Pesticide mixtures in the Swedish streams: environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation

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Abstract

This paper presents the ecotoxicological assessment and environmental risk evaluation of complex pesticide mixtures occurring in freshwater ecosystems in southern Sweden. The evaluation is based on exposure data collected between 2002 and 2013 by the Swedish pesticide monitoring program and includes 1308 individual samples, detecting mixtures of up to 53 pesticides (modal=8). Pesticide mixture risks were evaluated using three different scenarios for non-detects (best-case, worst-case and using the Kaplan-Meier method). The risk of each scenario was analyzed using Swedish Water Quality Objectives (WQO) and throphic-level specific environmental thresholds.

Using the Kaplan-Meier method the environmental risk of 73% of the samples exceeded acceptable levels, based on an assessment using Concentration-Addition and WQOs for the individual pesticides. Algae were the most sensitive organism group. However, analytical detection limits, especially for insecticides, were insufficient to analyze concentrations at or near their WQO's. Thus, the risk of the analyzed pesticide mixtures to crustaceans and fish is systematically underestimated. Treating non-detects as being present at their individual limit of detection increased the estimated risk by a factor 100 or more, compared to the best-case or the Kaplan-Meier scenario.

Pesticide mixture risks are often driven by only 1-3 compounds. However, the risk-drivers (i.e., individual pesticides explaining the largest share of potential effects) differ substantially between sites and samples, and 83 of the 141 monitored pesticides need to be included in the assessment to account for 95% of the risk at all sites and years.

Single-substance oriented risk mitigation measures that would ensure that each individual pesticide is present at a maximum of 95% of its individual WQO, would also reduce the mixture risk, but only from a median risk quotient of 2.1 to a median risk quotient of 1.8. Also, acceptable total risk levels would still be exceeded in more than 70% of the samples.

Introduction

Multiple studies have demonstrated that complex pesticide mixtures are present in surface waters globally, in the US (e.g. Gilliom, 2001. Stone et al., 2014a. Stone et al., 2014b), Europe (e.g. Moschet et al., 2014; Schreiner et al., 2016; Ccanccapa et al., 2016) and elsewhere (e.g. in South America (Hunt et al., 2016), Australia (Allinson et al., 2015) and China (Zhang et al., 2011)). Empirical evidence univocally shows that the combined toxic effects of such pesticide mixtures exceed the effect of each individual compound (e.g. Faust et al. 2001; Faust et al. 2003; Knauert et al., 2009 and Porsbring et al. 2010, see also reviews by Belden et al., 2007; Verbruggen & van den Brink, 2010 and Rodney et al., 2014).

Studies have repeatedly demonstrated that Concentration Addition (CA) describes the joint toxicity of pesticide mixtures well (reviewed by Belden et al., 2007; Rodney et al., 2014). This implies that all components contribute to the overall mixture toxicity, independently of whether they are present at concentrations above or below their individual No Observed Effect Concentration (NOEC) or Environmental Quality Standard (EQS). Mixtures might therefore cause toxic effects even if all components are present at concentrations below which an individual effect is detectable (e.g. Carvalho et al., 2014, Faust et al., 2001).Taken together, the available body of evidence thus clearly shows that pesticide mixtures warrant specific consideration during environmental risk assessment, monitoring and management.

Environmental risks of pesticides and pesticide mixtures are assessed in the European regulatory system from two perspectives. First, active ingredients and whole formulated pesticide products are evaluated for their environmental hazard, exposure and risk during market authorization (EFSA, 2013), following the legal frameworks that are laid down in Regulations EC 1107/2009 on the placing of plant protection products on the market (European Parliament, 2009) and EC 546/2011 on uniform principles (European Commission, 2011a). However, 'coincidental' pesticide mixtures, i.e. mixtures of active ingredients that result from farmers applying different pesticide products in close proximity to a given water body or because of sequential spraying of different pesticides on the same field, are not considered in Regulation EC 1107/2009 nor in Directive 2009/128/EC (European Parliament, 2009b). However, it has been argued that the uniform principles in Regulation 546/2011/EC require authorization of plant protection products to be based on the "proposed conditions for use" and consequently – given common agricultural practice – to consider the environmental impact of the resulting pesticide mixtures (Frische et al. 2014).

Second, the impact of mixtures of pesticides (and other hazardous chemicals) on the ecological status of an aquatic system is assessed from the perspective of the Water Framework Directive (WFD) (European Parliament, 2000). In order to be classified as having a good ecological status, a water body also needs to have good chemical status, which requires that the concentrations of each of 45 priority pollutants, which are currently listed in Directive 2013/39/EC (European Parliament, 2013), do not exceed European-wide thresholds, so-called Environmental Quality

Standards (EQS). In addition, in order to track progress towards the national goal of a "non-toxic environment" (adopted in 1999), Sweden also developed national Water Quality Objective(s) (WQO) for pesticides, defined as concentrations which are not expected to cause any adverse effects in the aquatic environment (Norberg, 2004. Lindström & Kreuger, 2015). These values are similar to EQS values and serve as a tool to evaluate surface water quality based on monitoring results, but are not legally binding. WQO's are derived using a method that closely follows the REACH approach for deriving Predicted No Effect Concentrations (PNEC) values, based on single species data and assessment factors between 10 and 1000, depending on the underlying ecotoxicological endpoints (Andersson et al. 2009, Andersson & Kreuger 2011, KEMI 2008).

Concentration Addition based mixture risk assessments

Risk assessment of chemical mixtures is routinely performed using CA (Kortenkamp et al., 2009; Bopp et al., 2015). CA has also been suggested specifically for the assessment of pesticide mixtures (EFSA, 2013) and it is the recommended approach for setting EQS values for chemical mixtures within the context of the WFD (European Commission, 2011b).

According to CA the risk quotient (RQ) of a mixture, RQ_{CA}, is defined as:

$$RQ_{CA} = \frac{c_{mix}}{ECx_{mix}} = \sum_{i=1}^{n} \frac{c_i}{ECx_i} = \sum_{i=1}^{n} TU_i \qquad \text{(eqn. 1)}$$

were c_{mix} is the total concentration of the mixture, ECx_{Mix} is the mixture concentration causing x% effect, 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). Although the concept is rooted in the idea of the mixture components sharing the same mode of action, as well as not taking possible synergistic (or antagonistic) effects into account (Cedergreen, 2014), CA has been successfully used for the risk assessment of heterogeneous mixtures (Belden et al., 2007; Kortenkamp et al., 2009; Verbruggen & van den Brink, 2010; Rodney et al, 2014; Bopp et al., 2015). The toxicity estimates in eq 1 (ECx_{mix} and ECx_i) in principle refer to the same ecotoxicological endpoint recorded for the same species under identical exposure conditions. However, in practice CA is often applied in a broader setting, e.g. by using data from different algal species in order to predict the toxicity to algae in general.

In the present paper, we have applied CA in order to separately calculate the risks for algae, crustaceans and fish. The corresponding CA-based mixture RQs are termed RQ_{Algae}, RQ_{Crust} and RQ_{Fish}. Moreover, by substituting the ECx_i with the WQO_i and c_i with the Measured Environmental Concentration (MEC_i) we determined ecosystem-wide RQ_{WQO} values as the sum of the individual MEC/WQO ratios, following the rational outlined by (Backhaus & Faust, 2012):

$$RQ_{WQO} = \sum_{i=1}^{n} \frac{MEC_i}{WQO_i} = \sum_{i=1}^{n} \frac{MEC_i}{\min(Toxicity\ data_i) * AF_i} \qquad \text{(eqn. 2)}$$

Comparing the trophic-level specific RQs with RQ_{WQO} is difficult, as the latter is calculated using assessment factors to account for the different amounts of data available for each compound, while RQ_{Algae}, RQ_{Crust} and RQ_{Fish} are calculated without using any assessment factors. In order to bridge these two approaches, we therefore also calculated a mixture RQ for the most sensitive trophic level (RQ_{MST}), defined as:

$$RQ_{MST} = \sum_{i=1}^{n} \frac{MEC_i}{\min(EC50_{Algae}, EC50_{Crustaceans}, EC50_{Fish})} \qquad \text{(eqn. 3)}$$

RQ_{MST} provides a measure for the risk across trophic levels, but is calculated without using any assessment factors. It thus takes an interim position and bridges the trophic-level specific RQs (RQ_{Algae}, RQ_{Crust} or RQ_{Fish}) to the ecosystem-wide RQ_{WQO}. The RQ_{MST} is conceptually identical to the point of departure index (PODI), frequently used in human toxicology (Wilkinson et al. 2000).

A RQ provides a yardstick for assessing the need to act. Values of RQ_{WQO} exceeding 1 indicate the need for either a more advanced mixture risk assessment, and/or for the implementation of risk mitigation measures. We defined the corresponding critical values for RQ_{Algae}, RQ_{Crust}, RQ_{Fish} as 0.1, 0.01 and 0.01, respectively, following the assessment strategy for individual pesticides (EFSA, 2013). Defining a critical value for RQ_{MST} is not feasible at the moment, as no strategy has been suggested yet on how an overall assessment factor should be calculated that reflects the overall uncertainty in eq 2. The RQ_{MST} will always be higher than any of the organism-group specific RQs (Backhaus & Faust, 2012) and, because no assessment factors are applied, lower than the RQ_{WQO}.

The Maximum Cumulative Ratio and its role in mixture risk assessments

The ratio between the total RQ of a mixture and the maximum RQ of its components has been termed the maximum cumulative ratio (MCR, Price & Han 2011). That is,

$$MCR = \frac{RQ_{CA}}{\max_{i=1..n}(RQ_i)} \qquad \text{(eqn. 4)}$$

If all components of a mixture are contributing equally to the predicted mixture risk, the MCR equals the number of compounds in the mixture. In a mixture whose TU distribution is dominated by one compound, the MCR approaches 1. Therefore, the MCR has been suggested as a tool to assess the value of performing mixture toxicity assessments (Price & Han 2011).

The problem of Non-Detects

Chemical risk assessment is in general based on comparing relevant exposure estimates (measured or modeled) with hazard estimates, such as NOEC's, EC50's and EQS values. Such estimates are straightforward to calculate on the basis of monitoring results, as long as detected environmental concentrations are quantified, either above the chemical-analytical

limit of quantification (LOQ) or the limit of detection (LOD). However, sometimes when the detection is below the LOQ but still above the LOD the concentration is not quantified (only given as 'trace') in order to save time in the laboratory. Nevertheless, reasonable assumptions on the trace concentrations present can be made using (LOQ+LOD)/2 as a surrogate for unquantified detections between the LOQ and the LOD, as long as these two parameters are stated in the analytical protocol.

However, the situation becomes problematic if a monitored chemical is not detected. Such a result does not prove that the compound is not present, it only shows that the concentration is somewhere between zero and the LOD. Assuming a zero concentration for all non-detects will therefore underestimate the total risk, if no additional knowledge about e.g. emission or use pattern is available.

On the other hand, assuming that all non-detected compounds are present just below their LOD – the worst-case scenario that is still compatible with the recorded values – is also unrealistic. Such an approach immediately leads to the logical inconsistency that the estimated risk becomes simply dependent on the number of compounds analyzed. The same is true for setting the concentration used for the risk assessment *a priori* to any other value above zero.

Parametric and non-parametric statistical methods are available for data with "less-than" values, i.e. findings of concentrations < LOD. They allow the estimation of the likely contribution of non-detects to the total RQ. In this paper we used the non-parametric Kaplan-Meier (KM) method (Helsel, 2010, 2012; Bolks et al., 2014), because it is not possible to ensure that the distributional assumptions of parametric alternatives are fulfilled in the analyzed data. A KM-adjusted sum of RQs lies between the sum of RQs that result from substituting all non-detects with their respective LOD and the sum of RQ that results from substituting all non-detects with zero.

The KM-method ignores the potential risk contribution of a compound, if its potential RQ exceeds the maximum of the RQs that are based on a quantified concentration. For such compounds, better analytical data are required for a reliable quantification of their risk contribution.

Aims of the study

The southern part of Sweden is an area of intense agricultural activity and pesticide residues have been systematically monitored at six sites since 2002 (Lindström, 2015; Lindström & Kreuger, 2015). In this paper, we applied CA-based risk assessment approaches in order to estimate and characterize the environmental risks from the detected pesticide mixtures, using RQ_{Algae}, RQ_{Crust}, RQ_{Fish}, RQ_{MST} and RQ_{WQO}. The results will then be used for a broader discussion on the impact of non-detects on component-based mixture risk assessments. Finally, we explore the consequences of a single-substance oriented risk management, i.e. assuming that risk mitigation measures ensure that all individual concentrations are below their corresponding WQO's.

In order to explore how the different possibilities to incorporate (or ignore) concentrations below the LOD influence the final mixture risk estimates, we calculated all RQs for three different exposure scenarios (table 1). Scenario 1 and 2 assumes that non-detects are present at a concentration equal to their LOD or at zero, respectively. Scenario 3 uses the KM-adjustment for compounds present <LOD.

Material and Methods

Pesticide monitoring data

As part of a continuous Swedish pesticide monitoring program the Swedish University of Agricultural Sciences publishes data on pesticide concentrations in four streams draining 8-16 km^2 and two rivers draining 102-488 km^2 at http://jordbruksvatten.slu.se/pesticider_start.cfm (Agricultural land, 2017; Lindström & Kreuger, 2015). The chemical monitoring data was quality-checked and is made publically available as a downloadable datafile for a broader audience via GitHub (https://github.com/ThomasBackhausLab/Swedish_Pesticide_Data.git), see also S.I. table 1. The data comprises more than 128 000 analytical measurements from 308 weekly samples for between 76 and 131 pesticides and pesticide degradation products (Lindström & Kreuger, 2015). The inclusion of pesticides in the monitoring program was based on use information (past and present) from the four catchments, but the program also includes pesticides identified as a priority substance within the European water policy. Individual pesticides requiring specific analytical methods were excluded, with the exception of glyphosate and AMPA. Pesticide concentrations were either in quantifiable concentrations (conc ≥ LOQ), in trace concentrations (LOD ≥ conc < LOQ) or in non-detectable concentrations (conc < LOD). Trace concentrations detected during 2002-2008 were not quantified and for the risk assessment it was assumed that the compounds were present at a concentration of (LOQ+LOD)/2. From 2009 and onwards trace concentrations were quantified (although with a somewhat lower precision compared to concentrations ≥ LOQ) and used directly for the risk assessment.

Compilation of toxicity data and water quality standards for the individual pesticides

Ecotoxicity data were collected from the US EPA ECOTOX database (US EPA, 2016), the eChemPortal (OECD, 2016), the ePesticide Manual (ePesticde Manual, v5.2), background documents on Swedish WQO as published by the Swedish Chemicals Agency (KEMI, 2008) and the Swedish University of Agricultural Sciences (Andersson et al. 2009, Andersson & Kreuger 2011).

The following data were retrieved independently from the ECOTOX database and eChemPortal: (i) EC50 values for fish mortality after an exposure between one and four days. (ii) EC50's for crustacean mortality (immobilization) after an exposure between one and four days. (iii) EC50 values for algae (population growth rate, biomass or reproduction) exposed between one and four days. No correction to account for the different exposure durations was performed prior to using the data in the following steps. If several EC50 values were available for the same species, its arithmetic mean was calculated within each data source. Finally, the geometric mean within each taxonomic group was calculated per data source and used for the subsequent risk estimations (EFSA, 2013).

All exposure durations and endpoint measurements were included for data retrieved from the ePesticide Manual (ePesticde Manual, v5.2) and the WQO linked sources. Limit data (i.e. EC50 and NOEC values given as "greater than" values) were included if no other data were available. This produced a final dataset based primarily on experimental data, rather than having to resort to modelling approaches.

Additionally, data for flamprop were retrieved from a New Zeeland report (ERMA, 2009), and ECOSAR version 1.11 (US EPA, 2016) was used to estimate the fish toxicity of two atrazine breakdown products (atrazine-desisoproyl, atrazine-desethyl) and the fish and algal toxicity of quinoxyfen.

The full dataset of pesticide ecotoxicity data is also available via GitHub (https://github.com/ThomasBackhausLab/Swedish_Pesticide_Data.git), see also S.I. Table 2.

Data Analysis

All data were analyzed using the statistical software R, version 3.2.5 (R Core Team, 2016), in order to calculate the RQs according to eqns. 1- 3, for three exposure scenarios each (table 1), as well as the corresponding MCR values (eqn. 4). The Kaplan-Meier adjustment was implemented using the NADA package for R, version 1.5 (Lee, 2015).

Results and Discussion

The ecotoxicological risk of the pesticide mixtures found in Swedish freshwater ecosystems was previously described by Bundschuh and coworkers (2014) for the timeframe from 2002 to 2011. In this paper, we analyze three additional issues: Firstly, we explore the relevance of non-detects for the overall mixture risk. Secondly, we compare the specific risks for the three main organism groups, i.e. algae, crustaceans and fish with ecosystem-wide risks. Finally, we analyze the impact of successful single-substance oriented risk mitigation measures on the overall risk of the monitored pesticide mixtures, as well as their implications for risk management strategies. Furthermore, in order for the analysis to cover the maximum available time-span we also included monitoring data recorded between 2011 and 2013.

Exposure profiles from the six monitored sites

Between 107 and 308 samples were analyzed from each site. This was done in weekly intervals during the growing season for the four agricultural streams, twice a month during May–June and monthly during July–November for the two rivers (summary in table 2). The samples taken in the four streams were time-proportional and are composites from samples taken every 90 minutes by automated samplers, while the river-samples are single grab samples. A total of 141 pesticides and pesticide degradation products (62 herbicides, 29 insecticides, 37 fungicides, 10 degradation products, 2 growth regulators and 1 impurity) were monitored at least once and 115 of these compounds were detected one or more times. At the start of the campaign in 2002 76 pesticides and degradation products were analyzed, increasing to a total number of 131 in 2013.

The presence of the monitored pesticides as mixtures is obvious: Up to 53 compounds were found per sample, with a modal (most common value) of 8 compounds per sample. A maximum of 42 pesticides per sample was found in quantifiable concentrations, i.e. at levels above the LOQ, with a modal value of 4 quantifiable compounds per sample (table 2). Given that mixture risks exceed single substance risks, these findings clearly demonstrate the need to consider the joint presence of pesticides for a realistic environmental risk assessment.

The analytical level of detection is insufficient for several insecticides

The ratio between the WQO and the LOD can be used to assess whether a chemical-analytical method is sufficiently sensitive and at the very least the LOD should be equal to the corresponding WQO. However, as also lower concentrations contribute to the overall toxicity of a mixture, LODs clearly below the corresponding WQO would be highly advantageous to adequately account for the contribution of all compounds to the mixture's toxicity.

Figure 1 depicts the pesticides with median LOD/WQO ratios higher than 0.1. 10 compounds, mainly pyrethroid and organophosphate insecticides, have a LOD/WQO ratio above 1, even exceeding 100 for Permethrin. Those compounds can currently not be analyzed for their presence at or near the respective WQO and, consequently, no reliable conclusions on whether these pesticides are risk drivers in Swedish surface waters can be drawn. Fungicides and herbicides have, in contrast, sufficiently low LODs.

Comparison of the different exposure scenarios

Table 1 lists the three different exposure scenarios implemented in this paper, with scenario 1 (assuming all non-detects being present at their corresponding LOD) being the most conservative and scenario 2 (assuming all non-detects being not present) being the least conservative. Risk estimates from scenario 3, using the KM adjustment of values below the LOD (non-detects), fall between the estimates from scenario 1 and 2.

Consequently, scenario 1 produces the highest risk estimates for the evaluated scenarios, and scenario 2 generates the lowest risk estimates (table 3). The differences are most prominent in situations that are dominated by compounds with insufficient LODs, i.e. when assessing the risks for crustaceans and fish, both of which are sensitive to insecticides which typically have a high LOD to WQO ratio (figure 1).

Interestingly, scenario 2 produces risk estimates that are very close to scenario 3, with the ratio between the median predicted RQ_{WQO} values being a mere 1.05 (table 3). This indicates that the non-detects whose potential risk contribution can be accounted for by the KM-adjustment add only marginally to the overall risk. However, given that the KM-adjusted RQ is more inclusive, it is considered the more realistic approach (Helsel, 2010. Helsel, 2012).

Trophic-level specific risks

Figure 2 gives an overview of the average risk estimates per site between 2002 and 2013 for each of the three exposure scenarios as described in table 1, separately for each trophic level

(algae, crustaceans, fish), and finally aggregated for the most-sensitive trophic level (MST) and for WQO values (see introduction for details). The corresponding numerical risk estimates are given in table 3.

For the scenario 2 and 3 the RQ for algae is the largest among all trophic level specific RQs (table 3). For scenario 3 it exceeds the RQ for crustaceans and fish on average by a factor of 17 and 54, respectively. This indicates that herbicides are the ecotoxicologically dominating group of the pesticide mixtures. This is in line with the findings by Schreiner et al. (2016), who identified herbicides as the most frequently detected compounds in monitoring data from Germany, France, the Netherlands and the USA. This pattern might also at least partly reflect that the mixture RQs for crustaceans and fish are calculated based on acute data (mainly mortality) while the RQ for algae is based on the results of algal growth and reproduction assays, i.e. chronic endpoints (see material and methods).

Correspondingly, the critical value for the RQ_{Algae} is 0.1 while the critical value for RQ_{Crust} and RQ_{Fish} is 0.01 (EFSA, 2013). These values are marked in figure 2 as horizontal red lines. For algae, it can be clearly seen that the median toxic RQs are below the corresponding critical values for all sites and exposure scenarios (table 3). Meanwhile, the median RQs for crustaceans and fish are close to, or even above, the critical value – but only in scenario 1. This reflects the largely insufficient LODs for insecticides (figure 1), which drive the overall toxicity towards crustaceans and fish in scenario 1. In contrast to fish and crustaceans, the risk estimates for algae, which are not affected by insecticides, are clearly less affected by the different approaches for handling non-detects (figure 2).

However, despite the median RQ being below 0.1 and 0.01, respectively, a certain percentage of samples from each site, trophic level and exposure scenario shows values above the critical threshold (table 4). Sites M42 and Skivarpsån have the highest percentage of unacceptable risks to algae with 0.6% and 0.9% of the samples (scenario 3), while crustaceans are most often put at risk at site N34 (in 9.5% of the samples, scenario 3).

Pesticide mixtures regularly put aquatic ecosystems at risk

Although the trophic-level specific RQs highlight the most sensitive trophic levels, they do not adequately describe the overall risks of a mixture for the exposed ecosystem. An alternative approach is to consider the most sensitive trophic level for each compound and sum the resulting RQs (Faust & Backhaus, 2012). The resulting RQ_{MST} indeed exceeds the trophic-level specific RQs by factors of 1.0-1.3 (RQ_{Algae}), 10.0-24.7 (RQ_{Crust},) and 26.1-143.4 (RQ_{Fish}) (table 3, scenario 3).

However, RQ_{MST} does not consider any assessment factors and only makes use of acute EC50 data for crustaceans and fish (see material and methods). It is therefore of only limited use to assess the final risks at the exposed sites. This is overcome by using the RQ_{WQO} which not only includes chronic toxicity data from invertebrates and fish as well as macrophyte responses, but also applies compound-specific assessment factors to account for the different types and

amounts of ecotoxicological data available for each compound (Andersson et al., 2009. Andersson & Kreuger, 2011. KEMI 2008).

The resulting WQO-based RQs indicate a substantial risk for the majority of samples, except at site O18 (figure 2 and table 3). Even scenario 2, which ignores the possibility that non-detects might not be actual zero concentrations, still results in median RQs of 0.7 at site O18 (table 3). Over all sites, the KM adjusted RQs (scenario 3) yield median RQs between 0.7 and 4.1, with a total of 73% of the analyzed samples indicating a risk of adverse effects (table 4).

These results confirm and provide further support to previous studies concluding that pesticide mixtures put exposed ecosystems at risk. For example, a series of mixture-oriented assessments in the Llobregat river (Köck-Schulmeyer et al., 2012. López-Doval et al., 2012) consistently found that the total loads with pesticides and other organic chemicals are unacceptably high. Similar findings are provided by Vaj et al. (2011) for the river Meolo.

Site specific ecosystem-wide risks do not change over time

Figure 3 visualizes the KM-adjusted RQ_{WQO} for the six sites between 2002 and 2013. Not only does the figure highlight the frequent risk of adverse effects, it also becomes obvious that no clear trends are visible: the risk at the six monitored sites is comparatively constant over time, despite EU Regulation No 1107/2009 replacing Council Directives 79/117/EEC and 91/414/EEC in 2009 (European Council, 1978. European Council, 1991. European Parliament, 2009a). This indicates a remarkably small impact of regulatory developments on the actual environmental risks. Most likely this is a consequence of pesticides leaving the market being replaced by an increased use (and hence risk contribution) from pesticides that remain on the market, and/or by risk contributions from new compounds. As a consequence, the overall environmental risk basically remains constant.

The contribution of individual compounds

The fact that up to 53 compounds were found in a single sample (table 2) does not imply that each compound contributes to a similar extent to the overall mixture risk. Table 5 provides the MCR, a measure for the evenness of the toxic unit distribution, for all sites, trophic levels and exposure scenarios. The median values for the three different trophic levels range from 1 to approximately 3, with the majority of values around 2. For all scenarios based on the WQO, the median MCR values never exceed 3. This indicates that, per sample, typically only very few compounds contribute substantially to the overall mixture risk.

These findings are consistent with previous studies. For instance, Verro et al. (2009) found that in the river Meolo in Italy one or a few compounds were usually responsible for more than 80% of the CA estimated mixture toxicity. More recently, Valloton and her co-workers analyzed the joint toxicity of pesticide mixtures detected in the water quality monitoring of the National Water-Quality Assessment (NAWQA) program of the U.S. Geological Survey (Vallotton et al., 2016). They concluded that the environmental risk for more than 90% of the samples analyzed is driven by just one compound.

Initially such results seem to indicate that mixture toxicity analyses are of only moderate importance for realistic environmental assessments. Furthermore, they also seem to open up for a substantial simplification of the exposure profiles, i.e. a focus of monitoring efforts and risk mitigation measures on those identified "mixture risk drivers". But those risk drivers are quite specific for each sample and site: site E21, for example, has a median MCR for the RQ_{WQO} of 2.9, indicating that only between 2 and 3 compounds dominate the estimated mixture risk. But in order to capture at least 95% of the overall risk of all 248 samples taken over the years, there are still 44 compounds that need to be monitored and the situation at the other monitored sites is very similar (table 2). This is due to the fact that the dominating compounds constantly fluctuate between samples.

Consequences of single-substance oriented risk management

It has been argued that a MCR close to 1 indicates that a given exposure situation is not relevant from a mixture perspective, but is instead a single substance issue (Price & Han, 2011). However, such an argumentation falls too short without taking a closer look at the consequences of single-substance oriented risk managements for the overall toxicity.

In order to analyze this issue in more detail, we assumed that single-substance oriented risk mitigation (RM) measures were successfully implemented, leading to a situation in which no individual RQ exceeds a value of 0.95. That is, each component is, after the implementation of risk mitigation measures, assumed to be present at a concentration of a maximum of 95% of its WQO. Under these circumstances, the analyzed aquatic ecosystem would be assessed as having a good chemical status according to the WFD. We then calculated the resulting mixture risk quotients for this situation, termed $RQ_{WQO(RM)}$, using WQO based RQs of the individual substances for scenario 1 and 2.

The results of the single-substance risk mitigations, using scenario 2, are shown in figure 3 as green bars for all sites, with the corresponding numerical values given in table 3. The assumed risk mitigations lower risks, but only from a median risk quotient of 2.1 to a median risk quotient of 1.8 (table 3). Overall, 70% of the sites still have an unacceptably high risk (risk mitigated scenario 2, see table 4).

Figure 4 provides a detailed example of the underlying RQ distribution for a complex sample with 27 compounds from site E21. It can be clearly seen that single-substance oriented risk mitigation substantially lowers the overall risks and such measures are thus a critical first step towards a non-toxic environment. But it is also obvious that single substance risk mitigation is unable to ensure that $RQ_{WQO(RM)}$ is below the critical value of 1.

As a consequence of the assumed successful implementation of single-substance oriented risk management measures, the average MCR values of the mixtures increase (table 5). This increased evenness is a consequence of introducing a ceiling for the maximum individual RQ at a value 0.95, simply attributing the same risk to all compounds which individually exceeded the WQO before the assumed risk mitigation.

Conclusions

The presented risk analysis concludes that pesticide residues frequently put aquatic ecosystems in Southern Sweden at risk. This is in line with previous studies in aquatic ecosystems elsewhere (see above). Using WQO values produced by the Swedish Chemicals Agency and the Swedish University of Agricultural Sciences as our basis we conclude that the risk posed by pesticide mixtures were unacceptably high in 73% of the analyzed samples (when using the KM adjustment for non-detects). The fact that the environmental risk at all monitored sites was quite constant over more than a decade indicates that the impact of new regulatory frameworks such as Regulation No 1107/2009 or Directive 2009/128/EC on the sustainable use of plant protection products (European Parliament, 2009a. European Parliament, 2009b) is limited. This goes together not only with previously published studies in the scientific literature (see above), but also with the recent assessment of the progress towards achieving the Swedish environmental objective of a “non-toxic environment”, where it was concluded that the environmental risk of pesticides in the Swedish environment is constant, or even slightly increasing (http://www.miljomal.se/Miljomalen/Alla-indikatorer/Indikatorsida/?iid=140&pl=1).

The ecotoxicology of the pesticide mixtures detected in the Swedish monitoring program is often dominated by only a few compounds – which fluctuate between sites and samples. This allows targeting risk mitigation measures at a subset of pesticides, but at the same time requires that the overall chemical complexity and dynamic at a site has been systematically explored and that this information is kept up to date. At the moment the detection limits of the methods used in multi-component monitoring programs seem to be insufficient for several insecticides . Additionally, purely chemical monitoring efforts will always be limited to an *a priori* selection of the compounds included in the analyses. That is, any component-based mixture risk analysis (as the one presented in this paper) will always be limited to a predefined mixture which is, inherently, only a fraction of the total toxic pressure that the organisms experience at a site. Additionally, such approaches currently do not account for potential synergistic interactions between the mixture components. Validated effect-based assessments (Brack et al., 2015. Altenburger et al. 2015) and in-situ experiments (Crane et al., 2007) might therefore be a necessary complement to chemical monitoring efforts.

The fact that the five different RQs for the three different scenarios differ substantially emphasizes the paramount importance of being clear about the aims of a mixture toxicity assessment and its underlying assumptions. For instance, while exposure scenario 1, in which we assumed that all non-detects are present just at their LOD, is useful for a first check on whether there is even the possibility that an exposed site is put at risk, it certainly overestimates the actual risks. The plethora of possibilities to use different ecotoxicological data, assessment factors and exposure scenarios also highlights the need to keep the data collection and condensation algorithms transparent and available for review and scrutiny.

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Figure 1: The LOD of the monitored pesticides has changed over time (2002-2103), giving a range of ratios of Limit of Detection (LOD) to WQO for each individual pesticide. Pesticides with a median ratio LOD / WQO >= 0.1 are included in the figure.

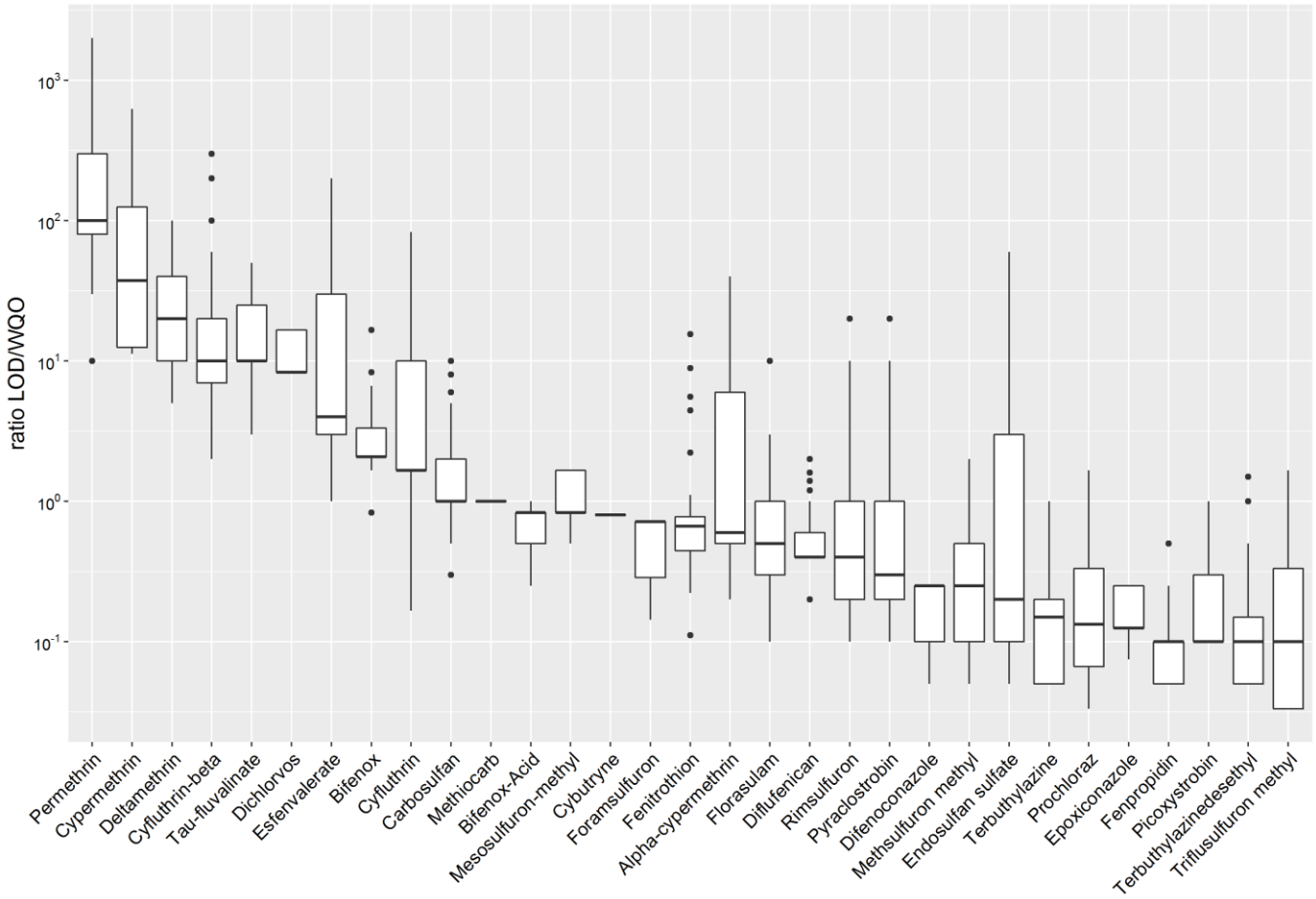


Figure 2: The summed risk quotients using algae, crustaceans, fish, the most sensitive trophic level (MST) and the WQO values for the six monitored site for 2002 – 2013, using four different exposure scenarios (see table 1). Horizontal bars indicate the critical threshold between the "no risk" and "risk" situation, which was set to 0.1 for algae and 0.01 for crustaceans and fish (EU Commission, 2002). For RQ_{WQO} the corresponding critical threshold is 1

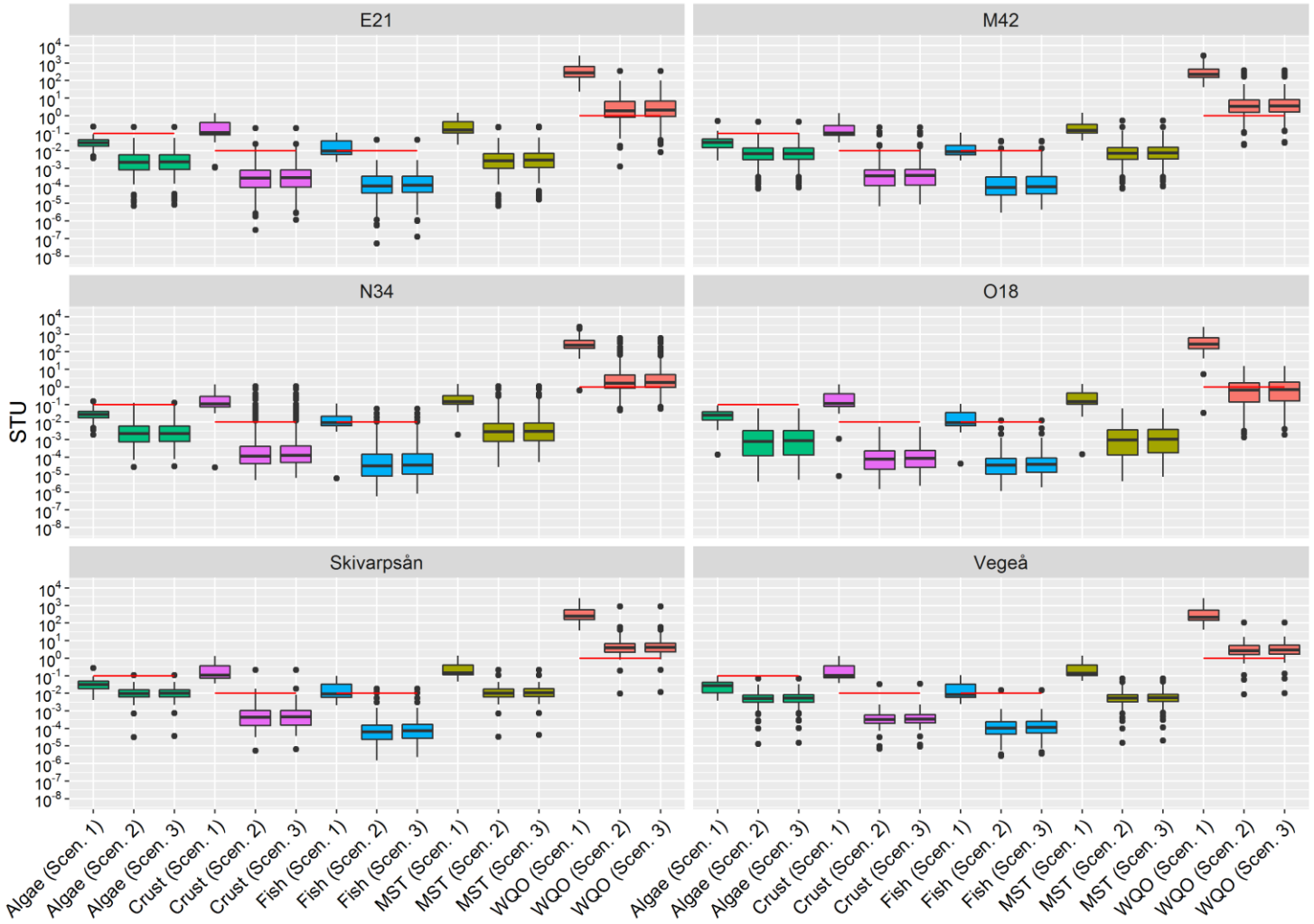
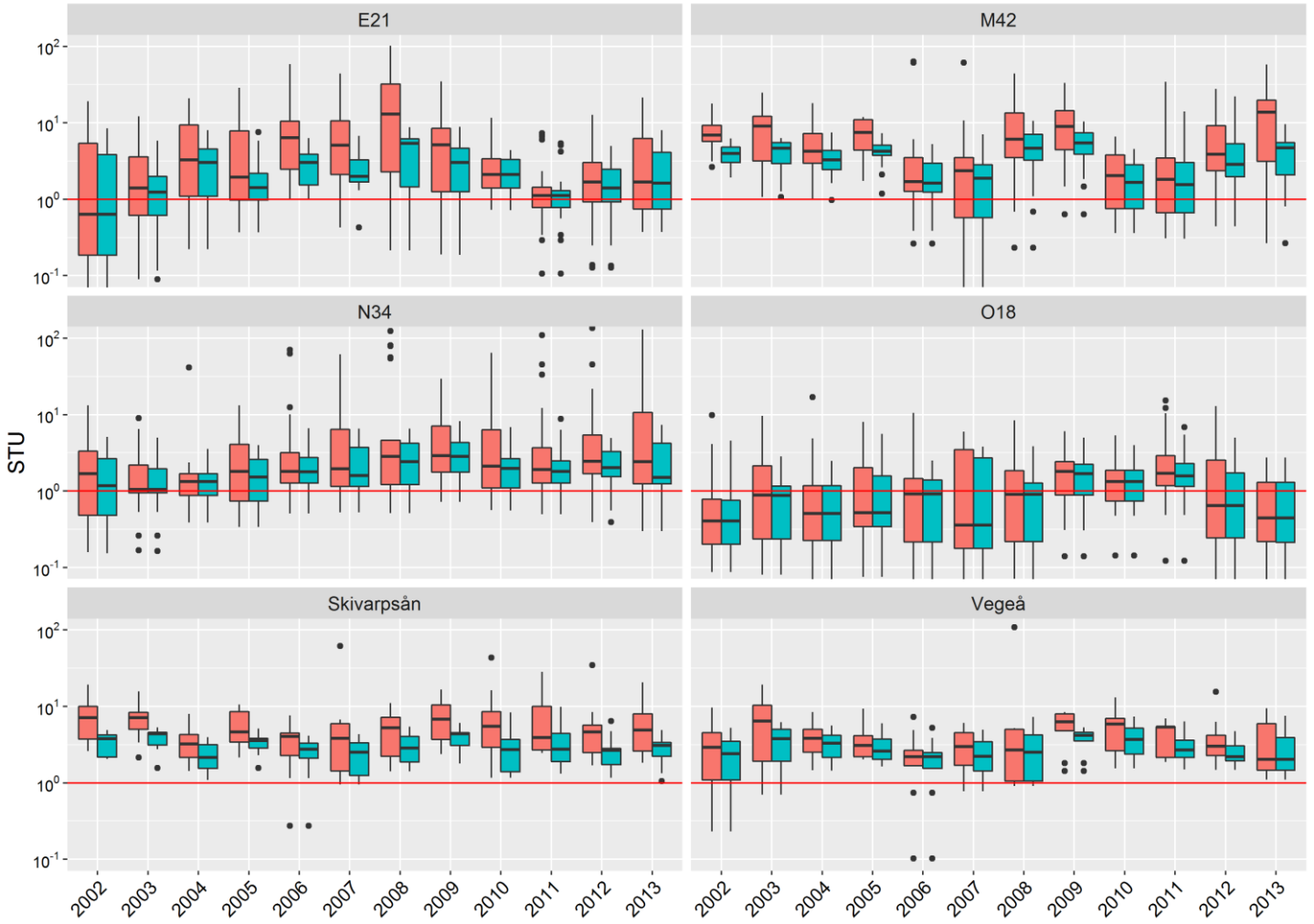
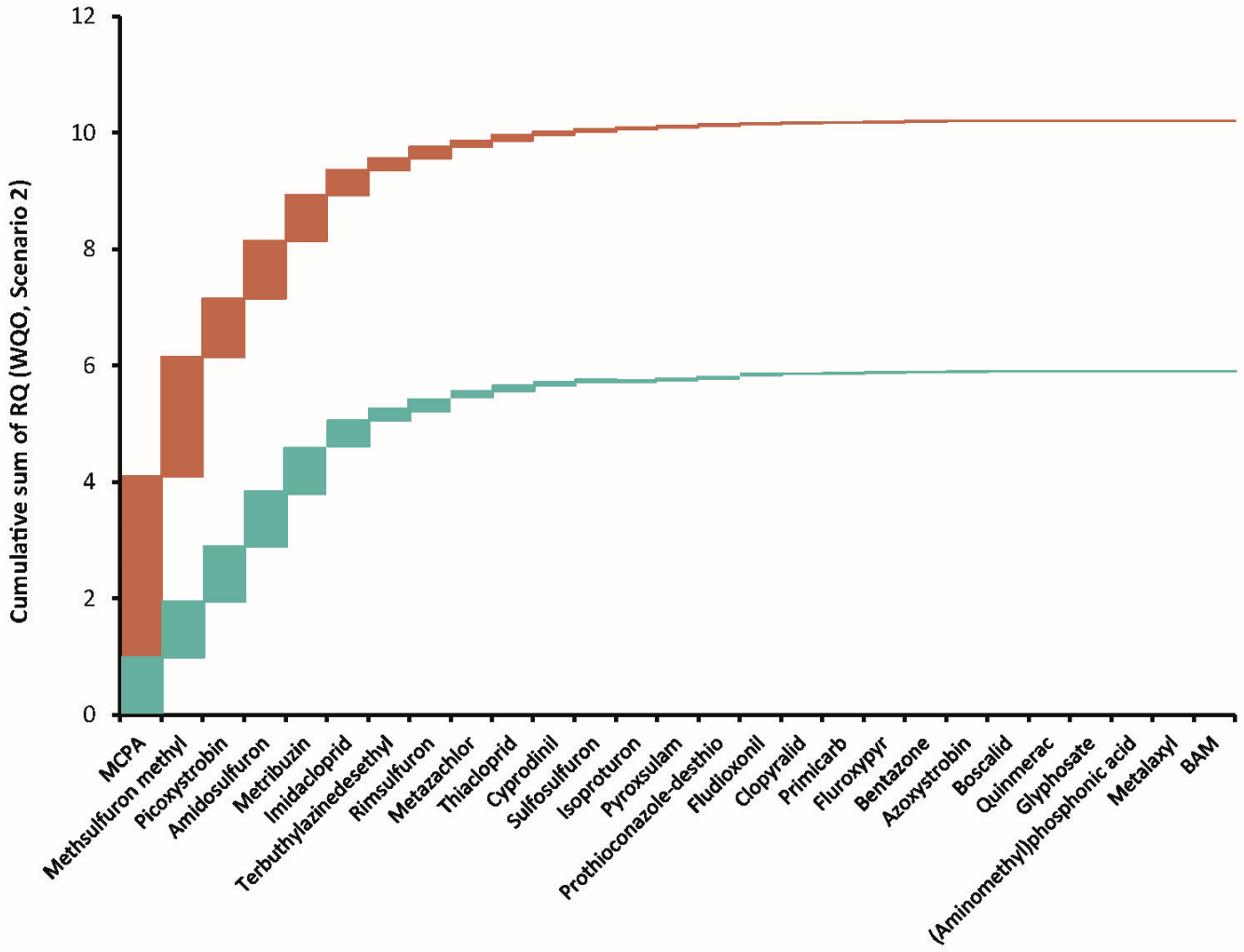


Figure 3: Ecosystem-wide risks (RQ_{WQO}) at the six monitored sites between 2002 and 2013 for scenario 2. The left bar in each pair displays the data from scenario 2, while the right bar displays the data from a risk mitigated scenario 2 (all compounds originally present above its WQO is assumed to be present at only 0.95% of its WQO). Horizontal bars indicate the critical threshold between the "no risk" and "risk" situation.



674 **Figure 4:** Example of a distribution of RQs from a typical sample from the site E21, before and after single-substance oriented risk management



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677 **Table 1:** The three evaluated exposure scenarios. LOD = Limit of Detection, LOQ = Limit of Quantification

	Analytical finding	**Concentration value used for the mixture risk assessment**
Scenario 1	Conc ≥ LOQ	Numerical value of the concentration detected
	Conc ≥ LOD and < LOQ	Before 2009, (LOD+LOQ)/2. From 2009 onwards, as recorded
	Conc < LOD	LOD
Scenario 2	Conc ≥ LOQ	Numerical value of the concentration detected
	Conc ≥ LOD and < LOQ	Before 2009, (LOD+LOQ)/2. From 2009 onwards, as recorded
	Conc < LOD	0
Scenario 3	Conc ≥ LOQ	Numerical value of the concentration detected
	Conc ≥ LOD and < LOQ	Before 2009, (LOD+LOQ)/2. From 2009 onwards, as recorded
	Conc < LOD	Kaplan-Meier adjustment (details see text)

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680 **Table 2:** Overview of occurrence frequencies. Average is calculated as the mode (most common number) of the compounds found per sample.
681 The minimum number of compounds analyzed at N34 and O18 are indicative of individual samples where technical problems have drastically
682 lowered the number of analyzed compounds.

683
684

	No of samples taken (total)	Number of compounds analyzed in each sample			Number of compounds found (≥LOD) per sample			Number of compounds quantified (≥LOQ) per sample			Number of compounds needed to cover 95% of RQ_{WQO} (scenario 2)
		Max	Min	Mode	Max	Min	Mode	Max	Min	Mode	
E21	248	131	68	131	37	2	11	25	1	6	44
M42	308	131	28	131	53	3	23	42	1	4	59
N34	295	131	15	131	43	3	15	30	1	3	58
O18	243	131	14	111	26	2	8	20	1	4	41
Skivarpsån	107	131	68	69	39	6	22	32	1	5	35
Vegeå	107	131	67	69	42	6	16	38	1	5	49
total	1308	131	14	131	53	2	8	42	1	4	83

685

686 **Table 3:** Summary statistics of the environmental risks at the six monitored sites (average over all years) given as median STU (25% percentile-
687 75% percentile). Scenarios refer to the three different mixture scenarios summarized in table 1. WQO = Water Quality Objective, RM = Risk
688 Management Measures (details see text). The critical value for risk exceedance for algae is 0.1, for crustaceans and fish it is 0.01 and for the
689 WQO analysis it is 1 (see text). Bold text is used when the median risk-estimate exceeds the corresponding critical value.

STU Medians		E21	M42	N34	O18	Skivarpsån	Vegeå	Total
Algae	Scenario 1	2.8E-2 (1.8E-2-4.3E-2)	3.0E-2 (1.5E-2-4.7E-2)	2.8E-2 (1.8E-2-4.1E-2)	2.4E-2 (1.3E-2-3.8E-2)	3.1E-2 (1.8E-2-5.0E-2)	2.8E-2 (1.1E-2-4.3E-2)	2.8E-2 (1.7E-2-4.4E-2)
	Scenario 2	2.2E-3 (8.1E-4-2.2E-3)	6.7E-3 (3.1E-3-1.4E-2)	2.7E-3 (7.5E-4-6.0E-3)	7.8E-4 (1.2E-4-3.3E-3)	9.8E-3 (6.1E-3-1.6E-2)	5.1E-3 (3.1E-3-8.1E-3)	3.8E-3 (9.3E-4-8.5E-3)
	Scenario 3	2.3E-3 (8.5E-4-5.9E-3)	6.8E-3 (3.2E-3-1.4E-2)	2.8E-3 (8.0E-4-6.0E-3)	8.5E-4 (1.3E-4-3.3E-3)	1.0E-2 (6.2E-3-1.6E-2)	5.2E-3 (3.2E-3-8.2E-3)	3.8E-3 (9.7E-4-8.6E-3)
Crustacean	Scenario 1	**1.1E-1 (7.9E-2-4.1E-1)**	**1.0E-1 (7.5E-2-2.8E-1)**	**1.1E-1 (7.4E-2-2.8E-1)**	**1.1E-1 (7.6E-2-4.1E-1)**	**1.1E-1 (7.5E-2-3.8E-1)**	**1.0E-1 (7.6E-2-3.8E-1)**	**1.1E-1 (7.5E-2-3.2E-1)**
	Scenario 2	2.8E-4 (8.2E-5-8.0E-4)	3.8E-4 (1.0E-4-8.3E-4)	1.2E-4 (4.4E-5-4.1E-4)	7.9E-5 (2.0E-5-2.3E-4)	4.4E-4 (1.5E-4-1.0E-3)	3.2E-4 (2.0E-4-5.8E-4)	2.1E-4 (6.5E-5-6.0E-4)
	Scenario 3	2.9E-4 (8.6E-5-8.1E-4)	4.0E-4 (1.1E-4-8.5E-4)	1.3E-4 (5.3E-5-4.4E-4)	8.5E-5 (2.6E-5-2.4E-4)	4.6E-4 (1.6E-4-1.1E-3)	3.3E-4 (2.1E-4-6.0E-4)	2.3E-4 (7.1E-5-6.2E-4)
Fish	Scenario 1	9.6E-3 (6.3E-3-3.6E-2)	8.9E-3 (6.0E-3-2.1E-2)	9.0E-3 (6.1E-3-2.1E-2)	9.2E-3 (6.1E-3-3.5E-2)	9.3E-3 (5.9E-3-3.4E-2)	8.3E-3 (5.9E-3-3.4E-2)	9.1E-3 (6.1E-3-3.0E-2)
	Scenario 2	1.0E-4 (3.8E-5-3.5E-4)	8.3E-5 (3.0E-5-3.3E-4)	3.2E-5 (9.4E-6-1.5E-4)	3.4E-5 (1.1E-5-8.4E-5)	6.3E-5 (2.4E-5-1.5E-4)	1.0E-4 (4.7E-5-2.4E-4)	6.5E-5 (1.9E-5-2.0E-4)
	Scenario 3	1.1E-4 (4.2E-5-3.6E-4)	9.1E-5 (3.5E-5-3.5E-4)	3.7E-5 (1.2E-5-1.6E-4)	3.8E-5 (1.4E-5-9.1E-5)	7.3E-5 (2.8E-5-1.7E-4)	1.1E-4 (5.4E-5-2.5E-4)	7.1E-5 (2.1E-5-2.1E-4)
MST	Scenario 1	1.5E-1 (1.0E-1-4.5E-1)	1.4E-1 (1.0E-1-3.2E-1)	1.4E-1 (1.0E-1-3.1E-1)	1.4E-1 (1.0E-1-4.5E-1)	1.4E-1 (1.2E-1-4.1E-1)	1.4E-1 (1.0E-1-4.1E-1)	1.4E-1 (1.0E-1-3.7E-1)
	Scenario 2	2.7E-3 (1.0E-3-6.8E-3)	7.2E-3 (3.2E-3-1.5E-2)	3.0E-3 (7.9E-4-8.7E-3)	9.3E-4 (1.3E-4-3.6E-3)	1.0E-2 (6.3E-3-1.8E-2)	5.4E-3 (3.2E-3-8.3E-3)	4.3E-3 (1.1E-3-9.9E-3)
	Scenario 3	2.9E-3 (1.1E-3-7.1E-3)	7.5E-3 (3.4E-3-1.6E-2)	3.2E-3 (9.2E-4-8.9E-3)	1.1E-3 (1.8E-4-3.8E-3)	1.1E-2 (6.5E-3-1.8E-2)	5.7E-3 (3.4E-3-8.7E-3)	4.4E-3 (1.2E-3-1.0E-2)
WQO	Scenario 1	**2.7E+2 (1.6E+2-6.3E+2)**	**2.3E+2 (1.5E+2-4.6E+2)**	**2.3E+2 (1.6E+2-4.4E+2)**	**2.7E+2 (1.5E+2-6.4E+2)**	**2.5E+2 (1.6E+2-5.9E+2)**	**2.2E+2 (1.5E+2-5.5E+2)**	**2.5E+2 (1.5E+2-5.3E+2)**
	Scenario 2	**1.9E+0 (8.1E-1-6.6E+0)**	**3.4E+0 (1.5E+0-7.8E+0)**	**1.7E+0 (8.7E-1-5.0E+0)**	6.6E-1 (1.4E-1-1.7E+0)	**3.9E+0 (2.2E+0-6.8E+0)**	**2.7E+0 (1.6E+0-5.4E+0)**	**2.1E+0 (8.5E-1-5.6E+0)**
	Scenario 3	**2.0E+0 (8.9E-1-6.9E+0)**	**3.6E+0 (1.7E+0-8.2E+0)**	**1.9E+0 (9.5E-1-5.2E+0)**	7.1E-1 (1.6E-1-1.9E+0)	**4.1E+0 (2.3E+0-7.2E+0)**	**2.9E+0 (1.7E+0-5.6E+0)**	**2.2E+0 (9.3E-1-5.9E+0)**
WQS+RM	Scenario 1	**1.8E+1 (1.5E+1-2.0E+1)**	**1.8E+1 (1.5E+1-2.0E+1)**	**1.8E+1 (1.5E+1-1.9E+1)**	**1.7E+1 (1.4E+1-1.9E+1)**	**1.6E+1 (1.4E+1-1.9E+1)**	**1.6E+1 (1.4E+1-1.9E+1)**	**1.8E+1 (1.5E+1-1.9E+1)**
	Scenario 2	**1.5E+0 (8.1E-1-3.4E+0)**	**2.7E+0 (1.4E+0-4.3E+0)**	**1.5E+0 (8.7E-1-2.7E+0)**	6.6E-1 (1.4E-1-1.4E+0)	**2.6E+0 (1.7E+0-3.7E+0)**	**2.3E+0 (1.5E+0-3.7E+0)**	**1.8E+0 (8.5E-1-3.3E+0)**

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Table 4: Percentage of risk exceedances, scenarios refer to the three different mixture scenarios summarized in table 1. WQO = Water Quality Objective, RM = Risk Management Measures (details see text). The critical value for risk exceedance for algae is 0.1, for crustaceans and fish it is 0.01 and for the WQO analysis it is 1 (see text).

	Algae			Crustaceans			Fish			WQO			WQO+RM	
Scenario	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**
Site														
E21	0.4	0.4	0.4	99.2	1.6	1.6	46.8	0.4	0.4	100	67.7	71.8	100	67.7
M42	2.9	0.6	0.6	100	3.2	3.2	39.9	1.3	1.3	100	82.1	84.7	100	81.8
N34	0.7	0.3	0.3	99.7	9.2	9.5	40.7	2.4	2.4	99.7	69.2	73.2	99.7	69.2
O18	0.0	0.0	0.0	99.2	0.0	0.0	42.8	0.4	0.4	99.6	41.6	42.8	99.6	39.5
Skivarpsån	0.9	0.9	0.9	100	1.9	1.9	43.9	2.8	2.8	100	94.4	96.3	100	94.4
Vegeå	1.9	0.0	0.0	100	0.9	0.9	40.2	0.9	0.9	100	88.8	89.7	100	88.8
Total	1.1	0.4	0.4	99.6	3.4	3.7	42.3	1.3	1.3	99.8	70.5	73.2	99.8	70.0

696 **Table 5:** Median maximum cumulative ratios (MCR) for all sites, trophic levels and exposure scenarios

		E21	M42	N34	O18	Skivarpsån	Vegeå	total
Algae	**Scenario 1**	2.27	2.45	2.33	2.22	2.33	2.27	2.31
	Scenario 2	1.69	1.34	1.58	1.31	1.23	1.44	1.41
	Scenario 3	1.82	1.37	1.67	1.38	1.24	1.46	1.46
Crustacean	**Scenario 1**	2.85	2.86	2.73	2.76	2.85	2.75	2.79
	Scenario 2	1.46	1.91	1.91	1.78	1.79	2.17	1.81
	Scenario 3	1.58	2.04	2.13	2.05	1.86	2.28	1.98
Fish	**Scenario 1**	3.26	3.19	3.26	3.14	3.20	3.25	3.24
	Scenario 2	1.57	1.97	1.79	1.64	1.97	1.66	1.77
	Scenario 3	1.79	2.24	2.11	1.95	2.44	1.79	2.01
MST	**Scenario 1**	3.26	3.38	3.24	3.12	3.48	3.34	3.30
	Scenario 2	1.92	1.39	1.57	1.39	1.25	1.49	1.46
	Scenario 3	2.09	1.44	1.72	1.61	1.30	1.57	1.59
WQO	**Scenario 1**	2.18	2.19	2.10	2.12	2.17	2.18	2.16
	Scenario 2	1.95	2.12	1.87	1.54	1.73	2.17	1.90
	Scenario 3	2.86	3.17	2.56	1.98	2.84	2.92	2.65
WQO+RM	**Scenario 1**	19.07	18.99	18.63	18.16	17.32	17.22	18.56
	Scenario 2	2.61	3.00	2.37	1.70	2.74	2.74	2.43

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