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Initial environmental risk assessment of combined effects of plant protection products in six different areas in Norway

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REPORT

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Abstract

The combined effect of plant protection products (PPPs) in Norwegian streams, rivers and shallow ground water was assessed for the potential risk to non-target organisms by cumulative risk assessment. Risk quotients were calculated based on available effect data for algae, crustaceans, fish and aquatic plants, and measured environmental concentrations of PPPs at six different sampling sites in Norway. Four out of the six sites obtained a risk quotient above one which is indicative of risk. The risk at each site appeared to be driven by a few compounds and these toxicity drivers for the risk of combined toxicity of PPPs were identified for future consideration of mitigation measures.

4 keywords, Norwegian	4 keywords, English
1. Plantevernmidler	1. Plant Protection Products
2. Kummulativ risikovurdering	2. Cumulative risk assessment
3. Kombinerte effecter	3. Combined effects

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Preface

This report 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 a collaboration between NIVA and Bioforsk. Marianne Stenrød from Bioforsk retrieved data from the JOVA program. Karina Petersen retrieved effect data for different organisms and performed the cumulative risk assessment. Marianne Stenrød, Karina Petersen and Knut Erik Tollefsen all contributed to the writing of the report.

Oslo, 14.11.2013

Knut Erik Tollefsen

Contents

Summary

As plant protection products (PPPs) are risk assessed individually but applied in combinations on the same crop, there is a need to consider the cumulative risk of environmentally relevant mixtures of PPPs to protect organism in the vicinity of agricultural areas from untargeted effects. In this study the combined effect of plant protection products in Norwegian agricultural streams was assessed for identification of potential risk to non-target organisms living in these water recipients. This was performed by a cumulative risk assessment based on available effect and exposure data. Effect data for algae, crustaceans, fish and aquatic plants were collected from various databases and used for calculating predicted no effect concentrations (PNEC). Measured environmental concentrations (MEC) of PPPs at six different monitoring sites in Norway were obtained through the JOVA program. The composition of compounds and concentrations varied between the different monitoring sites and also with the different sampling time points at each site.

In a first approach, a risk quotient based on the sum of the MEC/PNEC ratios of the detected PPPs in each sample ($RQ_{MEC/PNEC}$) was calculated. The $RQ_{MEC/PNEC}$ was found to be a suitable first approach as the data requirements for effect and exposure concentrations are similar to that of traditional environmental risk assessments of single compounds. Risk of cumulative effects was identified when the $RQ_{MEC/PNEC}$ was above 1. In addition, taxa-specific risk was calculated by summation of the toxic units (TU) to obtain the risk quotient RQ_{STU} after application of an appropriate assessment factor. An assessment factor of 100 and a RQ_{STU} limit of ≥1 for indication of risk were applied as a generic approach in order to make the limit for RQ_{STU} similar to the trigger value for toxicity/exposure ratio (TER, TER_{acute} ≥100).

There were large differences in the toxicity of the different PPPs and in the sensitivity between species. Based on the values used for calculation of PNECs, the most toxic PPP was carbendazim with a $NOEC_{crustaceans}$ of 1.5 µg/L, and the least toxic was fluroxypyr with an EC_{50} of 12300 µg/L for aquatic plants. Kresoxim obtained the lowest PNEC (0.024 µg/L), whereas the highest PNEC was observed for fluroxypyr (1230 µg/L). The highest and lowest PNEC differed by more than 4 orders of magnitude.

Of the total 56 samples, eight had a calculated $RQ_{MEC/PNEC}$>1; two samples from Hotranelva (Nord-Trøndelag county), four samples from Mørdrebekken (Akershus county), one sample from Skuterudbekken (Akershus county) and one sample from Vasshaglona (Aust-Agder county). These samples were typically collected from late June to mid-August. The cumulative risk was lowest at Timebekken (Rogaland county, $RQ_{MEC/PNEC}$ of less than 0.121) and highest at Skuterudbekken ($RQ_{MEC/PNEC}$ of up to 32.5). 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. Calculation of RQ_{STU} for aquatic plants provided challenging due to lack of effect data for several compounds and could potentially lead to an underestimation of the risk. However, as the $RQ_{MEC/PNEC}$ was considered to be more conservative than the RQ_{STU}, emphasis was placed on the $RQ_{MEC/PNEC}$ in cases where data were missing for the calculation of RQ_{STU}.

The risk at each site appeared to be driven by a few compounds and these toxicity drivers for the risk of combined toxicity of PPPs were identified for future consideration of mitigation measures. As risk assessment of combined toxicity of complex mixtures is still in early phases of development, a need for in-depth experimental and theoretical effort to improve both the data to support and verify the predictive modelling approaches were identified. However, the results obtained in this study and previous studies utilizing similar approaches suggest that summation of MEC(PEC)/PNEC ratios appear to be an acceptable approach for initial risk assessments of complex mixtures of PPPs.

Sammendrag

En rekke plantevernmidler brukes på samme område i løpet av vekstsesongen i Norge. Ettersom de vanligvis risikovurderes enkeltvis men brukes i kombinasjoner på samme avling, er det et behov for å vurdere den kumulative risiko av miljørelevante blandinger av plantevernmidler for å beskytte organismer i nærheten av jordbruksområder mot uønskede effekter. I denne studien ble risikoen for organismer i miljøet av kombinerte effekter av plantevernmidler i norske bekker og elver undersøkt. En kumulativ risikovurdering ble utført basert på tilgjengelig effekt- og eksponeringsdata for plantevernmidler. Effektdata for alger, krepsdyr, fisk og akvatiske planter ble samlet inn fra ulike databaser og benyttet for å beregne predikerte konsentrasjoner for ingen effekt (predicted no effect concentration, PNEC). Målte miljøkonsentrasjoner (MEC) av plantevernmidler på seks forskjellige lokaliteter i Norge ble innhentet gjennom JOVA programmet. Sammensetningen av plantevernmidler og konsentrasjoner varierte mellom de ulike lokalitetene og mellom de ulike prøvetidspunktene innenfor hvert område.

I en første tilnærming ble MEC/PNEC ratioene for alle detekterte plantevernmidler i en prøve summert for å gi en risikokvotient, $RQ_{MEC/PNEC}$. Beregning av $RQ_{MEC/PNEC}$ er en passende første tilnærmingen ettersom det nødvendige datagrunnlaget er likt som ved miljørisikovurdering av individuelle forbindelser. Minimal risiko var identifisert som $RQ_{MEC/PNEC}<1$. I tillegg ble det beregnet en risikokvotient for hver artsgruppe (RQ_{STU}) ved å summere opp de toksiske enhetene (TU) og applisere en sikkerhetsfaktor. En sikkerhetsfaktor på 100 og en RQ_{STU}-grense på ≥ 1 for indikasjon på risiko ble benyttet.

Det var store forskjeller i effekten av de forskjellige plantevernmidlene og i følsomhet mellom arter. Basert på toksisitetsverdiene benyttet til PNEC beregninger var det mest toksiske av de detekterte plantevernmidlene karbendazim med en $NOEC_{krepsdyr}$ på 1,5 µg/l, og den minst giftige var fluroksypyr med en EC_{50} på 12300 µg/l for akvatiske planter. Kresoksim hadde den laveste PNEC-verdien (0,024 µg/L), mens den høyeste PNEC-verdien ble observert for fluroksypyr (1230 µg/L), en forskjell på over 4 størrelsesordener.

Av de totalt 56 prøvene fordelt på 6 lokaliteter hadde åtte en beregnet $RQ_{MEC/PNEC}>1$; to prøver fra Hotranelva (Nord-Trøndelag), fire prøver fra Mørdrebekken (Akershus), en prøve fra Skuterudbekken (Akershus) og en prøve fra Vasshaglona (Aust-Agder). Disse prøvene var tatt fra slutten av juni til midten av august. Den akkumulerte risikoen var lavest for Timebekken (Rogaland, $RQ_{MEC/PNEC}$ på mindre enn 0,121) og høyest for Skuterudbekken ($RQ_{MEC/PNEC}$ opp til 32,5). De identifiserte risikoscenariene basert på $RQ_{MEC/PNEC}$ ble bekreftet av RQ_{STU}-verdier>1 for akvatiske planter basert på prøvene fra Hotranelva, Mørdrebekken og Vasshaglona, og for alger basert på prøvene fra Skuterudbekken og Vasshaglona. Beregning av RQ_{STU} for akvatiske planter var utfordrende på grunn av manglende effektdata for flere plantevernmidler som potensielt kan føre til en undervurdering av risiko. Ettersom $RQ_{MEC/PNEC}$ er ansett å være mer konservativ enn RQ_{STU}, kan man legge større vekt på $RQ_{MEC/PNEC}$ i tilfeller der det er datamangler for beregning av RQ_{STU}, noe som er i tråd med føre var-prinsippet.

Den identifiserte risikoen ved Hotranelva, Mørdre, Skuterudbekken og Vasshaglona så ut til å være forårsaket av noen få av de påviste plantevernmidlene. Risikovurdering av kumulativ toksisitet av komplekse blandinger er fortsatt i en tidlig fase av utviklingen, og det er et betydelig behov for inngående eksperimentelt og teoretisk arbeid for å forbedre datagrunnlag og for å verifisere prediktiv modellering av kumulativ risiko. Basert på resultatene i denne studien og tidligere studier som har vurdert den kumulative risikoen av blandinger av kjemikalier, virker imidlertid summering av MEC(PEC)/PNEC-ratioer som en fornuftig tilnærming for en innledende risikovurdering.

Abbreviations

CA	concentration addition
EC_{50}	concentration causing 50% effect
EU	European Union
EQS	Environmental Quality Standard
ha	hectares
LC_{50}	concentration causing 50% lethality
MEC	Measured Environmental Concentration
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
$RQ_{MEC/PNEC}$	Risk Quotient based on sum of MEC/PNEC ratios
RQ_{STU}	Risk Quotient based on sum of toxic units
STU	Sum of Toxic Units
TER	Toxicity Exposure Ratio
TU	Toxic Unit, here defined as MEC divided by EC_{50}

1. Introduction

A vast number of different plant protection products (PPPs) are used in Norwegian agriculture. Plant protection products are used to control fungal diseases, insect pests and weed within the crop, and are thus consisting of a broad range of chemicals with different properties and toxic mode of actions (MoAs). In 2013, more than 100 active compounds are approved for use in Norway by the Norwegian food safety authority (http://landbrukstilsynet.mattilsynet.no/plantevernmidler/godk.cfm). The environmental concentrations of many of the used PPPs are monitored in water recipients in agricultural areas through the Norwegian Agricultural Environmental Monitoring Program, JOVA (www.bioforsk.no/jova). The JOVA program was started in 1993 and has since then performed routine monitoring of PPPs in surface water from agricultural catchments during the growing season for plants. These studies conducted in agricultural areas have shown the presence of several PPPs in surface water (streams, rivers, shallow ground water) in concentrations ranging from ng/L to low µg/L (Hauken et al., 2012). In 2012, 99 active compounds and 17 metabolites of PPPs were included in the chemical analysis, of these approximately 35% are no longer in use but are monitored due to long persistence in the environment (http://www.bioforsk.no/ikbViewer/page/fagomrader/fagomrade/omrade/tema/artikkel?p_document_id=45590&p_dimension_id=15111: analytical methods M15, M60 and M91). Ecotoxicity testing for effects of single active ingredients in PPPs are required for the regulatory approval process, and acute and chronic effect data can be accessed at the Pesticide Properties DataBase (PPDB) (University of Hertfordshire 2013) and the EU Pesticides database (2013) among others. Risk assessment of PPPs is performed on the individual active compounds and commercial formulations, and environmental quality standards (EQS) for individual active compounds have been developed. Environmental risk assessment of plant protection products involves calculating the Toxicity Exposure Ratio (TER), the ratio between the toxicity (LC_{50}, EC_{50}, NOEC etc.) for different groups of organisms and the predicted (PEC) or measured environmental concentration (MEC). For effects on aquatic organisms the EU trigger values for acute (TER_{acute}) and long term ($TER_{long\text{-}term}$) exposure are ≥100 and ≥10, respectively (The European Commission 2011), meaning that there is minimal risk when the TER is larger than these trigger values.

However, several PPPs are sprayed within 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 (Hauken et al., 2012). There is little knowledge of how PPPs act together in mixtures in the Norwegian environment, as environmental risk assessment is normally performed on individual compounds. Even though the environmental concentrations of most PPPs are below the reported no observed effect concentrations (NOECs) and 50% effective concentrations (EC_{50}), effects on organisms in the aquatic environment might occur through combined toxicity as co-occurrence of several PPPs in water samples from agricultural streams are more the rule than the exception. Typical exposure scenarios of freshly sprayed PPPs 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 in the aquatic environment (JOVA-database, Bioforsk 2013).

Due to the increased focus and knowledge on combined effects over the last years, several approaches for how to include assessment of combined effects in the environmental risk assessment have been proposed. A study using the concept of concentration addition (CA) to predict the pesticide mixtures load in surface waters was performed by Finizio et al in 2005. The CA concept is also included in the first of two tiers for environmental risk assessment of combined effects (Backhaus and Faust, 2012). In that approach, the risk of combined effects was assessed using available baseline data (EC_{50} values), PEC or MEC and the resulting predicted no effect concentration (PNEC) values. A vast amount of studies have shown good correlation between the observed and CA predicted effects of mixtures in different organisms and for different endpoints (Belden et al., 2007; Cedergreen et al., 2008; Coors et al., 2012; Deneer, 2000; Faust et al., 2003; Petersen et al., 2013; Schnell et al., 2009; Silva et al., 2002; Thorpe et al., 2003; Tollefsen et al., 2012). As it has been shown that combined effects are more often additive than synergistic or antagonistic

(Belden et al., 2007; Deneer, 2000), there is a general acceptance of application of CA for initial assessment of combined effects of mixtures.

As PPPs are risk assessed singly but applied in combinations on the same crop, there is a need to consider the cumulative risk of environmentally relevant mixtures of PPPs to protect organism in the vicinity of agricultural areas from untargeted effects. In this study the combined effect of plant protection products in Norwegian agricultural streams were assessed for potential risks to aquatic organisms. This was performed by a cumulative risk assessment based on available effect data for algae, crustaceans, fish and aquatic plants, and measured environmental concentrations of PPPs at six different monitoring sites in Norway.

2. Materials and Methods

2.1 Data compilation

Monitoring data of occurrence and environmental concentrations of plant protection products were obtained from the JOVA program (www.bioforsk.no/jova). For the complete list of analyses see specification under the analysis method specifications M60, M15 and M91 (http://www.bioforsk.no/ikbViewer/page/fagomrader/fagomrade/omrade/tema/artikkel?p_document_id=45590&p_dimension_id=15111). Six agricultural catchments in Norway (Table 1) are currently monitored for PPPs in stream water through the JOVA program. During 2012, flow proportional sampling was performed throughout the growing season with samples analyzed for PPPs at 9 different time periods.

Table 1. Information of the six investigated sampling sites, modified from bioforsk.no (2013). The respective county is shown in brackets.

Sampling site	Catchment area (hectares)	Cultivated area (%)	Average yearly temperature (°C)	Precipitation (mm)	Crops
Heiabekken (Østfold)	170	62	5.6	829	potatoes, cereal, vegetables
Hotranelva (Nord-Trøndelag)	1940	58	5.3	892	cereal, grass
Mørdrebekken (Akershus)	680	65	4.3	665	cereal
Skuterudbekken (Akershus)	449	61	5.5	785	cereal
Timebekken (Rogaland)	91	94	7.1	1189	grass/pasture
Vasshaglona (Aust-Agder)	65	62	6.9	1230	vegetables, potatoes, cereal

Effect data for acute (EC_{50}) and chronic (NOEC) toxicity for algae, crustaceans, fish and aquatic plants were collected from The Pesticide Properties DataBase (PPDB) (University of Hertfordshire 2013), the EU/EFSA Review reports for active substances in the EU Pesticides database (2013) and from the OPP Pesticide Ecotoxicity Database (http://www.ipmcenters.org/Ecotox/DataAccess.cfm, data for prothioconazole-desthio and trifloxystrobin metabolite CGA 321113). Data for the pyridate metabolite CL9673 were collected from the review report from the European Commission (2001). For each compound, a PNEC was calculated by applying an assessment factor (Table 2) to the effect data for the most sensitive species.

Table 2. Assessment factors for calculating aquatic PNECs (European Chemicals Bureau 2003).

Available effect data	Assessment factor
At least one short-term LC_{50} or EC_{50} for each trophic level (base set: algae, daphnia, fish)	1000
One long term NOEC (fish or daphnia) in addition to base set	100
Two long term NOECs from two trophic levels (fish, daphnia, algae) in addition to base set	50
Three long term NOECs from three trophic levels (fish, daphnia or algae) in addition to base set	10

2.2 Risk assessment of mixtures of PPPs

Plant protection products detected in the same sample were considered to constitute one mixture. The risk assessment of mixtures of PPPs was performed based on the study by Finizio et al. (2005), the approach used by Andersen et al. (2012) and the approach presented by Backhaus and Faust (2012). 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. The $RQ_{MEC/PNEC}$ is a suitable first approach as the data requirements for effect and exposure concentrations are similar to that of traditional environmental risk assessment of single compounds. In addition, Backhaus and Faust (2012) showed that summation of PEC/PNEC ratios might serve as a justifiable CA-approximation in a first tier assessment. In addition, the risk for the different 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 ($STU_{(algae,crustacean,fish,aquatic\ plants)}$ * assessment factor = RQ_{STU}). Currently there is no guideline for how to determine the assessment factor to be used for calculating the RQ_{STU}, so 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 TER_{acute} (≥100). 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 aquatic organisms $TER_{acute} \geq 100$ and $TER_{long\text{-}term} \geq 10$, correspond to a STU_{acute} and $STU_{long\text{-}term}$ of ≤0.01 and ≤0.1, respectively. To be able to directly compare $RQ_{MEC/PNEC}$ and RQ_{STU}, the TU-values were multiplied by an assessment factor of 100 and a $RQ_{STU} \leq 1$ was used for indication of minimal risk. The sample from each site with the highest $RQ_{MEC/PNEC}$ was selected for this study, and results only from these samples are presented in the text.

3. Results

3.1 PPPs in the Norwegian environment

Results from the JOVA program (Stenrød et al., 2013) show that herbicide use dominates in cereal production (Skuterudbekken and Mørdrebekken catchments), followed by fungicide use. While the sprayed areas are quite stable, the amounts sprayed vary considerably between years, and the amounts of fungicide sprayed in cereal are occasionally similar to the amounts of herbicides used. The need for pesticides is to a large degree governed by management practices (e.g. tillage) and weather conditions. The total use of insecticides is generally low but in certain years the area sprayed has been similar or even larger than areas sprayed with fungicides. However, the low maximum allowed doses for insecticides results in very low amounts used. Within the catchments dominated by a combination of potato and cereal production (Heiabekken) and vegetable and potato production (Vasshaglona) the area sprayed with pesticides has been quite stable throughout the individual seasons, but with large variations in amounts sprayed between years. Also in these catchments, herbicide use dominate over fungicide use with regard to the area sprayed, but quite often the amount of fungicides sprayed is similar or even higher than the amounts of herbicides used. Areas dominated by meadows and pasture require less use of pesticides, and the main use is often herbicides in these areas.

3.1.1 Reported use of PPPs

The occurrence of pesticides in the aquatic environment is ultimately connected to their use. The reported pesticide use during 2012 within the monitored catchments (JOVA-database, Bioforsk, 2013) showed that for Heiabekken, 43 different pesticides were used in the catchment during the growing season. The most used (area sprayed) pesticides included the herbicides fluroxypyr (40 hectares (ha)) and sulfonylureas (e.g. tribenuron methyl) (37 ha), and the fungicide prothioconazole (34 ha). The herbicide metribuzin that in later years has been detected in concentrations that might cause effects on non-target organisms in the aquatic environment (Hauken et al., 2012), was sprayed on 15 ha. Thirty-six different PPPs were sprayed in the Mørdrebekken catchment during 2012. Prothioconazole (161 ha) and cyprodinil (61 ha) were the more widely used fungicides. Other much used pesticides include low dose sulfonylurea herbicides (340 ha), fluroxypyr (147 ha), and glyphosate (99 ha), which were not analyzed in the JOVA program due to costly analytical methods and economical restraints. The herbicide aclonifen, recently detected in concentrations that may potentially cause effects on non-target organisms (JOVA-database, Bioforsk, 2013) was only sprayed on 0.1 ha, while metribuzin was sprayed on 12 ha. In the Skuterud catchment, 23 different PPPs were sprayed, including the herbicides fluroxypyr (150 ha), mcpa and clopyralid (129 ha) and sulfonylureas (>90 ha), the fungicides prothioconazole (98 ha) and trifloxystrobin (98 ha), and the insecticides lambda-cyhalothrin (36 ha), thiacloprid (26 ha) and imidacloprid (14 ha). In general, less pesticides are sprayed in the Timebekken catchment due to a predominance of husbandry and grazing areas. Data from 2012 show that five different herbicides were sprayed in the catchment, with the most used being glyphosate (10 ha) and the sulfonylurea tribenuron methyl (4 ha), both of which are not analysed for in the JOVA program. In the Vasshaglona catchment, 33 different PPPs were used during the growing season of 2012. The most used herbicides included glyphosate (10 ha), clopyralid (8 ha) and metamitron (8 ha), while metribuzin was sprayed on 4 ha. Spraying of fungicides included fenamidon (7 ha), mandipropamide (6 ha) and cyazofamid (5 ha). No data on pesticide use was available for the Hotranelva catchment as this is not collected through the JOVA program.

3.1.2 Environmental conentrations

The environmental concentrations of PPPs were monitored throughout the growing season (appendix A). In general, the detected PPPs corresponded to the reported use, and with previously used PPPs detected only occasionally and at considerably lower concentrations than that expected to cause effects on non-target organisms in the aquatic environment. The combinations of compounds and concentrations varied

between the different monitoring sites and also with the different sampling time points at each site. The measured concentrations of PPPs in the sample with the highest $RQ_{MEC/PNEC}$ from each site are presented in Table 3. As many as 13 compounds were detected in a single sample (site Mørdrebekken). Single PPPs were present in concentrations up to 0.78µg/L (kresoxim). There was a variation in occurrence of different PPPs among the different sites and sampling time-points. Based on the chosen sample from each site, MCPA was found in five out of the six sites, whereas fluroxypyr, metribuzin and prothioconazole-desthio were found in three of the six sites.

Table 3. Concentrations (µg/L) of detected plant protection products in the sample with the highest resulting $RQ_{MEC/PNEC}$ from the studied sites in 2012. Sampling period indicated in brackets.

Sampling site / Compound	Heiabekken (25.07-07.08)	Hotranelva (01.07-15.07)	Mørdre-bekken (26.06-10.07)	Skuterud-bekken (10.08-28.08)	Timebekken (29.05-11.06)	Vasshaglona (11.06-25.06)
Aclonifen			0.23			
Azoxystrobin						
Bentazone					0.018	
Cyazofamid						0.03
Cyprodinil			0.021			
Dichlorprop				0.12		
Dimethoate			0.053			
Fenamidone	0.021					0.68
Fluroxypyr	0.059		0.26		0.056	
Imazalil					0.037	
Imidacloprid			0.021			
Iprodione						
Clopyralid	0.053		0.099			
Kresoxim				0.78		
Mandipropamid						0.053
MCPA	0.01	0.71	0.18	0.02	0.016	
Mecoprop			0.087			
Metalaxyl	0.038		0.037			
Metamitron						0.23
Metribuzin			0.077		0.027	0.28
Pencycuron			0.026			
Pinoxaden			0.029			
Prothioconazole-desthio	0.056	0.55	0.12			
Pyridate metabolite (CL9673)						0.024
Pyrimethanil						
Trifloxystrobin metabolite (CGA 321113)		0.076		0.33		
Detected PPPs	**6**	**3**	**13**	**4**	**5**	**6**

3.2 Effects of PPPs on aquatic organisms

The different databases used provided a relatively high number of acute and chronic data for algae, crustaceans, and fish (Table 4). Only acute effects were reported for aquatic plants (general exposure time 7 days). Assessment factors for calculating the PNEC varied between 10 and 1000, with only two compounds (kresoxim and pinoxaden) obtaining an assessment factor of 1000 due to lack of chronic ecotoxicity data for fish and crustaceans. There were large differences in the toxicity of the different PPPs and in the sensitivity between species. Based on the values used for calculation of PNECs, the most toxic PPP was carbendazim with a $NOEC_{crustaceans}$ of 1.5 µg/L, and the least toxic was fluroxypyr with an EC_{50} of 12300 µg/L for aquatic plants. Kresoxim obtained the lowest PNEC (0.024 µg/L), whereas the highest

PNEC was observed for fluroxypyr (1230 µg/L). The highest and lowest PNEC differed by more than 4 orders of magnitude.

Table 4. Effect data compilation (µg/L), applied assessment factors and resulting PNECs for the detected plant protection products (PPPs).

	Acute EC50				Chronic NOEC				
Substance	Algae	Crustacean	Fish	Aquatic plants	Algae	Crustacean	Fish	Assessment factor	PNEC
2,4-D	24200	100000	63400	**580**	100000	46200	27200	10	58
Aclonifen	470	1200	670	6		16	**5**	50	0.1
Azoxsystrobin	360	55	470	3200	800	**10**	147	10	1
Bentazone	10100	64000	100000	**5400**	25700	120000	48000	10	540
Boscalid	3750	5330	2700			1300	**125**	50	2.5
Carbendazim	>7700	150	190			**1.5**	3.2	50	0.03
Clopyralid	30500	>99000	>99000	89000	17000	17000	**10800**	10	1080
Cyazofamid	**25**	190	560	33		110	130	50	0.5
Cyprodinil	2600	220	2410	7710		**8.8**	83	50	0.176
Dicamba	1800	>41000	>100000	**450**	25000	97000	180000	10	45
Dichlorprop	26500	100000	>109000	**4100**	180000	>100000	100000	10	410
Dimethoate	90400	2000	30200		32000	**40**	400	10	4
Fenamidone	3840	190	740	880		**12.5**	310	50	0.25
Fenhexamid	>26100	>18800	1240	2300	5360	1000	**101**	10	10.1
Fluroxypyr	49800	>100000	14300	**12300**	56000	56000	100000	10	1230
Imazalil	870	3500	1480				**43**	100	0.43
Imidacloprid	>10000	85000	211000		10000	**1800**	9020	10	180
Iprodione	1800	660	3700	1000	3200	**170**	260	10	17
Kresoxim	**24**	186	150					1000	0.024
Mandipropamid	>19800	7100	>2900	>4400		870	**500**	50	10
MCPA	32900	>190000	50000	**152**	60000	50000	15000	10	15.2
Mecoprop	16200	>91000	>100000	**1600**	56000	22200	>50000	10	160
Metalaxyl	36000	100000	100000	85000	10000	**1200**	9100	10	120
Metamitron	400	5700	>190000	400	**100**	10000	7000	10	10
Metribuzin	20	49000	74600	**8**	19	320	5600	10	0.8
Pencycuron	>300	>300	>300		100	**50**	>300	10	5
Phenmedipham	86	410	1710	230		**61**	320	50	1.22
Pinoxaden	**910**		10300	3500				1000	0.91
Prothioconazole-desthio	70	5500	6630	39		100	**3.4**	50	0.068
Pyridate metabolite (CL 9673)	**>4930**	26100	20000			5000	20000	50	98.6
Pyrimethanil	1200	2900	10560	7800		**940**	1600	50	18.8
Trifloxystrobin metabolite (CGA 321113)	77100	95300	>106000		15700	**3200**		50	64

Data were collected from PPDB (University of Hertforshire, 2013), EFSA reports (European Food Safety Authority 2005; 2010a; 2010b; 2010c) in the EU Pesticides database (2013), the OPP Pesticide Ecotoxicity Database (http://www.ipmcenters.org/Ecotox/DataAccess.cfm) and review reports from the from the European Commission (2001). Blue bold numbers indicate effect data used for PNEC calculations.

Categorizing the PPPs according to use and MoA led to grouping of 14 herbicides, 16 fungicides and 2 insecticides (Table 5). For nine out of the 14 herbicides (64%), photosynthesizing organisms (algae and aquatic plants) were the most sensitive followed by crustaceans (21%) and fish (14%). For nine out of the 16 fungicides (56%), crustaceans were the most sensitive species group followed by fish (37.5%) and algae (12.5%). Only two insecticides were detected in the samples and crustaceans were the most sensitive to both of these PPPs.

Table 5. Usage and Mode of Action of the detected plant protection products (adapted from Pesticide Properties DataBase. University of Hertfordshire, 2013)

Substance	Use	Mode of Action
2,4-D	Herbicide	Selective, systemic, absorbed through roots and increases biosynthesis and production of ethylene causing uncontrolled cell division and vascular tissue damage. Synthetic auxin.
Aclonifen	Herbicide	Systemic and selective. Inhibition of carotenoid biosynthesis.
Azoxystrobin	Fungicide	Respiration inhibitor.
Bentazone	Herbicide	Selective action, absorbed by foliage with very little translocation. Inhibits photosynthesis (photosystem II).
Boscalid	Fungicide	Protectant, foliar absorption, translocates, inhibits spore germination and germ tube elongation.
Carbendazim	Fungicide	Systemic with curative and protectant activity. Inhibition of mitosis and cell division.
Clopyralid	Herbicide	Selective, systemic, absorbed through leaves and roots. Synthetic auxin.
Cyazofamid	Fungicide	Foliar and soil preventative action with some residual activity. Respiration inhibitor.
Cyprodinil	Fungicide	Systemic, absorbed through foliage. Inhibits protein synthesis.
Dicamba	Herbicide	Selective, systemic, absorbed through leaves and translocates throughout plant. Synthetic auxin.
Dichlorprop	Herbicide	Selective, systemic, absorbed through leaves and translocates to roots. Synthetic auxin causing stem and leaf malformations leading to death.
Dimethoate	Insecticide, acaricide	Systemic with contact and stomach action. Acetylcholinesterase inhibitor.
Fenamidone	Fungicide	Protective and curative action. Respiration inhibitor.
Fenhexamid	Fungicide	Foliar applied with protective action. Disrupts membrane function. Inhibits spore germination.
Phenmedipham	Herbicide	Selective, systemic, absorbed through leaves and translocated. Inhibits photosynthesis (photosystem II).
Fluroxypyr	Herbicide	Foliar uptake causing auxin-type response. Synthetic auxin.
Imazalil	Fungicide, veterinary treatment	Systemic with curative and protective properties. Disrupts membrane function.
Imidacloprid	Insecticide, veterinary treatment	Systemic with contact and stomach action. Acetylcholine receptor agonist.
Iprodione	Fungicide	Contact action with protectant and some eradicant activity. Signal transduction inhibitor.
Kresoxim	Metabolite of kresoxim-methyl, a fungicide and bacteriacide	Kresoxim-methyl: Protective, curative, eradicative action and long residual effects. Acts by binding to Quinone outer site blocking electron transfer and respiration of the fungi
Mandipropamid	fungicide	Inhibits spore germination with preventative action.
MCPA	Herbicide	Selective, systemic with translocation. Synthetic auxin.
Mecoprop	Herbicide	Selective, systemic, absorbed by leaves with translocation. Synthetic auxin.
Metalaxyl	Fungicide	Systemic with curative and protective action, acts by suppressing sporangial formation, mycelial growth and the establishment of new infectons
Metamitron	Herbicide	Selective, systemic, absorbed mainly by roots and translocated. Inhibits photosynthesis (photosystem II).
Metribuzin	Herbicide	Selective, systemic with contact and residual activity. Inhibits photosynthesis (photosystem II).
Pencycuron	Fungicide	Non-systemic with protective action. Inhibition of mitosis and cell division.
Pinoxaden	Herbicide	Systemic. Acetyl coenzyme A carboxylase inhibitor, inhibiting fatty acid synthesis.
Prothioconazole-desthio	Metabolite of prothioconazole, a fungicide	Prothiokonazole: Systemic with protective, curative and eradicative action. Long lasting activity.
Pyridate metabolite (CL 9673)	Metabolite of pyridate, a herbicide	Pyridate: Selective with contact action, absorbed mainly by the leaves.
Pyrimethanil	Fungicide	Protective action with some curative properties.
Trifloxystrobin metabolite (CGA 321113)	Metabolite of trifloxystrobin, a fungicide	Trifloxystrobin: Broad spectrum with preventative and curative action. Respiration inhibitor.

3.3 Risk assessment of mixtures of PPPs

The risk of environmentally relevant mixtures of PPPs were assessed in a two tiered approach and revealed a potential risk for aquatic organisms at four out of the six sampling sites with RQs above 1 (Table 6). A complete list of $RQ_{MEC/PNEC}$ values for all 9 sampling times at each site can be found in Appendix B.

3.3.1 Cumulative risk of PPPs based on $RQ_{MEC/PNEC}$

Out of the total 56 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. The cumulative risk was lowest at Timebekken ($RQ_{MEC/PNEC}$ of less than 0.121) and highest at Skuterudbekken ($RQ_{MEC/PNEC}$ as high as 32.5). 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 in samples from Skuterudbekken and Vasshaglona. For calculation of $RQ_{MEC/PNEC}$ effect data were available for all detected compounds. Lack of chronic effect data for some compounds led to the use of a higher assessment factor when calculating the PNEC.

Table 6. RQ_{STU} and $RQ_{MEC/PNEC}$ for worst case sample from each location sampled in 2012. Situations where the risk quotient is higher than 1 are presented by bold text.

	Heiabekken 25.07-07.08	Hotranelva 01.07-15.07	Mørdre-bekken 26.06-10.07	Skuterud-bekken 10.08-28.08	Timebekken 29.05-11.06	Vasshaglona 11.06-25.06
RQ_{STU} algae	0.081	0.788	0.620	**3.251**	0.140	**1.596**
RQ_{STU} crustacean	0.012	0.011	0.043[b]	0.420	0.001	0.379
RQ_{STU} Fish	0.004	0.01	0.049	0.520	0.003	0.100
RQ_{STU} aquatic plants	0.155	**1.877**[a]	**5.231**[c]	0.016[d]	0.349[e]	**3.727**[f]
$RQ_{MEC/PNEC}$	0.909	**8.136**	**4.344**	**32.507**	0.121	**3.159**

For calculation of RQ_{STU} an assessment factor of 100 was applied. [a]missing effect data for trifloxystrobin metabolite, [b]missing effect data for pinoxaden, [c]missing effect data for dimethoate, imidacloprid and pencycuron, [d]missing effect data for kresoxim and trifloxystrobin metabolite, [f]missing effect data for imazalil, [f]missing effect data for pyridate metabolite.

Based on the $RQ_{MEC/PNEC}$, four of the six different sampling sites (Hotranelva, Mørdrebekken, Skuterudbekken and Vasshaglona) were predicted to represent a risk scenario for combined effects of PPPs to non-target organisms (Figure 1). Of the three compounds detected in Hotranelva, prothioconazole-desthio was responsible for the majority (99%) of the risk, whereas MCPA and the trifloxystrobin metabolite were only minor contributors. The sample from Mørdrebekken contained the highest number of detected compounds (13). However, only four compounds (aclonifen, prothioconazole-desthio, cyprodinil and metribuzin) had a MEC/PNEC equal to or above 0.1 (Figure 1). Together these 4 compounds accounted for 98.6% of the risk of combined effects of PPPs at this location. The two largest contributors, aclonifen and prothioconazole-desthio alone accounted for 93.5% of the risk of combined effects and both had individual MEC/PNEC ratios above 1. Of the four compounds detected in the worst case sample from Skuterudbekken, only kresoxim represented a risk of effects in non-target organisms. This compound accounts for about 99.99% of the estimated risk at this location. The calculated PNEC for kresoxim was 0.024 µg/L (assessment factor of 1000). The $RQ_{MEC/PNEC}$ for the sample from Vasshaglona, calculated on the basis of 6 detected compounds, was

largely caused by fenamidone and metribuzin (97%), whereas cyazofamid, metamitron, mandipropamid and the pyridate metabolite accounted for a minor portion of the risk.

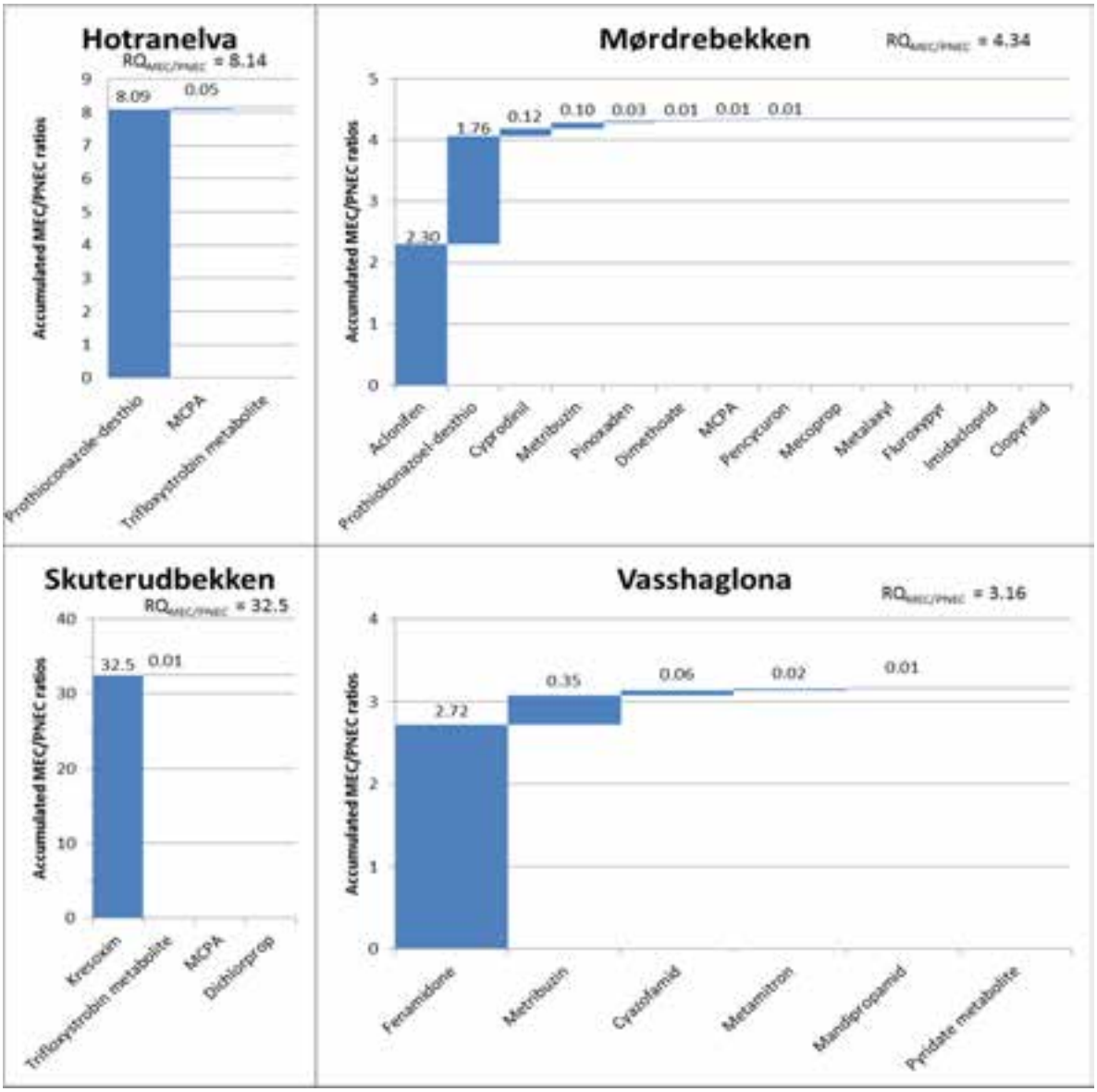

Figure 1. Contribution plot of the individual (blue column) and cumulative (value on the y-axis) MEC/PNEC ratios for the PPPs detected in, Hotranelva (01.07.12- 15.07.12), Mørdrebekken (26.06.12-10.07.12), Skuterudbekken (10.08.12-28.08.12) and Vasshaglona (11.06.12-25.06.12). The individual MEC/PNEC equal to or larger than 0.01 are given above each column.

Prothioconazole-desthio was among the largest contributors to the cumulative risk at Hotranelva and Mørdrebekken, contributing with 99% and 40.5% to the cumulated risk, respectively. However, the main contributors to the cumulated risk varied between the different sites. At the two sampling sites, Heiabekken and Timebekken, the $RQ_{MEC/PNEC}$ was below 1 (Table 6, Appendix B, Appendix C), indicating minimal risk of combined effect of PPPs on organisms in the aquatic recipients at these sites.

3.3.2 Cumulative risk of PPPs based on RQ_{STU}

The RQ_{STU} for the different species groups for the Mørdrebekken sample are shown in Figure 2 as an example of the RQ_{STU} approach. The RQ_{STU} for the most sensitive group of species for the sample taken in Mørdrebekken was $RQ_{STUaquatic\ plants}$ of 5.2. In the sample from Mørdrebekken, the herbicides aclonifen and metribuzin was the largest contributor to the risk of combined effects in aquatic plants accounting for 73.7% and 18.5% of the $RQ_{STUaquatic\ plants,}$ respectively. Metribuzin was also a main contributor to the $RQ_{STUalgae}$ accounting for 63% of the total risk. The herbicide aclonifen was the largest contributor to the risk of combined effects in crustaceans and fish accounting for 46.5% and 61% of the risk, respectively (Figure 2). Aquatic plants were the only taxa with a RQ_{STU} above 1 for the Mørdrebekken sample. Taxa-specific RQ_{STU} values for the sample from Timebekken and Skuterudbekken were all <1 and can be found in appendix D.

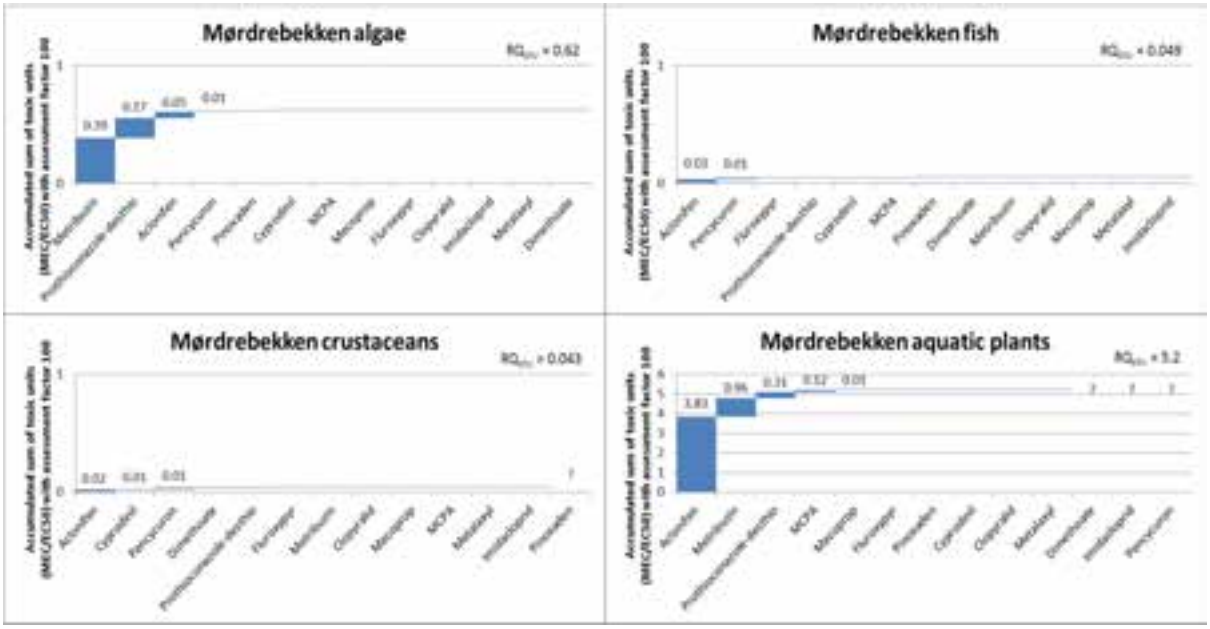

Figure 2. Contribution plot of the individual (blue column) and cumulative (value on the y-axis) MEC/EC_{50} ratios * assessment factor (100) for the PPPs detected in Mørdrebekken for algae, crustaceans, fish and aquatic plants. The individual MEC/EC_{50} * assessment factor equal to or larger than 0.01are given above each column. "?" indicate compounds where effect data is missing for the species in questions.

3.3.3 Comparison between $RQ_{MEC/PNEC}$ and RQ_{STU} assessment

The contribution of the different substances to the total $RQ_{MEC/PNEC}$ and RQ_{STU} for the four sites with an $RQ_{MEC/PNEC}$ larger than one is shown in Figure 3. In the sample from Hotranelva, prothioconazole-desthio appeared to be the largest contributor of the RQ_{STU} for all species contributing with 99.7% (algae), 95.7% (crustaceans), 84.8% (fish) and 75.1% (aquatic plants) to the RQ_{STU}. However the TU*assessment factor for this compound was only above 1 in aquatic plants indicating that this taxa was the most susceptible to prothioconazole-desthio. Aquatic plants were also the most sensitive taxa toward the total load of PPPs in the sample from Hotranelva with a $RQ_{STU(aquatic\ plants)}$ of 1.88 which was approximately four times lower than the calculated $RQ_{MEC/PNEC}$ (8.14).

In the sample from Mørdrebekken, aquatic plants were clearly the most sensitive taxa towards most of the compounds with a RQ_{STU} of 5.23 which was slightly higher than the $RQ_{MEC/PNEC}$ (4.34). In Vasshaglona, the largest contributor to the total RQ_{STU} for algae and aquatic plants was metribuzin, accounting for 93.9% (aquatic plants) and 87.7% (algae) of the $RQ_{STU.}$ The fungicide fenamidone was the largest

contributor to the total RQ_{STU} for crustaceans and fish (94.4% of the $RQ_{STUcrustaceans}$ and 92.2% of the $RQ_{STUfish}$). Aquatic plants appeared to be the most sensitive taxa towards the assembly of PPPs ($RQ_{STUaquatic\ plants}$ =3.73), which was 1.2 times higher than the calculated MEC/PNEC (3.16). Algae appeared to be the second most sensitive taxa with an $RQ_{STUalgae}$ of 1.60, whereas the $RQ_{STUcrustaceans}$ and $RQ_{STUfish}$ were 0.379 and 0.0996, respectively.

For the sample from Skuterudbekken, algae were the most sensitive taxa with a $RQ_{STUalgae}$ of 3.25, a value 10 times lower than the $RQ_{MEC/PNEC}$. The major contributor to the cumulative risk was kresoxim with a contribution of 100% ($RQ_{STUalgae}$), 99.9% ($RQ_{STUcrustaceans}$) and 99.9 % ($RQ_{STUfish}$). The RQ_{STU} for crustaceans and fish was 0.42 and 0.52 respectively. The risk to aquatic plants (RQ_{STU} of 0.02) might be underestimated at this site due to missing effect data on aquatic plants for kresoxim and trifloxystrobin metabolite.

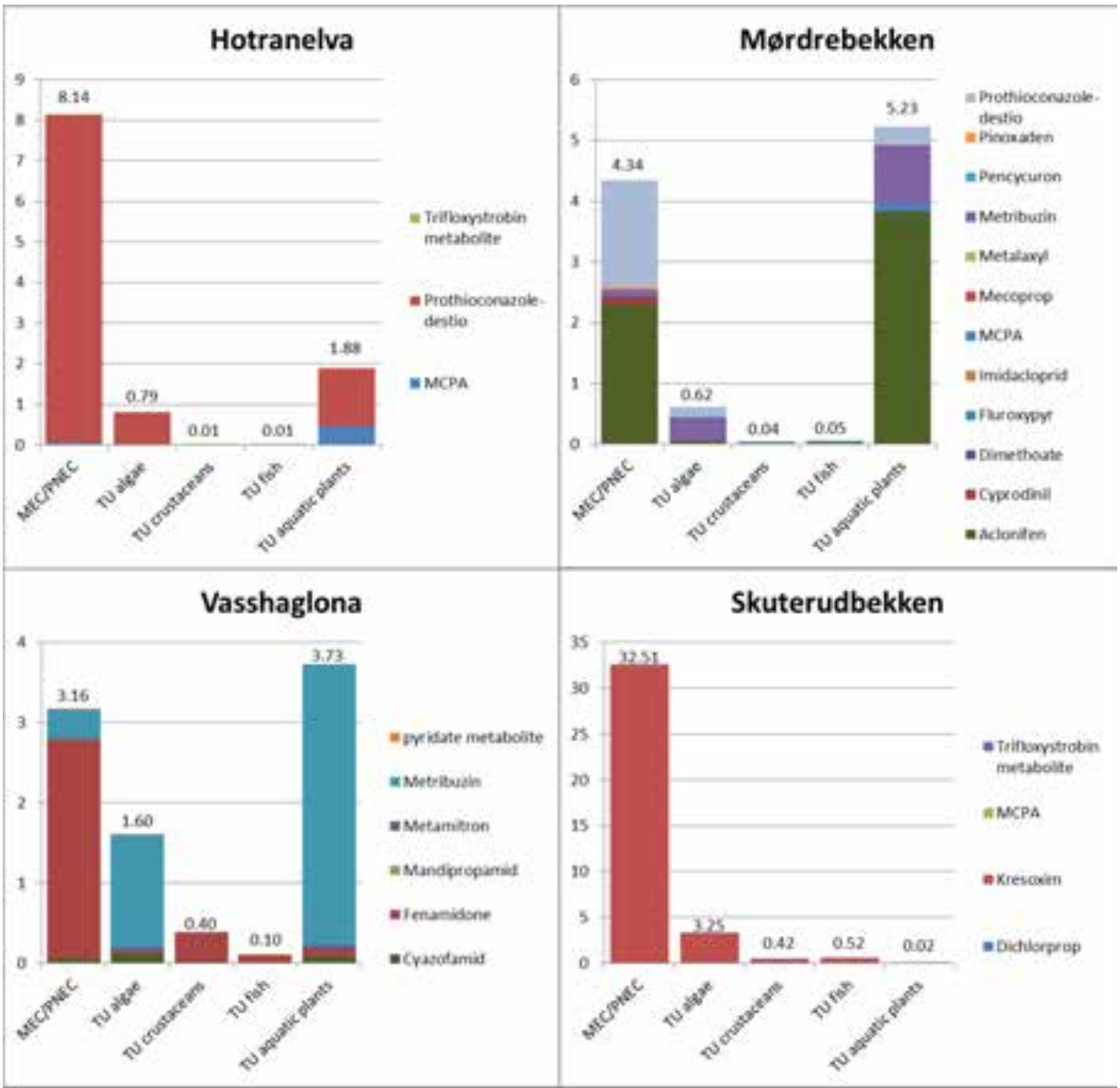

Figure 3. The individual PPP contributions to the $RQ_{MEC/PNEC}$, $RQ_{STUalgae}$, $RQ_{STUcrustacean}$, $RQ_{STUfish}$ and $RQ_{STUaquatic\ plants}$ for the samples from Hotranelva, Mørdrebekken, Vasshaglona and Skuterudbekken. The RQ for the different approaches are given above the respective column. The TU is calculated based on an assessment factor of 100.

4. Discussion

Although several authorities have started evaluating combined effects of chemicals, there is currently no common guideline in use for how to perform risk assessment of combined effects. However, both regulatory authorities and research institutes have shown interest in risk assessments of environmentally relevant mixtures and a few publications are available that address this issue within a regulatory context (Andersen et al., 2012; Finizio et al., 2005). In addition, there have been published papers presenting theoretical approaches for environmental risk assessment of mixtures and how these can be implemented in the regulative framework (Backhaus and Faust, 2012; Backhaus et al., 2013). In this study, which to our knowledge is the first to risk assess environmentally relevant mixtures of PPPs in Norway, summing up PEC/PNEC ratios and the calculation of taxa-specific risk quotients were performed to screen for potential risk scenarios due to combined effects of PPPs (insecticides, herbicides and fungicides) in surface waters (agricultural streams) in Norway.

4.1 Effect data and environmental concentrations of PPPs

Due to the ongoing monitoring of PPPs in Norway through the JOVA program, environmental concentrations of PPPs for the year 2012 were easily accessible. In general, pesticide use varies considerably between years. In addition to weather conditions determining the need for and actual use of plant protection measures, variation in pesticide use may also in part be explained by approval (bans, reduced recommended doses, new approvals etc.), pricing and taxation of PPPs. Although as many as 99 active compounds and 17 metabolites of PPPs were monitored in the JOVA program in 2012, a few widely used PPPs, such as sulfonylurea herbicides and glyphosate were not included in the analysis due to costly analytical methods and economical restraints (Hauken et al., 2012). Glyphosate is a broad spectrum/non-selective herbicide that inhibits growth by inhibition of lycopene cyclase, an enzyme involved in the synthesis of aromatic amino acids in actively growing plants. Glyphosate is commonly used to control perennial (and annual) weeds both in agriculture, railway and road maintenance, as well as in parks and private gardens, and is used regularly at all monitoring sites investigated in this study (Pedersen et al., 2013, JOVA-database, Bioforsk, 2013). Glyphosate was monitored through the JOVA program during the early 2000's and was detected in about 90% of the samples analyzed, but only in low concentrations not expected to have effects on non-target organisms (mean conc. 0.15 μg/L, maximum conc. 4 μg/L) (Hauken et al., 2012). Similarly, the on-going Swedish pesticide monitoring program has detected glyphosate only at low concentrations in about 70% of the samples from the period 2002-2010 (Datavärdskap Jordbruksmark, 2012). Chronic and acute toxicity data for glyphosate (EU Pesticide Database, 2013) do not indicate that this will be an important factor for the risk assessment in the aquatic environments. However, the ever increasing use of glyphosate in Norway and worldwide demand has resulted in the continued focus on this compound's possible role in environmental ecotoxicology.

In addition, lack of detection of certain PPPs due to higher limits of quantification than the concentrations encountered in the present set of surface waters, may have led to under-prediction of the total RQs. This is applicable to potent substances used in low concentrations with a potential untargeted effect on aquatic organisms at very low environmental concentrations, including some insecticides, a few fungicides and the sulfonylurea herbicides (Hauken et al., 2012). The sulfonylurea herbicides entered the Norwegian market towards the end of the 1990's, with a subsequent rapid increase in use. The sulfonylureas have not been included in the continuous pesticide monitoring in the JOVA-catchments due to economical restraints, hence, we have no data showing the environmental challenges connected to their use. Recent research does however illustrate the potential leaching/runoff of such substances from soil to water in environmentally relevant concentrations (Cessna et al., 2010; Almvik et al., 2011). The Swedish pesticide monitoring program shows detections of the sulfonylurea herbicides in concentrations that might cause effect on non-target organisms in the aquatic environment (Datavärdskap Jordbruksmark, 2013). The much used tribenuron-methyl is detected in about 5 % of the samples in the period 2002-2011. Although most detected concentrations are below 0.1 μg/L which would be the PNEC considering a full

dataset (the NOEC for aquatic plants for this substance is 1 μg/L, European Commission, 2003) occurrence and the occasional detected concentrations above 0.1 μg/L might contribute to the combined effect of PPPs on non-target organisms. The sulfonylurea herbicides rimsulfuron and metsulfuron-methyl have lower NOECs for aquatic plants (0.12 and 0.16 μg/L, respectively; EU Pesticide Database, 2013) resulting in PNECs of 0.012 and 0.016 μg/L, respectively. These values will be close to or below quantification limits for chemical analysis, and for the Swedish pesticide monitoring program these substances were detected at ecologically relevant concentrations in 1.5% (rimsulfuron) and 2% (metsulfuron-methyl) of all samples analyzed.

Effect data were found for all detected PPPs in the databases and documents used in the present study. However, taxa-specific data for all taxa were not successfully retrieved for all the PPPs (Table 4). The amount of compiled effect data have an impact on the applied assessment factor (Table 2), and hence the calculated PNEC (Table 4). The NOEC value from the 72h algal growth inhibition test that provides the EC_{50} for acute effects are often considered as chronic effect data. However, these data were not easily available, and could have reduced the applied assessment factor if made available. The assessment factor used for calculating the PNEC for Imazalil could have been 50 instead of 100 if chronic data for algae was available, whereas the assessment factors for aclonifen, boscalid, carbendazim, cyazofamid, mandipropamide, phenmedipham, prothioconazole-desthio, pyridate metabolite and pyrimethanil could have been reduced from 50 to 10 if sufficient effect data had been available. The PNECs calculated for these compounds are therefore considered "worst-case" scenarios and might be reduced by available chronic data for algae in the future. A wider search for effect data was not considered relevant as the applied databases and documents were believed to contain the most updated information regarding the effects of the detected pesticides, but existence of more relevant effect data cannot be ruled out.

4.2 **Risk of combined effects of PPPs**

In this study, cumulative risk assessment of environmentally relevant mixtures of PPPs were performed by calculation of two different risk quotients based on the proposed framework by Backhaus and Faust (2012) for assessing combined effect of mixtures. The approach of summing up MEC/PNECs for assessment of combined effects has previously been performed in Norway on substances in sewage sludge (Andersen et al., 2012), and summation of toxic units has been performed on mixtures of pesticides (Finizio et al., 2005). The calculation of RQ_{STU} is considered more vulnerable to missing data than $RQ_{MEC/PNEC}$ as effect data for all compounds for each species group are required for the assessment. The most sensitive of the available effect data are used to calculate the PNEC and derive a $RQ_{MEC/PNEC,}$ and the $RQ_{MEC/PNEC}$ is thus less sensitive to missing data (Backhaus and Faust 2012).

The environmental risk assessment of environmentally relevant mixtures of PPPs at six different sites in Norway was based on the compiled data for environmental concentration and biological effect. Two of the investigated sites, Heiabekken and Timebekken had a $RQ_{MEC/PNEC}$ below 1, indicative of minimal risk of effects on the aquatic organisms in these areas. The RQ_{STU} for the most sensitive species group (aquatic plants) for the sample with the highest $RQ_{MEC/PNEC}$ from these two sites was also below one, confirming predictions of minimal environmental risk at these sites. At four out of the six sampling sites a potential risk of the combined effect of PPPs were identified, typically in samples collected from late June to mid-August. The temporal and spatial variation in RQs could be a result of weather conditions like temperature and rainfall, or different time of PPPs application for different crops. As shown from the reported pesticide use in the catchment areas, both widely used pesticides and less sprayed compounds may contribute significantly to the environmental risk, with aclonifen sprayed on only 0.1 ha in Mørdrebekken representing the latter. Interestingly, as previously observed by Finizio et al. (2005) only a few compounds were drivers of risk at each site by having high individual RQs.

In Hotranelva, prothioconazole-desthio contributed to the majority of the potential risk and of the 9 sampling dates, two had a resulting $RQ_{MEC/PNEC}$ above 1. All other $RQ_{MEC/PNEC}$ values for this site were below 0.05 and prothioconazole-desthio was not detected in these samples, potentially due to lack of

usage in this period. The high risk of prothioconazole-desthio could be understood in light of the low PNEC for this compound. The PNEC for prothioconazole-desthio was in the ng/L range (applied assessment factor 50). Differences in sensitivity between different taxa were observed, with the algae and aquatic plants being the most sensitive. In addition, aquatic plants were the most sensitive taxa towards MCPA, a synthetic auxin analogue (herbicide) acting like a plant hormone in growth and development of plant organs (leaves and flowers).

Mørdrebekken was the site with most samples resulting in a $RQ_{MEC/PNEC}$ above 1 (4 out of 9). Organisms at this site were at potential risk for effects of PPPs for a longer time period than organisms at the other sampling sites. The most affected taxa at this site was aquatic plants with an $RQ_{STUaquatic\ plants}$ of 5.23, only 1.2 times higher than the calculated $RQ_{MEC/PNEC}$. Aclonifen, a herbicide which inhibits carotenoid biosynthesis, was the largest contributor to both the $RQ_{MEC/PNEC}$ and $RQ_{STUaquatic\ plants}$. Aclonifen seemed to affect aquatic plants to a considerable larger degree than algae, which suggest that uptake or toxic MoA differ between the two taxa being reliant on pigments for photosynthesis. Another major contributor to the RQ_{STU} for aquatic plants and algae was the herbicide metribuzin, a selective photosystem II inhibitor. The presence of these two herbicides in the samples from Mørdrebekken can explain why the RQ_{STU} values of algae (0.62) and aquatic plants (5.23) were higher than those for crustaceans (0.04) and fish (0.05).

Of the samples taken at Vasshaglona, only one sample was assigned a $RQ_{MEC/PNEC}$ above one, indicating that aquatic organisms were potentially at risk only during a short period. Algae and aquatic plants were the species groups at highest potential risk at this site, probably due to the presence of the herbicide metribuzin. The RQ_{STU} for crustaceans and fish were below one, and the largest contributor was fenamidone, a fungicide that seems to affect crustaceans and fish to a larger degree than aquatic plants and algae based on available effect data.

One sample from Skuterudbekken was assigned a $RQ_{MEC/PNEC}$ above one (32.5). This was the highest RQ observed in this study and was mainly due to the fungicide kresoxim. As kresoxim is the major contributor to the risk for all taxa, the difference of RQ_{STU} is reflective of the sensitivity difference for kresoxim between taxa. Algae was the most sensitive taxa with a RQ_{STU} 10 times lower than the $RQ_{MEC/PNEC}$. This can be explained by the difference in assessment factors used for the two methods. For calculating the PNEC for kresoxim, an assessment factor of 1000 was used, whereas an assessment factor of 100 was used for calculating the RQ_{STU}. Due to missing effect data for aquatic plants, the risk for this taxa could not be properly assessed.

The environmental concentrations of PPPs measured in the JOVA program gives an overview of some of the most important active PPPs used in the Norwegian environment. However, some of the active compounds are expected to cause biological effects at concentrations lower than the quantification limits for the analytical methods applied, and other compounds like glyphosate and some sulfonylurea herbicides were not included in the JOVA program. This indicates that the risk assessment based on MEC might exclude contributions from certain PPPs that are not included in the chemical analysis of various reasons. This might pose a problem if these compounds have a high potential for toxic effects in the receiving water bodies and thus represent toxicity drivers in the mixture risk assessment.

Calculation of RQ_{STU} may provide a challenge when there are substantial effect data gaps and those compounds are toxicity drivers in the mixture (see Table 4 for identification of data gaps). This could potentially lead to underestimation of the risk for one or more species group, and lead to large discrepancies between the two RQs derived. However, as the $RQ_{MEC/PNEC}$ is considered to be more conservative than the RQ_{STU} (Backhaus and Faust 2012), a larger emphasis could be placed on the $RQ_{MEC/PNEC}$ in cases where such data are missing for the calculation of RQ_{STU}. A larger reliance on $RQ_{MEC/PNEC}$ in data-poor mixture risk assessment procedures represents adoption to the precautionary principle, until sufficient data can support a more complete risk assessment.

The approach taken in this study has been thoroughly discussed in previous publications (Backhaus and Faust 2012; Finizio et al., 2005) and the summation of MEC/PNEC is strongly supported by combined toxicity predictions adopting to CA predictions as a default approach (Backhaus and Faust, 2012). In this risk assessment process the compounds have been assumed to act concentration additively despite the fact that the PPPs have different MoA (Table 5). However, it has been shown that more than 77 of 85 investigated combinations of compounds with presumably dissimilar MoA were well predicted by CA (Deneer 2000). Although this is generally applicable, both antagonistic and synergistic effects of binary mixtures of PPPs have been observed (Santos et al., 2010). Nevertheless antagonism and additivity were observed far more often than synergy (Deneer, 2000; Santos et al., 2010), indicating that risk assessment based on additivity expectations likely provide a conservative risk estimate and suggest that the present approach was appropriate as an initial risk assessment of PPP mixtures under ecologically relevant Norwegian conditions.

4.3 Knowledge gaps, future advice and suggestions

Risk assessment of combined toxicity of complex mixtures is still in early phases of development and there is a substantial need for in-depth experimental and theoretical effort to improve both the data support and verify the predictive modelling approaches taken. The present study has, as an initial approach to risk assessment of complex mixtures of PPPs under ecologically relevant exposure scenarios in Norway, detected a number of limitations that contributes to the uncertainty of the risk assessment approach used and are addressed below.

4.3.1 Uncertainty related to sampling and analysis

Uncertainties in sampling and analysis could lead to uncertainties in the MEC values used for cumulative risk assessment. The sampling of the six locations investigated in this project was performed with flow proportional composite sampling of stream water with a sampling period of approximately 14 days, and the details and uncertainties connected to this sampling method has been dealt with in Deelstra et al (2013). This type of sampling is believed to reduce the uncertainties by sampling over a larger time-period and adjusting to the total water flow, however, the actual peak concentrations of pesticides will rarely be obtained due to dilution during the sampling period and possible degradation in the sampling container. The uncertainty of sampling and analysis can be calculated by available tools like http://www.samplinghelper.com/

4.3.2 Missing taxa- and life stage-specific effect data

The applied assessment factor for calculation of PNEC is dependent on the amount of available effect data. For the calculation of $RQ_{MEC/PNEC}$ the uncertainty in risk contribution of compounds with poor data support is larger than that of compounds with substantial amount of effect data. Compound-specific effect data was not available for all species in this study, thus resulting in lack of data for calculation of the RQ_{STU}. There are often also limited data describing the sensitivity of different life stages, and in many cases the PPPs MoA in non-target organisms are not fully characterized. A wider knowledge of the MoA in non-target organisms would give a broader platform to identify mechanisms that would lead to synergistic or antagonistic interactions, and thus provide important input to employing more advanced approaches to assess the risks of PPP mixtures. For calculation of RQ_{STU}, only acute data were used and thus an assessment factor of 100 was applied. Additional chronic effect data would make it possible to calculate the RQ_{STU} for chronic effects and thus reduce the assessment factor from 100 to 10 in line with the $TER_{chronic} \geq 10$. The outlined limitations could successfully be overcome by conducting targeted ecotoxicity tests to fill data gaps and perform more in-depth characterization of the MoA of PPPs.

4.3.3 Missing exposure data for PPPs

The PPPs not included in the chemical analysis due to lower effect concentrations than analytical detection limits could lead to an underestimation of the risk in areas where such compounds are used. Approval of new PPPs and introduction of highly efficient PPPs on the Norwegian market challenges the analytic detection towards developing methodological approaches with lower limit of detection (LOD) and limit of quantification (LOQ). Successful implementation of a balance between advanced sampling methods such as time-weighted passive sampling and high-volume solid-phase extraction approaches with development of more sensitive analytical approaches for polar and non-polar PPPs may improve our ability to determine low concentrations of PPPs in the environment. Dispersion and fate modelling based on emission data could additionally aid the calculation of PEC values at different time-periods to support the cumulative risk assessment.

4.3.4 Lack of knowledge of potential RQ contribution from other pollutants

In addition to PPPs, organisms at the investigated sites could be exposed to pollutants from other local sources than pesticide use, and/or by long transport contaminants. This initial risk assessment has only focused on the risk of combined effect of PPPs, but contribution from other contaminants cannot be ruled out. An investigation of sources of pollutants in the respective areas and dispersion and fate modelling of these could potentially give an indication of presence and concentrations of additional pollutants. Non-target analytical approaches, such as that provided by GC-ToF-MS and LC-q-TOF, may provide unbiased detection of potential unknown toxicants that may be additional drivers of toxicity and thus affect the overall risk to organisms living in the recipient.

4.3.5 Lack of experimental evidence for the predictive modelling approaches used

The cumulative risk assessment based on summation of MEC/PNEC and calculation of taxa-specific RQs is based on the concept of CA. Although the CA approach has been successful in predicting the effect of mixtures in a variety of species and for different endpoints (Belden et al., 2007; Cedergreen et al., 2008; Coors et al., 2012; Deneer, 2000; Faust et al., 2003; Petersen et al., 2013; Schnell et al., 2009; Silva et al., 2002; Thorpe et al., 2003; Tollefsen et al., 2012), no verification has been performed to validate the approach used for cumulative risk assessment. Although verification studies for environmental risk assessment procedures could be challenging to perform, some approaches for providing such support can be proposed. Identified risk scenarios or hot spots could be verified by performing ecotoxicity studies directly on field samples or extracts of these, conduct ecotoxicity studies of synthetic mixtures defined from field monitoring or even caging organisms in recipient during high exposure events.

5. Conclusions/summary

In this initial risk assessment of cumulative risk of PPPs, compiled data for effect and environmental concentrations were used to calculate the risk quotients $RQ_{MEC/PNEC}$ and the species specific RQ_{STU} for six different sites in Norway. The composition of PPPs and environmental concentrations varied between the six sampling sites and also with the different sampling periods at each site. The performed cumulative risk assessment identified that aquatic organisms at four of the six investigated sites were potentially at risk for combined effects of PPPs. The sites Hotranelva, Mørdrebekken, Skuterudbekken and Vasshaglona had a composition and environmental concentrations of PPPs that resulted in $RQ_{MEC/PNEC}>1$ and $RQ_{STU\text{aquatic plants or algae}}>1$. Minimal risk was predicted for Heiabekken and Skuterudbekken as RQ was below 1. The risk at each site appeared to be driven by a few compounds and the MoA of these compounds were predominantly consistent with identification of susceptible taxa. The most sensitive taxa towards combined effect of PPPs of the four investigated groups appeared to be aquatic plants, although algae

were also identified to be potentially affected at two sites. The risk was highest from late June to mid-August, and the risk was mostly confined to 1-4 of the 9 sampling periods. The present study has shown that the approach of calculating $RQ_{MEC/PNEC}$ and RQ_{STU} is applicable to cumulative risk assessment of PPPs. Limitations that contributes to the uncertainty of the risk assessment approach used and measures to reduce these were also identified to aid in developing cumulative risk assessment in future initiatives.

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Appendix A.

Concentrations of detected plant protection products in all samples

Table A1. Concentrations (µg/L) of detected plant protection products at the investigated sites at different sampling periods

Sampling site	Sampling period	2,4-D	Aclonifen	Azoxystrobin	Bentazone	Boscalide	Cyazofamid	Cyprodinil	Dicamba	Dichlorprop	Dimethoate	Fenamidone	Fenhexamid	Phenmedipham	Fluroxypyr	Imazalil	Imidacloprid
Heiabekken	17.04.2012 - 03.05.2012																
Heiabekken	18.05.2012 - 04 06 2012			0.048													
Heiabekken	04.06.2012 - 25.06.2012			0.081									0.047				0.15
Heiabekken	25.06.2012 - 05.07.2012			0.044				0.022							0.1		0.25
Heiabekken	05.07.2012 - 25.07.2012				0.019												0.53
Heiabekken	25.07.2012 - 07.08.2012											0.021			0.059		
Heiabekken	07.08.2012 - 28.08.2012																0.075
Heiabekken	28.08.2012 - 21.09.2012				0.022												
Heiabekken	21.09.2012 - 08.10.2012																
Hotranelva	30.04.2012 - 13.05.2012																
Hotranelva	13.05.2012 - 02.06.2012																
Hotranelva	17.06.2012 - 24.06.2012														0.11		
Hotranelva	24.06.2012 - 01.07.2012																
Hotranelva	01.07.2012 - 15.07.2012																
Hotranelva	15.07.2012 - 30.07.2012																
Hotranelva	30.07.2012 - 10.08.2012																
Hotranelva	08.09.2012 - 16.09.2012																
Hotranelva	18.10.2012 - 01.11.2012																
Mørdrebekken	30.04.2012 - 07.05.2012	0.013															
Mørdrebekken	07.05.2012 - 21.05.2012																
Mørdrebekken	21.05.2012 - 18.06.2012														0.27		
Mørdrebekken	26.06.2012 - 10.07.2012		0.23					0.021			0.053				0.26		0.021
Mørdrebekken	10.07.2012 - 16.07.2012				0.013										0.11		
Mørdrebekken	16.07.2012 - 31.07.2012														0.18		
Mørdrebekken	31.07.2012 - 08.08.2012			0.045													1.1
Mørdrebekken	08.08.2012 - 28.08.2012																
Mørdrebekken	28.08.2012 - 24.09.2012														0.409		
Skuterudbekken	17.04.2012 - 03.05.2012																
Skuterudbekken	18.05.2012 - 11.06.2012																
Skuterudbekken	11.06.2012 - 25.06.2012	0.013													0.27		
Skuterudbekken	25.06.2012 - 06.07.2012														0.46		
Skuterudbekken	06.07.2012 - 25.07.2012	0.042			0.016				0.043						0.22		
Skuterudbekken	25.07.2012 - 10.08.2012														0.09		
Skuterudbekken	10.08.2012 - 28.08.2012									0.12							
Skuterudbekken	28.08.2012 - 21.09.2012																
Skuterudbekken	21.09.2012 - 12.10.2012																
Timebekken	16.04.2012 - 30.04.2012				0.016												
Timebekken	14.05.2012 - 29.05.2012																
Timebekken	29.05.2012 - 11.06.2012				0.018										0.056	0.037	
Timebekken	25.06.2012 - 09.07.2012				0.013												
Timebekken	23.07.2012 - 06.08.2012				0.024										0.44		
Timebekken	06.08.2012 - 20.08.2012				0.019										0.063		
Timebekken	03.09.2012 - 16.09.2012																
Timebekken	17.09.2012 - 30.09.2012																
Timebekken	11.06.2012 - 25.06.2012				0.025										0.065		
Vasshaglona	16.04.2012 - 30.04.2012				0.011	0.33											
Vasshaglona	30.04.2012 - 14.05.2012					0.14							0.086				
Vasshaglona	14.05.2012 - 29.05.2012																
Vasshaglona	29.05.2012 - 11.06.2012												0.044	0.034			
Vasshaglona	11.06.2012 - 25.06.2012						0.03					0.68					
Vasshaglona	17.09.2012 - 01.10.2012					0.081											
Vasshaglona	01.10.2012 - 15.10.2012																
Vasshaglona	15.10.2012 - 29.10.2012					0.079											
Vasshaglona	29.10.2012 - 12.11.2012					0.045											

Table A1 continued

Sampling site	Sampling period	Iprodione	Clopyralid	Kresoxim	Mandipropamid	MCPA	Mecoprop	Metalaxyl	Metamitron	Metribuzin	Pencycuron	Pinoxaden	Prothiokonazole-desthio	Pyridate metobolitt (CL 9673)	Pyrimethanil	Trifloxystrobin metabolite (CGA 321113)
Heiabekken	17.04.2012 - 03.05.2012															
Heiabekken	18.05.2012 - 04 06 2012					0.021					0.031					
Heiabekken	04.06.2012 - 25.06.2012	0.67				0.06		0.064		0.15	0.15				0.11	
Heiabekken	25.06.2012 - 05.07.2012	0.52	0.094			0.27		0.1		0.15	0.42		0.022		0.064	
Heiabekken	05.07.2012 - 25.07.2012	0.044			0.085			0.03		0.043	0.04				0.017	
Heiabekken	25.07.2012 - 07.08.2012		0.053			0.013		0.038					0.056			
Heiabekken	07.08.2012 - 28.08.2012	0.028								0.025	0.028					
Heiabekken	28.08.2012 - 21.09.2012							0.039			0.04					
Heiabekken	21.09.2012 - 08.10.2012															
Hotranelva	30.04.2012 - 13.05.2012															
Hotranelva	13.05.2012 - 02.06.2012															
Hotranelva	17.06.2012 - 24.06.2012					0.65	0.65									
Hotranelva	24.06.2012 - 01.07.2012					0.022	0.016									
Hotranelva	01.07.2012 - 15.07.2012					0.71							0.55			0.076
Hotranelva	15.07.2012 - 30.07.2012					0.13							0.16			
Hotranelva	30.07.2012 - 10.08.2012															
Hotranelva	08.09.2012 - 16.09.2012															0.13
Hotranelva	18.10.2012 - 01.11.2012															0.16
Mørdrebekken	30.04.2012 - 07.05.2012															
Mørdrebekken	07.05.2012 - 21.05.2012															
Mørdrebekken	21.05.2012 - 18.06.2012		0.32			0.98	1.7	0.024			0.023					
Mørdrebekken	26.06.2012 - 10.07.2012		0.099			0.18	0.087	0.037		0.077	0.026	0.029	0.12			
Mørdrebekken	10.07.2012 - 16.07.2012					0.014	0.021	0.046					0.076			0.062
Mørdrebekken	16.07.2012 - 31.07.2012					0.011		0.049					0.084			
Mørdrebekken	31.07.2012 - 08.08.2012				0.24			0.29		0.12	0.35		0.067			
Mørdrebekken	08.08.2012 - 28.08.2012															
Mørdrebekken	28.08.2012 - 24.09.2012					0.505		0.029								
Skuterudbekken	17.04.2012 - 03.05.2012															
Skuterudbekken	18.05.2012 - 11.06.2012					0.027	0.031									
Skuterudbekken	11.06.2012 - 25.06.2012		0.14			0.55	0.42									
Skuterudbekken	25.06.2012 - 06.07.2012		0.18			0.89	0.17						0.022			
Skuterudbekken	06.07.2012 - 25.07.2012		0.11			0.1	0.065						0.027			
Skuterudbekken	25.07.2012 - 10.08.2012					0.013	0.012						0.033			0.46
Skuterudbekken	10.08.2012 - 28.08.2012			0.78		0.02										0.33
Skuterudbekken	28.08.2012 - 21.09.2012															0.24
Skuterudbekken	21.09.2012 - 12.10.2012															0.219
Timebekken	16.04.2012 - 30.04.2012															
Timebekken	14.05.2012 - 29.05.2012															
Timebekken	29.05.2012 - 11.06.2012					0.016				0.027						
Timebekken	25.06.2012 - 09.07.2012															
Timebekken	23.07.2012 - 06.08.2012															
Timebekken	06.08.2012 - 20.08.2012					0.011										
Timebekken	03.09.2012 - 16.09.2012															
Timebekken	17.09.2012 - 30.09.2012					0.025										
Timebekken	11.06.2012 - 25.06.2012					0.17										
Vasshaglona	16.04.2012 - 30.04.2012										0.023					
Vasshaglona	30.04.2012 - 14.05.2012										0.023					
Vasshaglona	14.05.2012 - 29.05.2012															
Vasshaglona	29.05.2012 - 11.06.2012								0.49	0.048						
Vasshaglona	11.06.2012 - 25.06.2012				0.053				0.23	0.28				0.024		
Vasshaglona	17.09.2012 - 01.10.2012															
Vasshaglona	01.10.2012 - 15.10.2012															
Vasshaglona	15.10.2012 - 29.10.2012															
Vasshaglona	29.10.2012 - 12.11.2012															

Appendix B.

$RQ_{MEC/PNEC}$ for all time periods at each site

Table B1. Calculated $RQ_{MEC/PNEC}$ values for the different sampling periods at each site. Bold text indicates a risk quotient above 1.

Site	Sampling period	$RQ_{MEC/PNEC}$
Heiabekken	17.04.2012-03.05.2012	0.0000
Heiabekken	18.05.2012-04.06.2012	0.0556
Heiabekken	04.06.2012-25.06.2012	0.3537
Heiabekken	25.06.2012-05.07.2012	0.8182
Heiabekken	05.07.2012-25.07.2012	0.0770
Heiabekken	25.07.2012-07.08.2012	0.9088
Heiabekken	07.08.2012-28.08.2012	0.0389
Heiabekken	28.08.2012-21.09.2012	0.0084
Heiabekken	21.09.2012-08.10.2012	0.0000
Hotranelva	30.04.2012-13.05.2012	0.0000
Hotranelva	13.05.2012-02.06.2012	0.0000
Hotranelva	17.06.2012-24.06.2012	0.0469
Hotranelva	24.06.2012-01.07.2012	0.0015
Hotranelva	01.07.2012-15.07.2012	**8.1361**
Hotranelva	15.07.2012-30.07.2012	**2.3615**
Hotranelva	30.07.2012-10.08.2012	0.0000
Hotranelva	08.09.2012-16.09.2012	0.0020
Hotranelva	18.10.2012-01.11.2012	0.0025
Mørdrebekken	30.04.2012-07.05.2012	0.0002
Mørdrebekken	07.05.2012-21.05.2012	0.0000
Mørdrebekken	21.05.2012-18.06.2012	0.0804
Mørdrebekken	26.06.2012-10.07.2012	**4.3437**
Mørdrebekken	10.07.2012-16.07.2012	**1.1202**
Mørdrebekken	16.07.2012-31.07.2012	**1.2366**
Mørdrebekken	31.07.2012-08.08.2012	**1.2828**
Mørdrebekken	08.08.2012-28.08.2012	0.0000
Mørdrebekken	28.08.2012-24.09.2012	0.0338
Skuterudbekken	17.04.2012-03.05.2012	0.0000
Skuterudbekken	18.05.2012-11.06.2012	0.0012
Skuterudbekken	11.06.2012-25.06.2012	0.0394
Skuterudbekken	25.06.2012-06.07.2012	0.3837
Skuterudbekken	06.07.2012-25.07.2012	0.4060
Skuterudbekken	25.07.2012-10.08.2012	0.4935
Skuterudbekken	10.08.2012-28.08.2012	**32.5068**
Skuterudbekken	28.08.2012-21.09.2012	0.0038
Skuterudbekken	21.09.2012-12.10.2012	0.0034
Timebekken	16.04.2012-30.04.2012	0.0000
Timebekken	14.05.2012-29.05.2012	0.0000
Timebekken	29.05.2012-11.06.2012	0.1209
Timebekken	25.06.2012-09.07.2012	0.0000
Timebekken	23.07.2012-06.08.2012	0.0004
Timebekken	06.08.2012-20.08.2012	0.0008
Timebekken	03.09.2012-16.09.2012	0.0000
Timebekken	17.09.2012-30.09.2012	0.0016
Timebekken	11.06.2012-25.06.2012	0.0113
Vasshaglona	16.04.2012-30.04.2012	0.1366
Vasshaglona	30.04.2012-14.05.2012	0.0691
Vasshaglona	14.05.2012-29.05.2012	0.0000
Vasshaglona	29.05.2012-11.06.2012	0.1412
Vasshaglona	11.06.2012-25.06.2012	**3.1585**
Vasshaglona	17.09.2012-01.10.2012	0.0324
Vasshaglona	01.10.2012-15.10.2012	0.0000
Vasshaglona	15.10.2012-29.10.2012	0.0316
Vasshaglona	29.10.2012-12.11.2012	0.0180

Appendix C.

Contribution plot for $RQ_{MEC/PNEC}$ Heiabekken and Timebekken

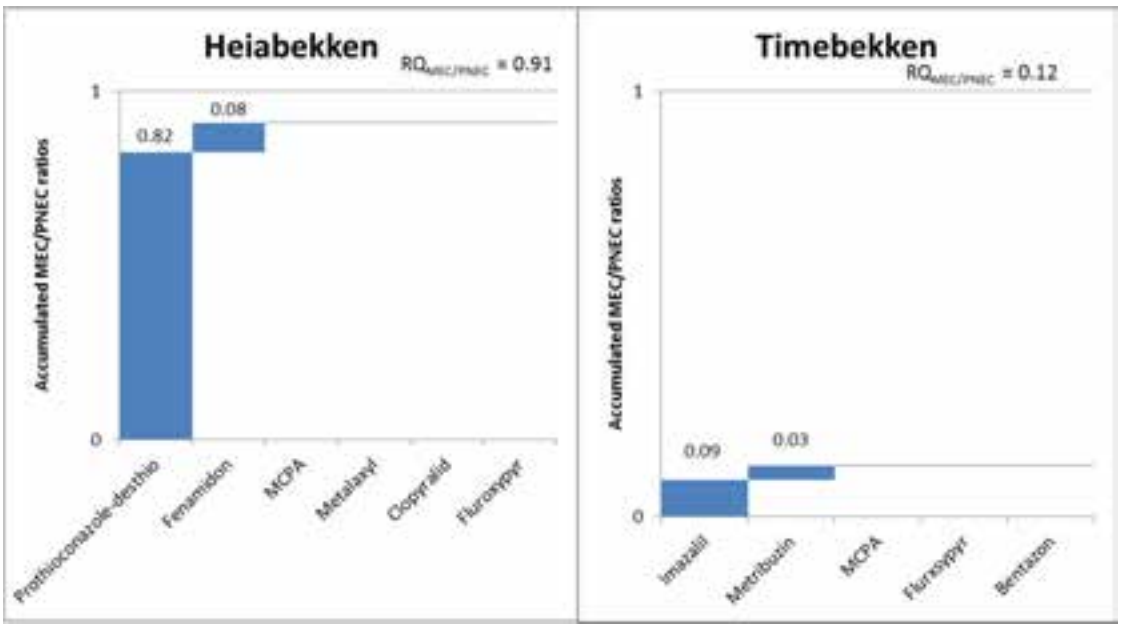

Figure C1. Contribution plot of the individual (blue column) and cumulative (value on the y-axis) MEC/PNEC ratios for the PPPs detected in Heiabekken 25.07-7.08 and Timebekken 29.05-11.06. The individual MEC/PNEC equal to or larger than 0.01 are given above each column

Appendix D.

Taxa-specific risk quotients at Heiabekken and Timebekken

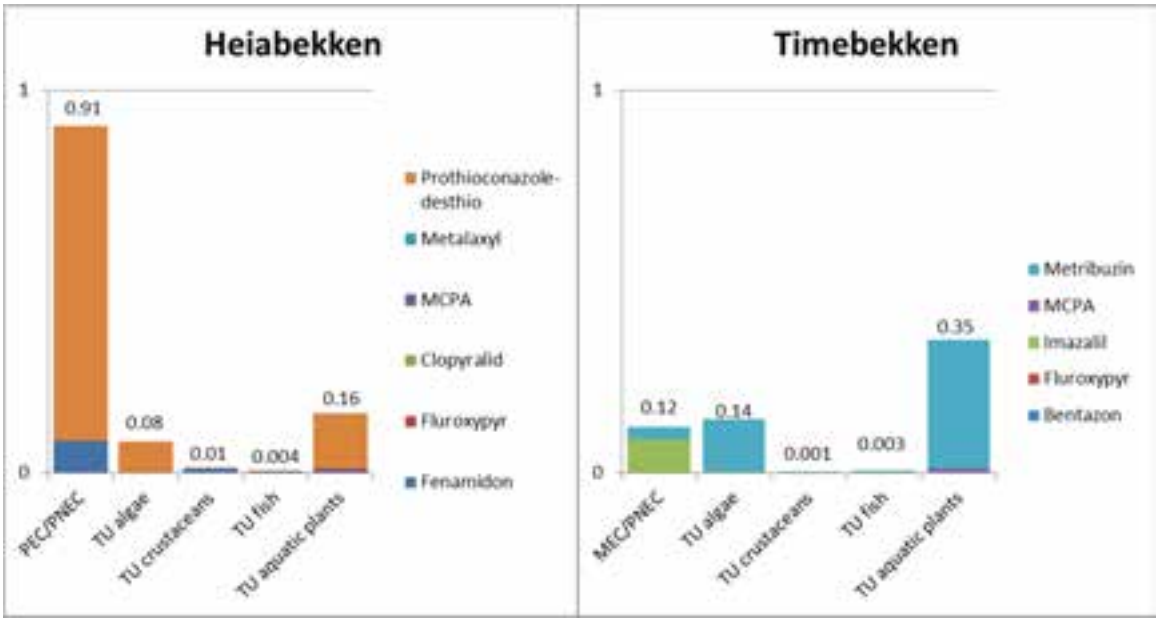

Figure D1. The individual contributions to the $RQ_{MEC/PNEC}$, $RQ_{STUalgae}$, $RQ_{STUcrustacean}$, $RQ_{STUfish}$ and $RQ_{STUaquatic\ plants}$ for the plant protection products in samples from Heiabekken and Timebekken. The RQ for the different approaches are given above the respective column. The TU is calculated based on an assessment factor of 100.

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