below their individual NOEC not contributing to the mixture effect. However, the NOEC -being the highest tested concentration where no statistically significant difference from the controls could be determined- typically cause between 10-30% effect (Warne et al., 2008), making such an interpretation overly simplistic.

In order to determine the full concentration-response curve of a mixture according to both CA and IA the dose-response curves of all compounds present in the mixture must be known.

It is highly unlikely that any environmental mixture contains components with only strictly similar or dissimilar modes of action. The concepts thus provide the boundaries with CA describing the situation as if all compounds have a similar mode of action and IA describes the situation as if all compounds have a dis-similar mode of action.

1.6.2 Mixture modelling in risk assessment

Despite CA originally being proposed as a model for mixtures where the components share a similar mode of action the concept is in practice used for mixtures with compounds with both similar and dissimilar modes of action (Belden et al., 2007; Kortenkamp et al., 2009; Rodney et al., 2013; Verbruggen and Brink, 2010). CA is also suggested in the guidance for setting of EQS values for chemical mixtures and for the assessment of pesticide and biocide products (ECHA, 2014; EFSA, 2013; European Commission, 2011)

As CA both has a lower data demand and provides a more conservative risk estimate than IA, it has also been proposed as a standard tool for Tier one mixture risk

assessment within the REACH context (Backhaus and Faust, 2012) and is then expressed as:

$$RQ_{tot} = \sum_i^n \frac{PEC_i}{PNEC_i} \qquad \text{eq:8}$$

This method most likely overestimates the risk as the PNEC is always based on the most sensitive species group. If the RQ_{tot} is above one, the assessment can be refined by basing it on individual species groups. This means obtaining one environmental threshold per specific species group and then recalculating the RQ for each group (herein referred to as RQ_{STU}, Sum of Toxic Units). However, if the RQ_{tot} is below one there is no need for further refinement of the mixture risk estimate.

Table 3: Background data for a hypothetical binary mixture of compound A and B. The RQ_{tot}, the species-group individual RQs as well as the final RQ_{STU} are calculated as an example of the first and second step in the risk assessment of chemical mixtures as proposed by Backhaus and Faust, 2012.

	CompoundA [nmol/L]	**CompoundB [nmol/L]**
EC50 Algae	12000	3500
EC50 Aquatic Invertebrate	7000	7000
EC50 Fish	3500	12000
PEC	2	2.5
AF for PNEC	1000	1000

$$\mathrm{RQ_{tot}} = \frac{\mathrm{PEC_A}}{\mathrm{PNEC_A}} + \frac{\mathrm{PEC_B}}{\mathrm{PNEC_B}} = \frac{2}{\left(\frac{3500}{1000}\right)} + \frac{2.5}{\left(\frac{3500}{1000}\right)} = 1.3$$

$$\mathrm{RQ_{Algae}} = \frac{\mathrm{PEC_A}}{\mathrm{EC50_{Algae}}} + \frac{\mathrm{PEC_B}}{\mathrm{EC50_{Algae}}} = \frac{2}{12000} + \frac{2.5}{3500} = 0.0009$$

$$\mathrm{RQ_{AI}} = \frac{\mathrm{PEC_A}}{\mathrm{EC50_{AI}}} + \frac{\mathrm{PEC_B}}{\mathrm{EC50_{AI}}} = \frac{2}{7000} + \frac{2.5}{7000} = 0.0006$$

$$\mathrm{RQ_{Fish}} = \frac{\mathrm{PEC_A}}{\mathrm{EC50_{Fish}}} + \frac{\mathrm{PEC_B}}{\mathrm{EC50_{Fish}}} = \frac{2}{3500} + \frac{2.5}{12000} = 0.0007$$

$$\mathrm{RQ_{STU}} = \max\left(\mathrm{RQ_{Algae}}, \mathrm{RQ_{AI}}, \mathrm{RQ_{Fish}}\right) * \mathrm{AF} = 0.0009 * 1000 = 0.9$$

In paper II the RQ_{MST} (Most Sensitive Trophic level) was also determined as:

$$RQ_{MST} = \sum_{i=1}^{n} \frac{PEC_i}{\min(EC50_{\text{Algae}}, EC50_{Crustaceens}, EC50_{\text{Fish}})} \qquad \text{eq:9}$$

The RQ_{MST} would in the above example be 0.0013 as it simply provides an RQ estimate without using an AF.

Only if the RQ_{STU} is above one is an assessment using IA needed. Further guidance as to the value of performing an IA assessment can then be found using the MCR (see below).

If compounds with similar modes of action would have roughly parallel dose-response curves it would simplify the data gathering needed to perform IA (and CA) assessments. If so, whole 'mode of action groups' of compounds could have their individual dose-response curves extrapolated from a single full curve from one compound and a single point on the dose response-curve of all other compound of interest. However, little evidence is present supporting that notion (see for instance Arrhenius et al. 2004 for an example of un-parallel dose response curves for similarly acting chemicals) and currently the only way to perform IA risk-assessments is therefore to know the relevant part of the dose response curve for each individual compound.

Also, if an assessment using IA indicates that the product would pose a risk under normal use whole product testing can be performed and used within the authorisation process.

The Maximum Cumulative Ratio and its role in mixture risk assessments

After an RQ has been determined it is possible to determine the maximum cumulative ratio (MCR, Price and Han, 2011). The MCR can be expressed as:

$$MCR = \frac{RQ}{\max_{i=1...n}(TU_i)} \qquad \text{eq:10}$$

and is used to describe how evenly the different components of a mixture contribute to the total mixture risk. The MCR reaches it theoretical maximum n, the number of mixture components, if all components contribute equally to the risk, and approaches one with increasing dominance of the largest contributor. Thus, an MCR close to one indicates a mixture where the uncertainty in exposure and environmental threshold for all compounds besides the main contributor has a minor influence on the estimated total risk. The MCR has therefore been proposed as an assessment-tool for (retrospectively) judging the value of performing mixture risk assessment (Price and Han, 2011).

The MCR is also equal to the maximum difference between a risk assessments based on the CA or IA concept (Junghans et al., 2006) and thereby describes the maximum possible overestimation when using CA on a mixture where all components have dissimilar modes of action. The MCR can therefore be used to estimate the value of expending additional efforts in order to also determine the risk in accordance to IA. Finally, it should be noted that the MCR is simply the inverse of the percentage of risk contribution from the largest risk contributor.

1.6.3 Synergy, antagonism and mixture modelling

Neither CA nor IA account for synergistic or antagonistic effects (that is, effects caused by exposure to two or more chemicals which results in effects that are respectively larger or smaller than predicted). However, CA has been shown to predict the effect concentration of pesticide mixtures within a factor two for 90% of 202 mixtures (Deneer, 2000). On the other hand, mixtures containing organophosphorous compounds, carbamates and pyrethroids deviate from the pattern as synergistic effects appear to occur (Deneer, 2000). Synergistic effects of pyrethroid insecticides have also been shown for *Daphnia* co-exposed to an ergosterol fungicide (Nørgaard and Cedergreen, 2010). Synergism was in that experiment defined as increasing the effect by a factor more than two. Yet another review concluded that out of more than 70 binary mixtures less than 6% of the studies showed synergy larger than a factor two as compared to CA or IA (Cedergreen et al., 2008). Additional reviews have shown that although antagonism and synergism

occur, 80% of mixtures tested for these interactions did not show any interaction effect, despite a testing bias towards mixtures where these effects were more likely (Belden et al., 2007). Also, even for mixtures where synergistic effects have been seen in laboratory tests it has been suggested that the environmental concentrations is usually too low for synergistic potentiation to occur (Cedergreen, 2014; Rodney et al., 2013).

From a risk assessment perspective antagonism causes less concern as the combined effect usually would be lower than anticipated, and thus err on the side of caution. However, antagonism is still relevant from a societal perspective, as overprotection leads to less efficient use of resources.

1.6.4 Understanding mixture modelling results

Only when RQ_{CA} is equal to one is it possible to infer which effect the mixture is likely to cause, as it is equal to the ECx used to derive the RQ_{CA}. For example, if EC50s have been used to calculate the RQ_{CA}, a RQ_{CA} of one means that the mixture is predicted to have 50% effect. However, it is not possible to calculate the expected effect from any other value of RQ, for instance implying that an RQ equal to 0.1 TU indicates an effect of 5%. As can be seen in figure 4 three hypothetical dose-response curves with the same $EC50_{mix}$ can have markedly different effects, if the mixtures are present at lower concentrations.

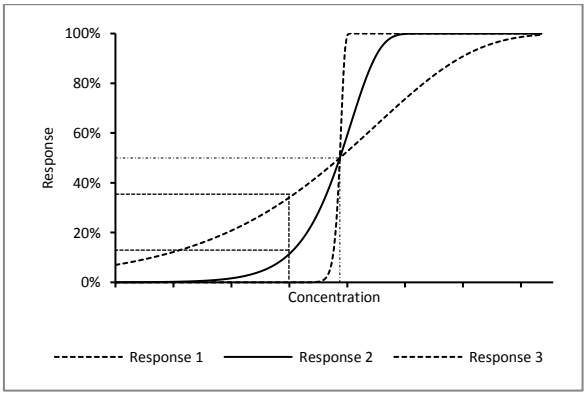

Figure 4: Three hypothetical curves sharing a common EC50. The divergence between the three curves greatly increase the further one moves from the EC50 thus showing the insecurity in extrapolating from a known to an unknown value on a curve with unknown steepness. The concentration axis is logarithmical and decreasing the concentration 10-fold from the concentration needed to reach 50% effect would lead to response 1: 38% effect, response 2: 10% effect and response 3: <0.1% effect

The same problem with inferring an effect from an individual value exist for the RQ_{tot} (eq:8) and RQ_{STU} (see Table 3 with accompanying example), with the additional complication that the RQ_{tot} and RQ_{STU} may be based on different organism groups, and different assessment factors for the different mixture components. Only under the special circumstance that the same organism group, as well as the same assessment factor is used and the RQ_{tot} or RQ_{STU} is equal to 1*AF can a direct inference to an effect be made. Similarly the RQ_{MST} must also be equal to one and be based only on the same organism group for direct inference on effect to be made. Such situations would be exceedingly rare.

1.6.5 Understanding risk contributions

The components in the mixtures analyzed in paper II-IV seldom contribute equally to the risk. Figure 5 shows a typical scenario where, although eleven components are present in the mixture, five of those account for more than 80% of the estimated risk (MCR = 3.4).

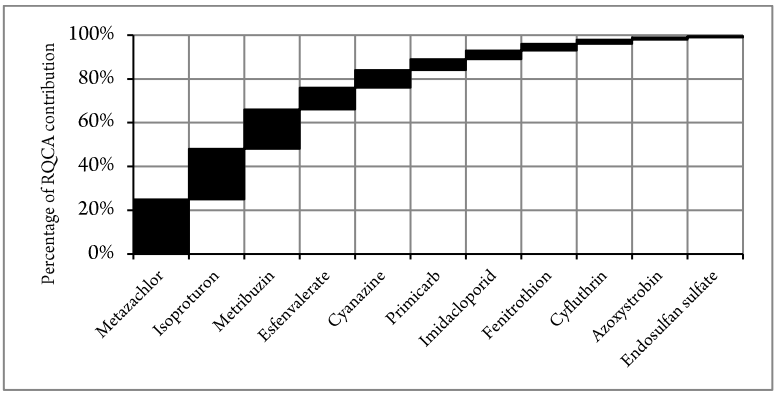

Figure 5: The RQ distribution towards the RQ_{CA}. The figure shows a typical pattern of a pesticide mixture from in Paper II (MCR = 3.4).

The pattern where a sub-set of chemicals account for a high proportion of the risk indicates that risk management should be focused on finding and managing such compounds. Which specific compounds these are might however differ substantially both between location, sampling-time and which organism group that is being considered (paper II-IV; Backhaus and Karlsson, 2014).

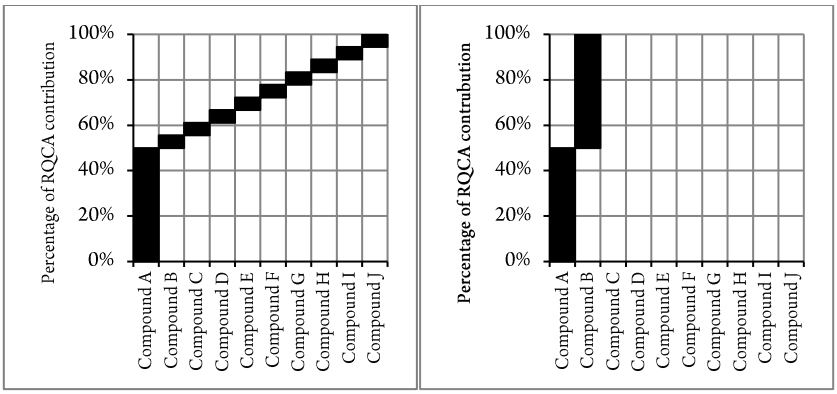

Figure 6: Two scenarios where the contribution to the total mixture risk is markedly different. This types of differences are not captured by the MCR which in both cases would be equal to 2.

The difficulty in easily measuring the importance of mixture assessment through the MCR is displayed in figure 6. Two different scenarios are shown where the MCR in both cases would be equal to two despite a completely different contribution from the mixture.

1.6.6 How to handle compounds which have not been detected

For the risk assessment of single compounds, non-detects are only problematic if the limit of detection (LOD) is higher than the environmental threshold. In such cases it is impossible to tell if the non-detect poses a risk or not. For risk assessment of mixtures, non-detects are of importance no matter the ratio between the LOD and the individual threshold, this as the non-detect can be present in a sample at any concentration between zero and the LOD.

Assuming that all non-detects are present at a zero-concentration is a best-case scenario which likely underestimates the total risk. Assuming that all non-detects are present at their individual LOD is a worst-case scenario which likely over-estimates the total risk. One statistical method suggested for dealing with non-detects is the Kaplan-Meier (KM) method (Helsel, 2009). The KM method is non-parametric, which means that it does not assume an underlying distribution in the data. The KM method estimates the contribution of the non-detects based on a ranked order of the risk of both detected and non-detected compounds in an individual sample (Bolks et al., 2014; Helsel, 2012, 2010).

Using the KM method, the average risk-contribution of all compounds is calculated and can then be multiplied with the number of mixture components to estimate the total risk from the mixture. The method can most easily be understood by a direct example. Table 4 outlines a five component mixture where the respective TU (MEC/ECx) contribution is based either on a measured concentration (compounds A, C and E), or based on worst-case estimates (i.e. non-detects are present at their individual LOD) (compounds B and D). The percentiles needed to determine the total risk is furthermore determine for three different scenarios (corresponding to the scenarios used in paper II and III). In scenario one all non-detects are assumed to be present at their corresponding LOD (the worst-case scenario). In the second scenario the KM method is used to include non-detects in the risk estimate, and in scenario three non-detects are assumed to have a concentration of zero (the best-case scenario).

The percentiles are in turn calculated; for the 'non-detect = LOD' by dividing 1 with the number of analyzed components in the mixture (5), for the 'non-detect = 0' by dividing 1 with the number of detected compounds (3). With the KM method the percentile is determined by the number of observation at or below the specific determined TU, and multiplied with the previous percentile value. That is, e.g. for the second detect there are three observations which are known to be at, or below, that specific TU-value. Therefore the percentile is determined as the number of TUs below the specific value (2) divided with the number of TUs below or at that specific value (3) multiplied with the previous percentile (0.8), (2/3*0.8≈ 0.53). From the percentiles the average risk contribution is then calculated.

In order to visualize the process figures 7a-c shows the distribution as determined in Table 4 for the three different scenarios, and the average contribution is reported as the area under the curve (AUC) in Table 5.

Table 4. A hypothetical five compound mixture where compound B and D are non-detects. The percentiles used to determine the average risk contribution under a worst-case, KM, or best-case scenario are also given.

Compound	Sign	TU	nondetect = LOD	nondetect = KM	nondetect = 0
A	=	0.50	0.80	0.80	0.67
B	<	0.25	0.60	-	-
C	=	0.15	0.40	0.53	0.33
D	<	0.10	0.20	-	-
E	=	0.05	0.00	0.00	0.00

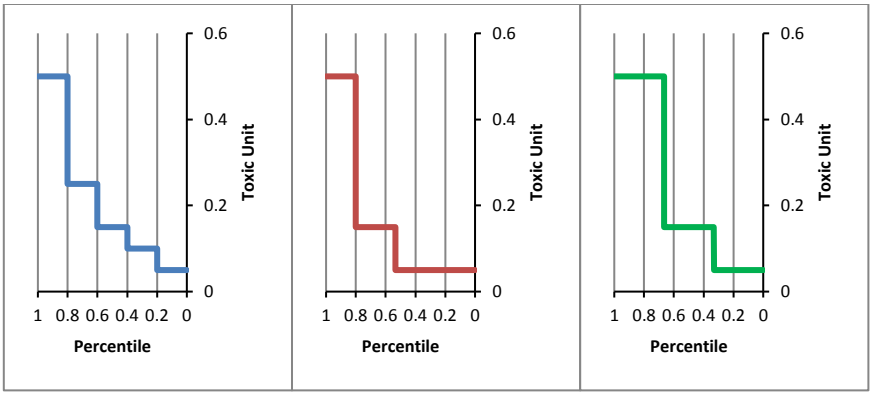

Figure 7a-c: A graphical display of the risk distributions from the hypothetical mixture in table 4 under a) worst-case b) KM, and c) best case scenario.

It should be noted that the KM method excludes all non-detects which (if present at their individual LOD) would have contributed more to the total risk than the largest risk contribution from a detected compound. Such situations calls either for lowered LODs through improved analytical methods, or concentration estimates based on exposure modelling.

Table 5: The average risk contribution per compound, number of compounds and total estimated risk for the hypothetical mixture defined in table 4 for the worst-case, KM and best-case scenario.

	Area Under Curve (Average risk contribution)	n	Sum of TU
Non-Detect Present at LOD	§0.21	5	1.05
Non-Detect KM Adjusted	0.17	5	0.83
Non-Detect Not Present	0.23	3	0.70

2 Aims of this thesis

The overarching goals of this thesis are i) to provide a risk assessment for mixtures found in the Swedish marine and freshwater environment and ii) to assess the available single substance data and explore which factors that limit risk assessment of chemical mixtures.

The specific aims of the presented work were to:

1. Characterize and compare the environmental hazard of chemicals on the European market, with a special focus on chemicals registered under REACH (Paper I).
2. Assess the environmental risk of pesticide mixtures in streams and rivers in the southern parts of Sweden (Paper II).
3. Quantify the differences between ecosystem-wide and trophic-level specific assessments (Paper II).
4. Assess the environmental risk of chemical mixtures detected in the waters along the Swedish west coast (Paper III).
5. Assess the mixture risk from fish when considering human consumption (Paper IV).
6. Analyse and describe the toxic unit distribution for overarching patterns (Papers II-IV).
7. Explore the quantitative consequences of three different strategies for handling non-detects in mixture risk assessment (Paper II & III).
8. Analyse whether single-substance oriented risk mitigation measures are sufficient to ensure a good ecological status in aquatic ecosystems (Paper II).
9. Assess the public data availability and documentation in the context of risk assessment of chemical mixtures (Papers I-IV).

3 Most significant findings

Aim 1: Characterize the environmental hazard of chemicals on the European market, with a special focus on chemicals registered under REACH (Paper I).

Paper I compares the hazard of chemicals which are authorized or registered under different regulatory frameworks. It is demonstrated that the median environmental thresholds follow the order: Industrial chemicals (237.8 ng/L) >> Pharmaceuticals (7.0 ng/L) > Pesticides (4.5 ng/L) > WFD-priority pollutants (0.9 ng/L) > Biocides (0.6 ng/L) (table 6). Thus, the difference in median hazard between the industrial chemicals and the pharmaceuticals, being the least and second least hazardous class, is a factor 34. Meanwhile, the pesticides, WFD-priority pollutants and biocides are only slightly more hazardous than the pharmaceuticals with a factor 12 between the second least and most hazardous class.

Table 6: Summary statistics for the five different regulatory chemical classes: maximum, minimum and median environmental threshold per class.

Group	Number of chemicals	Max [nmol/L]	Min [nmol/L]	Median [nmol/L]
Industrial Chemical	2244	4166667.0	2.4E-03	237.8
Pharmaceutical	142	3332.5	2.9E-05	7.0
Pesticide	298	57921.6	9.9E-06	4.5
Priority Pollutant	33	19427.8	6.8E-04	0.9
Biocide	76	46929.6	1.3E-04	0.6

However, the difference in hazard between the least and the most hazardous industrial chemical covers nine orders of magnitude. Also, there were 280 industrial chemicals with a lower environmental threshold than the median of the pesticide group and 73 industrial chemicals have a lower environmental threshold than the median biocide (figure 8, Paper I, S.I table 1).

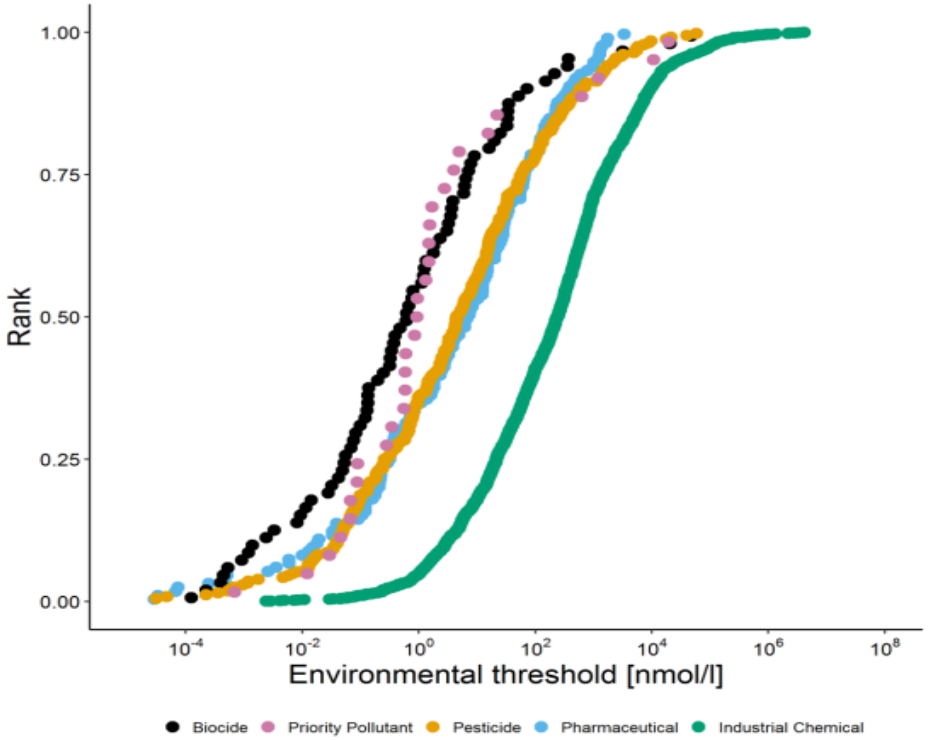

Figure 8: The cumulative distributions of environmental threshold values for biocides, WFD priority pollutants, pesticides, pharmaceuticals and industrial chemicals.

Of the regulatory classes mentioned above, the industrial chemicals, biocides and pesticides are all put on the market only after a risk assessment has shown that their environmental concentrations will not exceed environmental thresholds under normal use. However, the authorization or registration is performed without considering the chemical load already present in the environment. That chemical load has in turn already been suggested to 'jeopardize the health' of European freshwater ecosystems (organic chemicals, main risk contributor is pesticides, Malaj et al., 2014) and to 'threaten surface water at a global scale' (insecticides, Stehle and Schulz, 2015), thus effectively exceeding a global chemical boundary (Rockström et al., 2009, see figure 1). It should be noted that authorization or registration of products under REACH, and authorization of pesticides and biocides, may be reviewed if the release of a compound or product will endanger an environmental objective set within the WFD (Nilsson, 2013).

Aim 2: Assess the environmental risk of pesticide mixtures in streams and rivers in the southern parts of Sweden (Paper II).

In paper II the mixture risks from pesticides is estimated in four agricultural streams and two rivers located in southern Sweden. The data on measured environmental concentrations were gathered by the Swedish pesticide monitoring program and includes 1308 individual samples with on average 98 chemicals analysed per sample (table 7).

Table 7: Overview of occurrence frequencies. The average is calculated as the mode of the compounds found per sample. The minimum number of compounds analysed at M42, N34 and O18 are indicative of individual samples where technical problems have drastically lowered the number of analysed compounds.

		Compounds analysed in each sample			Compounds found (>LOD) per sample			No. compounds needed to cover 95% of RQ_{WQO} (non-detect = 0)
	No. of samples	Max	Min	Mode	Max	Min	Mode	
E21	248	131	68	131	37	2	11	44
M42	308	131	28	131	53	3	23	59
N34	295	131	15	131	43	3	15	58
O18	243	131	14	111	26	2	8	41
Skivarpsån	107	131	68	69	39	6	22	35
Vegeå	107	131	67	69	42	6	16	49
total	1308	131	14	131	53	2	8	83

Using environmental thresholds (water quality objectives, WQO) defined by the Swedish Chemicals Agency and the Swedish University of Agricultural Sciences it was estimated that in 73% of the samples the chemical mixtures put the aquatic ecosystems at risk. The RQ_{tot}, has a median value of 2.2 and 50 % of all RQs fall between 0.9 and 5.9 (paper II, table 3). It should also be noted that in 18 samples the RQ_{tot} is larger than 100 (from five out of six locations) and the maximum exceedance is by a factor of 900. Furthermore, there is no trend over time, indicating that no improvement in risk has occurred over the 10 years included in the analysis (figure 9).

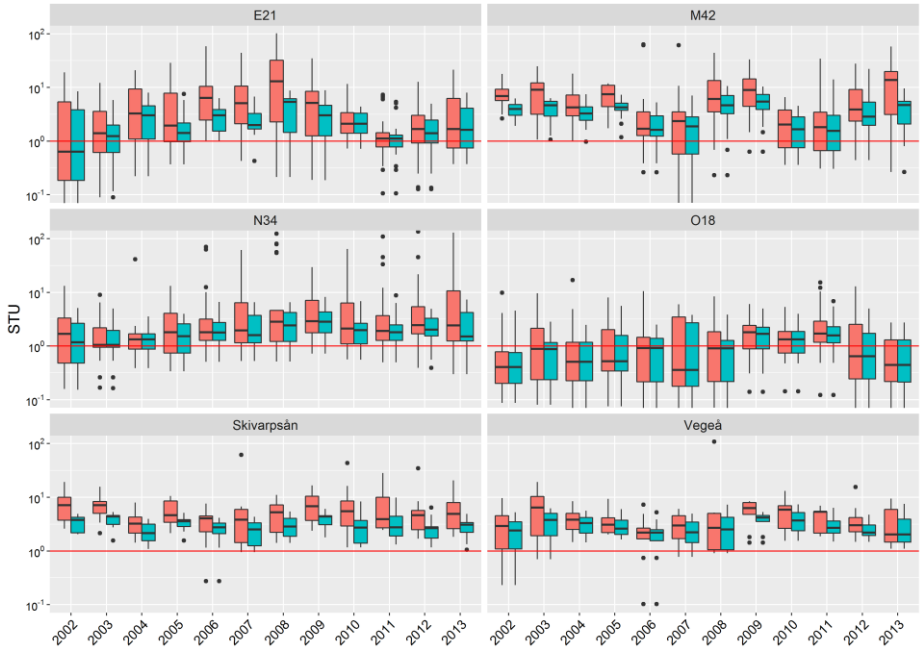

Figure 9: RQ_{CA} at the six monitored sites between 2002 and 2013 for a scenario where non-detects are set to a zero concentration. The left bar in each pair (red) displays the data from scenario two, while the right bar (teal) displays the data from a risk mitigated scenario (all compounds originally present above its WQO is assumed to be present at only 0.95% of its WQO). Horizontal lines indicate the critical threshold between the "no risk" and "risk" situation (see aim 8 for details).

The risk within paper II is most likely underestimated for aquatic invertebrates and fish as 11 compounds, primarily insecticides, have LODs which exceed their WQO (paper II, figure 1).

These results are in line with previous studies which have shown that pesticide mixtures put ecosystems at risk, for instance in four Iberian river basins (Kuzmanović et al., 2015) and the river Meolo in northern Italy (Vaj et al., 2011).

Aim 3: Quantify the differences between ecosystem-wide and trophic-level specific assessments (Paper II).

The WQOs used in paper II were derived in accordance to the 2003 technical guidance document on risk assessment of chemicals which aims to protect against 'adverse effects' (TGD, 2003), the WQO are thereby in practice a PNEC. As outline in Backhaus et al. 2012, a second step in the risk assessment process is to use group-specific environmental thresholds rather than PNECs (or WQOs) which are set to cover all species groups. Therefore the species-group individual RQs (RQ_{STU}) were calculated using a secondary dataset. That dataset contained specific environmental

thresholds for algae, aquatic invertebrates and fish derived from acute EC50 data in accordance to current pesticide guidelines for edge of field effects (EFSA, 2013). Using those environmental thresholds only 4.0% of the samples exceeded their corresponding critical values (paper II, table 4). The decrease in risk is partly due to the separation into individual species groups, which means that the risk is no longer determined by the most sensitive species group per mixture component. However, the different methodologies used to derive the environmental thresholds play a major role.

The WQOs are derived using the AFs from table 2 (for instance, 1000 if only acute EC50-data is present) while the current pesticide thresholds uses a trigger (in practice an AF) of 10 for algae and 100 from aquatic invertebrates and fish if only acute EC50 data is available (EFSA, 2013). The environmental thresholds used in paper II to determine the RQ_{STU} is based only on acute EC50 data. If an AF of 1000 rather than 100 and 10 is used, 75% of all samples exceed the critical value. This is primarily driven by the algae where the change in AF from 10 to 1000 increases the number of risk exceedances from 5 to 976.

Given that herbicides are the most frequently detected compounds in monitoring data from Germany, France, the Netherlands and the USA (Schreiner et al., 2016) the level at which the protection is set likely has a strong influence on the risk mitigation efforts for pesticide pollution in Europe.

Aim 4: Assess the environmental risk of chemical mixtures detected in the waters along the Swedish west coast (Paper III).

Paper III estimates the environmental risk from mixtures of 172 analyzed chemicals in water samples from the Swedish coastal zone (paper III, S.I. table 1). Even when assuming that non-detects are present at a concentration of zero all five locations are deemed to be at risk from the detected chemicals (RQ between 1.7 and 9.1) (paper III, table 4).Triclosan was the individual largest contributor at three sites, and exceeded its individual environmental threshold at four sites (figure 10). (Note that the triclosan PNEC data is from the corresponding 2014 REACH dossier (6.9 ng/L), see specific discussion on triclosan data in section 4.1)

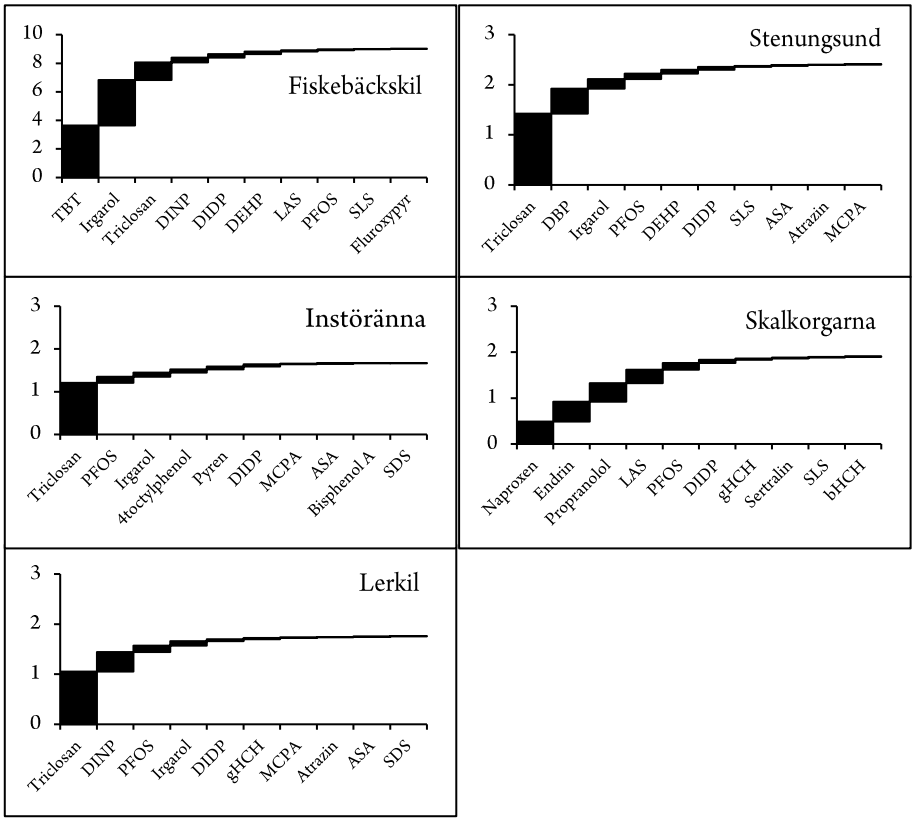

Figure 10: The RQ contribution from the 10 largest risk-contributors per site plotted in a cumulative manner. The sampling site is indicated in the top right corners. The y-axis displays the individual compounds contribution to the total RQ. As the combined RQ is above 1 it indicates risk at all locations. Note that the y-axis of *Fiskebäckskil* (top left) has a different scaling.

The assessment is based on ecosystem-wide environmental thresholds (PNECs or similar thresholds) and trophic-level specific assessments would estimate a lower risk at the individual sites. However, as individual compounds exceed their thresholds at four out of five sites a switch from an ecosystem-wide to a trophic-level specific threshold would still demonstrate risk exceedances at, at least, four out of five sites. These results are consistent with a similar study of the Belgian coastal zone where a widespread exceedance of environmental thresholds where determined (Ghekiere et al., 2013).

Aim 5: Assess the mixture risk from fish considering human consumption (Paper IV).

Paper IV ties the risk assessment of chemical mixtures to food provisioning and the MSFD descriptor nine of a good environmental status ("Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards", MSFD, Annex I). However, is should be noted that the fish species that has been sampled (eelpout, *Zoarces viviparus*) is typically not consumed by humans, and the species rather works as a proxy for other, more palatable species. It is shown that fish fillet samples taken from three locations along the Swedish all exceed the WFD threshold for human consumption (RQ 13.7, 11.4 and 2.3) (figure 11).

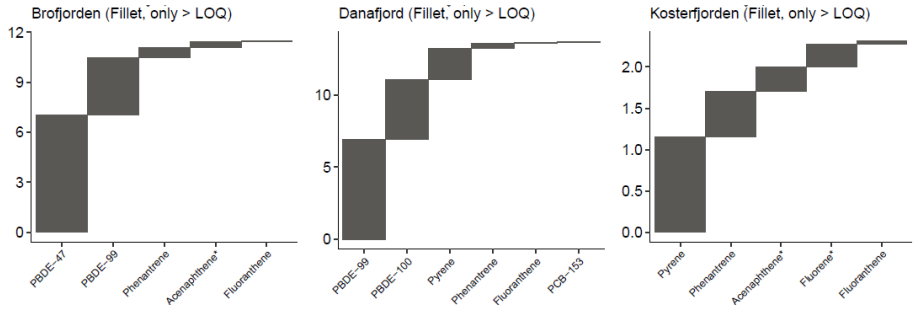

Figure 11: Distribution of individual RQ >0.1 (y-axis) in tissue of Eelpout from three sampling locations along the Swedish west coast.

At the two locations where the risk is highest it is dominated by polybrominated diphenyl ethers (PBDEs, commonly used as flame-retardants. 80 and 91% of the total risk) while at the site with the lowest risk no PBDE was detected in the samples. Similar concentrations of PBDEs have previously been detected in herring and sprat caught in the Skagerrak (see for example Carlsson et al., 2011).

Aim 6: Analyse and describe the toxic unit distribution for overarching patterns (Papers II-IV).

In paper II- IV a small number of mixture components dominate the estimated risk within the individual samples. For instance, in paper II (1308 samples) the MCR ranges between 1.0 and 7.0, with a median of 1.7 (50% of all MCRs fall between 1.3 and 2.3) for the scenarios where non-detects are assumed to be at a concentration of zero (WQO and species-group specific risk estimates, paper II, table 5). In paper III (5 samples) the MCR ranges between 1.4 and 4.1 for the corresponding non-detect approach (paper III, table 4). In paper IV the risk in two out of three samples are dominated by PBDEs, which contribute 80%-90% of the total risk (figure 11).

However, in paper II the risk drivers differ between sites, years and individual samples. Even when considering only the WQO scenario 83 of the 144 different

chemicals monitored, must be included in the assessment in order to account for 95% of the risk in all samples (table 7).

Although too few samples have been analyzed along the Swedish West Coast to draw conclusions on whether or not the risk drivers are different over time, it should be noted that they at least are different between the different matrices (water and fish-tissue, figure 10 and figure 11). For instance, likely due to their high lipophilicity, none of the PBDEs driving the risk towards human consumption (figure 11) was found in the water samples analyzed in paper III (figure 10, paper III, S.I. Table 1). Together with the differences in risk drivers determined in paper II, it demonstrates that choosing which compounds that should be the target of risk management changes with the considered location, matrix, species and timeframe.

Aim 7: Explore the quantitative consequences of three strategies on how to handle non-detects in mixture risk assessment (Paper II & III).

In paper II and III non-detects are treated using three different approaches. Non-detects are either; set to a concentration of zero (best-case scenario); set to a concentration equal to the LOD (worst-case scenario) or included through the application of the KM-method. It is shown that the inclusion of non-detects through the KM-method increase the estimated risk on average by 11% in paper II (for the WQO scenarios, paper II, table 3) and 5% in paper III (paper III, table 4), as compared to the assumption that all non-detects are present at a concentration of zero. In paper II the corresponding absolute increase in RQ between the KM and best-case is at maximum 8.9 (RQ increased from 603.6 to 612.5) with a median average increase of 0.1. In about 3% of the cases does the inclusion of non-detects through the use of the KM method increase the total RQ from below one to above one, i.e. a sample previously considered as not at risk is perceived as at risk.

It is possible to argue both for and against the use of the KM method in paper II and III. On the one hand it may be perceived as increasing the realism of the risk estimate as it includes compounds which might be present, but which we cannot detect. On the other hand, it can be argued that the KM method decreases the realism for just the same reason. Additionally, for samples where a large proportion of the compounds are non-detects, the final risk estimate is highly dependent on how high up in a LOD/WQO ranking that the first detect is. As all compounds that have a higher LOD/WQO than the highest MEC/WQO are discarded by the KM method, the number of compounds which the final KM-adjusted mean risk is multiplied with to yield the KM-adjusted sum, may vary substantially. However, the low impact on the estimated risk demonstrated in paper II indicates that the use or non-use of the KM-method, is actually a non-issue. Particularly if compared to the compounds whose LOD is substantially larger than their environmental threshold.

In both paper II and paper III it is also shown that the LOD might be far higher than the environmental thresholds, primarily for pyrethroid insecticides (e.g. in paper II the LOD/WQO ratio of permethrin exceeds a factor 100, in paper III it exceeds a factor 97000 for cypermethrin) and hormones (for ethinylestradiol by a factor 71 and for estriol by a factor 56 in paper III). Assuming that they are present at their LOD rather than at a concentration of zero increases, on average, the risk with close to 13 400% (paper II) and 3 500 000% (paper III). For these compounds there is clearly a need either for improved analytical tools, or for other approaches to assess their concentrations in the aquatic environment. This problem also indicates a fundamental problem with risk assessment based solely on monitoring data, as a large proportion of the potential risk cannot be estimated (this issue is further discussed below).

Aim 8: Analyse whether single-substance oriented risk mitigation measures are sufficient to ensure a good ecological status in aquatic ecosystems (Paper II).

In paper II a simulated risk management of single substances was performed. This was done by artificially lowering the concentration of each compound which exceeded its individual environmental threshold to a concentration of 95% of its WQOs (figure 9). This lowered the percentage of times the whole mixture exceeded acceptable concentration from 70.5% of the times to 70.0% of the times if all non-detects are assumed to be at a concentration of zero (paper II, table 4). The median reduction in RQ for is in that case equal to 0.05, with 50% of the reduction falling between 0.0 and 2.1. This demonstrates that risk management, in order to successfully achieve acceptable risk must focus not only on individual compounds, but on the total chemical load.

Aim 9: Assess the public data availability and documentation in the context of risk assessment of chemical mixtures (Papers I-IV).

Paper I demonstrate a high reliance on a small number of species for setting the environmental thresholds of industrial chemicals (table 8). *Daphnia magna* is the most tested species (*Daphnia magna* data was available for 97% of all compounds for which a PNEC could be determined), and also the species which most often leads to a PNEC (44% of all PNECs matched a test performed with *Daphnia magna*). This is predictable from the guidance document where *Daphnia* is pointed out as the preferred species for invertebrate testing, while no specification on species is present for the groups algae and fish (ECHA, 2008). For the algae group two species (*Selenastrum capricornutum* and *Desmodesmus subspicatus*) dominate the dataset while for fish the three most commonly tested species (*Oncorhynchus mykiss*, *Danio rerio* and *Pimephales promelas*) are comparatively evenly used (table 8). Together this indicates that those species should be evaluated for their average position in

species sensitivity distributions as they represent all species within their group in the risk assessment process (Gustavsson, 2016).

Table 8: Most commonly used test species from each organism group (algae, invertebrates, fish). Total number of chemicals analyzed is 1 666. The column 'No of chemicals tested' shows how often each species has been tested (in absolute numbers). 'Identification as PNEC Driver' shows how often a species was identified as PNEC driver, in absolute and relative numbers (as percentage of the number of chemicals tested with each species). For further details see paper I. A.I. = Aquatic Invertebrate.

Species	Taxa	Chemicals tested	Identification as PNEC driver	
			Absolute	Percentage
Selenastrum capricornutum	Algae	1013	293	29%
Desmodesmus subspicatus	Algae	770	203	26%
Skeletonema costatum	Algae	159	8	5%
Sum within group	-	1942	504	
Daphnia magna	A.I.	1609	701	44%
Ceriodaphnia dubia	A.I.	164	56	34%
Americamysis bahia	A.I.	129	5	4%
Sum within group	-	1902	762	
Oncorhynchus mykiss	Fish	739	152	21%
Danio rerio	Fish	705	103	15%
Pimephales promelas	Fish	577	101	18%
Sum within group	-	2021	356	

Paper I would not have been possible without manually curating the database re-built from the individual REACH dossiers. Manual retrieval of data was also required when gathering data on WFD priority pollutants, biocides and PPPs. While gathering the data needed for paper I it became apparent that the difference between making data available, and making it accessible, is very large. Furthermore, the data availability for industrial chemicals has been proved to be below regulatory requirements. In 2016 ECHA performed a compliance check with 184 compounds (produced at more than 100 tonnes per year) and in 168 cases information was missing. The missing data mostly related to pre-natal developmental toxicity, mutagenicity/genotoxicity, reproduction toxicity and long-term aquatic toxicity (ECHA, PR/17/03). Similarly the German environmental protection agency demonstrated that only 26% of 1814 HPVCs provided REACH-compliant ecotoxicological data (Springer et al., 2015). It should be noted that both the examined groups (≥ 100 and ≥ 1000 tonnes per year, respectively) will most likely have a better compliance rate as compared to compounds produced or imported at lower volumes. Finally, for chemicals registered under REACH there are also

indications that data from the scientific literature is not always included in the compound dossiers (see triclosan example below).

It was much harder to find environmental thresholds for paper III and paper IV as compared to paper II. Pesticide data (paper II) was readily available through three different reports (Andersson et al., 2009; Andersson and Kreuger, 2011; Norberg, 2004) as well as from the US EPA ECOTOX database. In contrast, paper III and paper IV analyzed compounds from a large number of different chemical classes covered by different, or no, legislations. This means that the derivation of the environmental thresholds was originally performed using different methodologies, by different organizations, sometimes working within different juridical systems. Although it would be unreasonable to expect a comprehensive database where every perceivable compound was given a transparent environmental threshold, it would at least seem reasonable that a central database existed which stored all data from the authorization and registration processes, irrespective of under which regulation the data was collected. For instance, as mentioned in paper I, ecotoxicological data on pharmaceuticals are produced as a part of the authorization process, but that data is not made available to the public. Similarly, data on pesticide and biocide products is available in individual reports, but are not made available in a more manageable format. Initiatives exist which collects environmental thresholds, (for example the UBA ETOX database), but that database is still considerably smaller than the corresponding database of REACH registration data. For instance ETOX contains 55000 ecotoxicological entries, compared to the more than 300 000 ecotoxicological entries available in 2014 in the REACH database (https://github.com/ThomasBackhausLab/Environmental-Thresholds).

4 Complications in the risk assessment process

As the component-based mixture assessment process is dependent on the single substance risk assessment (i.e. the individual environmental thresholds and exposure estimates) any problems in the single compound assessment becomes incorporated into the mixture risk assessment. The following sections will deal with specific issues which complicate the mixture risk assessment process.

4.1 Determining environmental thresholds, triclosan as an example

Triclosan, the dominant risk driver in paper III, can be used as an example on how single substance risk may vary depending on the environmental threshold used. This is then reflected in the risk estimates for the full mixture.

When we originally gathered the data from the REACH dossiers in March 2014, the $PNEC_{marine}$ for triclosan was set to 6.9 ng/L, likely derived from a study on *Desmodesmus subspicatus* where the NOEC for growth inhibition was determined to 690 ng/L (paper I, REACH triclosan dossier, 2014). Triclosan was also evaluated in a European biocide assessment report, where the $PNEC_{freshwater}$ was determined to be 50 ng/L. This translates into 5 ng/L for the $PNEC_{marine}$ (BPC, 2015), when the standard approach from REACH (dividing with an additional factor of 10 to account for the greater biodiversity in the marine environment) is employed. The $PNEC_{freshwater}$ was in turn based on a NOEC for *Desmodesmus subspicatus* of 500 ng/L (BPC, 2015). Another PNEC of 4.7 ng/L for freshwater was suggested by von der Ohe et al. 2012. That PNEC was derived using only an EC50 measure on *Selenastrum capricornutum* and an assessment factor of 1000, and labeled as a $PNEC_{acute}$. However, if one were to take the $PNEC_{acute}$ at face value the $PNEC_{marine}$ would be 0.47 ng/L (ECHA, 2008). All of the $PNEC_{marine}$ mentioned so far are within a factor 15 of each other.

However, during a re-check of the REACH dossiers in December 2016, the dossier lists a $PNEC_{marine}$ of 169 ng/L. This is derived, using an AF of 5, from the $PNEC_{freshwater}$ (843 ng/L), which in turn is determined from a species sensitivity distribution using an AF of 1. (An AF of 5 deviates from the more common AF of 10 for freshwater to marine water extrapolation, but no clear guidance on such extrapolations is provided. Also, although SSDs are allowed for marine organisms, the current guideline for marine SSDs only refers back to the section for freshwater with no added information (ECHA, 2008)). This change since 2014, from 6.9 to 169

ng/L, could be indicative of the risk assessment process working as intended, with increasing amounts of ecotoxicological data leading to a decreasing AF, which in turn leads to a more realistic estimate of the environmental threshold (higher than the initial estimate, if the initial AF was sufficiently protective). However, a closer examination of the reported ecotoxicity data shows that multiple tests, even using the same species, have reported lower NOEC/EC10 values than 843 ng/L.

For algae and cyanobacteria (which are the most sensitive species group when testing triclosan) the triclosan dossier reports a total of 37 NOEC/EC10 values and 31 EC50 values with a Klimisch reliability score of 2 or 1 (Klimisch et al., 1997). Only three of these values come from a single study with a reliability score of 1.

In the top pane of figure 12 only NOEC/EC10s from tests performed on the same species (*Desmodesmus subspicatus*), and the same measurement (growth rate), from studies of similar duration (72-96 hours) have been included (15 values in total). The data clearly demonstrates that all the NOEC/EC10s from assays which measure the effect of triclosan on the growth-rate of *Desmodesmus subspicatus* are within a factor of 4 (0.46-1.84 µg/L), with the exception of the data with a reliability score of 1, where the NOEC is 40 µg/L and the EC10 is 76 µg/L. This either means that the reliability 2 experiments systematically overestimate the real toxicity of triclosan, or that the least sensitive test has been assigned the highest reliability in order to disregard all other tests.

Including other species, but still limiting the dataset to only growth rate of algae and cyanobacteria and test durations of 72-96 hours, shows that 11 NOEC/EC10 values can be found which are below the PNEC (from four different species, figure 12 lower panel). Also, one EC50 is below the PNEC, and 12 EC50 values are within a factor five of the PNEC (data not shown).

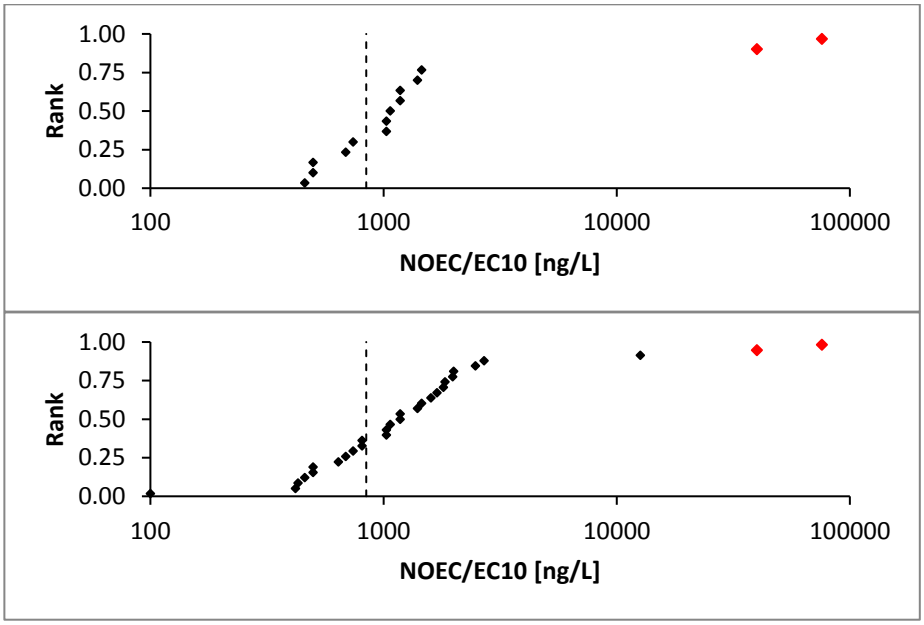

Figure 12: The EC10 or NOEC data from the REACH dossier on Triclosan for algae and cyanobacteria, only growth rate data is included. The top pane shows only data from test on *Desmodesmus subspicatus* with a reliability score of 2 (black) or 1 (red). The bottom pane shows all NOEC/EC10 data for algae and cyanobacteria with a reliability score of 2 (black) or 1 (red). The dotted line shows the $PNEC_{freshwater}$ as determined in the 2016 dossier.

Finally, within the online the version of the REACH dossier the 'rationale for reliability incl. deficiencies' is also reported. By far the most common comment for reliability 2 studies is that a recalculation of the growth-rate endpoint was needed as it was not originally reported (22 of 27), one had an ambiguous statement claiming that it was acceptable for assessment and meeting scientific principles and four had the same comment as the reliability 1 study. These last four studies also contained four out of the six lowest reported NOEC/EC10s for growth rate.

In comparison the US EPA ECOTOX database lists the lowest NOEC and EC50 values at 15 ng/L (*Chroococcus* sp. and *Chlamydomonas* sp. from natural assemblages of algae) and 530 ng/L (*Pseudokirchneriella subcapitata*), for measurements on populations or reproduction (Wilson et al., 2003; Yang et al., 2008). These studies further demonstrate that the determined $PNEC_{freshwater}$ reported in the 2016 REACH dossier is unlikely to be protective.

Together this demonstrates the difficulties in choosing which environmental threshold value to use in the risk assessment process. The PNECs determined by official sources is currently either not available any more (REACH 2014, 6.9 ng/L),

was never reported for the marine environment (Biocide Assessment Report, 5 ng/L) or is likely under-protective (REACH 2016, 169 ng/L). Also one PNECs reported in the primary literature is based on unconventional use of AFs and underlying data (von der Ohne, 2012, 4.7 ng/L). The highest detected concentration from paper III (9.8 ng/L) would yield a RQ between 0.1 and 20.9 depending on the environmental threshold used. On its own this is a problem with a single substance risk assessment. However, as the mixture assessment is simply the sum of all the components single substance assessments, any errors will be included also in a mixture issue. As the number of mixture components grow so does the number of incorporated single substance assessments which might be questioned. Triclosan is a well-studied compound and that different sources come to different conclusions on the environmental threshold does not induce confidence in the assessment of any less studied compound.

4.2 Determining environmental thresholds, WQOs as an example

In paper II the Swedish environmental thresholds for pesticides (WQOs) have been determined either by the Swedish Chemicals Agency, or by the researchers from the Swedish University of Agricultural Sciences, who also manage the monitoring campaign the paper is based on, (Andersson et al., 2009; Andersson and Kreuger, 2011; KEMI, 2008). For 91 of the 130 PPPs included in paper II environmental thresholds can also be derived from the 'conclusion on pesticides' reports published by the European Food Safety Authority (EFSA) (by determining the most sensitive bioassay and its corresponding trigger-value/AF) used in paper I. Figure 13 compares the environmental thresholds of PPPs from paper I and paper II. For 74% of the compounds the retrieved environmental thresholds are within a factor 10 for the same compound, and in 74% of all cases is the WQO lower than the corresponding environmental threshold from the EFSA-reports.

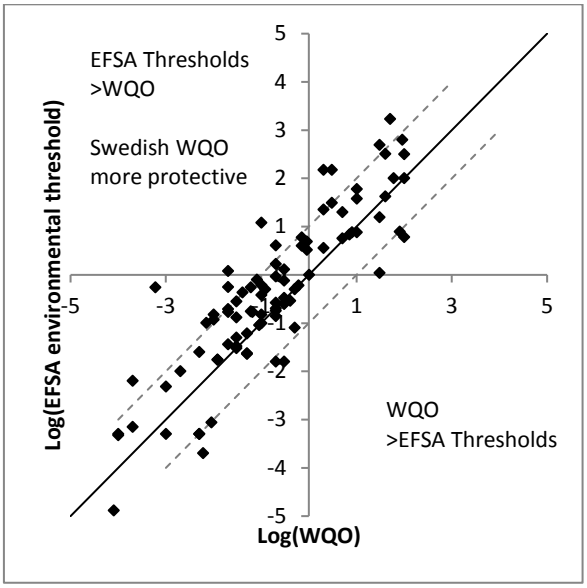

Figure 13: A comparative plot of Swedish WQOs and environmental thresholds collected from reports on 'conclusions on pesticides' as reported by EFSA. The full line depicts a 1:1 ratio (i.e. the WQO is equal to the EFSA threshold) while the dashed lines show the limits of a factor 10 difference.

As briefly discussed in paper II the datasets have been derived using two different methods (EFSA, 2013; Norberg, 2004) which may explain some of the differences as different assessment factors may be used based on similar datasets. However, at least the largest difference between a WQO and EFSA threshold (for the now banned insecticide dichlorvos the WQO is a factor 917 lower than the corresponding EFSA threshold) can be attributed to differences between datasets used to derive the threshold. The EFSA conclusion report (EFSA, 2006) lacks data for both algae and aquatic invertebrates and none of the data included in the KEMI 2008 document is present. The most sensitive species in the KEMI report is *Daphnia pulex* while no invertebrate data is present in the EFSA report (EFSA, 2006). The threshold is instead based on a test on *Oncorhynchus mykiss*. The lack of aquatic invertebrate data in the conclusion report is justified by the compound only being used to treat flower-bulbs and hence no environmental exposure is expected (EFSA, 2006). In the Swedish monitoring the compound is only found once, in 1308 samples, at a concentration of 26 ng/L (the WQO is 0.6 ng/L and the LOD is 7.5 ng/L on average).

The median difference between the WQO and the environmental thresholds from the EFSA reports is within a factor 4.6 and 76% of all differences are within a factor 10. As the thresholds were derived using different methodologies it is not surprising that differences exist, but it once again opens the question which environmental

threshold that should actually be used. As no site-specific information has been included in the Swedish WQOs there is no obvious reason for preferring the WQO rather than the EFSA thresholds in a Swedish environment.

4.3 Can we discontinue monitoring after market approval is removed?

Within the pesticide monitoring data analyzed in paper II 456 samples were taken between 2010-01-01 and 2013-12-31. The number of detects of compounds which are not approved under Reg. (EC) No 1107/2009 'concerning the placing of plant protection products on the market', are listed in table 9. Table 9 also list the final date for a PPP containing the active substance to be placed on the Swedish market, as obtained from the Swedish pesticides register (KEMI, pesticide registry).

Table 9: Compounds not approved under EC 1107/2009, but detected in the Swedish monitoring of pesticides between 2010 and 2013 (see paper II).

Compound	Detections	Percent of total	Swedish Approval Ended
Isoproturon	453	99.3%	2007
Atrazine	260	57.0%	1989
Methabenzthiazuron	116	25.4%	2005
Lindane	88	19.3%	1989
Dichlorprop	32	7.0%	2011
Bitertanol	24	5.3%	2014
Terbutryn	15	3.3%	2003
Metolachlor	14	3.1%	Not Found In Registry
Simazine	9	2.0%	1994
Cyanazine	5	1.1%	2007
Carbofuran	2	0.4%	Not Found In Registry
Hexazinone	1	0.2%	1994
Dichlorvos	1	0.2%	1990

The monitoring performed along the Swedish West Coast detected pesticides for which no current market approval within Sweden exist (KEMI, 2016). Out of five sampled locations atrazine was detected in two (Last approval ended 1989), irgarol in four (last approval ended 2010), gamma-HCH in two (last approval ended 1989) and pentachlorophenol in two (last approval ended 1978). Both gamma-HCH and pentachlorophenol are also listed under the Stockholm convention as products that

should be eliminated from use and production, as well as being priority substances under the WFD (Directive 2008/105/EC Appendix I; Stockholm Convention, Annex A-C).

It should be noted that the longest half-life of gamma-HCH reported in the WFD background document is 47 days (WFD SDS No. 18, 2005). Similarly the WFD background document for pentachlorophenol lists a half-life of between 6 and 34 days for anaerobic or aerobic degradation (WFD SDS No. 27, 2005). Using the respective longest reported half-lives of the two compounds (47 and 34 days), the lowest detected compound concentrations (0.06 and 0.07 ng/L) and their respective thresholds (2 and 350 ng/L) and solving for a first-order reaction indicates that the environmental concentrations of the compounds would have been at their respective thresholds 275 and 108 days previous to the sampling. As this is, most likely, not the case it demonstrates that half-lives reported in the background documents either have a very weak connection to the half-life's in the environment, or that the compounds are slowly released into the environment from a standing stock of products, buildings, structures etc. This removes the option of discontinuing monitoring efforts simply after market-removal, and demonstrates the need for caution during the authorization of products, as it may take a considerable time for hazardous chemicals to be removed from the environment. It should also be remembered that a ban is in practice the final risk mitigation method before active clean-up.

4.4 Non-detects and un-analyzed compounds

As discussed in paper II and paper III risk assessment of chemical mixtures based on chemical monitoring needs to consider four groups of chemicals: i) compounds which were found at quantifiable concentrations; ii) compounds included in the monitoring but which were below the LOD, for which we have a tool for estimating the risk (for instance the KM-method); iii) compounds included in the monitoring but not found, for which we do not have a tool for estimating the risk contribution; iv) compounds not included in the monitoring, for which no risk estimates can be made.

As shown in paper II and III, chemicals from group ii increased the risk with 11 and 5% respectively. Group iii and iv cannot, in an informed manner, be included in a risk assessment based only on chemical monitoring data. Thus, any such risk assessment only provides the lower boundary of the underlying risk. This has been shown experimentally when risk assessment based on chemical analytics explained only 0.1% of the observed effects (oxidative stress) against specific cells-lines (Escher et al., 2013) or only parts of the toxicity of contaminated sediments towards *Vibrio fischeri* (luminescence, Lahr et al., 2003). However, contrasting results have been

demonstrated for algae and reconstituted mixtures where the detected herbicides dominated the observed effects (photosystem inhibition and growth rate, Tang and Escher, 2014). It seems reasonable that the tighter the connection between the endpoint (e.g. photosynthetic efficiency) and the mode of action of a compound (e.g. herbicide specifically tailored to inhibit photosynthesis), the easier it will be to identify the responsible chemical compound or group.

Risk assessment of chemical extracts (effect directed analysis) has been proposed as an alternative to direct chemical monitoring (Altenburger et al., 2015; Brack et al., 2016). Extracts may for instance be collected from passive samplers placed at the site of interest (Tlili et al., 2017), or from enriched water samples. Both enrichment-methods are conceptually similar, but differ mainly on a temporal scale, as passive sampling gives an integrative extract based on the pollution over time, while enrichment of water-samples may be performed on a volume of water which might be collected in an number of ways (for instance from grab, time-integrated or flow-integrated samples).These extracts can in turn be tested against a range of different bio-assays. Such methods may also provide a causal link to observed or suspected effects, as compared to risk assessment of chemical monitoring data which only suggest that risk exist.

5 Outlook

One method put forth for determining the chemical carrying capacity of Europe is to determine the amount of water needed to dilute compounds to a safe concentration. It has been shown that that the dilution capacity of Europe, with the exclusion of Ukraine, Sweden and Finland, has been superseded (most commonly by a factor 1-5, but up to above a factor 100 for Slovenia, Belgium and Luxenburg due to low dilution capacity (Bjørn et al., 2014)using a subset of only 173 chemicals (Laurent et al., 2011). The paper by Bjørn et al. further assumes that the dilution capacity of the coastal zone is unlimited and consequently assumes a low impact from many metropolitan areas which emit directly into the ocean. This work is contrasted by the work by (Zijp et al., 2014) which concludes that only 1.1% of the water in the EU would be needed to dilute 630 organic pollutants to a safe level. A similar exercise was performed for the Rhine, Meuse-Scheldt basin where the risk from 343 high production volume chemicals (HPVCs) was evaluated using a Bayesian approach. The conclusion drawn was that the potentially affected fraction of species within the basin was 2.5%, with a confidence interval between 0.1% and 100% (Harbers et al., 2006). It has also been suggested that half of the waterbodies in Europe are threatened by chemical pollution (Malaj et al., 2014) and that insecticides are threatening surface water at a global scale (Stehle and Schulz, 2015). Similarly paper II concluded that using Swedish WQOs more than 70% of all samples taken exceeded their risk value, while only 4.3% of samples exceeded the critical value when the risk was determine per species-group and critical values were set in accordance to the current edge of field guidelines (EFSA, 2013). In contrast 75% of all samples still exceeded the critical value if the same method for deriving the critical values were used per species group, as was previously used for the WQO (basically by using a higher AF). Together this paints a picture where the estimated risk is highly dependent on the assumptions and operationalized protection goals used in the risk assessment process.

Paper III also indicates risk, but in the marine environment along the Swedish west coast, while paper IV demonstrate that the concentrations of organic pollutants in fish fillet exceed the thresholds set within the WFD for priority pollutants. Together the collected information indicates that the chemical use within Europe is putting the aquatic ecosystems at risk and most likely, the more studies that are presented the clearer the picture will become. It should be stressed that we do not want to be 100% certain that the chemical pollution is having an effect before we take action. As a first step it would be prudent to acknowledge the chemical load already present in the environment within the authorization and registration processes of individual chemicals and products.

Hopefully the development and implementation of effect directed analysis (Altenburger et al., 2015; Brack et al., 2016) in European monitoring programs will provide more information on risk and effects from coincidental mixtures in European waters (Wernersson et al., 2015). The methods will however share certain limitations with chemical monitoring based risk assessment. These problems include the risk driver identification being dependent on the choice of bio-assay, sampling time and location, as well as problems with extrapolation to relevant ecological protection goals. Also, it must be remembered that if a compound is identified as a contributing to an unacceptably high level of risk when found in the environment the legislation has already failed. Society is then reliant on risk mitigation measures being available and successful. Effect directed analysis can only show that a problem exist, and will not actually solve it.

In all papers within this thesis the data accessibility, and availability, has not been fully satisfactory. Sometimes this has been due to data only being present within individual written reports (papers I-IV), making manual data collection necessary. Sometimes this has been due to language issues (paper II-III). Sometimes this has been due to a far from satisfactory structure of the databases which collects the data (paper I). It is remarkable that the S.I. for paper I is one of the largest publically available and easily accessible sources for environmental thresholds of industrial chemicals. The creation and maintenance of a database which contains all data, irrespective of which regulatory framework it was originally reported under, should lie in the EUs own interest as better information yields better decisions. More accessible data would also simplify quality checks of the data reported under different regulatory frameworks. As an indication of the need for such quality checks the German environmental protection agency demonstrated that only 26% of 1814 HPVCs provided REACH-compliant ecotoxicological data (Springer et al., 2015).

Finally, chemical pollution is by no means the only pressure put on the ecosystems. Halpern et al. 2008 described the impact from human activities and concluded that approximately 41% of earth's oceans had a medium-high to high impact score from human activities. These impacts must finally be weighed against the benefits that society gains from the activities that cause them. It has been shown that wealthy nations on average have a higher impact score than poor ones, and that only 32% of the countries score a lower impact than 50 on a scale from 1-100 (Halpern et al., 2012). Most of the currently performed assessments of multiple stressors are made based on assumptions and extrapolations and little data on the impact from combined stressors are present (Andersen et al., 2013; Robinson et al., 2014)s. Given that the MSFD tries to implement a multiple stressor assessment and management approach large, efforts in research are likely needed in the near future to answer questions of how to perform such assessments.

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