

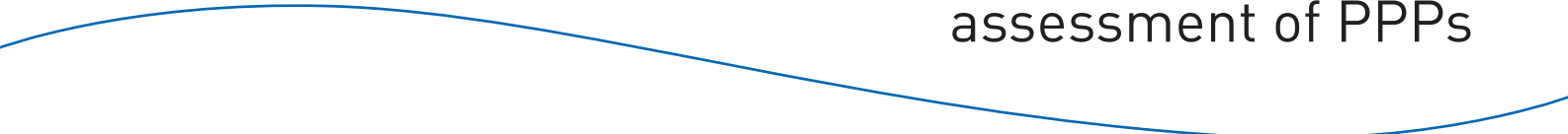
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Exposure and toxicity of mixtures of
plant protection products (PPPs) in
the environment under Norwegian
conditions. Evaluation of a
cumulative environmental risk
assessment of PPPs



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Abstract <p>This project was performed to improve the environmental cumulative risk assessment (CRA) of mixtures of plant protection products detected by the Norwegian Agricultural Environmental Monitoring Programme (JOVA) in Norwegian surface waters. Existing ecotoxicity data were compiled and reduced the assessment uncertainty compared to previous risk assessments. Ecotoxicity tests verified that the cumulative toxicity of ecologically-relevant environmental mixtures was fairly well predicted for algae, daphnia and aquatic plants. The results from the ecotoxicity tests were used to evaluate the assessment factor used in the risk assessment, and the improved data used in the CRA of plant protection products in the JOVA monitoring performed in 2013. Three of the six investigated sites had risk quotients indicative of environmental risk. Mitigation measures based on the identification of the main risk drivers were discussed and include consideration of no-spray zones, grassed buffer strips, reduced doses and patch spraying, and pesticide risk maps.</p>
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Exposure and toxicity of mixtures of plant protection products (PPPs) in the environment under Norwegian conditions. Evaluation of the cumulative environmental risk assessment of PPPs

Preface

This project was commissioned by the Norwegian Food Safety Authority and was funded by the action plan for reduced risk from use of plant protection products. The project was performed as collaboration between NIVA, Bioforsk and Faust & Backhaus consulting with Knut Erik Tollefsen (NIVA) as the project leader. Knut Erik Tollefsen (NIVA), Karina Petersen (NIVA), Marianne Stenrød (Bioforsk) and Thomas Backhaus (Faust & Backhaus consulting) were responsible for planning the ecotoxicity tests and chemical analysis. Tania Gomes, Lene Fredriksen, You Song, Li Xie and Ailbhe Macken at NIVA were responsible for performing the ecotoxicity tests, whereas Marianne Stenrød, Sven Roar Odenmarck, Agnethe Christiansen and Nina Oseth Svendsen at Bioforsk were responsible for the chemical analysis. Marianne Stenrød (Bioforsk) was responsible for retrieving data from the JOVA program and Karina Petersen (NIVA) was responsible for performing the environmental risk assessment with valuable input from the other partners. Employees from all three collaborative partners contributed to the writing of the report.

Oslo, 16.03 2015

Knut Erik Tollefsen

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Summary

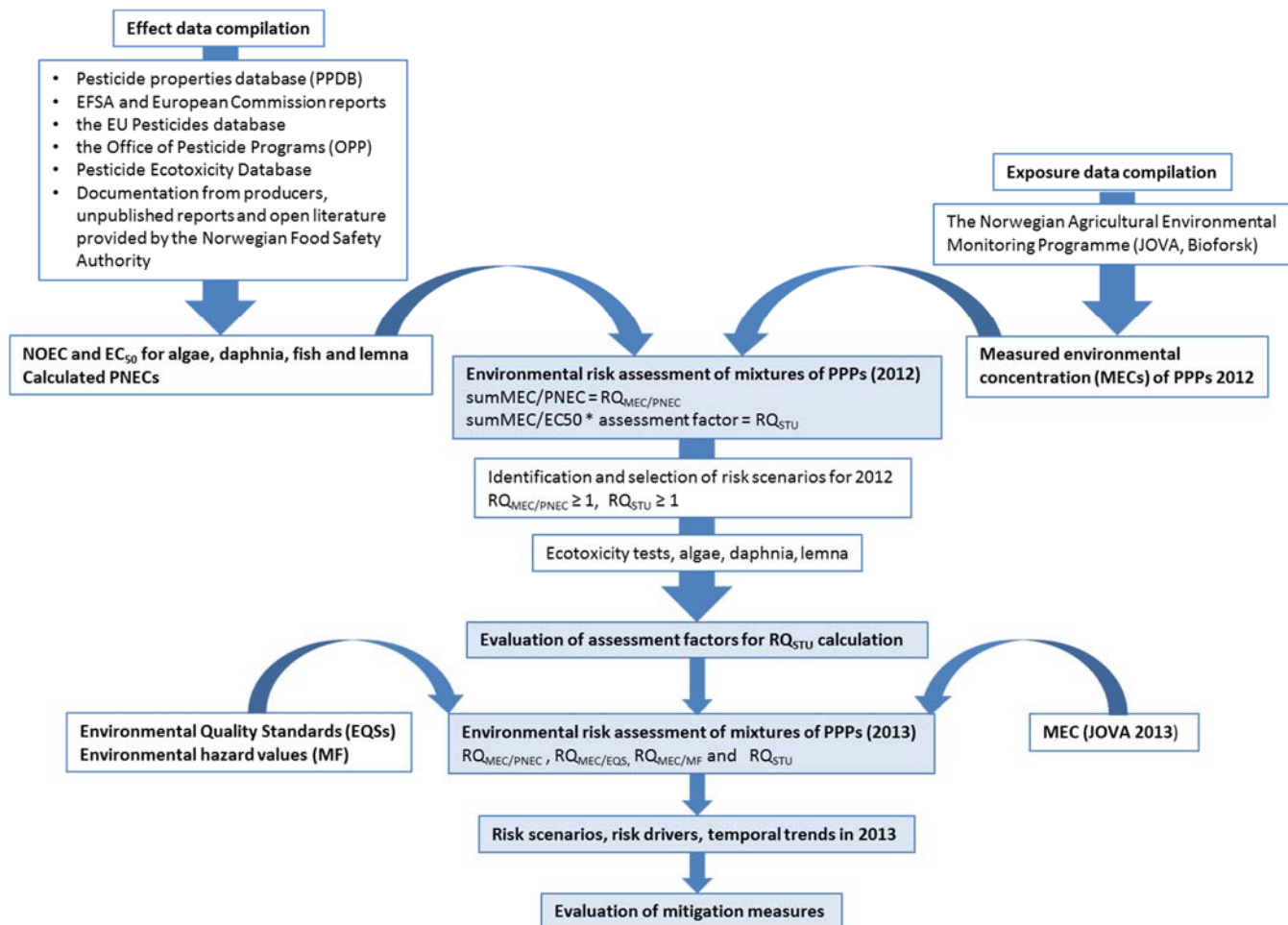
An environmental risk assessment of the active ingredients of plant protection product (PPP) mixtures was performed based on available effect and chemical monitoring data from 2012. Ecotoxicity data for algae, crustaceans, fish and aquatic plants were collected from various databases and used to calculate predicted no effect concentrations (PNEC). Measured environmental concentrations (MEC) of the PPPs at six different monitoring sites in Norway were obtained through the Norwegian Agricultural Environmental Monitoring (JOVA) Program.

In the first approach, a risk quotient (RQ) based on the sum of the MEC/PNEC ratios of the PPPs detected in each sample ($RQ_{MEC/PNEC}$) was calculated. A potential environmental risk of the mixture was identified when the $RQ_{MEC/PNEC}$ was above 1. In addition, taxa-specific risks were calculated by summing up the toxic units (TU) to obtain the risk quotient RQ_{STU} following the application of an appropriate assessment factor. In earlier risk assessments of PPP mixtures based on PPP occurrence data from 2012, a risk to the environment was predicted at four of the six investigated sites and the main risk drivers were identified. In addition, several knowledge gaps were recognized. The main knowledge gaps identified were related to exposure and toxicity data and to the assessment factors used for calculating RQ_{STU} .

The current project was performed in order to fill in the data gaps identified for exposure and toxicity data, and to evaluate the use of assessment factors for calculating the RQ_{STU} . The aim was to develop an improved approach for the environmental cumulative risk assessment of PPP mixtures, to apply it for assessing the environmental risks of PPPs measured during the JOVA 2013 monitoring campaign and to evaluate mitigation measures aimed to reduce the cumulative risk (summary figure 1). This work was performed in the following steps; (1) reduction of the uncertainty caused by missing exposure and toxicity data, (2) evaluation of assessment factors by performing ecotoxicity tests with algae, daphnia and aquatic plants for relevant PPP mixtures, (3) assessment of the environmental risks of PPP mixtures detected in 2013 at the various monitoring sites, and (4) evaluation of the need for and utility value of mitigation measures based on the identified risk drivers from the two investigated years.

Data gaps in exposure data were evaluated and toxicity data were compiled. The complemented toxicity data improved the models and resulted in reduced uncertainty in the calculation of $RQ_{MEC/PNEC}$. The re-calculation of the environmental risk of the samples taken in the JOVA 2012 monitoring program showed that the complemented toxicity data lead to a lower number of samples with RQs indicative of environmental risk and a reduction in the RQ value for some of the samples.

Laboratory results showed that the toxicity of environmental mixtures could be estimated quite well (within a factor of less than 4) for algae, daphnia and aquatic plants based on existing toxicity data. The results from the laboratory tests were used to evaluate the use of assessment factors for the calculation of risk quotients, and led to proposal of assessment factors for calculation the RQ_{STU} of 100 for algae and aquatic plants and 1000 for crustaceans and fish based on the existing data. These assessment factors were used in the environmental risk assessment of measured concentration of PPPs in the 2013 JOVA monitoring program. Based on the $RQ_{MEC/PNEC}$, 5 samples were identified as having an environmental risk. However, by using other reference values like environmental quality standards or environmental hazard values for calculating the RQ, 15 - 17 samples were identified as having an environmental risk. Common for all approaches was that three of the six investigated sites had RQs indicative of environmental risk (Mørdrebekken, Heiabekken and Vasshaglona). The main risk drivers differed between species groups, sites and samples. The main identified risk drivers in 2013 were alfa-cypermethrin, metribuzin, azoxystrobin and propiconazole.



Summary figure 1. Flow chart showing the workflow of the project. Main output of the environmental risk assessment of mixtures of PPPs are shown in blue boxes. The figure is described in more detail in section 2.

Mitigation measures based on the identification of the main risk drivers were discussed. As a general approach, any measure that reduces the use of pesticides will reduce the exposure. First and foremost, integrated pest management practices should be promoted. Current practices include widespread use of reduced pesticide doses and patch spraying, and ongoing research show promising results of precision spraying. Pesticide risk maps is another promising approach to enable farmers to choose pesticides of low environmental risk based on site-specific weather and soil data. These assessments are anticipated to contribute to identifying vulnerable areas with high risk of pesticide loss (e.g. areas with coarse material or rapid vertical leaching) that should be avoided when spraying mobile pesticides.

Abbreviations

AF	Assessment factor
CA	Concentration addition
CRA	Cumulative risk assessment
EC _x	Concentration causing X % effect
EFSA	European Food Safety Authority
EQS	Environmental quality standard
EU	European Union
ha	Hectares
IA	Independent action
LC _x	Concentration causing X% mortality (lethality)
MEC	Measure environmental concentration
MF	Environmental hazard value (miljøfarlighetsverdi)
NOEC	No observed effect concentration
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
PPDB	Pesticide properties database
PPP	Plant protection product
RQ	Risk quotient
STU	Sum of toxic units
TER	Toxicity exposure ratio
TU	Toxic Unit, here defined as MEC divided by EC ₅₀

1. Introduction

1.1 Approval, use and occurrence of plant protection products

Effective plant protection practices and the use of chemical plant protection products (PPPs) are a pre-requisite for maintaining yields of sufficient quality and quantity in conventional agriculture. Despite regulatory efforts to ensure safe use, reports have indicated that residual amounts of pesticides and their metabolites occur in surface and ground water and may have effects on non-target aquatic organisms (Malaj et al., 2014). The environmental concentrations of many of the active substances in the PPPs (hereafter referred to as PPPs) used are routinely monitored in water recipients in agricultural areas through the Norwegian Agricultural Environmental Monitoring Program, JOVA (www.bioforsk.no/jova). Through a 20 year period the JOVA program has compiled data on the occurrence of pesticides in surface water during the growing season, in selected agricultural catchments covering the variety of intensive agricultural practices in Norway. Concentration of PPPs in surface water (streams, rivers, shallow ground water) range from ng/L to low µg/L (Bechmann et al., 2014). Approval of PPPs for use in Norway is performed by the Norwegian Food Safety Authority. Norway's regulatory practices conform to national regulations for the approval and use of PPPs, however the EU legislation is expected to be implemented in Norway from 2015. The EU regulation concerning the placing of PPPs on the market (Regulation (EC) No 1107/2009), describes the approval criteria for PPPs that are based on risk assessment of the active ingredients in the products. In the regulation for evaluation and authorization of PPPs it is stated that member states shall evaluate potential exposure to aquatic organisms and evaluate the expected short term and long term risk. This is performed by the calculation of acute toxicity/exposure ratios (TER) for algae, daphnia and fish, defined as the quotient of acute 50% effect concentrations (LC₅₀ or EC₅₀) and the predicted short-term environmental concentration. When calculating these ratios the EU member states shall consider toxicity to the most sensitive relevant organism used in the tests. Calculation of the long-term TER for fish and daphnia shall be performed using the no observed effect concentration (NOEC) and predicted long-term concentrations. No authorization should be granted if the TER for fish and daphnia is less than 100 for acute exposure and less than 10 for long-term exposure, or the algal growth inhibition/exposure ratio is less than 10 (The European Commission, 2011). The new EFSA guidance document (EFSA, 2013) provides information of how to perform a tiered risk assessment for active ingredients in formulations. However, no framework for how to assess the environmental mixtures of active ingredients from different products and formulations is currently described in detail.

Several PPPs are sprayed on the same crop and within an agricultural catchment, and as many as 42 different PPPs have been detected at certain sites during the period from 1995 to 2010 (Bechmann et al., 2014). Even though the environmental concentrations of most PPPs are below the reported NOECs and EC₅₀, effects on organisms in the aquatic environment might occur through combined toxicity as co-occurrence of several PPPs in water samples from agricultural streams is more the rule than the exception. The typical aquatic exposure scenarios during the main spraying period involve the exposure to more than 5 substances during runoff events, whilst worst-case runoff events might result in concurrent exposure to more than 10 substances (Bechmann et al., 2014).

1.2 Assessment of chemical mixtures

In the environment, exposure to complex mixtures of chemicals is expected and will vary with time. The focus on a substance-by-substance assessment in most current regulatory strategies for environmental risk assessment therefore runs the risk of underestimating the actual toxic pressure that an ecosystem is exposed to. And even though the concentrations of individual pollutants might often be low, combined effects have been shown to occur even when the compounds are present in concentrations below their individual NOECs (Faust et al., 2001; Kortenkamp et al., 2009; Walter et al., 2002). The effects of chemical mixtures have been thoroughly assessed in laboratory studies over the years, and the general understanding of mixture toxicity is now well established (Kienzler et al.; Kortenkamp et al., 2009;

Scientific Committee on Health and Environmental Risks Scientific (SCHER) et al., 2012). Compounds in a mixture might interact, meaning that they can enhance (synergy) or decrease (antagonism) the toxicity of each other. If no interactions occur, the mixture is said to be additive. Additive toxicity can be predicted by the two prediction models for concentration addition (CA) and independent action (IA). The two models assume that the compounds act additively by similar MoA (CA) or independent MoA (IA). Review of data from mixture toxicity studies have shown that the effects of mixtures in most cases can be predicted by the CA model and that synergistic interactions only occur in a few cases (Belden et al., 2007; Cedergreen, 2014; Rodney et al., 2013; Verbruggen and Brink, 2010).

In order to assess whether a mixture is additive, synergistic or antagonistic, appropriate experimental designs are needed which includes fixed ratio ray designs, factorial designs and use of prediction models. A factorial design consists of two or more factors (e.g. chemicals), each with a set of values (e.g. concentrations) and the design consists of all possible combinations of these. In the fractional factorial design, a selection of combinations from the full factorial design is tested in order to reduce the workload and complexity and at the same time obtain as much information as possible. Fixed ratio ray designs are designs where the concentration ratio between the individual compounds is held constant for all tested mixture concentrations, and a concentration-response curve for the mixture can be obtained. This design is usually used in combination with the CA and IA prediction models.

1.3 Environmental risk assessment of chemical mixtures

Based on knowledge on mixture toxicity obtained from laboratory and field studies, a conceptual framework for the environmental risk assessment of chemical mixtures has been proposed by Backhaus and Faust (2012). The approach is based on an approximation of the concentration addition (CA) concept. A vast amount of studies has shown good correlation between the observed and CA-predicted toxicity of mixtures in different organisms and for different endpoints (Belden et al., 2007; Cedergreen, 2014; Deneer, 2000; Petersen et al., 2014; Tollefsen et al., 2012; Verbruggen and Brink, 2010). As it has been shown that combined effects are more often additive than synergistic or antagonistic (Belden et al., 2007; Cedergreen, 2014; Deneer, 2000), there is a general acceptance for applying the concept of CA for initial assessment of combined effects of mixtures. In the proposed conceptual framework, the risk of environmental effects of chemical mixtures is assessed using available effect data (NOEC and EC₅₀ values), the resulting predicted no effect concentration values (PNEC) and the predicted or measured environmental concentrations (PEC or MEC). In a first approach, a risk quotient based on the sum of (MEC or PEC)/PNEC ratios of the detected PPPs ($RQ_{MEC/PNEC}$), is calculated (figure 1). If the resulting $RQ_{MEC/PNEC}$ is equal to or above 1 there is a potential risk to the environment. The $RQ_{MEC/PNEC}$ is a pragmatic first approach as existing PNEC values, which have already undergone regulatory assessment, can be used directly, without the need to go back to the underlying studies with the various organisms. The shortcoming of this approach is that PNECs for the different components might be derived by different taxa, e.g. compound 1 of the mixture might be predominantly toxic to algae, compound 2 to invertebrates – under these conditions it is difficult to interpret the resulting sum of PEC/PNEC ratios. However, Backhaus and Faust (2012) showed that the summation of PEC/PNEC ratios might serve as a justifiable, i.e. slightly cautious, first-tier approximation of a conceptually more sound CA-based mixture toxicity assessment. Should the sum PEC/PNEC ratios exceed one, i.e. should a potential risk be indicated, the mixture toxicity can be assessed separately for each taxa or species group. This so-called toxic unit summation (toxic unit, $TU = MEC/EC_{50}$) results in taxa-specific risk quotients. Following the standard environmental risk assessment practice the overall risk for the environment is then based on the most sensitive taxa, termed RQ_{STU} (risk quotient, based on sum of toxic units). The critical issue when calculating the RQ_{STU} is the application of appropriate assessment factors (for details see Backhaus and Faust, 2012). As a risk to the environment is indicated if the RQ_{STU} is equal to or above 1, the assignment of an appropriate AF may be crucial for the final assessment of cumulative risk.

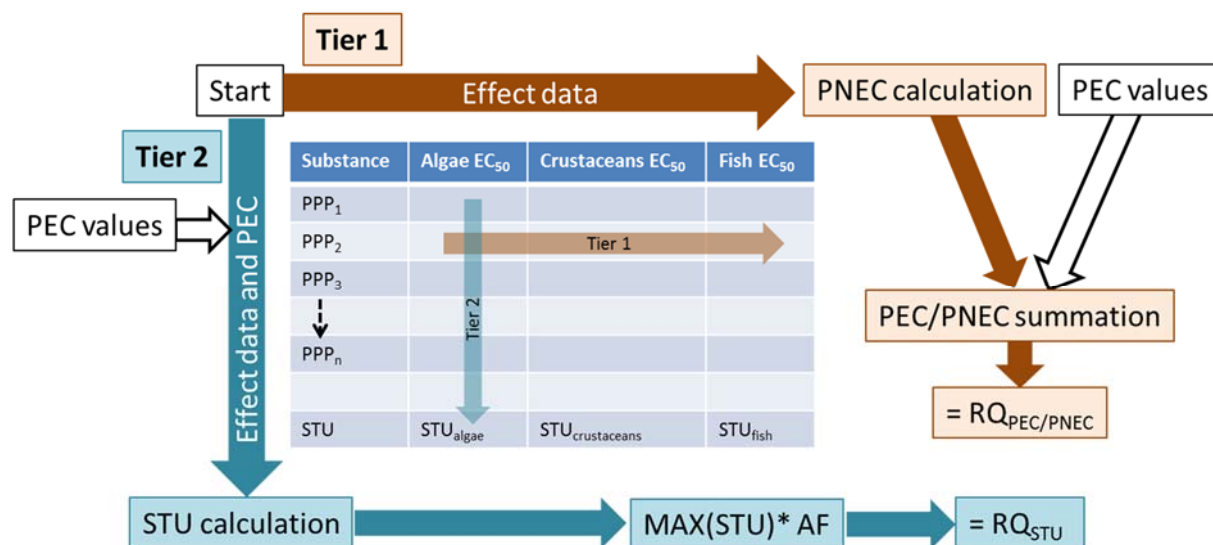


Figure 1. Visualization of the two-tiered approach for environmental risk assessment of chemical mixtures. In the first tier (brown), effect data for three trophic levels are used to calculate the predicted no effect concentrations (PNECs) for all substances (PPP₁ to PPP_n). The ratio of environmental concentrations (PEC) and PNECs are summed for all compounds giving the risk quotient $RQ_{MEC/PNEC}$. In tier 2 (blue), toxic units ($TU = PEC/EC_{50}$) for all substances are calculated for each trophic level individually, giving a sum of toxic units (STU) for each trophic level. The highest STU is then multiplied by an assessment factor (AF) to give the risk quotient RQ_{STU} . The figure has been modified from Backhaus and Faust (2012).

1.4 Environmental risk assessment of PPP mixtures

As PPPs are regulated and assessed individually in the current Norwegian regulation (FOR-2004-07-26-1138), presence of mixtures in the environment due to use of several PPPs in the same catchment area are often not adequately addressed. Consideration of the cumulative risk of environmentally relevant mixtures of PPPs is thus required in order to protect organisms in the vicinity of agricultural areas from unwanted toxic effects. A few studies have been performed to predict the risk of environmental mixtures of PPPs. In a cumulative risk assessment performed by Moschet et al. (2014), environmental quality standards (EQS values), from the EQS directive or EQSs calculated ad hoc from a limited dataset, were used together with the MEC values of PPPs. No grouping of the compounds was performed in the first tier assessment, which was performed by summing up the MEC/EQS ratios and thus in agreement with the approach presented by Backhaus and Faust (2012). A grouping of PPPs according to use groups (herbicides, fungicides, and insecticides) was performed in a second step, in order to analyse which group contributed most to the risk. It was found that in 44 out of 45 surface water samples the $RQ_{MEC/EQS}$ exceeded 1, indicating a potential environmental risk. An overview of contribution and composition of environmental pesticide mixtures based on predicted environmental concentrations of PPPs in runoff from agricultural areas in Italy has previously been performed by using toxic units for algae, crustaceans and fish (Finizio et al., 2005). A recent study by Bundschuh et al. (2014) assessed the impact of pesticide mixtures found in streams in Southern Sweden using similar toxic unit summation.

An initial environmental risk assessment of mixtures of PPPs measured in six different agricultural streams in Norway in 2012 was performed previously (Petersen et al., 2013). This cumulative risk assessment was based on available effect data for algae, crustaceans, fish and aquatic plants, and MECs of PPPs at six different monitoring sites in Norway. The environmental risk assessment was performed according to the first tier of the framework presented by Backhaus and Faust (2012) with MECs obtained from the JOVA 2012 monitoring campaign and PNECs calculated from compiled toxicity data. Of the total 56 investigated samples, eight had a calculated $RQ_{MEC/PNEC} > 1$; two samples from Hotranelva, four

samples from Mørdrebekken, one sample from Skuterudbekken, and one sample from Vasshaglona. These samples were typically collected from late June to mid-August (Petersen et al., 2013). The identified risk scenarios based on $RQ_{MEC/PNEC}$ were confirmed by RQ_{STU} values above 1 for aquatic plants based on the samples from Hotranelva, Mørdrebekken and Vasshaglona, and for algae based on samples from Skuterudbekken and Vasshaglona. The risk at each site was driven by a few compounds; aclonifen (Mørdrebekken), metribuzin (Mørdrebekken and Vasshaglona), prothioconazole-desthio (Hotranelva) and kresoxim (Skuterudbekken). Several knowledge gaps were identified in the study and these were expected to influence the predicted environmental risk. The main knowledge gaps were identified to be related to the lack of exposure data and toxicity data resulting in high assessment factors for calculating PNECs, lack of toxicity data for calculating the species-specific STUs and uncertainty related to use of assessment factors for calculating the RQ_{STU} .

1.5 Objectives of the present study

The overall aim of the present study was to investigate whether PPPs and mixtures of these in selected Norwegian agricultural streams have a potential for exerting toxicity to aquatic organisms (algae, aquatic plants, crustaceans and fish) that cannot be predicted by the toxicity of the individual PPPs alone. The work was built on the project performed in 2013 where predicted environmental risk of mixtures of PPPs based on MECs in the Norwegian environment from the 2012 JOVA monitoring campaign. The main objectives of the study was to

- Reduce the uncertainty connected to lack of exposure and toxicity data by complementing the existing data set compiled in 2012 (Petersen et al. 2013) and thereby revise the AFs for calculating PNEC values. A comparison of the calculated risk from the 2012 data with the calculated risk based on revised PNEC values will be performed to assess the impact of using a more complete toxicity data set.
- Evaluate the use of AFs for the calculation of RQ_{STU} by conducting experimental ecotoxicity studies with algae, aquatic plants and crustaceans.
- Use the complemented toxicity data set and revised AFs for calculation of RQ_{STU} to estimate the cumulative environmental risk of PPPs measured in Norwegian agricultural streams during the JOVA 2013 monitoring campaign. Identify risk scenarios, risk drivers and temporal trends, and compare the use of PNECs in relationship to other established reference values like EQSs and environmental hazard values (MF values, miljøfarlighetsverdier) in the cumulative risk assessment.
- Evaluate the need for, and utility value of, mitigation measures based on the identified risk drivers from the two investigated years.

2. Materials and methods

The overall course of the project is shown in the flow chart in figure 2, and is further described in detail in the preceding chapters.

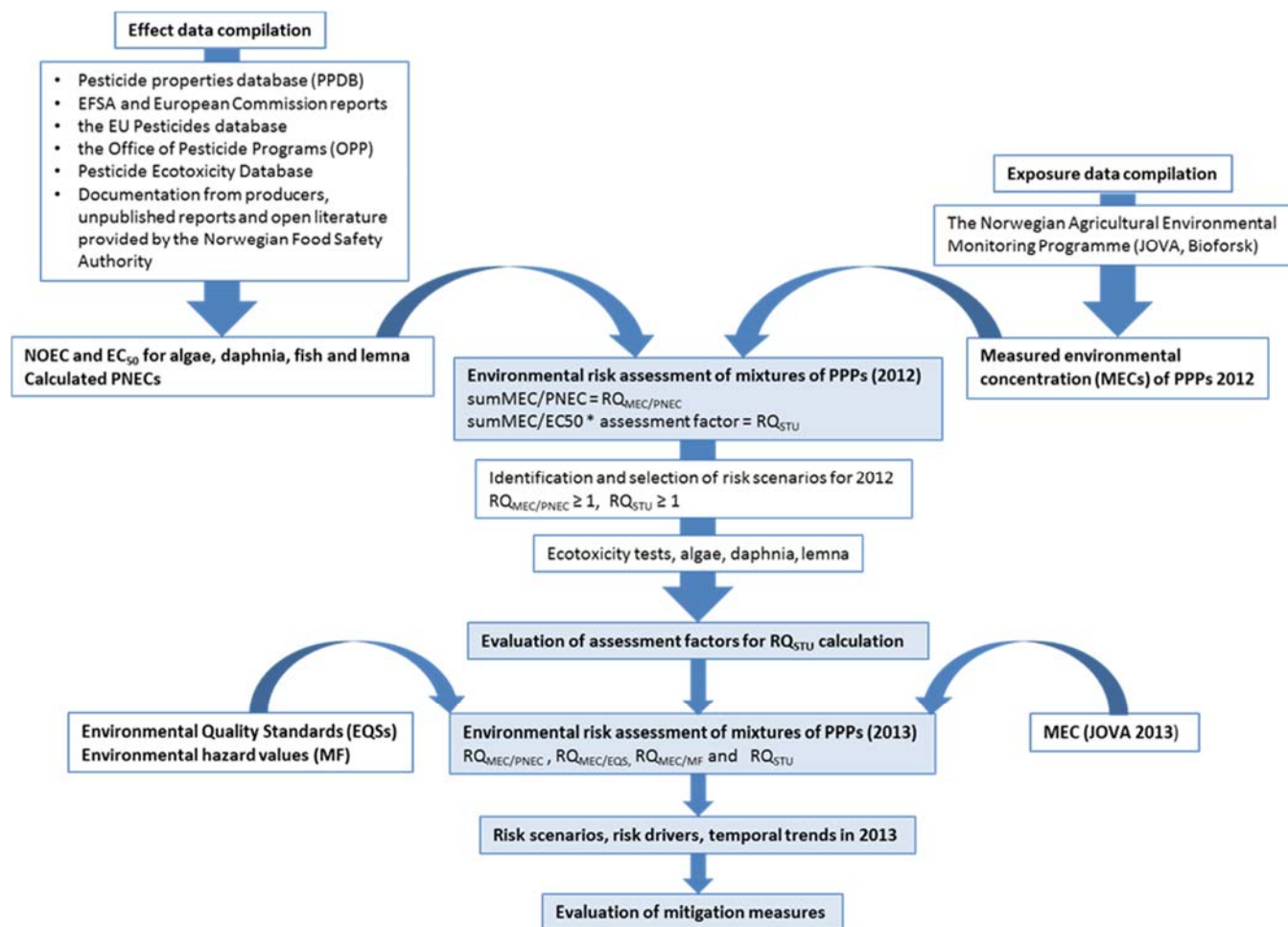


Figure 2. Illustration of the project work-flow. The first step of the project was to compile toxicity and exposure data for relevant PPPs. EC₅₀ and NOEC values for algae, crustaceans, fish and aquatic plants were used to derive PNECs. The MECs of PPPs in 2012 were obtained by the JOVA monitoring programme. The PNECs and MECs were used in the tier 1 of the environmental risk assessment of mixtures to calculate the $\text{RQ}_{\text{MEC/PNEC}}$, whereas EC₅₀ values and MECs were used in the tier 2 to calculate the RQ_{STU} for all trophic levels. Risk scenarios in 2012 were identified by $\text{RQ}_{\text{MEC/PNEC}}$ and $\text{RQ}_{\text{STU}} \geq 1$, and two scenarios were chosen for ecotoxicity testing in order to evaluate the use of assessment factors for calculating the RQ_{STU} . The resulting proposed AFs for calculation of RQ_{STU} were used when performing the environmental risk assessment of mixtures of PPPs detected in 2013. For the environmental risk assessment of mixtures of PPPs detected in 2013, the tier 1 was also calculated based on proposed EQS values (Kontiokari and Mattsoff, 2011) ($\text{RQ}_{\text{MEC/EQS}}$) and MF values from Bioforsk ($\text{RQ}_{\text{MEC/MF}}$). The final output of the project was the identification of risk scenarios in 2013, identification of risk driver and temporal trends and a final evaluation of the need for and utility value of mitigation measures.

2.1 Complementing exposure and toxicity data

The MECs from the initial environmental risk assessment of PPPs in Norwegian scenarios (Petersen et al., 2013) were obtained from the JOVA 2012 monitoring campaign. An assessment of the most relevant PPPs not included and/or detected in the JOVA monitoring was performed by investigating detected environmental concentrations in the Swedish monitoring programs and their predicted environmental behaviour.

The toxicity data from the initial environmental risk assessment were collected from the pesticide properties database, PPDB (University of Hertfordshire, 2013), EFSA (European Food Safety Authority) reports (EFSA 2005b; 2010a; 2010b; 2010c), the EU Pesticides database (2013) and the Office of Pesticide Programs (OPP) Pesticide Ecotoxicity Database (<http://www.ipmcenters.org/Ecotox/DataAccess.cfm>) and review reports from the European Commission (2001). The lack of toxicity data were complemented with data documentation from producers, draft to the EFSA draft assessment report (DAR) from UK, unpublished reports and open literature provided by the Norwegian Food Safety Authority, toxicity data from EFSA risk assessment documents and the PPDB (University of Hertfordshire, 2013).

2.2 Cumulative environmental risk assessment

The cumulative environmental risk assessment of mixtures of PPPs was performed using the framework presented by Backhaus and Faust (2012), figure 1. The PPPs detected together in a given sample were considered to constitute the mixture that was to be assessed. In a first approach a risk quotient based on the sum of MEC/PNEC ratios of the detected PPPs in each sample ($RQ_{MEC/PNEC}$) was calculated. In a second approach, the risk for the different trophic levels and species groups was assessed by calculating the sum of toxic units (STU) for relevant PPPs, to obtain a RQ_{STU} after application of an appropriate assessment factor ($STU_{(algae, crustaceans, fish, aquatic\ plants)} * assessment\ factor = RQ_{STU}$). The RQ_{STU} was calculated for all trophic levels to identify main risk contributors for a given trophic level. As there is no guideline for how to determine the assessment factor to be used for calculating the RQ_{STU} , use of an assessment factor of 100, and a RQ_{STU} limit of ≥ 1 for indication of risk was applied as a generic approach in order to make the limit for RQ_{STU} similar to the trigger value for risk identification for single PPPs, $TER \geq 100$ for daphnia and fish (see Petersen et al. 2013, for details). The TU (toxic unit) approach is related to the TER approach used for risk assessment of individual PPPs as shown in equation 1 and 2:

$$TER = EC_{50}/PEC \quad (1)$$

$$TU = PEC/EC_{50} \quad (2)$$

The trigger values for daphnia and fish $TER \geq 100$ correspond to a $STU \leq 0.01$. To be able to directly compare $RQ_{MEC/PNEC}$ and RQ_{STU} , the STU-values were multiplied by an assessment factor of 100 and a $RQ_{STU} \leq 1$ was used for indication of minimal risk (Petersen et al., 2013).

The environmental cumulative risk of PPPs monitored in the 2012 JOVA monitoring campaign (Petersen et al. 2013) was re-calculated based on the expanded toxicity dataset and the adjusted PNEC values with the same assessment factors to calculate the RQ_{STU} for direct comparison.

The environmental cumulative risk of PPPs monitored in the 2013 JOVA monitoring campaign was calculated based on the expanded toxicity dataset and use of appropriate assessment factors evaluated by experimental studies (see chapter 3.3).

In order to evaluate whether use of different datasets and methods for derivation of PNECs and other established reference values like EQSs (Kontiotkari and Mattsoff, 2011) and environmental hazard values (MF values, miljøfarlighetsverdier, derived by Bioforsk http://www.bioforsk.no/ikbViewer/page/prosjekt/tema/artikkel?p_dimension_id=18844&p_menu_id=18851&p_sub_id=18845&p_document_id=91227&p_dim2=18854) had an impact of the predicted risk, risk quotients based on sum of MEC/PNEC ($RQ_{MEC/PNEC}$), MEC/EQS ($RQ_{MEC/EQS}$) and MEC/MF ($RQ_{MEC/MF}$) were calculated for the 2013 JOVA monitoring data.

Grouping of PPPs according to their mechanistic or structural similarity prior to the cumulative environmental risk assessment was not performed. This approach would require different grouping to be provided for alga and plants, daphnia and fish on basis of their species-specific toxic mode of action

(MoA). In addition, the MoA of PPPs in non-target organisms are not always known. Grouping is very data-demanding and does currently not add a lot of extra value to the outcome.

2.3 Ecotoxicity tests

2.3.1 Test scenarios, experimental design and preparation of chemical mixtures

After recalculating the predicted environmental risk based on the 2012 monitoring data and the revised PNECs, one sample from Heiabekken (25.06.2012 - 05.07.2012) was chosen for ecotoxicity tests on algae, daphnia and aquatic plants. This mixture was selected as it contained a high number of different PPPs (12) and environmental risk identified by $RQ_{STUalgae}$ and $RQ_{STUaquatic\ plants} \geq 1$ (see chapter 3.2 and table 5 for more details). The measured concentrations and the MoA of the detected PPPs in the sample are presented in table 1.

Table 1. Measured environmental concentrations during the 2012 JOVA monitoring campaign of PPPs in $\mu\text{g/L}$ in the Heiabekken sample (25.06.2012 – 05.07.2012) and Mørdrebekken sample (31.07.2012 – 08.08.2012), pesticide class and mode of action (MoA).

Compound	Heiabekken environmental concentrations in $\mu\text{g/L}$	Mørdrebekken environmental concentrations in $\mu\text{g/L}$	Pesticide class	Mode of action
Azoxystrobin	0.044	0.045	Fungicide	Respiration inhibitor
Cyprodinil	0.022	n.d.	Fungicide	Systemic, absorbed through foliage. Inhibits protein synthesis
Imidacloprid	0.25	1.1	Insecticide	Systemic with contact and stomach action. Acetylcholine receptor agonist
Iprodione	0.52	n.d.	Fungicide	Contact action with protectant and some eradicant activity. Signal transduction inhibitor
Metalaxyl	0.10	0.29	Fungicide	Systemic with curative activity
Metribuzin	0.15	0.12	Herbicide	Selective, systemic with contact and residual activity. Inhibits photosynthesis (photosystem II)
Pencycuron	0.42	0.35	Fungicide	Non-systemic with protective action. Inhibition of mitosis and cell division.
Prothioconazole-desthio	0.022	0.067	Metabolite of prothioconazole, a fungicide	Systemic with protective, curative and eradicative action. Long lasting activity
Pyrimethanil	0.064	n.d.	Fungicide	Protective action with some curative properties
Fluroxypyr	0.10	n.d.	Herbicide	Foliar uptake causing auxin-type response. Synthetic auxin
Clopyralid	0.094	n.d.	Herbicide	Selective, systemic, absorbed through foliage. Inhibits protein synthesis
MCPA	0.27	n.d.	Herbicide	Selective, systemic with translocation. Synthetic auxin
Mandipropamid	n.d.	0.24	Fungicide	Inhibit spore germination with preventative action
Number of detected PPPs	12	7		

n.d. denotes compounds not detected in the respective sample.

To investigate whether commonly used compounds with known synergistic effects could affect the toxicity of the mixture of detected compounds, a fractionated factorial design was used with 3 concentrations of the Mørdre mixture (based on composition and concentrations detected in the sample from 31.07.2012 - 08.08.2012), and the two compounds propiconazole and lambda-cyhalothrin which are previously shown to have synergistic effects in insects (Cedergreen, 2014). The concentrations of the

detected PPPs in the Mørdrebekken sample are shown in table 1. The experimental design was prepared in MODDE v10.1.1 (Umetrics, AB, Umeå, Sweden) and is shown in figure 3. The chosen concentrations of propiconazole and lambda-cyhalothrin were based on reported EC₅₀ and NOEC concentrations in the acute 48h daphnia test (Barata et al., 2006; EFSA 2014; Mokry and Hoagland, 1990; Ochoa-Acuña et al., 2009; University of Hertfordshire, 2013) and can be found in appendix 5.

In order to identify appropriate test concentration ranges of the reconstituted mixtures in the different ecotoxicity tests, the concentration of each compound was expressed as toxic units (nominal concentration/EC₅₀), and the total mixture concentration expressed as sum of toxic units (STU) where a STU of 1 is predictive of a 50% effect. The mixtures were tested at increasing STUs in each test in order to cover the predicted 50% effect level (STU = 1).

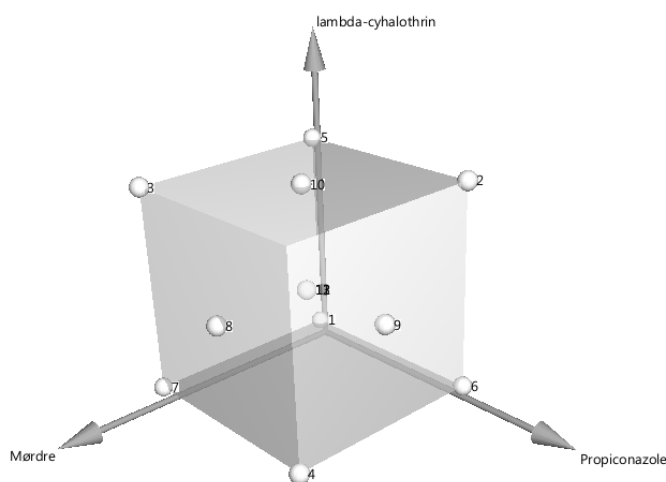


Figure 3. The experimental design of the mixture from Mørdrebekken in combination with propiconazole and lambda-cyhalothrin. Combinations are based on three different concentrations of each component; 0, low and high. For details, see appendix 5.

All of the test substances were purchased from Sigma-Aldrich and were of Pestanal® grade purity (usually with a purity of >99%). Stock-concentrations of the single compounds were prepared in DMSO before they were combined according to the environmental ratios to obtain a stock-solution of the synthetic environmental mixture. This stock was used to prepare a dilution stock-series of the mixture in DMSO. All stocks were diluted 1:10 000 in the respective test media to obtain a solvent concentration of 0.01% DMSO.

2.3.2 Ecotoxicity test with algae, crustaceans and aquatic plants

In order to evaluate the performance of the cumulative risk assessment approach, the sample chosen from Heiabekken was reconstituted in the lab and tested in increasing STUs using a battery of ecotoxicity tests; algae growth inhibition test, daphnia immobility test and lemna growth inhibition test.

A 72h algal growth inhibition test was performed according to ISO 8692 (ISO, 2012) and OECD Guideline for Testing of Chemicals No. 201: Freshwater alga and cyanobacteria, growth inhibition (OECD, 2011). The Heiabekken mixture was tested for toxicity on the freshwater species *Chlamydomonas Reinhardtii* which was incubated at 20±1°C as is the standard test temperature for this species.

Acute 48h immobilization tests with the Heiabekken synthetic mixture the Mørdrebekken synthetic mixture and the experimental design for potential synergy were conducted with *Daphnia magna*. The tests

was performed in accordance to ISO 6341 (ISO, 1996) and OECD guideline nr. 202; *Daphnia* sp. acute immobilization test (OECD, 2004). The test duration was extended from 48 to 72h in the tests of the experimental design as no effects were observed after 48h exposure.

A seven days *Lemna* sp. growth inhibition test was performed according to OECD guidelines 221 (OECD, 2006) to test the effects of the Heiabekken mixture on the growth of the aquatic plant *Lemna minor*. The test duration was seven days and the number of fronds was determined at days 0, 2, 5 and 7. The exposure media was replaced halfway through the exposure duration.

2.3.3 Chemical analysis of exposure concentrations

Stock solutions diluted in acetonitrile and exposure media from selected concentrations was sampled for chemical analysis at the start and end of selected tests. For the Heiabekken mixture, samples were split in two due to use of two separate analysis methods needed to measure all compounds in the mixture. One sub-sample was frozen and one sub-sample was added methanol and stored in the fridge until analysis (1-7 days). Samples from the test with the Mørdrebekken mixture and selected combinations from the experimental design were added methanol and stored in the fridge until analysis.

Extraction of pesticides from water samples

A modified QuEChERS-extraction of the water samples was used for the analysis of pesticides in the concentration range 10 – 1000 µg/L. For the analysis of pesticides in the concentration range 0.01 -10 µg/L, residues of pesticides were extracted from 200 mL of water sample using solid phase extraction (SPE). A detailed description of the extraction methods and chemical analysis are found in appendix 1.

Analysis of pesticides with LC-MS/MS

An Agilent 1200 series LC-system with binary pump, degasser and autosampler with cooling of samples at 5°C was used. The LC was equipped with an Agilent Zorbax Eclipse Plus C18 Rapid Resolution HT (2.1 mm x 100 mm, 1.8 µm particle size) for sample separation. The ionization and detection system consisted of an Agilent 6410B series triple quadrupole mass spectrometer equipped with an electrospray ionization source.

For quantification in the concentration range 10 – 1000 µg L⁻¹, calibration standards at 0.002, 0.005, 0.02, 0.05, 0.2, and 1 µg mL⁻¹ were prepared by diluting stock solutions of all pesticides (purchased from Dr. Ehrenstorfer, Augsburg, Germany) with acetonitrile. For quantification in the concentration range 0.01 - 10 µg L⁻¹, samples were quantified with samples of known concentration at 5, 10, 20, 50, 200, and 1000 ng L⁻¹ in tap-water with same sample extraction (SPE) method.

Analysis of pesticides with GC-MS

The measurements were performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass spectrometer using ChemStation Software version D.03.00. The gas chromatograph was equipped with a Gerstel (Mühlheim Ruhr, Germany) programmable temperature vaporizing (PTV) injector with a sintered liner. For quantification, calibration standards at 0.01, 0.05, 0.25, and 1 ng mL⁻¹ were prepared by diluting stock solutions of all pesticides (purchased from Dr. Ehrenstorfer, Augsburg, Germany) with phosphate buffer.

2.3.4 Data analysis

Results from the ecotoxicity tests with algae, daphnia and lemna after exposure to the Heiabekken mixture and Mørdrebekken mixture were analysed with Graphpad prism v 6 (Graphpad Software Inc., San Diego, CA, USA), whereas the results from the fractional factorial experimental design of the Mørdrebekken mixture in combination with lambda-cyhalothrin and propiconazole was analysed with MODDE v10.1.1 (Umetrics, AB, Umeå, Sweden). In Graphpad, data were fitted with a sigmoidal dose-response curve with variable slope (3) and because the data were normalized between 0 and 100,

constraints for top and bottom values were set at 100 and 0 respectively. The concentration was given as STU.

$$Y = \text{Bottom} + \frac{(\text{Top}-\text{Bottom})}{(1+10^{((\text{LogEC}_{50}-X)\cdot\text{slope}))}} \quad (3)$$

The STU₀₅ were used instead of NOEC values. Use of EC₀₅, or in this case STU₀₅ is applicable to all toxicological effects and makes use of all of the dose-response data to estimate the shape of the overall concentration-response relationship for a particular endpoint, and is thus often preferred to the use of NOEC. Parameters from the fitted concentration response curves (EC₅₀ and hillslope) were used to calculate the EC₀₅ by the graphpad calculator (<http://www.graphpad.com/quickcalcs/Ecanything1>).

Evaluation of the assessment factors by comparing the observed and predicted STU values (based on STU of nominal concentrations) of the tested mixtures was performed with the model deviation ratio (MDR) approach. The MDR is calculated as the ratio between the predicted effect concentration and the experimental effect concentration at a certain effect level. This ratio is often used to assess whether mixtures are additive or not. Additivity is assumed if the MDR is within a factor of two from the model prediction ($0.5 \leq \text{MDR} \leq 2$) as this ratio is within the expected inter-laboratory and inter-experiment deviation for most species (Belden and Lydy, 2006). As the predicted STU for 50% effect on the used test species were calculated by sum of toxic units for several different algae, crustaceans and aquatic plant species respectively, a deviation due to inter-species variation in sensitivity can be expected. Inter-clonal and inter-species variation of the sensitivity of cladocerans have been reported in the range of a factor of 10-12 (Baird et al., 1991; Bossuyt and Janssen, 2005). Inter-species variation in sensitivity of algae has been reported to be around 5-fold (Juneau et al., 2001). The inter-clonal variation in *Lemna gibba* have typically been reported to be a factor of 2 (Mazzeo et al., 1998), whereas inter-species variations of aquatic plants were reported to be up to a factor of 20 (Larsen et al., 1986). Therefore, deviation from predicted additive effects (50% effect at STU=1) and potential interactions were assumed only if the MDR were above a factor of 10 for daphnia, a factor of 20 for lemna and a factor of 5 for algae.

Results from the fractional factorial design was analysed with MODDE v10.1.1 (Umetrics, AB, Umeå, Sweden). The obtained model parameters R² (percentage of the variation in the response that is described by the model) and Q² (percentage of the variation in the response predicted by the model) were used to validate the model. A good biological model should have a R²>0.7 and Q² > 0.4 (Lundstedt et al., 1998).

3. Results and discussion

In the predictive cumulative environmental risk assessment presented in 2013 based on MECs of PPPs detected in 2012 (Petersen et al., 2013), eight of the 56 analysed samples had an RQ_{MEC/PNEC} ≥ 1. Four of these samples were from Mørdrebekken, two from Hotranelva, one from Skuterudbekken and one from Vasshaglona. During the cumulative environmental risk assessment several knowledge gaps were identified, including data gaps in toxicity data and uncertainties connected to the use of assessment factors for calculation of RQ_{STU}. The following work aimed to reduce the uncertainty in the cumulative environmental risk assessment by evaluating data gaps in exposure data, filling data gaps in toxicity data and evaluate the use of assessment factor for calculation of RQ_{STU} by experimental tests with algae, crustaceans and aquatic plants (lemna).

3.1 Potential exposure to non-detected PPPs

A list of heavily used, but not detected PPPs at the different investigated sites, as well as the detected PPPs were compared to a compiled list of known synergistic combinations (Cedergreen, 2014). No samples had

co-occurrence of compounds previously shown to have synergistic effects. However, at two sites; compounds that were reported to be involved in synergistic interactions were listed as “widely used” but were not detected above their respective LOQ in 2012. At one of these sites, Mørdrebekken, both propiconazole and lambda-cyhalothrin were among the PPPs used and have previously shown synergistic effects in insects (Cedergreen, 2014). The potential synergistic effect of these compounds was further investigated by laboratory studies with the crustacean *Daphnia magna* (see section 3.2).

The current monitoring data from the JOVA catchments does not provide the complete picture of the occurrence of pesticides in stream water due to the limits of the monitoring program (e.g. chemical analyses with incomplete coverage of pesticides and metabolites). Frequently used substances that are not monitored include glyphosate, diquat dibromide and sulfonylurea herbicides. Glyphosate has been detected in approximately 70 % of all assessed samples in the Swedish national pesticide monitoring program (Datavårdskap Jordbruksmark 2014), mostly at low concentrations that are not anticipated to affect aquatic organisms. The herbicide diquat dibromide is a desiccant that has been used in potatoes and other crops for several decades. It sorbs strongly to soil (BCPC 2011) but data demonstrating leaching and negative effects in soil are generally limited. Sulfonylurea herbicides are applied in low doses (10–60 g/ha) that are expected to constitute a low risk of leaching. However, recent national and international studies (Almvik et al., 2011; Cessna et al., 2010; Elliott and Cessna, 2010) have indicated that leaching can occur in concentrations that may have negative effects on the growth of aquatic plants (e.g. EFSA (2005a)), and monitoring results in Sweden have shown substantial detections of the widely used sulfonylurea herbicide tribenuron-methyl in slightly more than 5% of the samples analysed in 2002–2012 (Datavårdskap Jordbruksmark, 2014). Lambda-cyhalothrin is an insecticide sprayed in a range of crops, including grain, forage crops, vegetables, potatoes, berries, ornamentals and in forest nurseries. The limit of quantification of the chemical analysis performed in the JOVA program was 0.01 µg/L for lambda-cyhalothrin and hence, surpasses the estimated PNEC and MF-value by two orders of magnitude. Further, the maximum recommended dose for this active ingredient is at 7.5 g/ha, and hence, indicate that residual concentrations transported to agricultural streams will be low. Additional exposure to other PPPs than the ones provided in the JOVA program was not considered to have a large impact on the uncertainty reduction and was therefore not considered further. Despite so, development and expansion of the chemical analysis procedures to accommodate better characterization of the environmental exposure is warranted.

3.2 Complementing toxicity data

Most of the toxicity data gaps identified by Petersen et al. (2013) were complemented by toxicity data from the sources listed in the materials and methods. By complementing the dataset, the PNEC was adjusted for 12 of the 32 compounds with compiled toxicity data (appendix 2). For 9 compounds (aclonifen, carbendazim, cyazofamid, cyprodinil, fenamidone, mandipropamid, pyridate metabolite, pyrimethanil and trifloxystrobin metabolite) the assessment factor for calculating PNEC was reduced from 50 to 10, for imazalil the assessment factor was reduced from 100 to 10 and for kresoxim the assessment factor was reduced from 1000 to 50, leading to an overall reduction in uncertainty of the risk predictions made. The complemented toxicity data and reduction of assessment factor for these compounds resulted in a fold increase in PNEC for these compounds of 1.7 – 13.8 (with an average fold increase for these 12 compounds of 5.2). As two of these compounds (aclonifen and kresoxim) were identified as risk drivers in 2012 (Petersen et al. 2013), the importance of these as main risk drivers was reduced when recalculating the risk based on the adjusted PNEC values (table 2).

Of the eight samples identified as having a potential environmental risk in 2012 data (Petersen et al., 2013), seven samples still had a $RQ_{MEC/PNEC}$ above 1 when using the updated PNEC values. Of these seven samples (table 2), the $RQ_{MEC/PNEC}$ value was reduced by a factor of 1.02 – 3.33 for 4 samples. The effect of the revised PNECs on the calculated $RQ_{MEC/PNEC}$ in samples where risk drivers' PNECs were adjusted, highlights the need to ensure full toxicity data sets for identified main risk drivers in order to minimize the uncertainty of the risk predictions.

Table 2. Identified risk scenarios by $RQ_{MEC/PNEC}$ from the 2013 report (Petersen et al., 2013) and refined $RQ_{MEC/PNEC}$ values based on the complemented toxicity data set and revised PNECs. A fold decrease of 1 indicates no change in the $RQ_{MEC/PNEC}$ value before and after revising the PNECs.

Site	Sampling period	$RQ_{MEC/PNEC}$ from 2013 report	Revised $RQ_{MEC/PNEC}$	Fold decrease in $RQ_{MEC/PNEC}$
Hotranelva	01.07.2012 – 15.07.2012	8.14	8.14	1
Hotranelva	15.07.2012 – 30.07.2012	2.36	2.36	1
Mørdrebekken	26.06.2012 – 10.07.2012	4.34	2.84	1.5
Mørdrebekken	10.07.2012 – 16.07.2012	1.12	1.12	1
Mørdrebekken	16.07.2012 – 31.07.2012	1.24	1.24	1
Mørdrebekken	31.07.2012 – 08.08.2012	1.28	1.26	1.0*
Skuterudbekken	10.08.2012 – 28.08.2012	32.5	13.0	2.5
Vasshaglona	11.06.2012 – 25.06.2012	3.16	0.948	3.3
Number of samples with $RQ_{MEC/PNEC} \geq 1$		8	7	

*Slight change in $RQ_{MEC/PNEC}$ but not resulting in fold decrease higher than one due to the selected number of decimals.

The RQ_{STU} was calculated for all trophic levels and all samples based on the expanded effect data set and samples with $RQ_{STU} \geq 1$ are shown in table 3. Only two samples (Heiabekken 25.06.2012 - 05.07.2012 and Mørdrebekken 31.07.2012 - 08.08.2012) had RQ_{STU} values above 1 for both algae and aquatic plants with the use of AF of 100. These two samples were therefore chosen to be tested in laboratory ecotoxicity tests in order to evaluate the use of assessment factors for calculating the RQ_{STU} .

Table 3. Overview of the predicted risk based on sum of toxic units, RQ_{STU} , and the applied assessment factor (AF) for algae, crustaceans, fish and aquatic plants. Only samples with $RQ_{STU} \geq 1$ for one or more species groups are included in the table. Samples chosen as scenarios for laboratory tests are written in italics. $RQ_{STU} \geq 1$ is given in bold.

site	Sampling period	$RQ_{STU_{algae}}$ (AF 100)	$RQ_{STU_{crustaceans}}$ (AF 100)	$RQ_{STU_{fish}}$ (AF 100)	$RQ_{STU_{aquatic plants}}$ (AF 100)
Heiabekken	04.06.2012 – 25.06.2012	0.852	0.303	0.0906	1.99
<i>Heiabekken</i>	<i>25.06.2012 – 05.07.2012</i>	2.19	<i>0.312</i>	<i>0.167</i>	2.16
Hotranelva	01.07.2012 – 15.07.2012	0.0209	0.0105	0.00979	1.88
Mørdrebekken	26.06.2012 – 10.07.2012	0.214	0.0433	0.0486	5.23
<i>Mørdrebekken</i>	<i>31.07.2012 – 08.08.2012</i>	2.81	<i>0.205</i>	<i>0.136</i>	1.68
Skuterudbekken	10.08.2012 – 28.08.2012	0.0306	0.420	0.520	0.0161
Vasshaglona	11.06.2012 – 25.06.2012	0.464	0.379	0.100	3.73

3.3 Laboratory results and evaluation of assessment factors

The use of assessment factors for calculating the RQ_{STU} was evaluated by performing laboratory tests and comparing the observed STU causing 50% effect with the predicted STUs. The STU was calculated based on existing data for the respective species group for the Heiabekken mixture. The main risk drivers for this mixture are pencycuron for algae, pencycuron, azoxystrobin and iprodion for crustaceans and metribuzin for aquatic plants. Of these, only pencycuron (measured exposure concentrations) deviated by

more than 20% from nominal concentrations. All exposure and nominal concentrations are provided in appendix 4.

3.3.1 Growth inhibition of algae after 72h exposure

The experimentally observed STU for 50% effect was 1.6 for growth inhibition of algae, and is a factor of 1.6 higher than the predicted STU (figure 5). The difference between predicted and observed was within a factor of two thus within the reported factor for interspecies variation. The results therefore indicate that no strong synergistic or antagonistic interactions occurred for the current combination of chemicals and exposure concentrations. The main risk driver in this mixture was pencycuron which accounted for 96% of the total STU. The chemical analysis of the water samples showed a 67-85% reduction of exposure concentration compared to nominal start-concentrations of pencycuron and a concentration-dependent decrease from the start to the end of the studies for this compound (table A6 and A7). These deviations between nominal and measured concentrations might explain the discrepancy between observed and predicted STU.

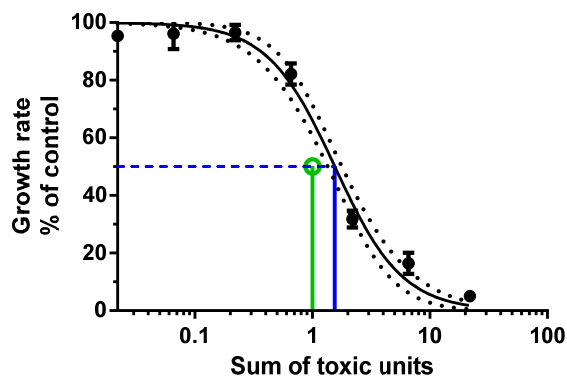


Figure 5. Concentration-response curve of the algal growth rate with 95% confidence interval after exposure to increasing concentrations of the synthetic Heiabekken mixture. The predicted sum of toxic units (STU) based on nominal concentrations are depicted by the green circle and line and the observed STU for 50% effect by the blue line.

3.3.2 Acute 48h daphnia immobility test

The observed STU for 50% effect on the acute toxicity for daphnia was 3.1, a factor of 3.1 higher than the predicted STU (figure 6). As inter-clonal and inter-species variation of crustaceans have been observed up to a factor of around 10, the observed deviation between observed and predicted STU did not give any indication of strong interactions in this mixture.

However, some uncertainty was related to the results as the mixture appeared to induce a stronger effect in a range finder test (results not shown), and may need verification. The main risk drivers for effects on daphnia in this scenario were pencycuron, azoxystrobin and iprodione. The lower than nominal concentrations of pencycuron (76.7% of nominal at start of the test) and iprodione (9.8% of nominal at start of the test) in the exposure solutions (table A8 and A9) might explain the difference in the predicted and observed STU. However, these samples were stored prior to chemical analysis for a longer time than the samples from the other ecotoxicity tests, thus higher degradation may have influenced the measured exposure concentrations. Despite these potential deviations, the combined toxicity of the mixture was considered additive.

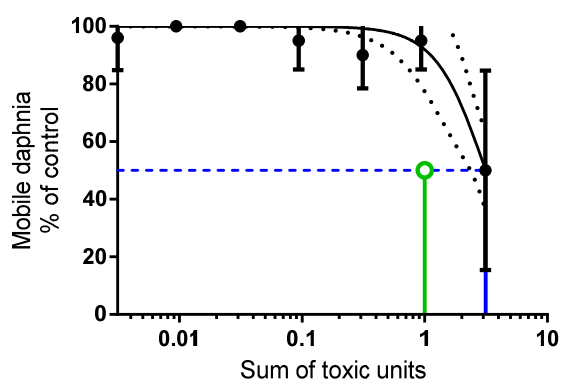


Figure 6. Mobile daphnia as percentage of the control after 48h exposure to the Heibekken mixture. The predicted sum of toxic units (STU) based on nominal concentrations are depicted by the green circle and line and the observed STU for 50% effect by the blue line.

3.3.3 Effect on the growth of Lemna

The observed STU causing 50% effect on the growth inhibition in lemna was a factor of 3.9 higher than the predicted STU (figure 7). The deviation is larger than what is expected based on inter-clonal sensitivity variations, but within the range observed for inter-species variation in sensitivity of aquatic plants. No large discrepancies between experimental and nominal concentrations of the main risk driver metribuzin were observed to indicate that the observed effects were due to experimental artefacts (table A10-A11). Thus the mixture was assumed to have an additive effect on the growth of lemna.

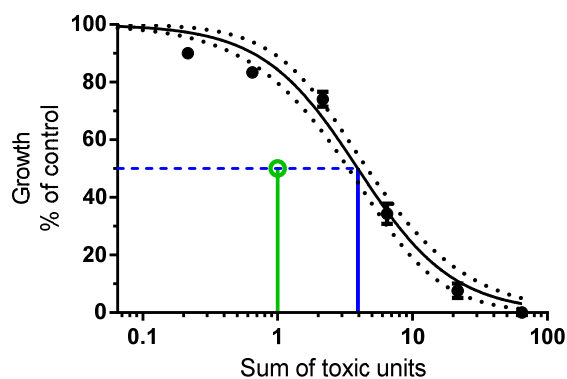


Figure 7. Growth of lemna minor expressed as percentage of control after exposure to increasing sum of toxic units of the Heibekken mixture. The predicted sum of toxic units (STU) based on nominal concentrations are depicted by the green circle and line and the observed STU for 50% effect by the blue line.

3.3.4 Potential for synergistic effects

In order to investigate if the potential for synergy could influence the toxicity of the mixture and should be considered when evaluating the use of assessment factors, an experimental design with the Mørdrebekken mixture in combination with the two potentially synergistic PPPs (lambda-cyhalothrin and propiconazole) widely used at this site was investigated with the daphnia immobilization test.

The results obtained were not optimal for fitting of a concentration-response curve, and the 50% effect was not reached within the tested concentration range (figure 8). The highest percentage of immobilization (35%) was reached at a STU of 0.205. No further increase in the percentage of immobile daphnia was observed at the higher test concentrations of up to a STU of 2.05. Some uncertainty of the actual toxicity of the mixture are linked to the effect data as the range finder study deviated from the final study.

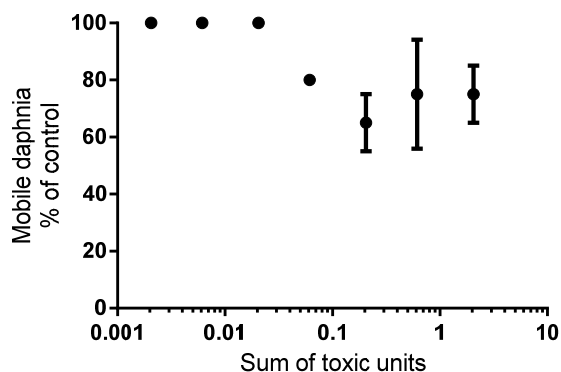


Figure 8. Effect on mobilization of *Daphnia* after 48h exposure to the synthetic Mørdrebekken mixture.

The results from the experimental design was modeled with the Modde software and the fitted model had a R^2 of 0.545 and a Q^2 of -0.094, which is lower than values indicative of good biological models ($R^2 > 0.7$ and $Q^2 > 0.4$) (Lundstedt et al., 1998). As a poor model fit was obtained, the information in the concentration-response surfaces was not assumed to explain much of the observed and predicted variation of the response. Based on the concentration-response surfaces, no clear indication of synergistic interactions occurring between the components of the mixture at the tested concentrations and combinations were observed. However, due to the poor model fit, large uncertainties were connected to the interpretation of the response surfaces and no reliable conclusion could be drawn in relation to potential synergistic interactions between the PPPs studied. An overview of the response of the different combination in the experimental design is found in appendix 5.

3.3.5 Evaluation of assessment factors for calculating RQ_{STU}

Interestingly, the observed STUs causing 50% effect in the ecotoxicity tests with *Chlamydomonas reinhardtii*, *Daphnia magna* and *Lemna minor* for the mixtures tested were within a factor of 4 from the predicted STU causing 50% effect which was based on available single chemical effect data from algae, crustaceans and aquatic plants (table 4). None of the tested species were more sensitive than predicted based on the STU calculations. This indicates that the use of cumulative risk assessment using CA-approximation as described by Backhaus and Faust (2012) provides a suitable estimate of the toxicity of the current environmental mixture. Of the tested species, algae was the most sensitive to the Heiabekken mixture (scenario 1) with a STU causing 50% effect of about 70 times the STU based on the environmental concentration, followed by lemna with a STU causing 50% effect of about 180 times the STU based on environmental concentration. *Daphnia* was the least sensitive species with a STU of 1005 times higher than the STU based on environmental concentration. The factor between the observed STU causing 50% effect and the observed STU causing 5% effect for *C. reinhardtii*, *D. magna* and *L. minor* were 7.0, 4.0 and 11.0 respectively.

Table 4. Summary of results from the ecotoxicity tests with the Heiabekken mixture showing the observed sum of toxic units (STU) causing 5% and 50%, predicted STU for 50% effect and STU of the mixture based on the measured environmental concentrations.

Species	STU for observed 5% effect	STU for observed 50% effect	STU for predicted 50% effect	STU of the mixture at environmental concentrations
Chlamydomonas	0.221	1.55	1.00	0.0219
Daphnia	0.787	3.14	1.00	0.00312
Lemna minor	0.357	3.93	1.00	0.0216

The AF proposed by Backhaus and Faust for calculating the RQ_{STU} is 1000 for algae, crustaceans and fish as only baseline data (EC_{50} values) are used to calculate the STU. In environmental risk assessment an AF of 1000 is usually used to account for the possible variations in values between acute and chronic conditions, inter- and intra-species variations and laboratory to field extrapolations. However, as the algae 72h growth inhibition test and the lemna 7 days growth inhibition test are considered chronic tests and the TER values for these in terms of approval of individual PPPs are a factor of 10 lower than the TER for crustaceans and fish, an assessment factor of 100 for calculating the RQ_{STU} of algae and lemna might be suitable. The reason for not using assessment factors similar to the TER values (TER >100 would correspond to an assessment factor of 100, whereas a TER >10 would correspond to an assessment factor of 10) is the larger uncertainty related to environmental mixtures than assessment of single PPPs. For instance, environmental samples are usually only screened for selected compounds by targeted analysis and compounds not included in the chemical analysis may thus still contribute to the risk.

The observed STU_{50} for *C. reinhardtii* was a factor of 1.6 higher than the predicted STU_{50} . The observed STU_{05} for *C. reinhardtii* was a factor of 7 lower than the observed STU_{50} . In addition, inter-species variation has been observed at a factor of around 5 (Juneau et al., 2001), meaning that the most sensitive algae species can have a STU_{05} being five-fold lower than the observed value. This results in a 13 fold difference in the overall variance (i.e. $7 \cdot 3 / 1.6$) between the observed STU_{50} in this study and the estimated STU_{05} for the most sensitive algae species. Thus an assessment factor of 100 for calculating $RQ_{STU_{algae}}$ appears to be protective for algae during the current exposure scenario.

The observed STU_{50} for *L. minor* was a factor of 3.9 higher than the predicted STU_{50} . The observed STU_{05} for *L. minor* was a factor of 11 lower than the observed STU_{50} . An inter-species variation of around 20 has been observed (Larsen et al., 1986), meaning that the most sensitive aquatic plant species can have a STU_{05} twenty-fold lower than the observed value. This results in a 56 fold difference in the overall variance (i.e. $11 \cdot 20 / 3.9$) between the observed STU_{50} in this study and the estimated STU_{05} for the most sensitive aquatic plant species. Thus an assessment factor of 100 for calculating $RQ_{STU_{aquatic\ plants}}$ appears to be protective for aquatic plants during the current exposure scenario.

The observed STU_{50} for *D. magna* was a factor of 3.1 higher than the predicted STU_{50} . The observed STU_{05} for *D. magna* was a factor of 4 lower than the observed STU_{50} . An inter-species variation of around 10 has been observed (Baird et al., 1991; Bossuyt and Janssen, 2005), meaning that the most sensitive crustacean species can have a STU_{05} ten-fold lower than the observed value. This results in a 13 fold difference in the overall variance (i.e. $4 \cdot 10 / 3.1$) between the observed STU_{50} in this study and the estimated STU_{05} for the most sensitive crustacean species. However, these effect concentrations are based on acute tests and the assessment factor should also take into account the extrapolation from acute to chronic effects which is usually a factor of 10 (Forbes and Calow, 2002), thus resulting in a 130 fold difference in the overall variance (i.e. $13 \cdot 10$). Thus an assessment factor of 1000 for calculating the $RQ_{STU_{crustaceans}}$ appears to be required to protect crustaceans during the current exposure scenario.

No strong synergistic interactions of the Mørdrebekken mixture in combination with propiconazole and lambda-cyhalothrin were observed in this study. In the review by Cedregreen et al (2014) the synergistic mixtures usually result in an MDR of within a factor of 10 from the CA prediction, and was only observed in 7% of the 194 binary pesticide mixtures investigated (Cedregreen, 2014). Thus the proposed assessment factors seem to be appropriately protective to also accommodate potential synergistic interactions of PPPs in the present exposure scenario. However, these assumptions are based on the test of only one mixture scenario, and more validation is needed to thoroughly assess the use of assessment factors in cumulative risk assessment.

No fish tests were performed in this study and no evaluation of the assessment factor for calculating $RQ_{STU_{fish}}$ was performed. An assessment factor of 1000 as proposed by Backhaus and Faust was therefore used for calculating $RQ_{STU_{fish}}$ in the environmental risk assessment of measured environmental concentrations of PPPs in the 2013 JOVA monitoring campaign.

3.4 Cumulative environmental risk assessment of PPPs detected in 2013

After filling data gaps in the toxicity data to reduce uncertainty and evaluate assessment factors for calculation of RQ_{STU} , a cumulative environmental risk assessment of PPPs detected through the JOVA monitoring campaign in 2013 was performed. In order to compare the outcome of the first step of the environmental risk assessment by use of different reference values (i.e. PNEC, MF, EQS), $RQ_{MEC/PNEC}$, $RQ_{MEC/MF}$ s and $RQ_{MEC/EQS}$ were calculated for all samples taken during the JOVA 2013 monitoring campaign. An overview of the different reference values are found in appendix 3. All samples with corresponding RQ values are found in appendix 6. The total numbers of samples with environmental risk from the mixtures expressed as $RQ_{MEC/PNEC}$ were lower than in 2012, with only 5 samples having a $RQ_{MEC/PNEC} \geq 1$ compared to 7 in 2012. The RQ values also appeared to be lower than the ones calculated for the 2012 samples except for one sample (Vasshaglona) where one compound with a very low PNEC (alpha-cypermethrin) constituted more than 99% of the total $RQ_{MEC/PNEC}$. The predicted environmental risk based on $RQ_{MEC/MF}$ identified 17 samples with potential environmental risk, whereas the $RQ_{MEC/EQS}$ identified 15 samples with potential environmental risk. This large discrepancy in risk predictions clearly indicate a need for evaluation of the use of different types of environmental reference values as the cumulative risk assessment largely depends on the data used for deriving PNECs, MFs or EQSs.

The environmental risk of mixtures of PPPs is highly variable in time as shown in figure 9 for all three sites. The potential environmental risk based on $RQ_{MEC/PNEC}$ varied several orders of magnitude, from the highest RQs in June for Vasshaglona ($RQ_{MEC/PNEC} = 3668$), in July for Mørdrebekken ($RQ_{MEC/PNEC} = 1.26$) and in August and October for Heiabekken ($RQ_{MEC/PNEC} = 1.43$ and 1.52). A similar pattern is observed when considering the $RQ_{MEC/MF}$ (figure 10), and $RQ_{MEC/EQS}$ (not shown). The date for the highest potential environmental risk was also different from site to site.

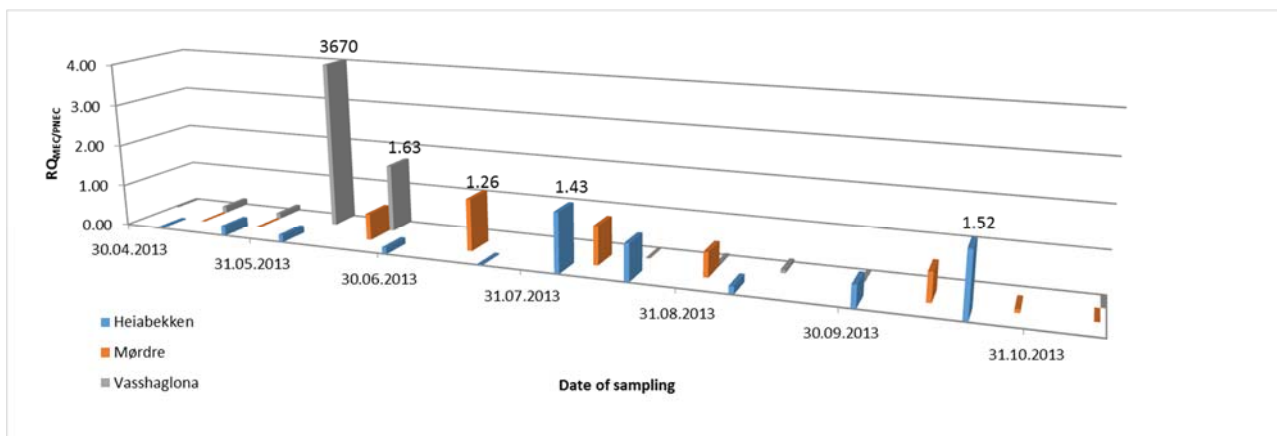


Figure 9. Temporal variation of risk calculated as $RQ_{MEC/PNEC}$

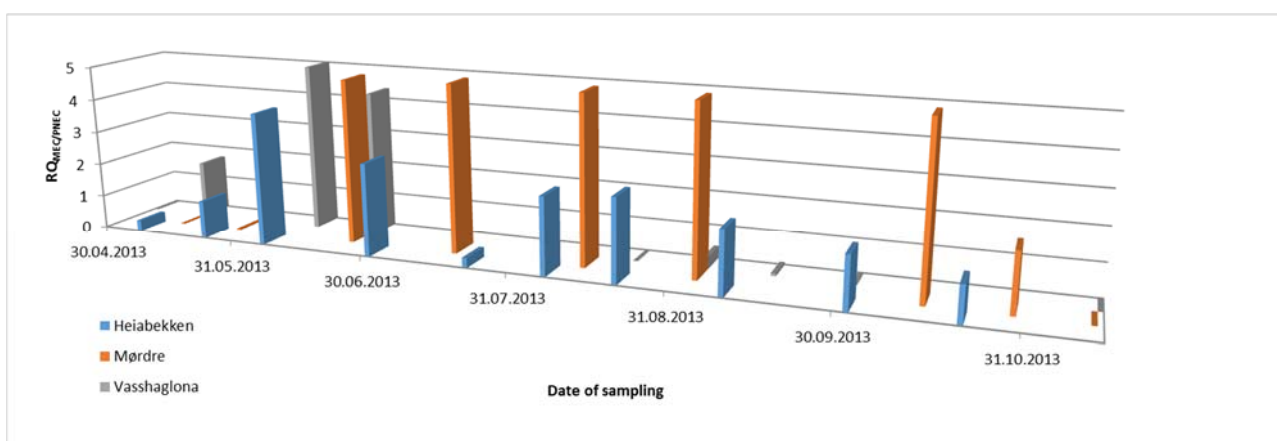


Figure 10. Temporal variation of risk calculated as $RQ_{MEC/MF}$

As many as 16 samples from the 2013 program were identified as having a predicted environmental risk based on the RQ_{STU} values. The most sensitive species vary from sample to sample and the RQ were clearly linked to the MoA of the main risk drivers in the different samples. Only two samples showed a predicted risk to all species groups based on RQ_{STU} values, one sample from Mørdrebekken and one sample from Vasshaglona. The main risk contributor in the sample from Mørdrebekken was propiconazole (78.7% of the $RQ_{STU_{algae}}$), whereas for aquatic plants the main risk driver was MCPA (93.5% of $RQ_{STU_{aquatic\ plants}}$). The main risk driver for crustaceans and fish was azoxystrobin with 99% of $RQ_{STU_{crustaceans}}$ and 81.7% of $RQ_{STU_{fish}}$. All samples with their corresponding RQ_{STU} s are shown in appendix 6.

The main contributor to the risk for algae in the sample from Vasshaglona was metribuzin (60.7 % of the total sum of risk quotients) and alfa-cypermethrin (19.4% of the total sum of risk quotients). The main contributor to the risk of aquatic plants in this sample was metribuzin (97% of the total sum of risk quotients). However, missing data for alfa-cypermethrin for aquatic plants might have led to an underestimation of the risk for aquatic plants. For crustaceans and fish, the main risk driver was alfa-cypermethrin which constituted >99% of the total sum of risk quotients in this sample. Predicted risk was observed for samples at three of the six investigated sites, Heiabekken, Mørdrebekken and Vasshaglona, with risk quotients in the order of 1.2-14.8 in Heiabekken, 2.4-18.1 in Mørdrebekken and 1.2-18647 in Vasshaglona.

3.5 Identification of need for and utility value of mitigation measures to reduce risk

Use of mitigation measures to reduce diffuse sources of pesticide contamination in agriculture is stimulated through national, regional and local subsidy programs/economical instruments. A variety of measures have proven to be effective (Reichenberger et al., 2007) and grassed buffer strips (Rasmussen et al., 2011; Syversen and Bechmann, 2004) is the main promoted measure in Norway (www.bioforsk.no/tiltak). The current Norwegian PPP regulations does not specify any specific requirements other than the specification of no-spray zones adjacent to water courses for individual PPPs. (However, several of the pesticide active ingredients included in the present risk assessment do not have any requirements regarding a no-spray zone (table 5).

Table 5. Selected characteristics of the studied pesticide active ingredients of relevance for evaluation of need for mitigation measures.

Compound	Environmental fate characteristics			Mandatory mitigation measure	Occurrence
	Mobility ^{1,2}	Persistence in soil ¹	Fate in water ¹	Required no-spray zone adjacent to water course ³	Detection frequency JOVA ⁴ (%)
Azoxystrobin (F)	Moderate	Moderate	Moderately fast photolysis	5-10 m (depending on crop)	10
Alfa-cypermethrin	Very low	Moderate	Stable	30 m	<1
Cyprodinil (F)	Low	Moderate	Moderately fast photolysis	5-30 m (depending on crop and compound mixture)	3
Fluroxypyr (H)	High	Low	Stable	-	5
Imidacloprid (I)	Moderate	High	Fast photolysis	- (tuber coating, indoor use)	9
Iprodione (F)	Moderate	Moderate	Fast hydrolysis	5-20 m (depending on crop)	3
Clopyralid (H)	Very high	Moderate	Stable	-	3
Mandipropamid (F)	Low	Moderate	Moderately fast photolysis	-	3
MCPA (H)	High	High	Fast photolysis	-	28
Metalaxyl-m (F)	Low	Moderate	Stable	0-20 m (depending on compound mixture)	11
Metribuzin (H)	High	Low	Fast photolysis	10 m	21
Pencycuron (F)	Very low	Moderate	Stable	- (tuber coating)	13
Prothioconazole-desthio (F-met)	Low	Low	-	- (metabolite. Parent prothioconazole: 0-10 m depending on compound mixture)	15
Pyrimethanil (F)	Moderate	Moderate	Stable	5 m	<1
Lambda cyhalothrin (I)	Very low	High	Stable	30 m	0
Propiconazole (F)	Low	High	Moderate hydrolysis	20-30 m (depending on compound mixture)	4

¹University of Hertfordshire, 2015. ²Assessed based on soil sorption parameters. ³Based on product label information. ⁴As percentage of samples assessed 1995-2012. Bechmann et al., 2014.

Most of these have not been identified as compounds of specific concern either from the JOVA monitoring program or the present environmental cumulative risk assessment and definition of a no-spray

zone may thus be irrelevant. There could however, be a need for further evaluation of the no-spray zones for prothioconazole, due to the wide-spread occurrence of the metabolite prothioconazole-desthio in aquatic environments, as well as challenges connected to the use of imidacloprid for potato tuber coating and in greenhouse production due to recent detections in agricultural streams (Bechmann et al., 2014; Roseth, 2012).

Among the pesticide active ingredients studied here, the fungicides pencycuron, azoxystrobin and iprodione, the herbicide metribuzin, and the insecticide alfa-cypermethrin were identified as risk drivers in the environmental exposure scenarios based on environmental concentrations from 2012 and 2013. The identification of pencycuron as an important risk driver further supports the need for an evaluation of the use of substances for potato tuber coating in relation to risk for aquatic organisms. Pencycuron is used alone or in compound mixtures with imidacloprid. The existing requirements for no-spray zone for the fungicides azoxystrobin and iprodione and the herbicide metribuzin suggest that an extension of these requirements could provide better protection of the aquatic environment. Alfa-cypermethrin is, as is also the case for lambda-cyhalothrin, very toxic to non-target aquatic organisms. The limit of quantification (0.01 µg/L) for the chemical analysis is two orders of magnitude above the estimated PNEC or MF-value, thus suggesting that the occurrence determined by the JOVA monitoring programme may underestimate the risk of this compound. Further studies to document the toxicity threshold and developing chemical analytical solutions with higher sensitivity is clearly valuable to improve our ability to identify risk scenarios and propose suitable mitigation measures.

Grassed buffer strips have in general shown effective in reducing pesticide runoff (Arora et al., 2010). During 1999-2002 this measure was tested on the plot scale under Norwegian field conditions (Syversen, 2005), focusing on moderately and strongly sorbing pesticides. However, the effect of grassed buffer strips has been shown to vary a lot (Reichenberger et al., 2007) assumedly due to site specific conditions. Pesticide fate research shows that Norwegian soil and climate conditions induce other challenges than in other parts of Europe, with slow degradation (Almvik et al., 2014) and risk of mobilization of sorbed pesticides in winter/spring (Stenrød et al., 2008). Topography will affect the transport of pesticides from soil to water and there is lack of knowledge on how this affects the efficacy of measures like grassed buffer strips (Tang et al., 2012), and this will be specifically important in countries like Norway with a large proportion of agricultural areas in relatively steep slopes. Hence, the current knowledge does not allow for specific recommendations as to whether such measures will effectively reduce the environmental risk from pesticide use under Norwegian topography, soil and climate conditions.

As a general approach, any measure that reduces the use of chemical pesticides will reduce the diffuse pollution from pesticides. First and foremost, integrated pest management practices should be promoted (Hofsvang, 2010). Current practices include widespread use of reduced pesticide doses and patch spraying, and ongoing research show promising results with regard to precision spraying (Berge et al., 2012). Further, pesticide risk maps is a developing tool aimed to enable farmers to choose pesticides of low environmental risk based on site specific weather and soil data, that will contribute to identifying vulnerable areas with high risk of pesticide loss (e.g. areas with coarse material or rapid vertical leaching) that should be avoided when spraying mobile pesticides (Eklo et al., 2009).

4. Summary and conclusions

The current project was performed to fill data gaps in exposure and toxicity data, and to evaluate the use of assessment factors for calculating RQ_{STU} . The aims were to improve the environmental cumulative risk assessment of mixtures of PPPs, and to use the improved approach to assess the environmental risk of PPPs measured during the JOVA 2013 monitoring campaign and finally to evaluate potential mitigation measures. This was achieved through the following objectives; (1) reduce uncertainty connected to lack of

exposure and toxicity data, (2) evaluation of assessment factors by ecotoxicity tests with algae, daphnia and aquatic plants, (3) environmental risk assessment of mixtures of PPPs detected in 2013 and (4) evaluate the need for and utility value of mitigation measures based on the identified risk drivers from the two investigated years.

Data gaps in exposure data were evaluated and toxicity data were compiled. The complemented toxicity data resulted in reduced uncertainty in the calculation of $RQ_{MEC/PNEC}$. A recalculation of the environmental risk of the samples taken in 2012 showed that the complemented toxicity data led to a lower number of samples with RQs indicative of environmental risk and a reduction in the RQ value for some samples.

Ecotoxicity tests showed that the toxicity of environmental mixtures was well predicted for algae, daphnia and aquatic plants based on existing toxicity data. The results from the laboratory tests were also used to evaluate the use of assessment factors for calculation of risk quotients, and led to a proposal for an assessment factor for RQ_{STU} calculation of 100 for algae and aquatic plants and 1000 for crustaceans and fish. These assessment factors were used in the environmental risk assessment of measured concentration of PPPs in 2013. Based on the $RQ_{MEC/PNEC}$, 5 samples were identified as having an environmental risk. However, by using other reference values like environmental quality standards or environmental hazard values for calculating the RQ, 15 - 17 samples were identified as having a potential environmental risk. Common for all approaches was that three of the six investigated sites had RQs indicative of environmental risk, Mørdrebekken, Heiabekken and Vasshalgona. The environmental risk of mixtures of PPPs was highly variable in time, with $RQ_{MEC/PNEC}$ as high as 3668 (June, Vasshalgona) and more moderate for Mørdrebekken in July ($RQ_{MEC/PNEC} = 1.26$) and for Heiabekken in August and October ($RQ_{MEC/PNEC} = 1.43$ and 1.52). The main risk drivers differed between species groups, sites and samples, but overall estimates identify that alfa-cypermethrin, metribuzin, azoxystrobin and propiconazole were the major risk contributors in 2013.

Mitigation measures based on identification of the main risk drivers were evaluated on basis of the current findings of risk. As a general approach, any measure that reduces the use of chemical pesticides will reduce the diffuse pollution from pesticides. First and foremost, integrated pest management practices should be promoted. Current practices include widespread use of reduced pesticide doses and patch spraying, and ongoing research shows promising results with regard to precision spraying. Pesticide risk maps is another promising approach to enable farmers to choose pesticides of low environmental risk based on site specific weather and soil data, that will contribute to identifying vulnerable areas with high risk of pesticide loss (e.g. areas with coarse material or rapid vertical leaching) that should be avoided when spraying mobile pesticides.

References

- Almvik, M., Riise, G., Bolli, R., Børresen, T., Christiansen, A., Odenmarck, S.R., and Holten, R. (2011). Multi-year transport studies of sulfonylurea herbicides from a barley field in Norway, 2007-2010 - Including development of LC-MS/MS methods for quantitative analysis of sulfonylurea herbicides and degradation products. *Bioforsk FOKUS* 6 (10). Ås (Norway): Bioforsk. ISBN 978-82-17-00750-0. (Bioforsk).
- Almvik, M., Bolli, R., Christiansen, A., Eklo, O., Holten, R., Senneset, G., and Waernhus, K. (2014). Nedbryting av soppmidler i norsk klima. *Bioforsk TEMA* vol. 9 nr 4.
- Arora, K., Mickelson, S.K., Helters, M.J., and Baker, J.L. (2010). Review of Pesticide Retention Processes Occurring in Buffer Strips Receiving Agricultural Runoff1. *JAWRA J. Am. Water Resour. Assoc.* 46, 618–647.
- Backhaus, T., and Faust, M. (2012). Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environ. Sci. Technol.* 46, 2564–2573.
- Baird, D.J., Barber, I., Bradley, M., Soares, A.M.V.M., and Calow, P. (1991). A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. *Ecotoxicol. Environ. Saf.* 21, 257–265.
- Barata, C., Baird, D.J., Nogueira, A.J.A., Soares, A., and Riva, M.C. (2006). Toxicity of binary mixtures of metals and pyrethroid insecticides to *Daphnia magna* Straus. Implications for multi-substance risks assessment. *Aquat. Toxicol.* 78, 1–14.
- BCPC, British Crop Protection Council. 2011. The e-Pesticide Manual (15th ed.) ver. 5.2 (2011-2012). Hampshire (UK): BCPC
- Bechmann, M., Stenrød, M., Pengerud, A., Grønsten, H.A., Deelstra, J., Eggestad, H.O., and Hauken, M. (2014). Erosjon og tap av næringsstoffer og plantevernmidler fra jordbruksdominerte nedbørfelt Sammendragsrapport fra Program for jord- og vannovervåking i landbruket (JOVA) for 1992-2013.
- Belden, J.B., and Lydy, M.J. (2006). Joint toxicity of chlorpyrifos and esfenvalerate to fathead minnows and midge larvae. *Environ. Toxicol. Chem. SETAC* 25, 623–629.
- Belden, J.B., Gilliom, R.J., and Lydy, M.J. (2007). How well can we predict the toxicity of pesticide mixtures to aquatic life? *Integr. Environ. Assess. Manag.* 3, 364–372.
- Berge, T.W., Goldberg, S., Kaspersen, K., and Netland, J. (2012). Towards machine vision based site-specific weed management in cereals. *Comput. Electron. Agric.* 81, 79–86.
- Bossuyt, B.T.A., and Janssen, C.R. (2005). Copper toxicity to different field-collected cladoceran species: intra- and inter-species sensitivity. *Environ. Pollut.* 136, 145–154.
- Bundschuh, M., Goedkoop, W., and Kreuger, J. (2014). Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept—experiences from long-term measurements. *Sci. Total Environ.* 484, 84–91.
- Cedergreen, N. (2014). Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies within Environmental Toxicology. *PLoS ONE* 9, e96580.
- Cessna, A.J., Elliott, J.A., and Bailey, J. (2010). Leaching of Three Sulfonylurea Herbicides during Sprinkler Irrigation. *J. Environ. Qual.* 39, 365.
- Datavårdskap Jordbruksmark (2014). Databas SLU Jordbruksvatten version 2014:5.
- Deneer, J.W. (2000). Toxicity of mixtures of pesticides in aquatic systems. *Pest Manag. Sci.* 56, 516–520.
- EFSA (2005a). Conclusion regarding the peer review of the pesticide risk assessment of the active substance rimsulfuron. European Food Safety Authority.
- EFSA (2005b). Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance Dichlorprop. Scientific Report 52, 1-67. European Food Safety Authority.
- EFSA (2010a). Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance Azoxystrobin. 8(4): 1542. European Food Safety Authority.
- EFSA (2010b). Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance Carbendazim. 8(5): 1598. Parma, Italy: European Food Safety Authority.
- EFSA (2010c). Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance Pencycuron. 8(10):1828. Parma, Italy: European Food Safety Authority.

- EFSA (2013). Guidance on the tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters.
- EFSA (2014). Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance lambda-cyhalothrin. 12(5):3677. Parma, Italy: European Food Safety Authority.
- Eklo, O.M., Bolli, R., Kværner, J., Sveistrup, T., Hofmeister, F., Solbakken, E., Jarvis, N., Stenemo, F., Romstad, E., Glorvigen, B., et al. (2009). Tools for environmental planning to reduce risks of leaching and runoff of pesticides to groundwater. 18th World IMACS / MODSIM Congress, Cairns, Australia.
- Elliott, J.A., and Cessna, A.J. (2010). Transport of two sulfonylurea herbicides in runoff from border dyke irrigation. *J. Soil Water Conserv.* 65, 298–303.
- European Commission, 2001. Review report for the active substance pyridate. 7576/VI/97-final. European Commission, Health and consumer protection directorate-general- Directorate E – Food safety. 42p.
- EU Pesticide Database, accessed 27.06.13,
http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.selection
- Faust, M., Altenburger, R., Backhaus, T., Blanck, H., Boedeker, W., Gramatica, P., Hamer, V., Scholze, M., Vighi, M., and Grimme, L.H. (2001). Predicting the joint algal toxicity of multi-component s-triazine mixtures at low-effect concentrations of individual toxicants. *Aquat. Toxicol. Amst. Neth.* 56, 13–32.
- Finizio, A., Villa, S., and Vighi, M. (2005). Predicting pesticide mixtures load in surface waters from a given crop. *Agric. Ecosyst. Environ.* 111, 111–118.
- Forbes, V.E., and Calow, P. (2002). Extrapolation in Ecological Risk Assessment: Balancing Pragmatism and Precaution in Chemical Controls Legislation Extrapolation is a practical necessity in ecological risk assessment, but there is much room for improvement in the extrapolation process. *BioScience* 52, 249–257.
- Hofsvang, T. (2010). Integrert plantevern. *Bioforsk TEMA* vol. 5 nr, 12.
- ISO (1996). Test No. 6341: Water quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustaceas) - Acute toxicity test. International Organization for Standardization.
- ISO (2012). Test No. 8692: Water quality - Fresh water algal growth inhibition test with unicellular green algae. International Organization for Standardization.
- Juneau, P., Dewez, D., Matsui, S., Kim, S.-G., and Popovic, R. (2001). Evaluation of different algal species sensitivity to mercury and metolachlor by PAM-fluorometry. *Chemosphere* 45, 589–598.
- Kienzler, A., Berggren, E., Bessems, J., Bopp, S., van der Linden, S., and Worth, A. Assessment of Mixtures - Review of Regulatory Requirements and Guidance (Joint Research Centre).
- Kontiokari, V., and Mattsoff, L. (2011). Proposal of Environmental Quality Standards for Plant Protection Products. Finnish Environment Institute (SYKE).
- Kortenkamp, A., Backhaus, T., and Faust, M. (2009). State of the art report on mixture toxicity.
- Larsen, D., DeNoyelles, F., Stay, F., and Shiroyama, T. (1986). Comparison of single-species microcosm and experimental pond responses to atrazine exposure. *Environ. Toxicol. Chem.* 5, 179–190.
- Lundstedt, T., Seifert, E., Abramo, L., Thelin, B., Nyström, Å., Pettersen, J., and Bergman, R. (1998). Experimental design and optimization. *Chemom. Intell. Lab. Syst.* 42, 3–40.
- Malaj, E., Ohe, P.C. von der, Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., and Schäfer, R.B. (2014). Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci.* 111, 9549–9554.
- Mazzeo, N., Dardano, B., and Marticorena, A. (1998). Interclonal variation in response to simazine stress in *Lemna gibba* (lemnaceae). *Ecotoxicology* 7, 151–160.
- Mokry, L.E., and Hoagland, K.D. (1990). Acute toxicities of five synthetic pyrethroid insecticides to *Daphnia magna* and *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 9, 1045–1051.
- Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., and Hollender, J. (2014). How a Complete Pesticide Screening Changes the Assessment of Surface Water Quality. *Environ. Sci. Technol.* 48, 5423–5432.
- Ochoa-Acuña, H.G., Bialkowski, W., Yale, G., and Hahn, L. (2009). Toxicity of soybean rust fungicides to freshwater algae and *Daphnia magna*. *Ecotoxicology* 18, 440–446.
- OECD (2004). Test No. 202: *Daphnia* sp. Acute Immobilisation Test. Organisation for Economic Co-operation and Development.
- OECD (2006). Test No. 221: *Lemna* sp. Growth Inhibition Test. Organisation for Economic Co-operation and Development.

- OECD (2011). Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Organisation for Economic Co-operation and Development.
- Petersen, K., Stenrød, M., and Tollefsen, K.E. (2013). Initial environmental risk assessment of combined effects of plant protection products in six different areas in Norway. NIVA report 6588-2013.
- Petersen, K., Heiaas, H.H., and Tollefsen, K.E. (2014). Combined effects of pharmaceuticals, personal care products, biocides and organic contaminants on the growth of *Skeletonema pseudocostatum*. *Aquat. Toxicol.* 150, 45–54.
- Rasmussen, J.J., Baatrup-Pedersen, A., Wiberg-Larsen, P., McKnight, U.S., and Kronvang, B. (2011). Buffer strip width and agricultural pesticide contamination in Danish lowland streams: Implications for stream and riparian management. *Ecol. Eng.* 37, 1990–1997.
- Reichenberger, S., Bach, M., Skitschak, A., and Frede, H.-G. (2007). Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; a review. *Sci. Total Environ.* 384, 1–35.
- Rodney, S.I., Teed, R.S., and Moore, D.R.J. (2013). Estimating the Toxicity of Pesticide Mixtures to Aquatic Organisms: A Review. *Hum. Ecol. Risk Assess. Int. J.* 19, 1557–1575.
- Roseth, R. (2012). Veksthus med produksjon av potteplanter - plantevernmidler i avrenning, avfall og grunnvann. Bioforsk report 7(26)2012.
- Scientific Committee on Health and Environmental Risks Scientific (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and Scientific Committee on Consumer Safety (SCCS) (2012). Final opinion on: Toxicity and Assessment of Chemical Mixtures.
- Stenrød, M., Perceval, J., Benoit, P., Almvik, M., Bolli, R.I., Eklo, O.M., Sveistrup, T.E., and Kværner, J. (2008). Cold climatic conditions: Effects on bioavailability and leaching of the mobile pesticide metribuzin in a silt loam soil in Norway. *Cold Reg. Sci. Technol.* 53, 4–15.
- Syversen, N. (2005). Cold-climate vegetative buffer zones as pesticide-filters for surface runoff. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* 51, 63–71.
- Syversen, N., and Bechmann, M. (2004). Vegetative buffer zones as pesticide filters for simulated surface runoff. *Ecol. Eng.* 22, 175–184.
- Tang, X., Zhu, B., and Katou, H. (2012). A review of rapid transport of pesticides from sloping farmland to surface waters: Processes and mitigation strategies. *J. Environ. Sci.* 24, 351–361.
- The European Commission (2011). Commission regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products.
- Tollefsen, K.E., Petersen, K., and Rowland, S.J. (2012). Toxicity of Synthetic Naphthenic Acids and Mixtures of These to Fish Liver Cells. *Environ. Sci. Technol.* 46, 5143–5150.
- University of Hertfordshire (2013). The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006-2013.
- Verbruggen, W., and Brink, P. (2010). Review of recent literature concerning mixture toxicity of pesticides to aquatic organisms.
- Walter, H., Consolaro, F., Gramatica, P., Scholze, M., and Altenburger, R. (2002). Mixture toxicity of priority pollutants at no observed effect concentrations (NOECs). *Ecotoxicol. Lond. Engl.* 11, 299–310.

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Appendix 1 – Chemical analysis

Extraction of pesticides from water samples

A modified QuEChERS-extraction of water samples was used for the analysis of pesticides in the concentration range 10 – 1000 µg L⁻¹. Residues of pesticides (listed in tables A1 and A2) were extracted from 10 mL of bioassay water mixing with 10 mL acetonitrile (Pestiscan, LAB-SCAN POCH SA, Gliwice, Poland) and Citratextraction tube, Supelco 55227-U in 50 mL centrifuge tubes. The samples were extracted by end-over-end shaking (10 min; Reax2, Heidolph). After centrifugation (3000 rpm, 5 min), 1.0 mL of the supernatant was filtrated into a LC-vial for analysis on LC-MS/MS.

For the analysis of pesticides in the concentration range 0.01 -10 µg L⁻¹, residues of pesticides were extracted from 200 mL of water sample using solid phase extraction (SPE). Pesticides listed in table A1 were extracted using solid phase extraction cartridges (Phenomenex, Strata X-CW 200 mg, 6 mL) mounted on a vacuum manifold (IST VacMaster) and conditioned with 10 mL acetonitrile and then 10 mL of Milli-Q water before the application of sample (pH 5-7). The sample was applied to the SPE using a 60 mL sample reservoir with a flow giving separate droplets out of the cartridge. After application of sample the SPE cartridges were washed with 4 mL 7% acetonitrile in Milli-Q water, and then dried for 30 min under suction with air. The samples were eluted with 4 mL acetonitrile with 5 % formic acid into a sample tube. The sample eluent was evaporated to dryness under a flow of nitrogen at 40 °C. The sample was dissolved in 250 µL of acetonitrile. A 190 µL portion of the sample was mixed with 140 µL of Milli-Q water and filtrated into a LC-vial with 400 µL insert for analysis on LC-MS/MS. Correspondingly, pesticides listed in table A2 were extracted using solid phase extraction cartridges (Isolute ENV+, 200 mg, 6 mL) mounted on a vacuum manifold (Gilson ASPEC XL) and conditioned with 5 mL methanol and then 5 mL of Milli-Q water before the application of sample (pH adjusted to 3 using 3M HCl). After application of sample the SPE cartridges were eluted with 5 mL 5% NH₃ in methanol. The methanol and NH₃ was evaporated from the sample eluent under a flow of nitrogen at 60 °C. Phosphate buffer (0.05 M, pH8, 4 mL) was added to the sample eluent. For derivatization, tetraheksylammoniumhydrogen sulphate (0.015 M in phosphate buffer, 150 µL) and penta-fluoro-benzyl-bromide (0.10 % in dichlormethane, 2.0 mL) was added to the sample. After mixing (20 min, shaker, max speed) the samples were left to settle for 5 min and then centrifuged (2500 rpm, 5-10 min). After removing the water phase, decan (20 g L⁻¹ in petrolether, 250 µL) was added to the remaining sample (approx.. 1.4 mL) before evaporation to dryness under a flow of nitrogen. The sample was dissolved in 1.4 mL iso-octane (2,2,4-trimethyl pentane). The sample was transferred to GC-vial with insert for analysis on GC-MS.

Analysis of pesticides with LC-MS/MS

An Agilent 1200 series LC-system with binary pump, degasser and autosampler with cooling of samples at 5°C was used. The LC was equipped with an Agilent Zorbax Eclipse Plus C18 Rapid Resolution HT (2.1 mm x 100 mm, 1.8 µm particle size) for sample separation. The ionization and detection system consisted of an Agilent 6410B series triple quadrupole mass spectrometer equipped with an electrospray ionization source. Pesticides listed in table A1 were analyzed as follows. Programmed injection was used with mixing 5 µL of sample with 25 µL of Milli-Q water in the injector. The mobile phase was 5 mM ammoniumformiate in Milli-Q water (A) and 5 mM ammoniumformiate in methanol (B) at a flow rate of 0.3 mL/min. A linear gradient of 10% methanol in 1 min, then linear gradient to 100 % methanol at 10 min and hold 100 % methanol for 5 min, before returning to initial conditions, was applied for the separation of the analytes on the column. Total runtime was 23 min. MS/MS was performed in the Multiple Reaction Mode (MRM) with positive electrospray ionization. Collision energy and fragmentor voltage were optimized for each compound (table A1). Two characteristic fragmentations of the protonated molecular ion [M+H]⁺ were monitored for each compound, the first and most abundant one being used for quantification, while the second one was used as a qualifier.

Pesticides listed in table A2 were analyzed as follows. Programmed injection was used with mixing 5 µL of sample with 20 µL of Milli-Q water in injector. The mobile phase was 5 mM formiate in Milli-Q water (A)

and methanol (B) at a flow of 0.3 mL min⁻¹. A linear gradient of 10% methanol to 95 % methanol at 8 min and hold 95 % methanol for 4 min, before returning to initial conditions was applied for the separation of the analytes on the column. Total runtime was 20 min. MS/MS was performed in the Multiple Reaction Mode (MRM) with positive and negative electrospray ionization (ES+ and ES-). Collision energy and fragmentor voltage were optimized for each compound (table A2). Two characteristic fragmentations of were monitored for each compound, the first and most abundant one being used for quantification, while the second one was used as a qualifier.

For quantification in the concentration range 10 – 1000 µg L⁻¹, calibration standards at 0.002, 0.005, 0.02, 0.05, 0.2, and 1 µg mL⁻¹ were prepared by diluting stock solutions of all pesticides (purchased from Dr. Ehrenstorfer, Augsburg, Germany) with acetonitrile. The calibrating standards contained triphenyl phosphate (TPP) as internal standard at 0.1 µg mL⁻¹ equal to the concentration in acetonitril used for extraction. Samples with analyte concentrations exceeding the calibration range, were reanalyzed after dilution. For quantification in the concentration range 0.01 -10 µg L⁻¹, samples were quantified with samples of known concentration at 5, 10, 20, 50, 200, and 1000 ng L⁻¹ in tap-water with same sample extraction (SPE) method. The calibrating standards contained a mixture of 2-brom bifenyl, ditalimfos, triphenyl phosphate and deca-chlor-bifenyl as internal standard at 1.0 µg mL⁻¹ equal to the concentration in acetonitril used for extraction.

Table A1. MRM conditions for LC-ESI-MS/MS analysis of the pesticides. Q1 = quantifier ion, Q2 = qualifier ion.

Compound	Precursor ES+ (m/z)	Product ions Q ₁ (Q ₂) (m/z)	Fragmentor (V)	Collision (V) Q ₁ (Q ₂)
Azoxystrobin	404	372 (344)	80	23 (30)
Lambda-cyhalothrin	467	225 (450)	110	20 (4)
Cyprodinil	226	93 (77)	110	35 (35)
Fluroxypyr	255	209 (181)	80	12 (22)
Imidacloprid	256	175 (209)	80	20 (15)
Iprodione	330	245 (56)	105	12 (44)
Mandipropamid	412	328 (356)	105	12 (4)
Metalaxyl	280	220 (192)	105	10 (15)
Metribuzin	215	187 (131)	130	15 (25)
Pencycuron	329	125 (99)	130	20 (50)
Propiconazole	342	159 (69)	140	25 (25)
Prothioconazole-desthio	312	125 (70)	140	40 (24)
Pyrimethanil	200	82 (107)	110	35 (35)
Triphenyl phosphate	327	152 (215)	185	52 (28)

Table A2. MRM conditions for LC-ESI-MS/MS analysis of the pesticides. Q1 = quantifier ion, Q2 = qualifier ion.

Compound	Precursor (m/z)	Product ions Q ₁ (Q ₂) (m/z)	Fragmentor (V)	Collision (V) Q ₁ (Q ₂)
Clopyralid	192 (ES+)	110 (174)	70	23 (30)
MCPA	199 (201) (ES-)	141 (143)	110	10 (10)

Analysis of pesticides with GC-MS

The measurements were performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass spectrometer using ChemStation Software version D.03.00. The gas chromatograph was equipped with a Gerstel (Mühlheim Ruhr, Germany) programmable temperature vaporizing (PTV) injector with a sintered liner. The separation was performed using a fused silica column (Chrompack CP-SIL 5CB MS, 50 m x 0.25 mm i.d., 0.40 µm film thickness, Varian Inc.). The temperature program was as follows; 80°C held for 1.0 min, 20°C min⁻¹ to 160°C, held for 0 min, 5°C min⁻¹ to 280°C, held for 5 min. Injection volume 5 µL. The mass spectrometer was operated in selected ion monitoring mode with target/qualifier ions as follows: clopyralid: m/z=308/310 and MCPA: m/z=380/382. For quantification, calibration standards at 0.01, 0.05, 0.25, and 1 ng mL⁻¹ were prepared by diluting stock solutions of all pesticides (purchased from Dr. Ehrenstorfer, Augsburg, Germany) with phosphate buffer. The calibrating standards contained fenoprop as internal standard at 0.2 µg mL⁻¹ equal to the concentration in phosphate buffer used for extraction.

Appendix 2 – Compiled toxicity data for algae, crustaceans, fish and aquatic plants

Table A3. Acute EC₅₀ and chronic NOEC toxicity data for algae, crustaceans, fish and aquatic plants and assessment factors used for calculating PNECs for compounds detected in 2012 and 2013.

Compound	Acute effects (µg/L)				Chronic effects (µg/L)			Assessment factor	Adhoc PNEC (µg/L)
	Algae	Crustaceans	Fish	Aquatic plants	Algae	Crustaceans	Fish		
2,4-D	24 200	100 000	63 400	580	100 000	46 200	27 200	10	58
Aclonifen	470	1 200	670	6	2.5	16	5	10	0.25
Azoxystrobin	360	55	470	3 200	800	10	147	10	1.0
Bentazone	10 100	64 000	100 000	5 400	25 700	120 000	48 000	10	540
Boscalid	3 750	5 330	2 700	n.f.	n.f.	1 300	125	50	2.5
Cyazofamid	25	190	560	33	10	110	130	10	1.0
Cyprodinil	2 600	220	2 410	7 710	570	8.8	83	10	0.88
Dicamba	1 800	41 000	100 000	450	25 000	97 000	180 000	10	45
Dichlorprop	26 500	100 000	109 000	4 100	180 000	100 000	100 000	10	410
Dimethoate	90 400	2 000	30 200	n.f.	32 000	40	400	10	4
Fenamidone	3 840	190	740	880	1 850	12.5	310	10	1.25
Fenhexamid	26 100	18 800	1 240	2 300	5 360	1 000	101	10	10.1
Phenmedipham	86	410	1 710	230	n.f.	61	320	50	1.22
Fluroxypyr	49 800	100 000	14 300	12 300	56 000	56 000	100 000	10	1 230
Imazalil	870	3 500	1 480	n.f.	457	<1 800	43	10	4.3
Imidacloprid	10 000	85 000	211 000	n.f.	10 000	1 800	9 020	10	180
Iprodione	1 800	660	3 700	1 000	3 200	170	260	10	17
Carbendazim	7 700	150	190	n.f.	2 500	1.5	3.2	10	0.15
Clopyralid	30 500	99 000	99 000	89 000	17 000	17 000	10 800	10	1 080
Kresoxim	24	186	150	n.f.	< 3	32		50	0.06
Mandipropamid	19 800	7 100	2 900	4 400	≥19 800	870	500	10	50
MCPA	32 900	190 000	50 000	152	60 000	50 000	15 000	10	15.2
Mecoprop	16 200	91 000	100 000	1 600	56 000	22 200	50 000	10	160
Metalaxyl	36 000	100 000	100 000	85 000	10 000	1 200	9 100	10	120
Metamitron	400	5 700	190 000	400	100	10 000	7 000	10	10
Metribuzin	20	49 000	74 600	8	19	320	5 600	10	0.8
Pencycuron	300	300	300	n.f.	100	50	300	10	5
Pinoxaden	5 000	8 300	10 300	13 900	630		3 200	50	12.6
Prothioconazole-desthio	70	5 500	6 630	39	n.f.	100	3.4	50	0.068
Pyridate metabolite	4 930	26 100	20 000	1 800 ^a	1 700	5 000	20 000	10	170
Pyrimethanil	1 200	2 900	10 560	7 800	1 000	940	1 600	10	94
Trifloxystrobin metabolite	77 100	95 300	106 000	n.f.	15 700	3 200	106 000	10	320
Alfa-cypermethrin	>100	0.3	28	n.f.		0.0015	0.03	50	0.00003
propiconazole	93	4800	4800	4828	51	310	95	10	5.1
Dimethomorph	29200	7900	3400		9800	5	56	10	0.5
Fluazinam	160	220	55	53600	48	12,5	12	10	1.2
Fludioxonil	24	35	230	920		2,5	39	50	0.05

We here define acute effect data as EC₅₀ values from acute tests on daphnia and fish and from the chronic tests on algae and aquatic plants. The chronic effect data are NOEC values preferably from long term studies on crustaceans and fish and chronic studies on algae. ^aNOEC value. n.f. are not found toxicity data.

Appendix 3 – Reference values; PNECs, MFs and EQSs

Table A4. Comparison of the different reference values; PNECs, the preliminary annual average proposed environmental quality standards (AA-EQS) and environmental hazard values (MF, miljøfarlighetsverdi) and limit of quantification (LOQ).

Compound	Ad hoc PNEC (µg/L)	AA EQS ¹ (µg/L)	MF (µg/L)	LOQ (µg/L)
2,4-D	58	27	2.2	0.01
Aclonifen	0.25	0.12	0.12	0.01
Alfa-cypermethrin	0.00003	0.0006	0.0001	0.01
Azoxystrobin	1	0.95	0.95	0.02
Bentazone	540	80	80	0.01
Boscalid	2.5	2.5	12.5	0.02
Cyazofamid	1	n.r.	0.25	0.02
Cyprodinil	0.88	0.18	0.18	0.01
Dicamba	45	n.r.	4.5	0.02
Dichlorprop	410	20	15	0.01
Dimethoate	4	n.r.	4	0.02
Dimetomorph	0.5	3.2	4	0.02
Fenamidone	1.25	1.25	0.25	0.02
Fenhexamid	10.1	10	28	0.02
Phenmedipham	1.22	n.l.	1	0.02
Fluazinam	1.2	0.29	2	0.02
Fludioxonil	0.05	0.1	0.5	0.02
Fluroxypyr	1230	0.46	123	0.05
Imazalil	4.3	n.r.	0.86	0.02
Imidacloprid	180	0.12	0.2	0.02
Iprodione	17	17	17	0.02
Carbendazim	0.15	0.15	0.03	0.02
Clopyralid	1080	0.72	71	0.05
Kresoxim	0.06	n.l.	100	0.02
Kresoxim-methyl	n.r.	n.l.	0.7	0.02
Mandipropamid	50	28	0.76	0.02
MCPA	15.2	1.62	1.4	0.01
Mecoprop	160	44	44	0.01
Metalaxyl	120	120	96	0.01
Metamitron	10	4	10	0.02
Metribuzin	0.8	0.058	0.058	0.02
Pencycuron	5	n.l.	4.96	0.02
Pinoxaden	12.6	n.r.	0.91	0.02
Prothioconazole-desthio	0.068	0.334	0.034	0.02
Propiconazole	5.1	1.8	0.13	0.02
Pyridate metabolite	170	n.r.	4.93	0.02
Pyrimethanil	94	n.r.	16	0.01
Trifloxystrobin metabolite	320	64	32	0.02
Lambda-cyhalothrin	0.000198	n.r.	0.0006	0.01

n.l. – not listed, n.r. - not relevant, not detected in the year of the analysis

Appendix 4 – Results of chemical analysis

For the stock concentrations of the Heiabekken mixture (table A5), the concentrations of iprodione, prothioconazole-desthio, clopyralid and MCPA deviated by more than 20% from the nominal concentrations. The concentration of iprodion was measured at almost half the nominal concentration, the concentration of prothioconazole-desthio was measured at almost double the nominal concentration, the concentration of clopyralid was measured at approximately 20% lower than nominal concentrations and MCPA was measured at 64.4% of the nominal concentration. The average deviation from the nominal concentrations was -5.3% with a standard deviation of 29.8. The main risk drivers for this mixture are pencycuron for algae, pencycuron, azoxystrobin and iprodion for crustaceans and metribuzin for aquatic plants. Of these, only pencycuron deviated by more than 20% from nominal concentrations. The chemical analysis revealed that pencycuron was lower than the nominal concentrations in both the highest and lowest test concentrations (table A6 and A7), and the start concentration deviated by more than 20% in the highest test concentration. Also the concentration decreased from the start of the test to the end of the test in both analyzed concentrations. The measured concentrations of iprodione deviated by more than 20% from nominal concentrations in both analysed test concentrations. The concentrations were much lower than nominal and decreased from the start of the test to the end of the test. Results from all the performed chemical analysis are shown in tables A5-A14 in this appendix.

Table A5. Nominal and measured stock concentrations of the Heiabekken mixture in g/L.

Compound	Nominal stock concentrations (g/L)	Measured stock concentrations (mg/L)	% deviation
Azoxystrobin	4.400	4.909	12
Cyprodinil	2.200	2.475	13
Imidacloprid	25.000	25.920	4
Iprodione	52.000	23.482	-55
Metalxyl	10.000	9.209	-8
Metribuzin	15.000	15.507	3
Pencycuron	42.000	44.831	7
Prothiokonazole-desthio	2.200	3.183	45
Pyrimethanil	6.400	6.314	-1
Fluroxypyr	10.000	10.472	5
Clopyralid	9.400	7.291	-22
MCPA	27.000	9.603	-64

Table A6. Nominal and measured concentrations of the algae exposure concentration of the Heiabekken mixture at a concentration of 0.0219 STU

Compound	Nominal concentration (µg/L)	Measured start concentration* (µg/L)	Measured end concentration* (µg/L)	Measured mean (µg/L)	% deviation
Azoxystrobin	0.044	0.050	0.042	0.046	-14
Cyprodinil	0.022	0.019	0.013	0.016	14
Imidacloprid	0.250	0.343	0.278	0.311	-37
Iprodione	0.520	0.219	0.081	0.150	58
Metalaxyl	0.100	0.140	0.107	0.123	-40
Metribuzin	0.150	0.417	0.178	0.297	-178
Pencycuron	0.420	0.355	0.183	0.269	15

Compound	Nominal concentration (µg/L)	Measured start concentration* (µg/L)	Measured end concentration* (µg/L)	Measured mean (µg/L)	% deviation
Prothiokonazole-desthio	0.022	0.045	0.017	0.031	-103
Pyrimethanil	0.064	0.059	0.038	0.048	8
Fluroxypyr	0.100	Not analysed	Not analysed		
Clopyralid	0.094	Not analysed	Not analysed		
MCPA	0.270	Not analysed	Not analysed		

*Average of 2 separate analysis.

Table A7. Nominal and measured concentrations of the algae exposure concentration of the Heiabekken mixture at a concentration of 21.9 STU, and % deviation between nominal and start concentrations.

Compound	Nominal concentration (µg/L)	Measured start concentration (µg/L)	Measured end concentration (µg/L)	Measured mean (µg/L)	% deviation
Azoxystrobin	44	40.344	44	42	8
Cyprodinil	22	14	14	14	37
Imidacloprid	250	237	221	229	5
Iprodione	520	86	42	64	84
Metalaxyl	100	83	86	85	17
Metribuzin	150	144	115	130	4
Pencycuron	420	280	300	290	33
Prothioconazole-desthio	22	29	29	29	-31
Pyrimethanil	64	53	54	54	17
Fluroxypyr	100	101	107	104	-1
Clopyralid	94	95	92	93	-1
MCPA	270	230	227	229	15

Table A8. Measured concentrations in the lowest test concentration in the daphnia test (µg/L) corresponding to STU of 0.00312, and % deviation between nominal and start concentrations.

Compound	Nominal concentration (µg/L)	Measured start concentration* (µg/L)	Measured end concentration* (µg/L)	Measured mean (µg/L)	% deviation
Azoxystrobin	0.044	0.067	0.053	0.060	-53
Cyprodinil	0.022	0.031	0.015	0.023	-39
Imidacloprid	0.250	0.438	0.253	0.346	-75
Iprodione	0.520	0.316	0.154	0.235	39
Metalaxyl	0.100	0.187	0.100	0.144	-87
Metribuzin	0.150	0.305	0.172	0.238	-103
Pencycuron	0.420	0.386	0.327	0.356	8
Prothiokonazole-desthio	0.022	0.071	0.039	0.055	-221
Pyrimethanil	0.064	0.092	0.049	0.070	-44
Fluroxypyr	0.100	0.107	0.064	0.085	-7
Clopyralid	0.094	0.049	0.056	0.052	48
MCPA	0.270	0.193	0.195	0.194	29

*Average of 2 separate analysis on refrigerated and frozen sample.

Table A9. Nominal and measured concentration of the highest mixture test concentration in the daphnia test ($\mu\text{g/L}$) corresponding to a STU of 3.12, and % deviation between nominal and start concentrations.

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% deviation
Azoxystrobin	44	43	30	37	1
Cyprodinil	22	22	16	19	2
Imidacloprid	250	242	180	211	3
Iprodione	520	51	26	39	90
Metalaxyl	100	85	64	75	15
Metribuzin	150	147	109	128	2
Pencycuron	420	322	230	276	23
Prothioconazol-destio	22	31	22	26	-39
Pyrimethanil	64	56	41	48	13
Fluroxypyr	100	107	77	92	-7
Clopyralid	94	72	59	66	23
MCPA	270	159	130	144	41

Table A10. Nominal and measured concentration of the lowest mixture test concentration in the Lemna test ($\mu\text{g/L}$) corresponding to a STU of 0.0648, and % deviation between nominal and start concentrations.

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% deviation
Azoxystrobin	0.132	0.178	0.234	0.206	-35
Cyprodinil	0.066	0.152	0.128	0.14	-130
Imidacloprid	0.75	1.47	1.07	1.27	-96
Iprodione	1.56	1.04	1.06	1.05	33
Metalaxyl	0.3	0.64	0.58	0.61	-113
Metribuzin	0.45	Not analysed	Not analysed		
Pencycuron	1.26	1.45	1.71	1.58	-15
Prothioconazol-destio	0.066	0.34	0.31	0.325	-415
Pyrimethanil	0.192	0.46	0.41	0.435	-140
Fluroxypyr	0.3	0.268	0.147	0.208	11
Clopyralid	0.282	0.405	0.314	0.360	-44
MCPA	0.81	0.818	0.706	0.762	-1

Table A11. Nominal and measured concentration of the highest mixture test concentration in the Lemna test ($\mu\text{g/L}$) corresponding to a STU of 64.8, and % deviation between nominal and measured mean concentrations.

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% deviation*
Azoxystrobin	132	105	134	120	9
Cyprodinil	66	53	68	61	8
Imidacloprid	750	565	743	654	13
Iprodione	1560	467	608	538	66

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% deviation*
Metalaxyl	300	200	260	230	23
Metribuzin	450	339	448	394	12
Pencycuron	1260	763	915	839	33
Prothioconazol-destio	66	69	88	79	-20
Pyrimethanil	192	137	174	156	19
Fluroxypyr	300	Not analysed	Not analysed		
Clopyralid	282	Not analysed	Not analysed		
MCPA	810	Not analysed	Not analysed		

Table A12. Nominal stock and measured stock concentration (g/L) of the Mørdrebekken mixture and % deviation between nominal and measured stock concentrations.

Compound	Nominal stock concentration (g/L)	Measured stock concentration (g/L)	% Deviation
Azoxystrobin	4.500	4.653	3
Imidacloprid	110.000	116.000	6
Mandipropamid	24.000	20.226	-16
Metalaxyl	29.000	24.004	-17
Metribuzin	12.000	11.808	-2
Pencycuron	35.000	28.273	-19
Prothioconazole-desthio	6.700	9.151	37

Table A13. Nominal and measured concentrations of the lowest mixture test concentration ($\mu\text{g/L}$) of the Mørdrebekken mixture tested on *Daphnia* corresponding to a STU of 0.00205, and % deviation between nominal and start concentrations.

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% Deviation
Azoxystrobin	0.045	0.073	0.050	0.620	-63
Imidacloprid	1.100	1.704	0.996	1.350	-55
Mandipropamid	0.240	0.338	0.214	0.276	-41
Metalaxyl	0.290	0.614	0.333	0.473	-112
Metribuzin	0.120	0.760	0.243	0.501	-533
Pencycuron	0.350	0.375	0.234	0.304	-7
Prothioconazole-desthio	0.067	0.229	0.120	0.174	-241

Table A14. Nominal and measured concentrations of the highest mixture test concentrations ($\mu\text{g/L}$) of the Mrdrebekken mixture tested on *Daphnia* corresponding to a STU of 2.05, and % deviation between nominal and measured start concentration. *Daphnia* mix 2

Compound	Nominal concentration ($\mu\text{g/L}$)	Measured start concentration ($\mu\text{g/L}$)	Measured end concentration ($\mu\text{g/L}$)	Measured mean ($\mu\text{g/L}$)	% Deviation
Azoxystrobin	45	43	45	44	4
Imidacloprid	1100	1104	1159	1132	-0.5
Mandipropamid	240	190	200	195	21
Metalaxyl	290	240	253	246	17
Metribuzin	120	118	125	121	2
Pencycuron	350	200	222	211	43
Prothioconazole-desthio	67	84	89	87	-26

Appendix 5 – Experimental design and responses of Mørdrebekken mixture in combination with lambda-cyhalothrin and propiconazole

Table A15. Experimental design and % immobilization of daphnia exposed for 72h.

Exp. nr	Mørdre mixture (STU)	Propiconazole (STU)	Lambda-cyhalothrin (STU)	% immobilization in daphnia after 72h
1	0	0	0	0
2	0	1	1	100
3	0.205	0	1	100
4	0.205	1	0	10
5	0	0	1	90
6	0	1	0	30
7	0.205	0	0	100
8	0.205	0.06	0.1	80
9	0.00205	1	0.1	65
10	0.00205	0.06	1	90
11	0.00205	0.06	0.1	65
12	0.00205	0.06	0.1	35
13	0.00205	0.06	0.1	20

STU concentrations of propiconazole and lambda-cyhalothrin are predicted based on previously reported EC₅₀ data for 48h immobilization in daphnia

Appendix 6 – Environmental risk assessment of PPP mixtures measured in 2013

Table A16. Calculated $RQ_{MEC/PNEC}$, $RQ_{MEC/MF}$ and $RQ_{MEC/EQS}$ based on measure concentrations of PPPs in the 2013 JOVA monitoring campaign. RQ values ≥ 1 is marked with bold.

Site	Sampling start date	Sample collection date	$RQ_{MEC/PNEC}$	$RQ_{MEC/MF}$	$RQ_{MEC/EQS}$
Heiabekken	24.04.2013	08.05.2013	0.030	0.32	0.48
Heiabekken	08.05.2013	24.05.2013	0.24	1.1	0.24
Heiabekken	24.05.2013	07.06.2013	0.17	4.0	5.1
Heiabekken	07.06.2013	01.07.2013	0.15	2.8	3.3
Heiabekken	01.07.2013	22.07.2013	0.027	0.29	0.60
Heiabekken	22.07.2013	07.08.2013	1.4	2.3	4.0
Heiabekken	07.08.2013	21.08.2013	0.88	2.5	3.1
Heiabekken	21.08.2013	10.09.2013	0.19	1.9	2.0
Heiabekken	10.09.2013	02.10.2013	0.52	1.5	2.0
Heiabekken	02.10.2013	21.10.2013	1.5	1.1	3.6
Hotrankanalen	28.04.2013	12.05.2013	0.00018	0.0018	0.00088
Hotrankanalen	12.05.2013	26.05.2013	0.0016	0.018	0.015
Hotrankanalen	26.05.2013	12.06.2013	0.0023	0.025	0.021
Hotrankanalen	12.06.2013	01.07.2013	0.015	0.17	0.14
Hotrankanalen	01.07.2013	25.07.2013	0.0046	0.049	0.04
Hotrankanalen	25.07.2013	06.08.2013	0.0018	0.019	0.015
Hotrankanalen	06.08.2013	21.08.2013	0.0015	0.016	0.012
Hotrankanalen	21.08.2013	14.09.2013	0.00044	0.0044	0.0022
Hotrankanalen	01.10.2013	20.10.2013	0.0021	0.021	0.011
Hotrankanalen	20.10.2013	04.11.2013	0.00030	0.0030	0.0015
Mørdrebekken	22.04.2013	13.05.2013	0.00019	0.0050	0.00041
Mørdrebekken	13.05.2013	27.05.2013	0.0024	0.026	0.022
Mørdrebekken	27.05.2013	23.06.2013	0.64	4.9	1.6
Mørdrebekken	23.06.2013	16.07.2013	1.3	11	6.2
Mørdrebekken	16.07.2013	12.08.2013	0.93	9.7	32
Mørdrebekken	12.08.2013	03.09.2013	0.59	6.5	3.5
Mørdrebekken	03.09.2013	14.10.2013	0.68	5.0	0.67
Mørdrebekken	14.10.2013	29.10.2013	0.085	1.8	0.18
Mørdrebekken	29.10.2013	11.11.2013	0.00040	0.0030	0.0017
Skuterudbekken	24.04.2013	08.05.2013	0.00029	0.0029	0.0015
Skuterudbekken	08.05.2013	24.05.2013	0.00024	0.0024	0.0012
Skuterudbekken	24.05.2013	07.06.2013	0.0066	0.071	0.062
Skuterudbekken	07.06.2013	01.07.2013	0.087	0.85	3.0
Skuterudbekken	01.07.2013	24.07.2013	0.0021	0.023	0.11
Skuterudbekken	24.07.2013	07.08.2013	0.0046	0.050	0.043

Site	Sampling start date	Sample collection date	RQ _{MEC} /PNEC	RQ _{MEC} /MF	RQ _{MEC} /EQS
Skuterudbekken	07.08.2013	21.08.2013	0.0018	0.019	0.016
Skuterudbekken	21.08.2013	12.09.2013	0	0	0
Skuterudbekken	12.09.2013	02.10.2013	0.3695	0.75	0.24
Skuterudbekken	02.10.2013	21.10.2013	0	0	0
Timebekken	27.05.2013	03.06.2013	0.000022	0.00015	0.00015
Timebekken	03.06.2013	10.06.2013	0.000020	0.00014	0.00014
Timebekken	10.06.2013	24.06.2013	0.0010	0.011	0.0095
Timebekken	24.06.2013	08.07.2013	0.0098	0.10	0.21
Timebekken	08.07.2013	22.07.2013	0.000096	0.00065	0.00065
Timebekken	22.07.2013	05.08.2013	0.0097	0.12	0.084
Timebekken	05.08.2013	19.08.2013	0.0059	0.063	0.24
Timebekken	19.08.2013	02.09.2013	0.00013	0.0010	0.14
Timebekken	02.09.2013	16.09.2013	0.53	0.00032	0
Timebekken	16.09.2013	30.09.2013	0.000041	0.00028	0.00028
Vasshaglona	15.04.2013	30.04.2013	0.017	0.0037	0.018
Vasshaglona	30.04.2013	13.05.2013	0.15	1.7	1.7
Vasshaglona	13.05.2013	27.05.2013	0.13	0.84	0.87
Vasshaglona	27.05.2013	10.06.2013	3700	1100	190
Vasshaglona	10.06.2013	24.06.2013	1.6	4.3	3.7
Vasshaglona	05.08.2013	20.08.2013	0	0	0
Vasshaglona	20.08.2013	03.09.2013	0.036	0.35	0.31
Vasshaglona	03.09.2013	16.09.2013	0.073	0.075	0.37
Vasshaglona	16.09.2013	01.10.2013	0.0014	0.014	0.012
Vasshaglona	28.10.2013	12.11.2013	0.015	0.0035	0.015

Values given as 0 indicates where no substances were measured or where an EQS was not found for the detected compound(s).

Table A17. Calculated RQ_{STU} based on data from algae, crustaceans, fish and aquatic plants. RQ_{STU} values ≥ 1 are marked with bold. An assessment factor of 100 was used for calculation of RQ_{STU}algae and RQ_{STU}aquatic plants, and an assessment factor of 1000 was used for calculation of RQ_{STU}fish and RQ_{STU}crustaceans. RQ_{STU} ≥ 1 are in bold.

Site	Sampling start date	Sample collection date	RQ _{STU} algae	RQ _{STU} crustaceans	RQ _{STU} fish	RQ _{STU} aquatic plants	
Heiabekken	24.04.2013	08.05.2013	0.051		0.50	0	
Heiabekken	08.05.2013	24.05.2013	0.0034		0.28	1.8	0.0035
Heiabekken	24.05.2013	07.06.2013	0.67		0.17	0.16	1.6
Heiabekken	07.06.2013	01.07.2013	0.58		0.28	0.28	1.4
Heiabekken	01.07.2013	22.07.2013	0.044		0.44	0.44	0.00069
Heiabekken	22.07.2013	07.08.2013	0.75		15	9.8	0.65
Heiabekken	07.08.2013	21.08.2013	0.61		9.0	1.8	1.0
Heiabekken	21.08.2013	10.09.2013	0.52		1.2	0.46	1.2
Heiabekken	10.09.2013	02.10.2013	0.29		7.9	0.93	0.43

Site	Sampling start date	Sample collection date	RQSTUalgae	RQSTUcrustaceans	RQSTUfish	RQSTUaquatic plants
Heiabekken	02.10.2013	21.10.2013	0.26	6.8	1.9	0.20
Hotrankanalen	28.04.2013	12.05.2013	0.000073	0.00059	0.00053	0
Hotrankanalen	12.05.2013	26.05.2013	0.000076	0.00013	0.00050	0.016
Hotrankanalen	26.05.2013	12.06.2013	0.00019	0.00092	0.0013	0.021
Hotrankanalen	12.06.2013	01.07.2013	0.00092	0.0016	0.0049	0.15
Hotrankanalen	01.07.2013	25.07.2013	0.00058	0.0020	0.0027	0.042
Hotrankanalen	25.07.2013	06.08.2013	0.00026	0.0017	0.0018	0.013
Hotrankanalen	06.08.2013	21.08.2013	0.00019	0.0012	0.0014	0.011
Hotrankanalen	21.08.2013	14.09.2013	0.00018	0.0015	0.0013	0
Hotrankanalen	01.10.2013	20.10.2013	0.00087	0.0070	0.0063	0
Hotrankanalen	20.10.2013	04.11.2013	0.00013	0.0010	0.00091	0
Mørdrebekken	22.04.2013	13.05.2013	0.000045	0.00011	0.00017	0.0019
Mørdrebekken	13.05.2013	27.05.2013	0.00011	0.00019	0.00072	0.024
Mørdrebekken	27.05.2013	23.06.2013	0.64	9.1	1.3	0.79
Mørdrebekken	23.06.2013	16.07.2013	1.4	18	2.6	1.3
Mørdrebekken	16.07.2013	12.08.2013	1.4	12	2.8	0.71
Mørdrebekken	12.08.2013	03.09.2013	0.94	8.6	1.4	0.090
Mørdrebekken	03.09.2013	14.10.2013	0.63	2.4	0.48	0.12
Mørdrebekken	14.10.2013	29.10.2013	0.25	0.97	0.20	0.0067
Mørdrebekken	29.10.2013	11.11.2013	0.00034	0.0013	0.0011	0.00039
Skuterudbekken	24.04.2013	08.05.2013	0.00012	0.00098	0.00088	0
Skuterudbekken	08.05.2013	24.05.2013	0.00010	0.00081	0.00073	0
Skuterudbekken	24.05.2013	07.06.2013	0.00030	0.00053	0.0020	0.066
Skuterudbekken	07.06.2013	01.07.2013	0.0072	0.083	0.088	0.71
Skuterudbekken	01.07.2013	24.07.2013	0.00031	0.00084	0.0013	0.021
Skuterudbekken	24.07.2013	07.08.2013	0.00021	0.00037	0.0014	0.046
Skuterudbekken	07.08.2013	21.08.2013	0.00016	0.00082	0.0011	0.016
Skuterudbekken	21.08.2013	12.09.2013	0	0	0	0
Skuterudbekken	12.09.2013	02.10.2013	0.036	0.0084	0.012	0.073
Skuterudbekken	02.10.2013	21.10.2013	0	0	0	0
Timebekken	27.05.2013	03.06.2013	0.00012	0.00019	0.00012	0.00022
Timebekken	03.06.2013	10.06.2013	0.00011	0.00017	0.00011	0.00020
Timebekken	10.06.2013	24.06.2013	0.00024	0.00039	0.00050	0.010
Timebekken	24.06.2013	08.07.2013	0.0067	0.011	0.013	0.098
Timebekken	08.07.2013	22.07.2013	0.00051	0.00081	0.00052	0.0096
Timebekken	22.07.2013	05.08.2013	0.0078	0.015	0.017	0.093
Timebekken	05.08.2013	19.08.2013	0.0015	0.0030	0.0088	0.059
Timebekken	19.08.2013	02.09.2013	0.00054	0.0013	0.0050	0.0013
Timebekken	02.09.2013	16.09.2013	0.13	0.17	0.21	0
Timebekken	16.09.2013	30.09.2013	0.00022	0.00034	0.00022	0.00041

Site	Sampling start date	Sample collection date	RQ _{STU} algae	RQ _{STU} crustaceans	RQ _{STU} fish	RQ _{STU} aquatic plants
Vasshaglona	15.04.2013	30.04.2013	0.0013	0.0084	0.016	0.00037
Vasshaglona	30.04.2013	13.05.2013	0.50	0.20	0.21	1.2
Vasshaglona	13.05.2013	27.05.2013	0.25	0.17	0.20	0.59
Vasshaglona	27.05.2013	10.06.2013	1.1	19000	280	1.7
Vasshaglona	10.06.2013	24.06.2013	1.5	3.6	0.92	2.2
Vasshaglona	05.08.2013	20.08.2013	0	0	0	0
Vasshaglona	20.08.2013	03.09.2013	0.0016	0.0045	0.039	0.32
Vasshaglona	03.09.2013	16.09.2013	0.031	0.30	0.33	0.044
Vasshaglona	16.09.2013	01.10.2013	0.000097	0.00024	0.00052	0.013
Vasshaglona	28.10.2013	12.11.2013	0.0014	0.0077	0.014	0.00085

Values given as 0 indicates where no substances were measured or where an EQS was not found for the detected compound(s).

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