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Evaluation of risk for aquatic organisms when realistic conditions of use are considered in risk assessments of plant protection products (PPPs)

- A risk assessessment of PPPs used in cultivation of spring rape (*Brassica napus*)


Photo: Henry von Platen

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Abstract

Before plant protection products (PPPs) are authorized and placed on the market, the applicant must demonstrate with a risk assessment that no unacceptable effects occur when products are used according to good plant protection practice and realistic conditions of use. Nevertheless, risk assessments are presently not considering realistic conditions of use, mainly for legislative and scientific reasons. Chemicals are generally regulated on a substance-by-substance approach, *i.e.* they are risk assessed individually and not in their actual context, including realistic exposure. The contribution to the total potential toxicity from each individual chemical is therefore not considered and how to restrict what chemicals should be considered to be "in the mixture" is not established**.** From a scientific perspective it is also under debate how, when and where chemicals should be considered as jointly affecting organisms since it is unknown until tested. A template for considering realistic exposure and risk has therefore not yet been established. The aims of this study were to investigate what realistic conditions of use for PPPs in cultivation of spring rape really are, and to perform cumulative risk assessments to aquatic organisms on such usage, *i.e.* to risk assess mixtures of PPPs. In order to see how much is missed when realistic conditions of use is not considered; individual risk assessment of the active substances was also performed.

First a literature study on the usage of PPPs in cultivation of spring rape was performed. Thereafter a temporal schematic overview on the use of different PPPs was made. When this was done, there were still gaps in the knowledge on what realistic conditions of use are. In order to fill the gaps, four questions were asked to 60 individuals representing different professionals within the area of PPPs in rape fields in Sweden. A qualitative compilation of the literature study and the interviewees' answers were carried out. Thereafter individual risk assessments of 21 active substances were performed. Three of the active substances were each present in two different products and were therefore risk assessed twice, in total, 24 individual risk assessments were performed. When the individual risk assessments were done, cumulative risk assessments of relevant mixtures were performed. The model used for the cumulative risk assessments was Concentration Addition.

The investigation of realistic conditions of use showed that several different PPPs are used during the same growing season in cultivation of spring rape. The usage differs between regions of Sweden but some general tendencies can be seen, for example that seed dressing is always required and the numbers of sprayings with insecticides are around two. Tank mixtures, *i.e.* the mixing of two PPPs in the same tank before spraying, are not very common in cultivation of spring rape, but do occur. The results from the individual risk assessments showed that ten out of the 24 active substances posed a risk to the aquatic environment. Hence, the cumulative risk

assessments also showed a risk when the ten substances that posed a risk were included, since an additive model was used. It was also shown that the cumulative risk assessments in this study were mainly driven by one or two active substances. When these substances were removed, the mixture was no longer considered a risk.

The conclusion of the present study is that in order to perform risk assessments where realistic conditions of use are considered, there must be a competent authority that keep track on the usage and publishes guidelines for what is considered realistic conditions of use. Another conclusion was that realistic conditions for the use of PPPs are the usage of several different PPPs during a growing season and which products that are used are continuously changing. Also, concentration addition is at the moment considered to be the best model for cumulative risk assessments. The study has shown that risk assessments of realistic conditions for the use of PPPs are complex and that further work is needed before methods of risk assessments where realistic conditions of use are taken into consideration can be implemented in the approval process of PPPs.

Abbreviations

CA – Concentration Addition

DG SANCO – Directorate-General for Health and Consumers (SANCO is an abbreviation of the French name: Santé et consommateurs)

DT_{50} – Degradation time for 50% of a compound.

EbC_{50} – Concentration at which 50% reduction in biomass is observed

EC_{50} – Median effective concentration, concentration that gives the preset effect to 50% of the tested population.

ECPA – European Crop Protection Association

EFSA – European Food Safety Authority

EFSA PPR – EFSA Panel on Plant Protection Products and their Residues

ErC_{50} – Concentration at which 50% inhibition of growth rate is observed

FOCUS - Forum for the Co-ordination of Pesticide Fate Models and their Use

GAP – Good Agricultural Practice

GPP – Good Plant Protection Practice

GV – Guideline Value

IA – Independent Action

KemI – Swedish Chemicals Agency

K_{OC} – Partition coefficient organic carbon - water

LC_{50} –Median lethal concentration, concentration that kills 50% of the population observed.

LoD – Limit of Detection

MFC – Measured Field Concentration

MoA – Mode of Action

NOEC –No observed effect concentration

PEC_{SW} - Predicted Environmental Concentration in surface waters.

PPP – Plant Protection Products

PRZM – Pesticide Root Zone Model

SCB –Statistics Sweden

SCCS - Scientific Committee on Consumer Safety

SCENIHR - Scientific Committee on Emerging and Newly Identified Health Risks

SCHER - Scientific Committee on Health and Environmental Risks

SJV – Swedish Board of Agriculture

SLU – Swedish University of Agricultural Sciences

SPG – Specific Protection Goals

SWAN- Surface Water Assessment eNabler

TER – Toxicity Exposure Ratio

TOXSWA – TOXic Substances in Surface Waters

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Introduction

Plant protection products (PPPs) are formulated chemical products used to protect plants or plant products against harmful organisms. PPPs are composed of active substances (chemicals that cause the pesticidal effect) and co-formulants. Risk assessments of plant protection products (PPPs) should according to Regulation (EC) No 1107/2009 consider realistic conditions for the use of PPPs. It is not obvious what realistic conditions of use are, since the usage does not follow a template. However, realistic conditions of use is often the usage of several different products for the same pest and/or the use of different compounds to combat several pests at the same time, for example both a fungus and an insect. Thereby, the PPPs can be mixed both in the field and already in the tank, before spraying. It is therefore quite uncommon that only one PPP is used in the same field during a whole growing season. Despite the well-known fact that PPPs are mixed in the fields, and that chemicals interact, each substance is risk assessed substance-by-substance, without taking into account the "mixture effects" (Backhaus and Faust 2012). This is a case of growing concern and it is therefore very important to try to find a feasible way to make risk assessments where combination effects are included.

The aims of this study are to investigate PPP-use under realistic conditions in spring rape (*Brassica napus*) and to estimate if risk assessments on product-by-product basis provide similar level of protection as if realistic conditions of use would be considered. All active substances in this study are therefore risk assessed both individually and in relevant combinations. The following hypotheses were tested in this study (1) realistic conditions for the use of PPPs are the usage of several different active substances during a growing season and (2) many products that are approved today would not be approved if realistic conditions for the use of PPPs were considered, *i.e.* in mixtures.

Cultivation of rape

Rape (*Brassica napus*), also called rapeseed, is the most common oilseed in Sweden and is cultivated on 95% of the oilseed areal. It is mainly cultivated in Götaland and the southern parts of Svealand, but fields have occurred as high up as the northern parts of Gästrikland (Mattson 1990, Larsson 2008). Rape is a domestic oilseed plant in Sweden but was not cultivated in a large scale until after World War II (Mattson 1990, DVF 2008). The plant is a one- or two-year herb which gets meter high and is slightly branched at the top. The leaves are bald and blue-green, often with a bluish dewy surface. Rape flowers in June-July with large, bright yellow flowers. The pods are up to 10 cm long and 0.5 cm wide, with a distinct whip (Figure 1) (DVF 2008). The seeds are dark brown or black, quite small, 1-2 mm, and very oil-rich (30-40%) (Mattson 1990, Jonsson 2001). The seeds also contain proteins (20-25%) and fibers (15%). Rape is both used as an oilseed plant and as a feed plant due to its high nutritional value (Jonsell and

Svensson 2010). The rapeseed oil can be used both in food and for example in biodiesel, depending on how it is treated. Biodiesel is produced via esterification where the glycerol in the oil is exchanged to methanol (SCC 2012).

Sowing of rape can be done both in the spring (spring rape) and in the autumn (winter rape). Autumn sowing normally provides a higher yield but is also associated with a higher risk since the crops might die during a cold winter (Mattson 1990). The autumn sowing takes place in August so the plants will have enough time to grow strong before the winter (Mattson 1990). The timing of spring sowing is difficult to assess. It should be done as early as possible since early sowing both increases the yield and the quality of the oil, but at the same time the seeds should not be exposed to severe cold. Cold can cause a delayed germination or in the worst case, the seeds will not germinate at all (Mattson 1990). Too early sowing in the spring can also lead to frost damage and problems with weeds, which grow better than oil plants at low temperatures. If the rape is sown too late in the spring, the plant does not have enough time to mature (Mattson 1990). Rape has a high oxygen demand and is therefore sensitive to moist soils, where the amount of oxygen is limited. Winter rape is particularly sensitive and it has to be grown in well-drained soils. Spring rape is not as sensitive and can be grown in most soils as long as the soil dries up early enough (Mattson 1990).

Figure 1. Rape (*Brassica napus*) is a one- or two-year herb which gets meter high. Rape is mainly recognized by its large yellow flowers. The pods are approximately 10 cm long and the seeds are small and dark brown or black (DVF 2008). Picture from: Köhler's Medizinal-Pflanzen.

Rape is mainly autogamous but 10-15% of the flowers cross-fertilize by air or pollinators (Mattson 1990). Pollination by insects increases the harvest and quality of the rapeseed oil. It is therefore recommended by the Swedish Board of Agriculture that farmers who cultivate rape have two to three hives with honeybees (*Apis mellifera)* per hectare in the rape field. This can increase the yield with 10-20% (SJV 2011).Unfortunately, there is a widespread decline of pollinators in the world (Biesmeijer *et al.* 2006, Klein *et al.* 2007, Blacquière *et al.* 2012). Not only wild pollinators are affected, also honeybees decline. The cause of the decline is still not completely understood but extensive use of insecticides is believed to have contributed to the loss of pollinators (Blacquière *et al.* 2012). The use of certain insecticides is therefore restricted. Usage of these products is not allowed when the crop blooms or other times when bees are foraging (SJV 2012a).

The quality of the harvested rapeseeds is very dependent on the conditions when harvesting. The seed is mature and ready to harvest when the water content of the seed is 15-20%. If the water content when harvesting is too high, there will be unnecessary costs for drying the seeds and if the water content is too low (<10%) the seed is easily damaged (Mattson 1990, Jonsson 2001). In 2011, a total area of 94 040 ha was cultivated with rape in Sweden and the preliminary statistics for 2012, show that there has been a 16% increase of cultivation areas for rape compared to 2011 (SJV 2012b, SJV 2012c).

Weeds, diseases and pests

It is very important to follow a crop rotation in cultivation of rape (Leino 2006). Crop rotation is a practice where different types of crops are grown within the same area in sequential seasons. Continuously growing of the same crop within an area often leads to that the natural pests of the crop propagate to an unacceptable level. This problem can be avoided or reduced if the farmer alternate between different crops in the area *i.e.* follows a crop rotation (Ohlander 1990). Rape is a good break crop but it should not be cultivated too often since several pests and diseases benefit from a more intense cultivation of rape (Leino 2006).

The most serious and widespread fungal diseases in rapeseed are black leafspot (*Alternaria brassicae*), cotton mildew (*Sclerotinia sclerotiorum*), dry rot (*Leptosphaeria maculans*) and verticillium wilt (*Verticillium longisporum*). Most fungal diseases, for example cotton mildew benefits from humid conditions. All four of the above mentioned diseases can lead to large decreases of the harvest (Leino 2006). Common pests are the grey field slugs (*Deroceras reticulatum)*, pollen beetles (*Meligethes aeneus*) and cabbage stem flea beetles (*Psylliodes chrysocephala*) (Andersson *et al.* 2012). Pollen beetles are a problem especially in spring rape since the pests are more active when it is warmer outside (SJV 2012).

Weeds are, however, a problem especially in winter rape. Cornflower (*Centaurea cyanus*), poppy (*Papaver sp.*), mayweed (*Tripleurospermum perforatum*) and cleavers (*Galium aparine*) are some examples of weeds that lower the yield. The field mustard (*Sinapis arvensis*) can be particularly troublesome since the most common herbicides have no effect on it (Gunnarsson 2012a). Another problem occur when the proceeding crop is cereal, because then the eventually spilled grains can, just like weed, compete with the rape and thereby decrease the yield (Gunnarsson 2011).

PPPs in surface water

PPPs are widely used all over the world and are therefore contaminating the environment, for example surface waters close to the fields (Ulén *et* al. 2002, Adielsson *et al*. 2009). Studies of the impact of agriculture on the environment with focus on PPPs have been performed within the framework of national monitoring program since 2002. The surveys are conducted on behalf of the Swedish Environmental Protection Agency (EPA) and are included in two program areas, "Farmland" and "Air" (Nanos *et al.* 2012). The monitoring program includes investigations of PPPs in surface water, ground water, storm water, sediment and air in agriculture-dominated areas in Sweden. In 2011, the chemical analyses included over 120 substances and particularly those substances that are widely used in Swedish agriculture. Some active substances included in the survey might be used to a less extent but are of importance anyway since they are prone to leak from fields to surface waters, have low guideline values or are included as priority substance in the Water Framework Directive (2000/60/EC) (Nanos *et al.* 2012). The guideline value indicates the highest concentration of a substance in surface water where no negative effect on organisms in the ecosystem can be expected (Nanos *et al.* 2012). What substances and the amount of substances included in the survey vary to some extent from year-to-year depending on what substances are available on the market (Adielsson *et al.* 2009). Each year, surface water samples are collected from agricultural streams in four type areas of Sweden. The type areas are situated in Västergötland, Östergörland, Halland and Skåne (Figure 2) (Nanos *et al.* 2012). In 2011, 95 summer surface water samples, 26 winter surface water samples, nine samples from the river Skivarp, nine samples from the river Vege, and 28 flow proportional samples from a stream in Skåne were analyzed. The flow proportional samples are taken every 80^{th} minute and are merged into weekly samples in the summer and two-week samples in the winter (Nanos *et al* 2012). During the annual sampling season in 2011, seven substances used in cultivation of rape were found above the guideline value in surface waters (Table 1) (Nanos *et al.* 2012). Note that the substances in Table 1 are also approved for use in cultivation of other crops than rape.

Figure 2. Within the national monitoring program**,** surface water samples are collected from agricultural streams in four type areas in Sweden and analyzed for plant protection products. The type areas are Västergötland (O 18), Östergötland (E 21), Halland (N 34) and Skåne (M 42). Samples are also collected from the river Vege and the river Skivarp (based on a figure by Nanos *et al.* 2012).

Table 1. Active substances used in cultivation of spring rape detected above the guideline value (GV) in samples from surface water from the annual sampling of selected Swedish streams and rivers in 2011, the number of times the substances have been detected above or close to the GV, maximum concentration measured and the ratio between maximum concentration and GV. Detection limit (DL) given as median (Nanos *et al.* 2012).

Substance	**GV (µg l^{-1})**	**LoD (µg l^{-1})**	**No. times ≥ LoD**	**Max conc. (µg l^{-1})**	**Ratio: max. conc/LoD**
beta-cyfluthrin	0.0001	0.0006^	1	*0.008*	80
Esfenvalerate	0.0001	0.0003^	3	0.004	40
Imidacloprid*	0.06	0.001	2	0.14	2
Metazachlor	0.2	0.001	2	0.42	2
Prochloraz	0.06¨	0.002	2	0.31	5
Tau-fluvalinate	0.0002	0.002^	2	0.012	60
Tiachloprid	0.03¨	0.001	3	0.051	2

^Limit of detection higher than guideline value.

¨Preliminary guideline value

*Approved for seed dressing – rarely used due to problems with resistance

The possible effects of the substances in table 1 are of concern since the active substances are present in the environment at concentrations where a negative effect on organisms in the ecosystem cannot be excluded. The potential toxicity of the mixtures that might occur in the surface waters is not included in the calculations of the guideline values and therefore the substances that do not reach their guideline value might also be of concern (Norberg 2004). The monitoring data is a good indication on what substances might be of greatest concern, but can also be misleading since only the substances that are included in the survey can be detected. It is therefore important to map the total use of PPPs in an area, in order to know what substances might pose a risk to the aquatic environment.

EU legislation regarding risk assessment of PPPs

Sweden became a member of the European Union (EU) in 1995 and due to this all Swedish laws had to be adapted to the legislation of the EU (Michanek 2008). During the years, different directives and regulations for the placing of PPPs on the market have been developed and applied. Since the approvals extend over several years, a great deal of the PPPs that are authorized for use today is authorized according to old risk assessment manners and old legislations. The current legislation, Regulation (EC) No 1107/2009 (from now on written as 1107/2009) should be applied on new authorizations since the 14th of June 2011. There is however a transition period for approximately five years when the old national legislation based on Council Directive 91/414/EEC (from now on written as 91/414) still can be applied. This is regulated in Article 80 in 1107/2009. It is also important to point out that in accordance with point 8 in 1107/2009 it is the industry that has to demonstrate that substances or products produced or placed on the market do not have any harmful effects on human or animal health or have any unacceptable effects on the environment. What counts as a PPP is listed in 1107/2009, Article 2 (1):

"1. This Regulation shall apply to products, in the form in which they are supplied to the user, consisting of or containing active substances, safeners or synergists, and intended for one of the following uses:

(a) protecting plants or plant products against all harmful organisms or preventing the action of such organisms, unless the main purpose of these products is considered to be for reasons of hygiene rather than for the protection of plants or plant products;

(b) influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient;

(c) preserving plant products, in so far as such substances or products are not subject to special Community provisions on preservatives;

(d) destroying undesired plants or parts of plants, except algae unless the products are applied on soil or water to protect plants;

(e) checking or preventing undesired growth of plants, except algae unless the products are applied on soil or water to protect plants."

Another important legislation is the Commission Regulation (EU) No 546/2011 (from now on written as 546/2011/EU) which in accordance with 1107/2009 regulates the uniform principles for evaluation and authorization of PPPs. For example, in part B, point 1.2. in 546/2011/EU it is stated that the Member States shall have regard to all normal conditions under which the PPPs may be used and to the consequences of its use when evaluating risk assessments of PPPs. The regulation also contains the requirements concerning the placing of PPPs on the market. The requirements set out in 546/2011/EU are for example that no authorization should be granted if the toxicity/exposure ratio for fish and *Daphnia* is less than 100 for acute exposure. This is called "trigger value" and is further described under the subtitle "Individual risk assessments of PPPs regarding aquatic organisms". The requirements in 546/2011/EU are the same requirements as set out in Annex VI to 91/414, with small modifications.

Regulation 1107/2009 applies to *products* which mean that all compounds in the product must be risk assessed in a combination. This is further expressed in Article 29 "Requirements for the authorization for placing on the market", where it is stated that interaction between the active substance, safeners, synergists and co-formulants shall be taken into account when assessing PPPs. Cumulative risk assessments for active substances in the same product is thus executed today, but what does the legislation say about considering the other chemicals co-occuring in the agricultural landscape in the risk assessments?

EFSA Panel on Plant Protection Products and their Residues (PPR) describes in a scientific opinion the legal basis for why combination effects should be taken into account when performing risk assessments of PPPs (EFSA PRP 2010). For example, in Article 4(3) in 1107/2009, it is stated that a PPP after application with "good plant protection practice" and under realistic conditions of use must not have any immediate or delayed harmful effects in humans and it should have no unacceptable effects on the environment. "Good plant protection practice" is defined in Article 3(18) in 1107/2009 as "a practice whereby the treatments with plant protection products applied to given plants or plant products, in conformity with the conditions of their authorised uses, are selected, dosed and timed to ensure acceptable efficacy with the minimum quantity necessary, taking due account of local conditions and of the possibilities for cultural and biological control;". As suggested by EFSA PPR, this can be interpreted as risk assessments of PPPs should be carried out based on how they are normally used for a certain crop. Since realistic conditions of use often are the use of several different PPPs on the same crop, this paragraph might give legal support for the idea that cumulative risk assessments must

be performed. In the scientific opinion, EFSA PRP further stated that "in order to meet the protection goals consistent with good plant protection practice (GPP) and having regard to realistic conditions of use, the risk assessment methodology should account for some degree of non-compliance to label instructions, for simultaneous use of products, and for variability in local conditions" (EFSA PPR 2010).

In Article 2 (3d) in 1107/2009 it is stated that the regulation also applies to products that are placed on the market in a form in which the user is recommended to mix it with another PPP. Such recommendations can for some products be found on the label and also The Swedish Board of Agriculture gives recommendations on which products that can be mixed for better effects (SJV 2013). This is, however, also not considered in risk assessments today. It is presently not established how risk assessments of mixtures due to realistic conditions of use should be performed. The EU Commission has commenced the work (COM/0252/2012) for consider cumulative effects in the chemical legislation.

Member State rapporteurs and The European Food Safety Authority (EFSA)

In recital 12 in 1107/2009 it is stated that in order to give an approval for an active substance, it is important that the approval follows a detailed procedure of evaluation. Since there is much work connected to an approval procedure as such, it is appropriate that a single Member State evaluate the information submitted by the interested parties and then act as a rapporteur for the community. It further states that to ensure consistency in the evaluation an independent scientific review has to be performed by EFSA.

EFSA is an independent European agency, established in 2002 and is funded by the EU budget. The establishments of EFSA as well as the general principles governing food and feed safety are regulated in Regulation (EC) No 178/2002. EFSA is responsible for risk assessment and risk communication regarding food and feed safety within the EU. The authority provides scientific opinions and advice in close collaboration with national authorities such as the Swedish Chemicals Agency (EFSA 2013a). The risk assessment is carried out independent from risk management and it is the European Commission that takes the management decisions as stated in 178/2002 and 1107/2009. The risk management is however based on the scientific advice from EFSA and thereby a large part of EFSA's work is scientific assessment requested by the European Commission, the European Parliament and member states (EFSA 2013b). EFSA publishes "Conclusion on the peer review of the pesticide risk assessment of the active substance XXX" on their website and these conclusions are used by the national authorities when evaluating applications for approval of PPPs (NZ 2011).

Risk assessment in a tiered approach

In the registration process of new PPPs, the environmental risk assessment is most commonly performed with a tiered approach as suggested in the Guidance document SANCO/3268/2001 (EC 2002). The idea with a tiered approach is that it is cost-effective for both the industry and for regulatory agencies but still protective for the environment (EFSA PPR 2010). The first tier is thereby a simple conservative assessment which is not very specific. If there is no risk at the first tier, no further work is necessary. If however a risk is identified at the first tier, additional work is needed. The additional work can for example be a more advanced exposure assessment or more studies on a certain organism (EFSA PPR 2010). The first tier can be used as an indication on which species the higher tier assessments need to focus on.

The exposure assessment follows the tiered approach and is divided into four steps and for each step, additional input data is required (EC 2002):

- Step 1 = Worst-case scenario
- Step 2 = Worst-case scenarios where dissipation between applications are taken into account.
- Step 3 = Realistic worst-case scenario where crop, climate, topography, water bodies, realistic worst-case soils and agronomy is taken into account.
- Step 4 = Localized risk assessment where mitigating factors are added to the calculations for Step 3.

Step 1-3 is performed with a set of standardized models that have been developed by The FOCUS Surface Water Scenarios Group (FOCUS is the acronym for Forum for the Co-ordination of Pesticide Fate Models and their Use) (EC 2002). The models calculate the predicted environmental concentration in surface waters (PEC_{SW}) which is used in the tiered risk assessment (FOCUS 2001). The model performing the calculations for Step 4 is developed by ECPA (European Crop Protection Association) and must be applied on top of calculations for step 3 (FOCUS 2007). For a more detailed description of the models, see “Surface water simulations” (page 19).

Acute- and chronic toxicity tests

In order to estimate the risk of an active substance, studies on organisms exposed to the active substance are used at all tiers of risk assessments. The studies are important since exposure to PPPs can cause both lethal and sub-lethal effects to organisms. Sub-lethal effects are for example biochemical, physiological, reproductive and behavioral effects (EC 2002a). The concentration where effects will occur is tested with both acute and chronic test methods. Acute toxicity testing tests the effects from short-term exposure, most often lethal effects (LC_{50}). Mortality is, however, difficult to define for crustaceans such as *D. magna* and the acute toxicity is instead stated as immobility (EC_{50}). Acute toxicity testing for fish usually lasts for 96

hours and for crustaceans it usually lasts for 48 hours (Norberg 2004). Short-term exposure can also cause sub-lethal effects but such effects are not covered in acute toxicity testing. If there are any concerns that short-term exposure might cause sub-lethal effects, further evaluations might be needed, *i.e.* higher tier studies (EC 2002a).

Long-term exposure can occur if static conditions predominate, when spraying is repeated or when the substance is stored in biota and sediment. Chronic toxicity tests are thus tests with repeated exposure, for 21 days or more for fish, and 21 days for crustaceans (Norberg 2004). The test parameters are for example growth, behavior and reproduction (EC 2002a). Chronic toxicity is presented as a No Observed Effect Concentration (NOEC). Chronic toxicity tests are the only measure of sub-lethal effects and are thus very important (EC 2002a).

The toxicity tests performed on algae are multigenerational tests. Therefore, both short- and long-term effects can be interpreted from the same study if the exposure is between 72 and 96 hours long. The test parameters are either growth measured by biomass (E_bC_{50}) or growth rate (E_rC_{50}). The test parameter is the same for both short- and long-term toxicity (Norberg 2004).

Combination effects of chemical mixtures

It has long been known that chemicals can react with each other and that the outcome of the reaction can differ substantially between different chemicals. It can, for example, be a physical reaction which causes the chemical mixture to stratify, froth, produce heat etc., *i.e.* reactions visible to the mere eye. The visible reactions are always risk assessed in compliance with the requirements set up in 546/2011/EU. However, all reactions are not directly visible and may be a cause of great concern since the toxicity of the chemicals might be enhanced (synergy, potentiating, supra-additive) or diminished (antagonistic, inhibitive, sub-additive) (Backhaus and Faust 2012). There is currently no requirement that studies on these effects in risk assessments of PPPs must exist. Synergy is when the toxic effect of two components is greater than the sum of their individual effects. This phenomenon is quite rare but can be of great importance in specific cases (Walker *et al.* 2006a, Backhaus and Faust 2012). An antagonistic effect is when the toxic effect of two components is less than the sum of their individual effects (Walker *et al.*2006a, Backhaus and Faust 2012). Antagonism might not be a big problem for the environment since the components become less toxic when mixed, but it is still an unwanted effect since the efficacy of the PPPs will be diminished. In the worst case, antagonism leads to the use of more PPPs.

Another aspect of combination effects is on the biological scale when organisms get exposed to several chemicals causing different effects. For example, if one chemical affects the reproduction by disturbing the reproductive behavior and another chemical affects the

reproduction by reducing the sperm count, the combined effect on the reproduction will be larger than the effect for each chemical alone. In those cases, the chemicals will not react with each other, but there will still be a combination effect.

There are several different methods for testing toxicity of chemical mixtures used in scientific studies today (Backhaus *et al.* 2010). Many of these test the mixture in its totality for example whole effluent testing of wastewater streams (OSPAR Commission 2007). These methods are however quite limited though experiments are needed on the specific mixture. Hence the methods are unusable in effect assessments since they are too costly and too time-consuming and most importantly, the whole mixture is seldom available (Backhaus *et al.* 2010). Due to the limitations of the whole mixture approach, methods that are based on specific knowledge of the mixture components have been developed. These methods can predict the mixture toxicity from a known (or assumed) chemical composition and the toxicity of the individual components. Concentration Addition (CA) and Independent Action (IA) are two classical mixture toxicity concepts which these methods most often are based on (Backhaus *et al.* 2010). Both CA and IA assume that there is no interaction between the components in the mixture and the uptake, distribution and elimination of single components is not influenced by other components in the mixture (Backhaus and Faust 2012).

Concentration Addition and Independent Action

When there is no evidence for an enhancing or diminishing effect, the estimates of the toxicity of a mixture can be made in a pure additive manner by adding the toxicity measures of each compound together. For example, if a certain concentration of a compound causes 5% mortality and the concentration of another compound causes 20% mortality, then the added toxicity will be 25% (Walker *et al*. 2006). CA assumes that the components in the mixture compete for the same receptor and hence have the same mode of action (MoA) (Backhaus *et al.* 2010). CA is mathematically formulated as (Kortenkamp *et al.* 2009):

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{P_i}{ECx_i}\right)^{-1} \qquad \text{(eq. 1)}$$

- n = number of mixture components
- p_i = relative fraction of chemical i in the mixture
- x = a common effect level
- ECx_i = total concentration of component i that causes x effect
- ECx_{mix} = total concentration of a mixture that causes x effect

The fraction $\left(\frac{P_i}{ECx_i}\right)$ of every component in the mixture is called a toxic unit and it gives the concentration of a compound, scaled for its relative potency. A chemical can thereby be

exchanged to equivalent concentrations by another chemical without a change in mixture toxicity, as long as the size of the toxic unit is constant (Kortenkamp *et al.* 2009, Backhaus *et al.* 2010, Backhaus and Faust 2012). If the sum of all toxic units within a mixture equals one, the mixture behaves according to CA. (Backhaus and Faust 2012). This means that all compounds in the mixture contribute to the mixture toxicity, in proportion to its toxic unit. The individual doses therefore do not need to be effective alone, which means that those mixtures with many components always will have an additive toxicity (Kortenkamp *et al.* 2009).

IA also assumes that all components in the mixture affect the same endpoint. However, in IA each component acts on different sub-systems, for example cells and molecular receptors. The endpoint is independently affected by the different sub-systems which are commonly interpreted as the compounds having different MoA (Backhaus and Faust 2012). IA follows the statistical concept of independent random events described by Bliss 1939 (referenced in Kortenkamp *et al.* 2009). To be able to calculate the IA-expected mixture effect, knowledge of the effect that each compound would elicit individually is needed at the concentration it is present in the mixture, *i.e.* the concentration-response curve for all individual compounds must be known (Backhaus and Faust 2012). Thereby, huge amounts of ecotoxicological data are needed for the calculations of mixture effects using IA. Such data is usually not available and that is a large limitation for the concept of IA (Backhaus and Faust 2012). Another limitation is that the components must be present at concentrations above their individual effect threshold to contribute to the joint effect of the mixture. This means that if none of the components is present at concentrations above the individual effect threshold, no combination effect will be seen (Kortenkamp *et al.* 2009).

Neither IA nor CA fully describes the biological reality since none of these concepts take into account the biology of the exposed organism or the chemical characteristics of the components in the mixture (Kortenkamp *et al.* 2009, Backhaus and Faust 2012). Several studies have therefore been carried out in order to predict the accuracy of the two concepts. The studies have compared CA- and/or IA-predicted mixture toxicities with experimental studies (Backhaus and Faust 2012). However, only a small fraction of the experimental studies investigated applied IA while almost all applied CA. In those cases where both concepts were compared in the same study, it could be seen that CA usually predicts slightly higher mixture toxicity or equal mixture toxicity than IA (Belden *et al.* 2007). Different predictions of mixture toxicity using CA and IA most often depend on whether the components in the tested mixture have similar or dissimilar MoA (Belden *et al.* 2007, Backhaus and Faust 2012). An environmentally realistic mixture however does not consist only of components with similar or dissimilar MoA. So which one of these models should be chosen?

CA is the concept suggested as the default approach (tier 1) for describing the mixture effect in risk assessments in a joint opinion by three scientific committees (SCHER/SCENIHR/SCCS) of DG SANCO (DG SANCO 2011). Also other scientists have suggested the same approach with CA as the default method (Coors and Frische 2011, Kortenkamp *et al.* 2012). The main advantages of CA compared to IA is that CA seldom underestimate the joint toxicity and that it does not require any additional data than what is used in risk assessments today (Coors and Frische 2011). Even though effects such as synergism or antagonism are not taken into account, the predictions based on CA are very good (Belden *et al.* 2007, Kortenkamp *et al.* 2009, Coors and Frische 2011). It has been shown that CA, regardless of MoA and pesticide target groups, predicts the toxicity of pesticide mixtures within a factor five from the observations (Belden *et al.* 2007, Kortenkamp *et al.* 2009, Backhaus *et al.* 2010). However, if a risk is indicated using CA, further assessment is needed and the assessor must proceed to what is called a "higher tier assessment". The higher tier should also take IA into consideration which often requires additional experimental studies due to the data demand of IA (Backhaus and Faust 2012).

In the present study, the active substances that will be assessed in combinations have both similar and dissimilar MoA. Since CA seldom underestimates the joint toxicity and since it does not require any additional data than what is used in risk assessments today, CA is the method that will be used in this study. No higher tier assessments with IA will be performed if CA indicates a risk.

Protection goals

Risk assessments are used to demonstrate that PPPs have no harmful effects on human or animal health or have any unacceptable effects on the environment, but what are unacceptable effects on the environment? What counts as an unacceptable effect can be interpreted by the broadly defined protection goals in 1107/2009. However, clear protection goals are needed and the Panel on Plant Protection Products and their Residues (PPR) has stated their view on the development on specific protection goals (SPG) in a scientific opinion. PPR suggests that the SPG should be based on seven "key drivers" of ecosystem services. The "key drivers" are microbes, algae, non-target plants (aquatic and terrestrial), aquatic invertebrates, terrestrial non-target arthropods including honeybees, terrestrial non-arthropod invertebrates, and vertebrates. The key drivers were chosen since they were believed to be the most important organisms potentially impacted by the agricultural use of PPPs (EFSA PPR 2010). The dimensions of the SPGs for each key driver are defined both by the effect and by the exposure. In the scientific opinion, it is defined what SPGs are suggested for each key driver (EFSA PPR 2010). The suggestions are today used in the revision of the guidance documents on aquatic and terrestrial ecotoxicology (EFSA PPR 2013).

Material and Methods

Investigating realistic conditions for the use of PPPs in cultivation of spring rape

There is no authority or other similar agency in Sweden that has an overview of the total use of PPPs. The Swedish Chemicals Agency is responsible for the sales statistics but specific data for individual products is classified and the municipalities are responsible for the supervision but they do not record the use. Therefore, none of them can provide information on the overall picture, *i.e.* what active substances are used and how they are used. The investigation of the use of PPPs in the cultivation of rape therefore started with a literature study of the information available from different sources: Statistics Sweden (SCB), Plant Protection Centers (the Swedish Board of Agriculture), the Swedish Chemicals Agency and the industry company Swedish Canola Council. The outcome of the literature study – at what point in time the different PPPs are used is summarized in figure 6. When the literature study was done, four questions emerged.

The questions were:

1. Which substances are used in the largest quantity in the cultivation of spring rape?
2. Which substances are always used during a growing season, independent of yearly variations such as precipitation?
3. Which substances are often mixed in the same tank before spraying?
4. It is common that seeds treated with seed dressing which is not approved in Sweden are imported. Which seed dressing is most common?

The above listed questions were then asked to 60 individuals representing different professionals within the area of PPPs in rape fields in Sweden:

1. advisors (25)
    a. HIR – Malmöhus AB
    b. Plant Protection Centers
    c. Swedish Canola Council
    d. Swedish Rural Economy and Agricultural Societies
    e. The Federation of Swedish Farmers (LRF)
    f. Visavi God Lantmannased AB
2. contractors (2)
    a. Agriöst AB
    b. Enköpings maskinstation
3. farmers (3)
    a. Halland
    b. Värmland

    c. Västra Götaland
    d.
4. supervisors (20)
    a. Municipalities
5. vendors (10)
    a. Lantmännen
    b. Svenska Foder

The interviewees were asked to answer the questions based on their own experiences, perceptions and observations. The answers varied in length and in the amount of details and no statistics could therefore be performed. Thus, a qualitative compilation of the answers was carried out instead (see “The use of PPPs in cultivation of spring rape”, page 26).

Surface water simulations

The surface water simulations were performed in compliance with the work of the FOCUS Surface Water Group (FOCUS SW) and the Guidance Document on Aquatic Ecotoxicology (FOCUS 2001, EC 2002a). FOCUS SW has examined the combination of soil, climate and slope characteristics across the European Union (member states that entered the EU after 1995 are not included) and from these identified 10 scenarios with realistic worst-case characteristics for drainage and run-off. When the scenarios were identified and selected, representatives “field sites” were chosen for each scenario (Figure 3). The field sites were in most cases chosen due to availability of extensive monitoring data at the specific site (FOCUS 2001). The properties and the names of the field sites of each scenario are presented in table 2**.**

Figure 3. Field sites in the European Union for ten representative worst-case scenarios used in calculations of predicted environmental concentration in surface water (D = drainage and R = run-off). The member states of the European Union are colored green (FOCUS 2001, based on a figure by EU 2013).

Table 2. Properties of the ten worst-case scenarios for the European Union selected by the FOCUS Surface Water Group (FOCUS 2001).

Name	Mean annual Temp. (°C)	Annual Rainfall (mm)	Topsoil	Organic carbon (%)	Slope (%)	Water bodies	Field site (weather station)	Country
D1	6.1	556	Silty clay	2.0	0 – 0.5	Ditch, stream	Lanna	Sweden
D2	9.7	642	Clay	3.3	0.5 – 2	Ditch, stream	Brimstone	Great Britain
D3	9.9	747	Sand	2.3	0 – 0.5	Ditch	Vreedepeel	Netherlands
D4	8.2	659	Loam	1.4	0.5 – 2	Pond, Stream	Skousbo	Denmark
D5	11.8	651	Loam	2.1	2 – 4	Pond, stream	La Jailliere	France
D6	16.7	683	Clay loam	1.2	0 – 0.5	Ditch	Thiva	Greece
R1	10.0	744	Silt loam	1.2	3	Pond, stream	Weiherbach	Germany
R2	14.8	1402	Sandy loam	4	20*	Stream	Porto	Portugal
R3	13.6	682	Clay loam	1	10*	Stream	Bologna	Italy
R4	14.0	756	Sandy clay loam	0.6	5	Stream	Roujan	France

*terraced to 5%

For the calculations of PEC_{SW}, FOCUS SWASH 3.1 was used. SWASH (Surface WAter Scenarios Help) is a software shell which contains four individual models; Spray Drift Calculator, MACRO, PRZM and TOXSWA (Figure 4). SWASH has four main functions; maintaining a central database for pesticide properties, give an overview of all Step 3 FOCUS runs for a specific pesticide and crop, calculate spray drift deposition and preparing input data for MACRO, PRZM and TOXSWA (FOCUS 2011, FOCUS 2013a). This means that the user must manually perform simulations with the individual models.

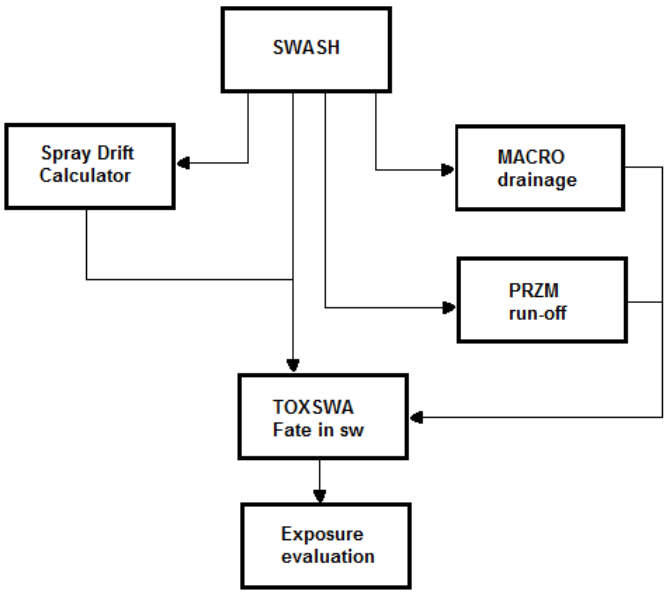


Figure 4. Illustration of the models used to calculate PEC_{SW} (Predicted Environmental Concentrations in surface waters). SWASH is a software shell and the other models in the boxes are tools within the shell (based on a figure by FOCUS 2001).

Spray Drift Calculator

The drift calculator is used for calculating drift values, for example the mean drift loading, mg/m^2 active substance of water body surface area, for use in TOXSWA simulation. The model runs automatically in the background when the other models in SWASH are used. The FOCUS group has set up default distances between the crop fields and the water bodies and thereby, the user only has to enter crop type, application rate and the number of applications per growing season in SWASH (FOCUS 2001, FOCUS 2013b).

MACRO 4.4.2

The MACRO model is used for calculating drainage from field soils to surface waters by calculating the unsaturated-saturated water flow in the field soils. In the model, the soil porosity is divided into two flow systems, macropores and micropores (FOCUS 1997, FOCUS 2001, FOCUS 2013c). Each system is characterized by a flow rate and a solute concentration. The exchange between the two systems is calculated with approximate physically-based expressions. The movement of water through the soil matrix is calculated by using Richards' equation (Richards 1931) and the transport of solutes is calculated with convection-dispersion equation (FOCUS 2001). The degradation of pesticides in both flow systems and the solid/liquid phases of the soil is calculated with first order kinetics (FOCUS 2001, FOCUS 2013c). A Freundlich isotherm (Ng *et al.* 2002) describes the sorption (FOCUS 2001).

PRZM_SW 1.5.6

PRZM (Pesticide Root Zone Model) is used for predicting the movement of chemicals in unsaturated soils within and directly below the root zone (FOCUS 2001). The model simulates runoff and erosion but also take plant uptake, leaching, decay, foliar wash-off and volatilization in account (FOCUS 2013d). Thereby, the model is dependent on physical and chemical input data for the active substances tested (FOCUS 2013d).

TOXSWA 2.2.1

TOXSWA (TOXic substances in Surface Waters) is used for simulating water flow and pesticide behavior in a small surface water system and its sediment, i. e. a ditch, pond or stream next to a single field. The model considers (FOCUS 2001, FOCUS 2013e):

- Transport – advection and dispersion in the water layer
- Transformation – hydrolysis, photolysis and biodegradation
- Sorption – Freundlich equation
- Volatilization

To calculate the pesticide behavior, TOXSWA uses files created by MACRO and PRZM regarding drainage, runoff and erosion as input. The available information in the files is water and mass fluxes as a function of time on an hourly basis. TOXSWA compiles the data and perform further calculations (FOCUS 2001). When all calculations are done, The PEC_{SW} for the selected scenarios is given in a TOXSWA report.

SWAN 1.1.4

SWAN (Surface Water Assessment eNabler) is a software tool developed by ECPA, i. e. it is developed independently and not by the FOCUS workgroup. The software supports Step 4 calculations through minor changes of the input files for the FOCUS models. Therefore, the user can perform simulations where buffer zones or other mitigating factors are added (FOCUS 2007).

The simulations

The simulations in this study were not performed in a tiered approach since the PEC_{SW} values needed to be comparable, *i.e.* all simulations performed with the same type of input values. Since Step 3 is the most realistic scenario, it was believed to be the most relevant step and therefore chosen for this study. However, for some PPPs, there is a condition of approval that the label on the package of the PPP must indicate that “the helper” should be used. “The helper” is a tool developed by the Swedish EPA and the Swedish University of Agricultural Sciences (SLU) and is used to determine the appropriate safety distance to surface waters when spraying PPPs (KEMI 2012a). In those cases where “the helper” is a condition of approval, Step 4

was also performed. The PEC_{SW} values for Step 3 and Step 4 is still comparable since step 4 uses the same input data as Step 3 but with mitigation factors added (FOCUS 2007).

The scenarios that represent Sweden are D1, D4 and R1 (Figure 3) and thereby these were the scenarios used in the simulations for this study. The input values for each active substance used in the simulations were (see Appendix I):

- Molar mass (g mol^{-1})
- Vapor pressure (Pa)
- Solubility in water
- K_{OC}
- DT_{50} for soil, water and sediment
- Crop type (spring oilseed rape)
- Application rate (g as ha^{-1})
- Time of application (interval, days)
- Number of applications per season
- Step 4: 15 m buffer zone

The input values for the active substances used in the simulations were primarily taken from EFSA's conclusion reports on pesticide peer reviews. When no conclusion report was available for the active substance, review reports from DG SANCO were used instead. For a few substances, no reports were available and the information was thereby taken from the registration report for the specific PPP. (For references regarding physical and chemical data of the active substances see Appendix I). The application rate, time of application and the number of applications per season were done according to Good Agricultural Practice (GAP). A table of GAP is included in every registration report and the approval of a PPP is always based on usage according to the GAP.

Individual risk assessments of PPPs regarding aquatic organisms

The individual risk assessments of the active substances were performed by calculating the TER (Toxicity Exposure Ratio) for fish, crustaceans and algae. For fish and crustaceans, both acute and chronic TER values were calculated. The values were calculated for the three Swedish scenarios and for the different water bodies (stream, ditch and pond) within the scenarios (see Appendix III). The formula used was:

$$TER = \frac{Measured\ toxicity}{PEC_{sw}} \qquad \text{(eq. 2)}$$

- Measured toxicity
  - Fish: LC_{50} and NOEC
  - Crustacea: EC_{50} and NOEC
  - Algae: ErC_{50}

- PEC_{SW} = Predicted Environmental Concentration in Surface Water

Trigger values are listed in 546/2011/EU, Appendix C, point 2.5.2.2., and are values that the TER values must exceed. The trigger values for fish and crustaceans are 100 for acute toxicity and 10 for chronic toxicity. The trigger value for algae is 10. When one TER value was below its trigger value, the active substance was considered to pose a risk to aquatic organisms regardless of how many TER values were above the trigger value.

The values for the measured toxicity were taken from EFSA (European Food Safety Authority), SANCO review reports, Draft Renewal Assessment reports and registration reports from applicants (Appendix I).

Cumulative risk assessments of PPPs regarding aquatic organisms

The cumulative risk assessment was carried out in compliance with the mixture toxicity concept Concentration Addition (CA). The cumulative risk assessment was performed on the same groups of organisms and the same scenarios as the individual risk assessment (see Appendix IV). The formula used in the risk assessment is derived from the basic formula describing CA (Equation 1):

$$TER_{mix} = \left(\frac{1}{TER_A} + \frac{1}{TER_B} \ldots \frac{1}{TER_n} \right)^{-1} \qquad \text{(eq. 3)}$$

- TER_{mix}: Theoretic TER-value for the mixture
- $TER_{A\text{-}n}$: TER value for the single components in the mixture
- Trigger value: See Individual risk assessment of PPPs

The combinations used in the cumulative risk assessment were the tank mixtures given in the answers in the interviews (see “The use of PPPs in cultivation of spring rape”, page 26), as well as a worst-case scenario combination. The worst-case combination was a mix of all PPPs used in a normal growing season, *i.e.* no consideration was taken to the timing of the recommended

use. The products used in the worst-case scenario mix were the products that according to the interviewees are most commonly used:

- Seed dressing: Elado (clothianidin + *beta*-cyfluthrin)
- Herbicide: Butisan Top (metazachlor + quinmerac)
- Insecticide: Bisacaya OD 240 (thiacloprid)
- Insecticide: Mavrik 2F (tau-fluvalinate)
- Fungicide: Cantus (boscalid)

Results

The use of PPPs in cultivation of spring rape

Out of the 60 interviewees, 24 could provide answers that could be used in this study. Below follows a qualitative compilation of the literature study and the answers from the interviews. Many of the interviewees gave more information than just direct answers to the questions; this information is also added to the compilation.

The literature study and also the answers from the interview showed that the use of PPPs varies substantially between years and also between different parts of Sweden. Nevertheless, some general tendencies were found.

Parts of the interviewees' answers were similar for all regions, mainly the answers regarding the usage of PPPs independent of annual variations. Almost 100% of all spring rape seeds are treated with seed dressing against flea beetles. There are only two approved seed dressings for rapeseeds in Sweden, Chinook FS 200 (*beta*-cyfluthrin and imidacloprid) and Force 20 CS (tefluthrin)(KEMI 2013). Force 20 CS were not mentioned in the literature or by the interviewees. It is therefore assumed that Force 20 CS is no longer used in rapeseeds. A study performed at SLU has shown that Chinook FS 200 no longer has a satisfactory effect on the fleas, and the plants get heavily affected. In the study, the damage on the cotyledons and the first two leaves caused by flea beetles did not differ at all between the treated seeds and the control seeds (Ekbom and Müller 2011). Due to the lack of effect, Chinook FS 200 is no longer used. Instead, Elado with the active substances clothianidin and *beta*-cyfluthrin is used. It is, however, an imminent risk that flea beetles will build up resistance against clothianidin as well, since both imidacloprid and clothianidin are neonicotinoids and have the same mode of action (Ekbom and Müller 2011). Elado is not approved in Sweden and the seeds are therefore most often treated in Germany and then imported to Sweden as commercial goods. That way, the unapproved substances can legally be used here in Sweden.

At least one spraying with an herbicide is often used. The most common herbicide seems to be Butisan Top (metazachlor and quinmerac), but all available herbicides are used to some extent. The largest pest in spring rape is pollen beetles and 1-2 sprayings with insecticides are always necessary. Pyrethroids of various sorts were for approximately twenty years, 1980-2000, the only insecticides used in the rape fields. This monotonous use did unfortunately cause resistance against pyrethroids in pollen beetles (Gustafsson and Lerenius 2010). The resistance has spread across the country and resistant pollen beetles are found in most regions of Sweden. There are two different types of resistance among the beetles today, metabolic and knock-down. Metabolic resistance is when the beetle starts to produce an enzyme that can degrade the PPP (Gustafsson and Lerenius 2010). The so-called knock-down resistance is a mutation where the sensitivity of the beetle's nervous system gets reduced (Soderlund and Knipple 2003). To avoid that more resistance is developed, it is now recommended to use several insecticides with different mode of action (Gustafsson and Lerenius 2010). Neonicotinoids such as Biscaya (thiacloprid) and Mospilan (acetamiprid) are now the most commonly used insecticides. Also the uses of other insecticides with different modes of action have increased in recent years. Pyrethroids are however still used in some areas of Sweden, but not in the same volumes as before. The pyrethroid mainly used is Mavrik (tau-fluvalinate), which is less toxic to bees than the other pyrethroids (SJV 2012a). Fungicides are not always necessary in cultivation of spring rape but when fungicides are used, Cantus (boscalid) seem to be the most common product.

Tank mixtures are not recommended in cultivation of rape since the different PPPs are recommended for use at different growth stages of the plants. Therefore, when tank mixtures are used, the spraying with the mixture takes place too late or too early, for one or for both of the mixed PPPs. There are, however, three products approved for use in spring rape that always have to be mixed with oil that aid/enhance the absorption of the PPP, namely Select (clethodim), Matrigon 72 SG (clopyralid) and Galera (clopyralid and picloram). However, tank mixtures with different PPPs still occur since mixing results in fewer spraying events and are thus are both cheaper and more time effective. Following are seven examples of specific mixtures of PPPs given in the answers to the interview:

1. Biscaya OD 240 + Cantus *(*thiacloprid + boscalid)
2. Butisan Top + Fastac 50 (metazachlor, quinmerac + alphacypermethrin)
3. Butisan Top + Focus Ultra (metazachlor, quinmerac + cycloxydim)
4. Butisan Top + Select/Renol (metazachlor, quinmerac + clethodim, penetrating oil)
5. Matrigon 72 SG + some sort of insecticide (clopyralid + insecticide)
6. Select/Renol + Mavrik 2F (clethodim, penetrating oil + tau-fluvalinate)
7. Select/Renol + Galera (clethodim + clopyralid, picloram)

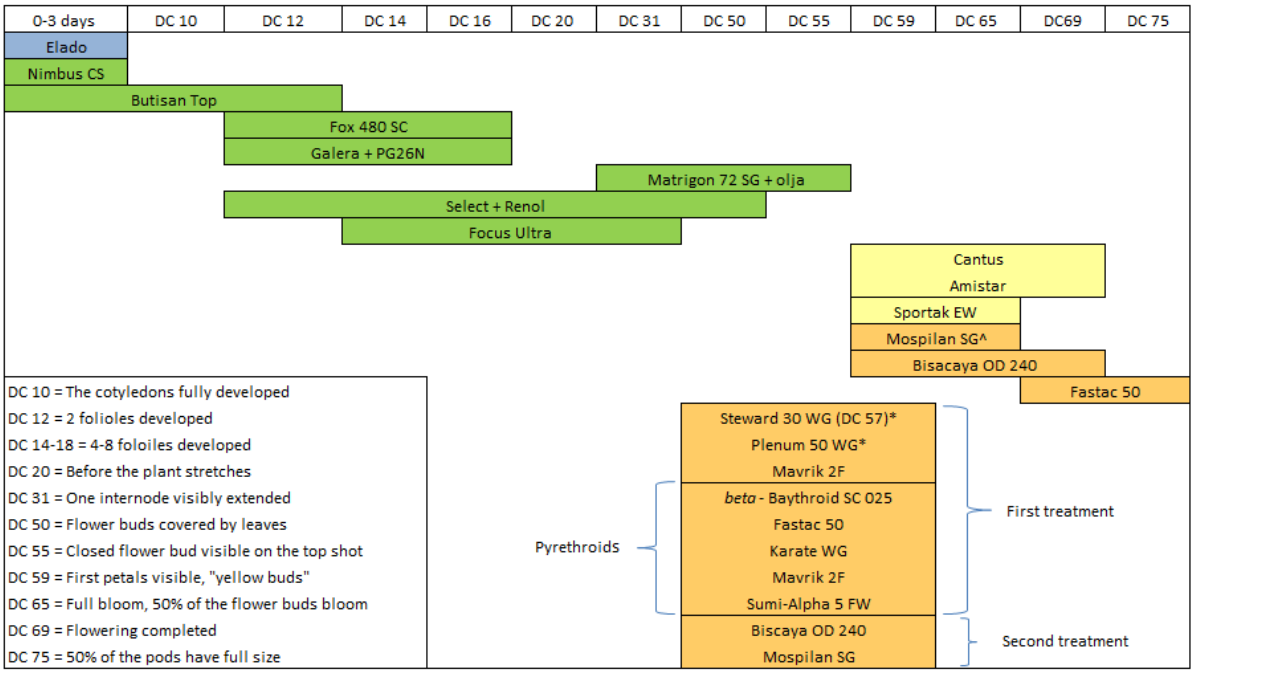
Several other interviewees gave more general answers, insecticide + herbicide and/or insecticide + fungicide. Many of the interviewees have answered that the most common mixture is not with another PPP, it is with micronutrients and especially boron.

In the manual for the herbicide Galera it is stated that "Galera cannot be mixed with herbicides, fungicides, insecticides, micronutrients or liquid fertilizers." Galera is despite this fact one of the PPPs that is given in the answer as an example of tank mixtures used in cultivation of spring rape. This is misuse and is not a mixture that the risk assessor can take into account. It is however important to note that such usage also occurs.

At what growth stage is PPPs used in cultivation of spring rape?

A schematic overview on at when during the growing season the different PPPs are used was illustrated (Figure 5). Seed dressing is marked in blue, herbicides in green, fungicides in yellow and insecticides are marked in orange. Note that the insecticides can be used earlier in the growing season as well, but such use is not considered to be normal. Sprayings with insecticides early in the growing season is often connected to bad efficacy of the seed dressing and seldom needed when using Elado (Gunnarsson 2012b). All pyrethroids except Mavrik 2F (tau-fluvalinate) are only used in the few regions of Sweden where pyrethroid resistance has not developed.

The figure further indicates that, usage of different PPPs overlap and tank mixtures with herbicide + insecticide and insecticide + fungicide are possible. The products' names are written in the boxes and each box marks in what growth stages of rape that spraying is allowed according to the GAP. The time of restraint, *i.e.* how many days before harvest the product can be used at the latest, is not added in the figure since the timing of harvest differs between different plants and fields (Mattson 1990). "First treatment" and "second treatment" refer to spraying against pollen beetles. For a full size figure see Appendix II.



* Do not use where bees are actively foraging. To protect bees and other pollinating insects, do not use this product to flowering crops when bees are actively looking for food.

^Treatment not allowed after flowering.

Figure 5. The boxes show at what growth stage different plant protection products are used in cultivation of spring rape. Seed dressing: blue; herbicide: green; fungicide: yellow; insecticide: orange. See Appendix II for a full size figure. DC describes at what growth stage the plant is.

Individual risk assessment of PPPs regarding aquatic organisms

24 risk assessments on single substances were performed. Three of the active substances were risk assessed twice but for different products, *i.e.* with different application rate and time of application. Of the 24 assessed substances, ten were considered to pose a risk to the aquatic environment according to 546/2011/EU (Table 3). For detailed information on the risk assessments, see Appendix III**.**

Table 3. Result of the risk assessment on single substances. 10 out of 24 substances were considered to pose a risk to the aquatic environment. For detailed information on the risk assessments, see Appendix III**.**

Active substance	**Product**	**Type of PPP**	**Risk? Y**es**/N**o	**Most sensitive organism(s)**	**Type of risk (acute or chronic effects)**
Azoxystrobin	Amistar	Fungicide	Y[1]	Crustacean	Acute and chronic
Boscalid	Cantus	Fungicide	N	-	-
Prochloraz	Sportak EW	Fungicide	Y	Fish, crustacean	Chronic
Bifenox	Fox 480 SC	Herbicide	Y[2]	Crustacean, algae	Chronic
Clethodim	Select	Herbicide	Y[3]	Crustacean	Chronic
Clomazone	Nimbus CS	Herbicide	N	-	-
Clopyralid	Galera	Herbicide	N	-	-
Clopyralid	Matrigon 72 SG	Herbicide	N	-	-
Cycloxydim	Focus Ultra	Herbicide	N	-	-
Metazachlor	Butisan Top	Herbicide	Y	Algae	Acute
Metazachlor	Nimbus CS	Herbicide	N[4]	-	-
Picloram	Galera	Herbicide	N	-	-
Quinmerac	Butisan Top	Herbicide	N	-	-
Acetamiprid	Mospilan SG	Insecticide	N	-	-
Alpha-cypermethrin	Fastac 50	Insecticide	N[4]	-	-
beta-Cyfluthrin	Beta-baythroid SC 025	Insecticide	Y[1]	Fish, crustacean	Acute and chronic
beta-Cyfluthrin	Elado	Insecticide	Y	Fish, crustacean	Acute and chronic

Clothianidin	Elado	Insecticide	N	-	-
Esfenvalerate	Sumi-alpha 5 FW	Insecticide	Y[2]	Fish	Acute
Indoxacarb	Steward 30 WG	Insecticide	N	-	-
Lambda-cyhalothrin	Karate 2.5 WG	Insecticide	Y[1]	Fish, crustacean	Acute and chronic
Pymetrozine	Plenum 50 WG	Insecticide	N	-	-
Tau-fluvalinate	Mavrik 2F	Insecticide	Y	Fish, crustacean	Acute and chronic
Tiachloprid	Biscaya OD 240	Insecticide	N[4]	-	-

[1]Not approved at step 4, 15 m buffer zone, [2]The approval is based on a mesocosm study, [3]Product + penetrating oil, [4] Step 4 required

Cumulative risk assessment of PPPs regarding aquatic organisms

Seven cumulative risk assessments were performed; six with the tank mixtures given in the answers to the interview questions (page 22) and one worst-case scenario where all PPPs in a whole growing season were considered a mixture. Of the seven mixtures assessed, five were considered to pose a risk to the aquatic environment (Table 4).

Table 4. Result of the cumulative risk assessments. Five of seven mixtures were considered to pose a risk to the aquatic environment. Active substances in bold were considered to pose a risk also when assessed alone. For detailed information on the risk assessments, see Appendix III and IV.

Combination of products	**Active substances**	**Risk? Yes/No**	**Most sensitive organism(s)**	**Type of risk (Acute or chronic effects)**
Biscaya OD 240 + Cantus	Thiacloprid, boscalid	N[1]	-	-
Butisan Top + Fastac 50	**Metazachlor**, quinmerac, alfacypermethrin	Y[1]	Algae	Acute
Butisan Top + Focus Ultra	**Metazachlor**, quinmerac, cycloxydim	Y	Crustacean, algae	Acute and chronic
Butisan Top + Select	**Metazachlor**, quinmerac, **clethodim**	Y	Crustacean, algae	Acute and chronic
Matrigon 72 SG + Mospilan SG	Clopyralid, acetamiprid	N	-	-
Select + Mavrik 2F	**Clethodim**, **tau-fluvalinate**	Y[2]	Fish, crustacean	Acute and chronic
Worst-case[3]	Seven substances[4]	Y	Fish, crustacean, algae	Acute and chronic

[1] Step 4 required, [2] Product + penetrating oil, [3]Elado, Butisan Top, Biscaya, Mavrik, Cantus, [4]Clothianidin, **beta-cyfluthrin**, **metazachlor**, quinmerac, thiacloprid**, tau-fluvalinate**, boscalid

To what extent is the risk under estimated?

It was only in one case when two products posing no risk to the aquatic environment on their own were found to pose a risk when mixed. Note that this was only for one organism in one scenario and one water body (Table 3, Appendix IV). It seems as if there often is one of the active substances that drive the outcome of the risk assessment on the organism level. This has been illustrated with figure 6 and 7. The scenario chosen for the graphs was D1 ditch. D1 is the only field site in Sweden and ditch is the water body that usually has the highest PECs (Figure 3, Table 2). If the substances that are considered to pose a risk on their own are removed from the cumulative risk assessment of the worst-case scenario, the mixture is no longer considered to pose a risk to the aquatic environment (Appendix IV).

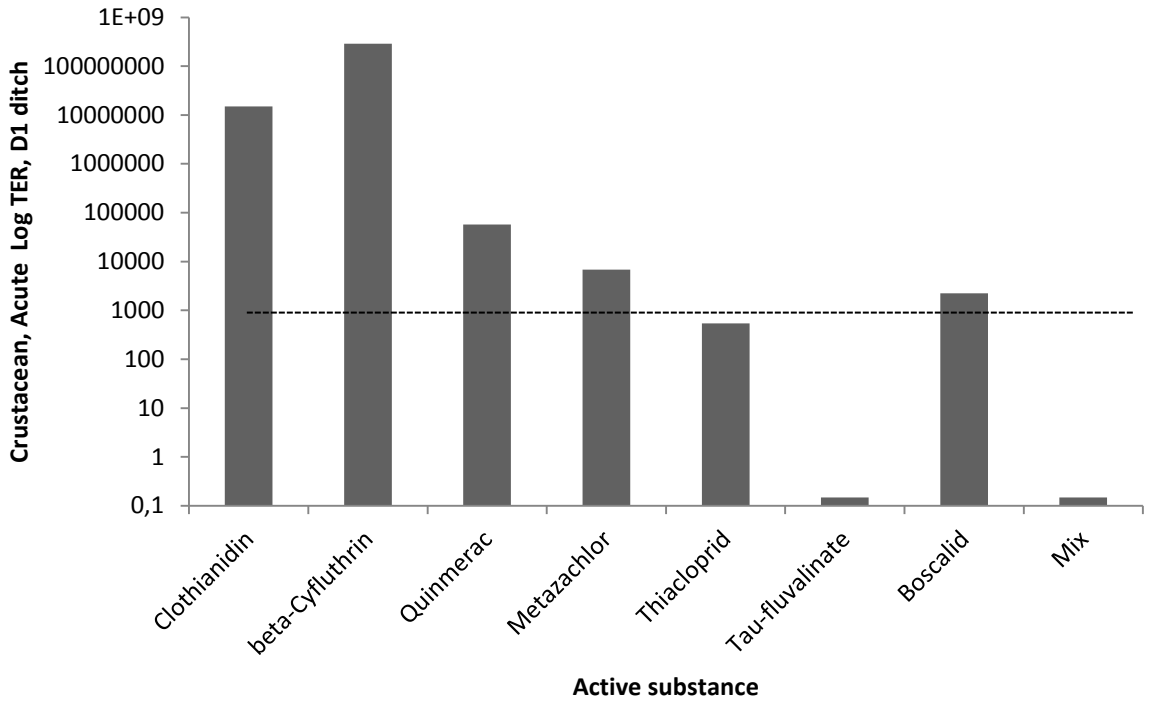


Figure 6. Toxicity Exposure Ratio (TER) for scenario D1 ditch, calculated from EC_{50} for crustaceans and PEC_{SW} for seven different active substances; clothianidin, *beta*-cyfluthrin, quinmerac, metazachlor, thiacloprid, tau-fluvalinate, boscalid and the mixture of them. The TER value for the insecticide tau-fluvalinate and the mixture is equal (0.15). The horizontal line marks the trigger value. Note that the TER scale is logarithmic.

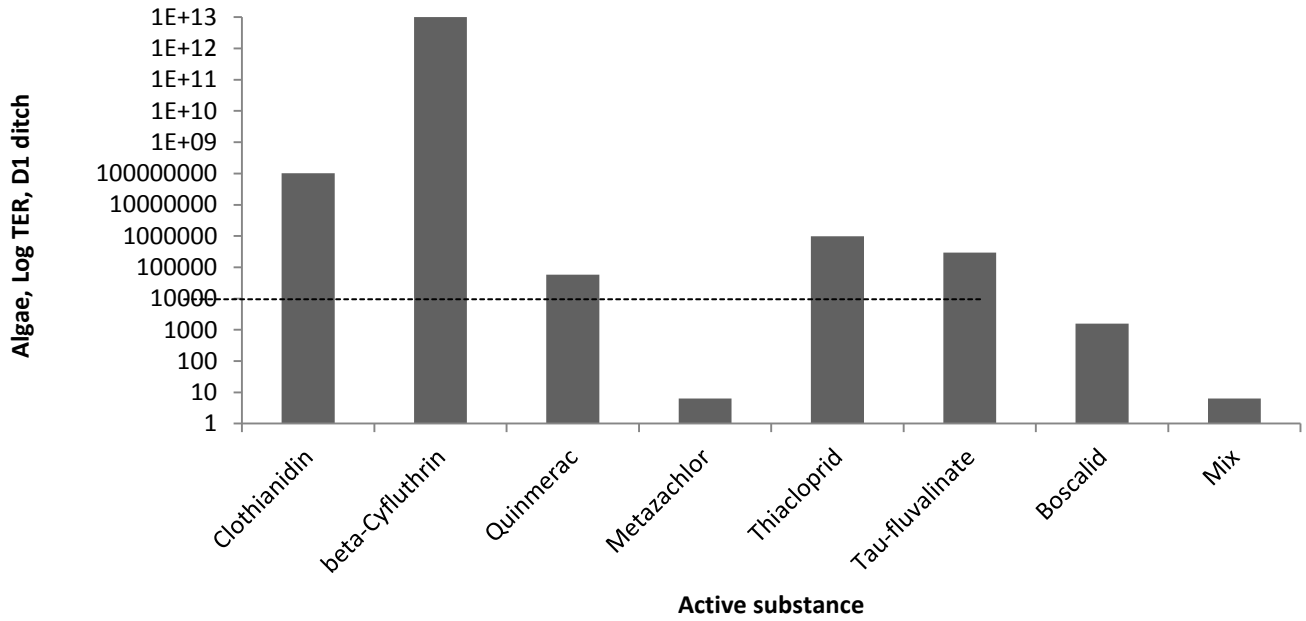


Figure 7. Toxicity Exposure Ratio (TER) for scenario D1 ditch, calculated from ErC_{50} for algae and PEC_{SW} for seven different active substances; clothianidin, *beta*-cyfluthrin, quinmerac, metazachlor, thiacloprid, tau-fluvalinate, boscalid and the mixture of them. The TER value for the herbicide metazachlor and the mixture is almost equal - 6.4 and 6.3, respectively. The horizontal line marks the trigger value. Note that the TER scale is logarithmic.

Discussion

Investigation of realistic conditions of use

The results from the investigation of realistic conditions for the use of PPPs in spring rape indicate the need for a competent authority that keeps track on how the authorised PPPs are used in all crops grown in Sweden. Each year, The Swedish Chemicals Agency (KemI) and SCB compile the sales statistics for PPPs, which means that information on what products that are mainly used do exist. The sales statistics, however, is classified and only the processed statistics is presented, *i.e.* the statistics is not presented at product level (SCB and KEMI 2012). Even if the raw data for the statistics would be available, it would not be enough to determine what realistic conditions of use really are. To really know what realistic conditions of use are and to be able to use it in the evaluation of risk assessments, an investigation of realistic conditions for the use of PPPs in all crops in Sweden would be needed. For example, the investigation could be

based on information from the farmers spray records. The information could for example be collected and compiled by the supervisors at the municipalities since they already control the spray records. The compiled information could then be presented for all different crops in a similar way as shown in figure 5. The figures could be used to see what other products are approved for the same period in the growing season and/or to see what products are approved for the whole growing season for a specific crop. In that way it would be easier to determine if there are any products or active substances that need further evaluation, for example a cumulative risk assessment. The compiled information would need to be updated annually since the usage of PPPs changes often due to changes in what products are available at the market and other factors such as resistance against active substances.

The recommendation that insecticides with different MoA should be used when spraying multiple times seems to be well-known. However, a question was raised when compiling the results: do farmers know which insecticides that have different MoA? The information is for example provided by advisors at the Plant Protection Centers, but do farmers contact advisors? This question was raised due to the fact that the most common insecticides, according to the interviewees' answers, are Biscaya OD 240 (tiachloprid) and Mospilan SG (acetamiprid). Both are neonicotionoids and have the same MoA which conflicts with the fact that two sprayings are usually needed in cultivation of spring rape. This may be due to that the first spraying is mainly with Biscaya OD 240 or Mospilan SG but for the second spraying, what insecticide the farmer use varies widely. This question has not been fully investigated in the present study and is a suggestion for further investigations.

Risk assessments – substance-by-substance

The most striking conclusion of the individual risk assessments is that ten out of 24 substances risk assessed were considered to pose a risk to the aquatic environment according to 546/2011/EU (Table 3). The authorizations for the products for six of the active substances that were considered a risk to the aquatic environment were based on old national legislations, Regulation (2006/1010) on plant protection products, and the products have thereby not been evaluated according to the current legislation. Amistar (azoxystrobin) has been assessed and approved according to legislation (91/414) but not for spring rape. A simulation was thereby performed for spring rape which led to different results of the risk assessment than the assessment performed in the approval process. Fox 480 SC (bifenox) and Sumi-alpha 5 FW (esfenvalerate) were considered to pose a risk in the present study, even though they have been risk assessed according to new legislation and in compliance with FOCUS Surface Water Scenarios. The reason why they are approved even though an acceptable risk to the aquatic environment according to 546/2011/EU has not been demonstrated is because the

authorizations of these products are based on refinements which the present study not has taken into consideration. In the case of Fox 480 SC and Sumi-alpha FW, mesocosm studies have been used where the trigger values are a case-by-case judging and lower trigger values can thereby be allowed (EC 2002a, KEMI 2009a, KEMI 2010). For example, in the approval of Sumi-alpha (esfenvalerate) a trigger value of 2.4 was accepted due to a case-by-case judging of the mesocosm study, performed by KemI (KEMI 2010). A mesocosm study is a controlled experimental study that should mimic natural conditions, and take into account aspects such as indirect effects, biological compensation and recovery, and ecosystem resilience (MESOAQUA 2013). Most mesocosms are aquatic and two examples are experimental ponds and simulated streams. Mesocosm can also be enclosures within natural water bodies, both in freshwater and marine environment (Walker *et al.* 2006b). Mesocosm studies were not included in the present study. Refinements need deeper evaluations and case-by-case judging and there was no time for such evaluations in the present study.

It can also be questioned if refinements should be accepted. The benefits from refinements are for example that assessments are more profound due to the case-by-case judging. Also, if the refinement is a well performed study, it will be closer to reality than simple studies (Walker *et al.* 2006b). The disadvantages with refinements are that it is both difficult and time consuming to fully investigate the studies (de Jong *et al.* 2008)**.** Moreover, the assessments of these can be less conservative due to the case-by-case judging (EC 2002a, de Jong *et al.* 2008), an advantage for companies but hardly for the environment. It might therefore be better to keep the assessments simple and conservative. A conservative approach is preferable when knowledge is limited and when the usage might be connected to large risks. As stated in 1107/2009, Article 1 (4), Member states can in those cases where there is scientific uncertainty regarding the risks to human or animal health or to the environment posed by PPPs, apply the precautionary principle, *i.e.* have a conservative approach when evaluating the risk assessments submitted by the applicant.

Another disadvantage with refinements and risk assessments in a tiered approach is that it does not work as expected. One major rationale for developing the tiered approach was to speed up the risk assessment process for less harmful substances. Hence, a less elaborated risk assessment and fewer studies are needed to demonstrate that the protection goals are maintained. Due to the nature of pesticides – they should kill unwanted organisms – lower tier risk assessments are generally not able to demonstrate that non-target organisms are unaffected since they are also exposed to the pesticides. Therefore higher tier risk assessments need to be performed for the majority of the pesticides and thus, the risk assessment process is on the contrary much more elaborated and time consuming than intended. For example, out of 29 insecticides risk assessed by EFSA, the risk estimation was based on FOCUS step 3 for four

compounds and on step 4 for 20 compounds, *i.e.* 24 of the 29 substances required a higher tier risk assessment (Knäbel *et al.* 2012).

In the present study it was only the parent compounds that were simulated according to the FOCUS Surface Water Scenarios and later risk assessed. This means that the ten substances were considered to pose a risk even without taking into account the metabolites. A wonder is thus how many of the 24 substances that would be considered to pose a risk if the metabolites were included in the risk assessments? It will be very interesting to see what happens with the substances that were considered to pose a risk in the present study when they are re-evaluated. Will they get a new approval? Will mesocosm studies be used in the risk assessments and will that be enough for a new approval? Will mitigating factors such as buffer zones be used? One thing is obvious, the TER values are at the moment far below the trigger values and this is a reason of concern (See Appendix III). Therefore, in order to get new approvals for those products, something new has to be added to the risk assessments.

Cumulative risk assessments

The results from the cumulative risk assessments are difficult to interpret since many of the single substances within the mixture were considered to pose a risk by themselves. In mixtures where one of the active substances is considered a risk; the whole mixture becomes a risk due to the formulas' construction (Equation 1 and 3, Table 3 and 4). This indicates that the risk assessments and approvals of all products must be done according to the same legislation, as a suggestion EU legislation 1107/2009. Also, it is not possible to make cumulative risk assessments if the approval for one product is based on a mesocosm study when no mesocosm studies are performed for the other products. Once again, refinements might not always make a risk assessment better. There are currently no internationally agreed method on how to risk assess mixtures of PPPs, but methodologies are developed by EFSA (Kortenkamp et al. 2012).

When the substances that are considered to pose a risk on their own in the cumulative risk assessment of the worst-case scenario in the present study are removed, the mixture is no longer considered to pose a risk (Appendix IV). This further indicates that in the present study, there are a few single substances that drive the outcome/result of the cumulative risk assessments (Figure 6 and 7). This is however not a reason to ignore cumulative risk assessments since there are also other aspects that must be taken into account, for example carry-over toxicity. It is a phenomenon when organisms get exposed to for example a PPP and survive, but the damage caused by the toxicant results in reduced fitness. If the organism is exposed once again before it has fully recovered, stronger effects than expected by a single application are possible (Ashauer *et al*. 2010). Carry-over toxicity can be caused by accumulated toxicant and/or accumulated damage and there are models available for explaining and

simulating such effects (Ashauer and Escher 2010). Also the toxicity of a mixture can be enhanced by carry-over toxicity, most often due to different recovery time for organisms exposed to different toxicants (Ashauer and Escher 2010). Carry-over toxicity is thus a good reason for why cumulative risk assessment always should be performed even if the PPPs are not sprayed at the same time. In this study, carry-over toxicity justifies the worst-case scenario, which extends over the entire growing season. Another aspect is if one substance is toxic to fish and another is toxic to algae, the mixture will be toxic to both fish and algae, *i.e.* the mixture is toxic to more organisms than the single substances on their own. The toxicity of the single substances is captured by the individual risk assessments but does this mean that the toxicity of the mixture is of less importance? Seen in a wider view, the ecosystem will be affected at a larger scale by the mixture than if the single substances were applied alone.

No laboratory experiments have been performed with the assessed mixtures in this study and it is therefore not possible to say how accurate the results of the cumulative risk assessment are. A large difference between risk assessment of individual substances and mixtures is that additional sources of uncertainty must be added to the mixtures (Backhaus *et al.* 2003). There will for example always be more chemicals present in the environment than considered in the risk assessment which means that the mixture is only an approximation of the "real" mixture. How good the approximation is can only be shown by laboratory studies (Backhaus *et al.* 2003). Also, both CA and IA assume that there are no physico/chemical reactions between components within the mixture or with the mixture and components outside the mixture. It is most unlikely that no such interaction would occur in the environment (Backhaus *et al.* 2003). These are just examples of uncertainty factors, and all factors that need to be taken into account might not even be known. The accuracy of the result in the present study can also be questioned due to "the funnel hypothesis". The funnel hypothesis presented by Warne and Hawker (1995) predicts that "as the number of components in a mixture increases, the range of deviation from toxic additivity decreases". In the present study, the largest number of components in a risk assessed mixture was seven. If the funnel hypothesis is correct, CA might not be the best model for predicting the risk of a mixture with such a small number of components (Warne and Hawker 1995). However, the seven active substances is not the only substances that the organisms are exposed to in the environment and also, at the moment, CA is the model suggested to be applied for any type of mixture (DG SANCO 2011, Backhaus and Faust 2012).

Synergistic effects are not very common, but they do occur (Backhaus and Faust 2012, Kortenkamp *et al.* 2012). Synergistic effects occur most often in binary mixtures (Backhaus and Faust 2012). Since tank mixtures often are binary mixtures it can be discussed if tank mixtures which have not been experimentally tested should be used. In a report by Belden and co-workers (2007) it was reported that in approximately 5% of the studies reviewed, a synergistic effect more than twofold larger than the effect estimated by CA was seen. It has also been

shown that the fungicide prochloraz enhance the toxicity of the insecticide esfenvalerate four to sixfold to copepods and three to sevenfold to cladocerans (Bjergager *et al.* 2011). These active substances are used in spring rape and in several other crops and both substances have been detected above their guideline value in the annual sampling of surface water in 2011 (Table 1) (Nanos *et al.* 2012). Prochloraz has also been shown to increase the toxicity of the insecticide alpha-cypermethrin to *D. magna* 12-fold compared to what was expected by using CA (Nørgaard and Cedergreen 2010). Alpha-cypermethrin is also an active substance used in the cultivation of spring rape. This further indicates that the uncertainties in cumulative risk assessments will always be there, as long as they are based on estimated data instead of results from experimental studies. This is, once again, a reason for having a conservative approach. Another aspect of this is that it is important that the findings in the environmental monitoring are seen as feedback on the risk assessments and that the information is used to improve the assessments. Such improvements can for example at a large scale be validation of the software for the surface water simulations. On substance level, the findings could for example lead to a situation where a mitigating factor such as buffer zones are demanded even if it is not needed due to what is shown by the surface water simulations.

The environmental monitoring and the evaluations of risk assessments are today not connected. Thus, risk assessments provide a false ground for safe use of PPPs since the risk assessments are based on models which are not validated based on field data. A study by Knäbel and co-workers (2012) showed that the FOCUS simulations do not provide reliable field estimates. When using the default FOCUS scenario assumptions for step 1 and 2, PEC_{SW} generally over predicted the measured field concentrations (MFC) while for step 3 and 4, PEC_{SW} underestimated the MFCs by 23% and 31% respectively (Knäbel *et al.* 2012). When the FOCUS scenario assumptions were adjusted to available realistic field study information (insecticide use, climatic conditions, water body characteristics, etc.) the step 3 PEC_{SW} underestimated the MFC even more, by 43% (Knäbel *et al.* 2012). This indicates that the FOCUS surface water scenarios needs to be modified and calibrated in order to provide reliable field estimates.

Conclusions

Risk assessments today do not consider treatment series with other PPPs, PPPs from other fields in the catchment areas or other stressors such as local weather conditions. At the same time, farmers do not always use the PPPs as specified in the approvals; hence the approvals do not consider realistic conditions of use. Realistic conditions for the use of PPPs in cultivation of spring rape are the usage of several different PPPs during a growing season. What products that are used and also what types of PPPs, differ between different regions of Sweden, but there are some general tendencies. For example, two sprayings is the average number of sprayings with

insecticides. Prior to this investigation, neither any authority nor any other organization/person had a complete overview of the use of PPPs in spring rape fields. Hence, the present study demonstrates the need for a coherent overview of the use of PPPs in agriculture. It is thus a great need for a competent authority that keeps track on the usage and provides guidelines for what realistic conditions of use are, and how it should be considered. A suggestion is to compile the information regarding realistic conditions of use for PPPs and produce a figure similar to figure 5 for all crops grown in Sweden. Such a figure is easy to grasp and the information is readily interpreted, *i.e.*it is easy to see what other products need to be considered in the risk assessments.

In the present study, it is not possible to conclude whether or not the products approved today would not be approved if they were risk assessed according to realistic conditions for the use of PPPs. However, ten out of the 24 assessed substances where considered to pose a risk to the aquatic environment according to 546/2011/EU even when assessed individually. Also, five out of seven assessed combinations of PPPs were considered to pose a risk to the aquatic environment. It will be interesting to see if the products with old approvals get new approvals when they are re-assessed.

In order to fully perform a risk assessment of mixtures of PPPs regarding aquatic organisms, additional work is needed than the work performed in the present study. The study has, however, elucidated the complexity of cumulative risk assessments and shed some light on when specific active substances are used in cultivation of spring rape. The present study can therefore be used for improving risk assessments by considering realistic conditions of use for PPPs for a more realistic estimate of the total potential risk for adverse effects in non-target organisms. As written in the introduction, there is no template for realistic conditions of use and there are not always obvious what combinations/mixtures should be considered in the risk assessments.

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Appendix I. Input data for the simulations

Table 1. Input data for simulations of predicted environmental concentration in surface waters (PEC_{SW}) at step 3 in FOCUS Surface Water Scenarios. Three of the active substances are simulated twice but with different application rate (*i.e.* different products).

Active substance	Systemic? Y/N (crop interception)	Molar mass (g/mole)	Vapor pressure (Pa)	Water solubility (mg/L, 20°C)	DT_{50} soil (days)	DT_{50} water (days)	DT_{50} sediment (days)	K_{OC}	Application rate[2] (kg as/ha)	Number of applications[2]	Reference
Acetamiprid	Y	223	$1 \cdot 10^{-5}$	2950	1.84	4.7	43.3	107	0.04	1	KEMI 2006
Alpha-Cypermethrin[1]	Y	416.3	$3.4 \cdot 10^{-7}$	0.004	41.9	16.7	1000	76344	0.015	2	KEMI 2009b
Azoxystrobin	Y	403.4	$1.1 \cdot 10^{-10}$	6.7	78	1000	205	427	0.25	1	EFSA 2010a
beta-cyfluthrin (B)	N	434.3	$4.95 \cdot 10^{-8}$	0.00165	54	270	1000	64300	0.0075	2	EC 2002b
beta-cyfluthrin (E)	N	434.3	$4.95 \cdot 10^{-8}$	0.00165	54	270	1000	64300	0.024[3]	1	EC 2002b
Bifenox[1]	N	342.1	$4.74 \cdot 10^{-8}$	0.1	8.3	0.11	0.11	7143	0.48	1	KEMI 2009a
Boscalid	Y	343.2	$1 \cdot 10^{-10}$	5	130	32	1000	771	0.25	1	EC 2008
Clethodim	Y	359.92	$2.08 \cdot 10^{-6}$	5450	0.56	19.70	1000	22.70	0.0960	1	EFSA 2011b
Clomazone	Y	239.7	0.0094	1102	167.5	52.53	1000	286.5	0.083	1	EFSA 2007
Clopyralid (G)	Y	191.96	$1.36 \cdot 10^{-3}$ (25°C)	143 000	34	148	1000	5.15	0.0801	1	EFSA 2005
Clopyralid (M)	Y	191.96	$1.36 \cdot 10^{-3}$ (25°C)	143 000	34	148	1000	5.15	0.12	1	EFSA 2005
Clothianidin	Y	249.7	$3.8 \cdot 10^{-11}$	0.304	545	49.8	1000	160	0.12[3]	1	EC 2005a
Cycloxydim	Y	325.5	$1 \cdot 10^{-5}$	53	1	20.80	20.80	59.10	0.6/0.4	2	EFSA 2010b

Esfenvalerate[1]	N	419.9	$1.17 \cdot 10^{-9}$	0.001	50	1000	67	251700	0.015	3	KEMI 2010
Indoxacarb	Y	528	$9.8 \cdot 10^{-9}$ (25°C)	0.02	19.90	2	1000	5125	0.0255	1	EC 2005b
Lambda-cyhalothrin	N	449.9	$2 \cdot 10^{-7}$	0.005	56	15	1000	157000	0.0075	2	dRAR 2013
Metazachlor (BT)	Y	277.8	$9.6 \cdot 10^{-5}$	450	6.80	137.6	4.60	110	0.75	1	EFSA 2008
Metazachlor[1] (N)	Y	277.8	$9.6 \cdot 10^{-5}$	450	6.80	137.6	4.60	110	0.75[4]	1	EFSA 2008, NZ 2012
Picloram	Y	241.46	$8 \cdot 10^{-8}$ (25°C)	560	82.80	1000	196.10	35	0.0201	1	EFSA 2009
Prochloraz	N	376.7	$1.5 \cdot 10^{-4}$ (25°C)	24.90	68.80	359	1000	1440.50	0.45	1	EFSA 2011a
Pymetrozine[1]	Y	217	0	270 (25°C)	4.4	1000	94.4	2245	0.075	1	KEMI 2012b
Quinmerac[1]	Y	221.64	$1 \cdot 10^{-10}$	220	10.4	2	138.8	1000	0.25	1	EFSA 2010c
Tau-fluvalinate	Y	502.9	$9 \cdot 10^{-11}$	0.00103	31	1.96	87.32	750746	0.0480	2	EFSA 2010d
Tiachloprid	Y	252.73	$3 \cdot 10^{-10}$	184	1.30	11	1000	615	0.0720	1	EBSF 2005

[1]FOCUS simulations were not carried out in this study, PEC_{SW} according to documentation.

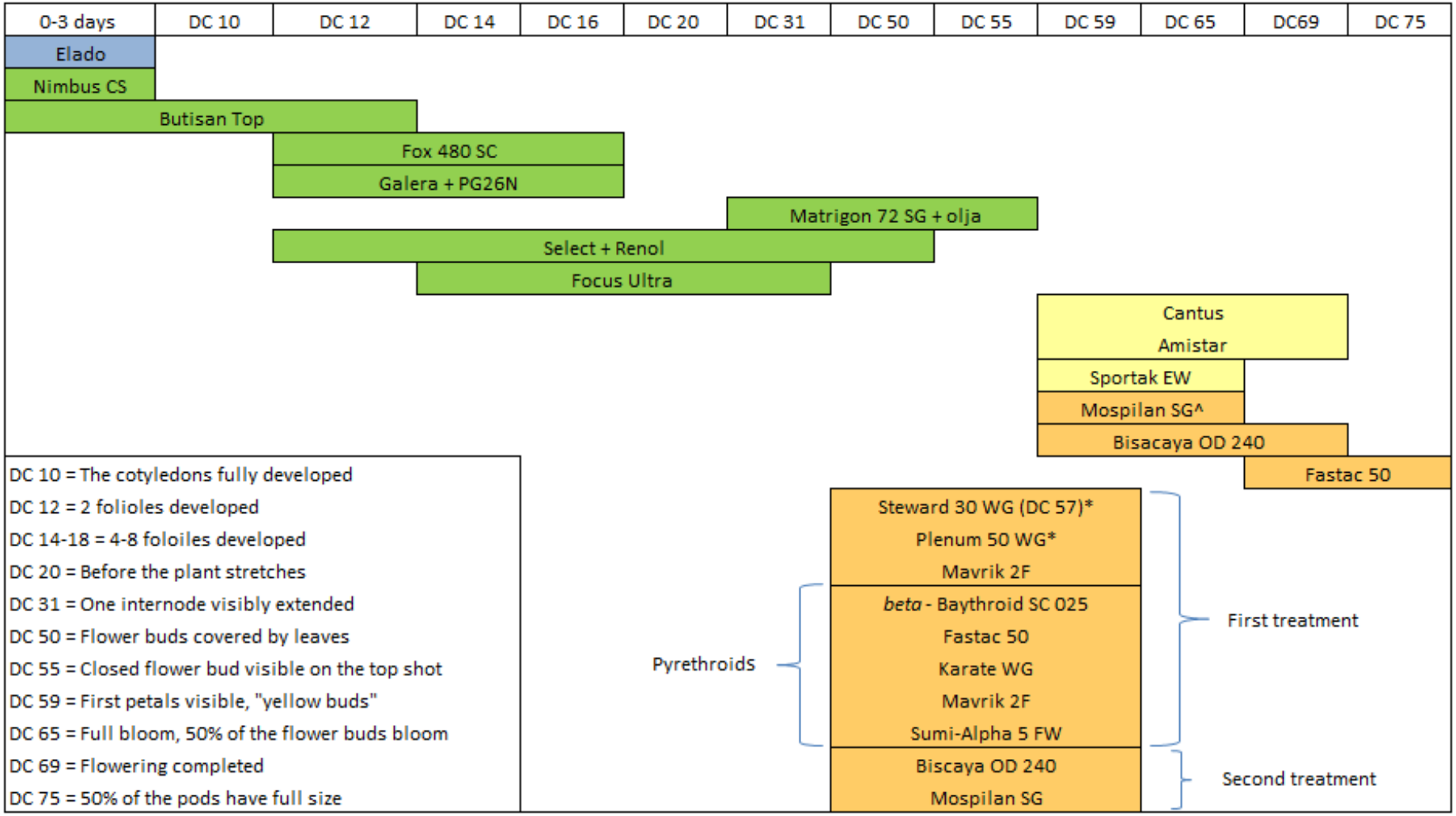
[2]Application rate and number of applications is according to Good Agricultural Practice (GAP) for each product. The approval of a product is based on applications in compliance with GAP; the GAP for each product is available at the Swedish Chemicals Agency.

[3]Elado (seed dressing with the active substances *beta*-cyfluthrin and clothianidin) is not approved in Sweden and the application rate is thereby a qualified estimation.

[4]The application rate in the simulations is not according to the GAP. The application rate according to the GAP is 0.63 kg as/ha.

Appendix II. At what growth stage are PPPs used in cultivation of spring rape?

Below is a figure of at what growth stage different PPPs are used in cultivation of spring rape. The products name and dose are written in the boxes and each box marks in which growth stages that spraying is allowed according to the GAP. “First treatment” and “second treatment” refer to spraying against pollen beetles. The time of restraint is not added in the figure since the timing of harvest differs between different plants and fields. Different types of PPPs are marked with different colors: seed dressing in blue, herbicide in green, fungicide in yellow and insecticide in orange. DC describes at what growth stage the plant is.



* Do not use where bees are actively foraging. To protect bees and other pollinating insects, do not use this product to flowering crops when bees are actively looking for food.

^Treatment not allowed after flowering.

Appendix III. Risk assessment of individual active substances used in spring rape

- The risk assessments are listed in alphabetical order on active substance.
- The products name is written in brackets.

Table 1. Risk assessment of the active substance acetamiprid (Mospilan SG). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Acetamiprid (Mospilan SG)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (µg/L)			0.258	0.224	0.00875	0.219	0.0247	0.18	KEMI 2006	
Tox. measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	>100000	*O. mykiss*	380000	440000	11000000	450000	4000000	560000	100	N
NOEC	19200	*P. promelas*	74000	86000	2200000	88000	770000	110000	10	N
NOEC	5000	*D. magna*	19000	22000	57000	23000	202000	28000	10	N
ErC_{50}	>983100	*S. subspicatus*	380000	4400000	110000000	4500000	40000000	5500000	10	N

Table 2. Risk assessment of the active substance alphacypermethrin (Fastac 50). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Alfacypermetrin (Fastac 50)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (µg/L)			0.001	0.001	0.001	0.001	<0.001	0.001	KEMI 2009b	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.93	*P. promelas*	930	930	930	930	930	930	100	N
NOEC	0.03	*P. promelas*	30	30	30	30	30	30	10	N
EC_{50}	0.3	*D. magna*	300	300	300	300	300	300	100	N
NOEC	0.03	*D. magna*	30	30	30	30	30	30	10	N
ErC_{50}	1000	*P. subcapitata*	1000000	1000000	100000	100000	100000	100000	10	N

Table 3. Risk assessment of the active substance azoxystrobin (Amistar). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Azoxystrobin (Amistar)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 m buffer zone PEC_{sw} (mg/L)			0.002454	0.001534	0.00042	0.000527	0.000601	0.004465	EFSA 2010a	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
EC_{50}	0.47	*O. mykiss*	190	306	1100	890	780	100	100	N
NOEC	0.147	*P. promelas*	60	96	350	280	240	33	10	N
EC_{50}	0.13	*M. fuscus*	**53**	**85**	310	250	220	**29**	100	**Y**
NOEC	0.044	*D. magna*	18	29	100	83	73	**9.9**	10	**Y**
ErC_{50}	2	*S. capricornutum*	150	230	860	680	600	33	10	N

Table 4. Risk assessment of the active substance bifenox (Fox 480 SC). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Bifenox (Fox 480 SC)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 m buffer zone PEC_{sw} (mg/L)			0.000439	0.000518	-	0.000485	-	0.000385	KEMI 2009a	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.27	*L. macrochirus*	620	520	-	560	-	700	100	N
NOEC	0.0091	*O. mykiss*	20	20	-	20	-	24	10	N
EC_{50}	0.66	*D. magna*	1500	1300	-	1400	-	1700	100	N
NOEC	0.00015	*D. magna*	**0.34**	**0.29**	-	**0.31**	-	**0.40**	10	**Y**
ErC_{50}	0.00019	*D. subspicatus*	**0.43**	**0.37**	-	**0.40**	-	**0.50**	10	**Y**

Table 5. Risk assessment of the active substance beta-cyfluthrin (*beta*-Baythroid). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

***beta*-Cyfluthrin (*beta*-Baythroid SC 025)**			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 m buffer zone PEC_{sw} (µg/L)			0.00311	0.0039	0.000498	0.00377	0.000515	0.0029	EC 2002b	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.068	*O. mykiss*	**22**	**17**	140	**18**	130	**23**	100	Y
NOEC	0.01	*O. mykiss*	**3.2**	**2.6**	20	**2.7**	19	**3.4**	10	Y
EC_{50}	0.29	*D. magna*	**93**	**74**	580	**77**	560	100	100	Y
NOEC	0.02	*D. magna*	**6.4**	**5.1**	40	**5.3**	39	**6.9**	10	Y
EC_{50}	>10	*S. subspicatus*	3215434	2564103	20080321	2652520	19417476	3448276	10	N

Table 6. Risk assessment of the active substance beta-cyfluthrin (Elado). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

***beta*-Cyfluthrin (Elado)**			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (µg/L)			1.00E-09	1.00E-09	1.00E-09	1.00E-09	0.00448	0.0888	EC 2002b	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.068	*O. mykiss*	6.80E+07	6.80E+07	6.80E+07	6.80E+07	**15**	**0.77**	100	Y
NOEC	0.01	*O. mykiss*	1.00E+07	1.00E+07	1.00E+07	1.00E+07	**2.2**	**0.11**	10	Y
EC_{50}	0.29	*D. magna*	2.90E+08	2.90E+08	2.90E+08	2.90E+08	**65**	**3.3**	100	Y
NOEC	0.02	*D. magna*	2.00E+07	2.00E+07	2.00E+07	2.00E+07	**4.5**	**0.23**	10	Y
EC_{50}	>10	*S. subspicatus*	1.00E+10	1.00E+10	1.00E+10	1.00E+10	2200	110	10	N

Table 7. Risk assessment of the active substance boscalid (Cantus). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Boscalid (Cantus)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.002369	0.001414	0.000236	0.001368	0.000456	0.002617	EC 2008	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	2.7	*O. mykiss*	1100	1900	11000	2000	6000	1000	100	N
NOAEC	0.125	*O. mykiss*	53	88	530	91	270	50	10	N
EC_{50}	5.33	*D. magna*	2200	3800	23000	3900	12000	2040	100	N
NOEC	1.31	*D. magna*	550	930	5500	960	2900	500	10	N
ErC_{50}	3.75	*P. subcapitata*	1600	2700	16000	2700	8200	1400	10	N

Table 8. Risk assessment of the active substance clethodim (Select). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Clethodim (Select)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.000612	0.000482	0.000021	0.000473	0.000021	0.000401	EFSA 2011b	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
EC_{50}	3.4	*O. mykiss*	5600	7100	160000	7200	160000	8500	100	N
NOEC	3.9	*O. mykiss*	6400	8100	190000	8200	190000	9700	10	N
EC_{50}	5.1	*D. magna*	8300	11000	240000	11000	240000	13000	100	N
NOEC	0.00084	*D. magna*	**1.4**	**1.7**	40	**1.8**	40	**2.1**	10	**Y**
ErC_{50}	48	*S. subspicatus*	78000	100000	2300000	101000	2300000	120000	10	N

Table 9. Risk assessment of the active substance clomazone (Nimbus CS). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Clomazone (Nimbus CS)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.002912	0.001819	0.000561	0.00064	0.0000726	0.000958	EFSA 2007	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	15.5	*O. mykiss*	5300	8500	28000	24000	210000	16000	100	N
NOEC	2.3	*O. mykiss*	790	1300	4100	3600	32000	2400	10	N
EC_{50}	0.57	*M. bahia*	200	310	1010	890	7900	590	100	N
NOEC	2.2	*D. magna*	760	1200	3900	3400	30300	2300	10	N
ErC_{50}	0.185	*N. pelliculosa*	64	100	330	290	2500	190	10	N

Table 10. Risk assessment of the active substance clopyralid (Matrigon 72 SG). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Clopyralid (Matrigon 72 SG)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.002871	0.001806	0.0000263	0.000631	0.000219	0.004175	EFSA 2005	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	53	*O. mykiss*	18000	29000	2000000	84000	240000	130000	100	N
NOEC	10.8	*P. promelas*	3800	6000	410000	17000	49000	2600	10	N
LC_{50}	>99	*D. magna*	34000	55000	3800000	160000	450000	24000	100	N
NOEC	17	*D. magna*	5900	9400	650000	27000	78000	4070	10	N
ErC_{50}	30	*green algae*	10400	17000	1100000	48000	140000	7200	10	N

Table 11. Risk assessment of the active substance clopyralid (Galera). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Clopyralid (Galera)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. Stream**	**Reference**	
PEC_{sw} (mg/L)			0.005147	0.003242	0.00039	0.000549	0.0000175	0.000335	EFSA 2005	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	53	*O. mykiss*	10000	16000	140000	97000	3000000	160000	100	N
NOEC	10.8	*P. promelas*	2100	3300	28000	20000	620000	32000	10	N
EC_{50}	>99	*D. magna*	19000	31000	250000	1800000	5700000	300000	100	N
NOEC	17	*D. magna*	3300	5200	44000	31000	970000	51000	10	N
ErC_{50}	30	*green algae*	5800	9300	77000	55000	1700000	90000	10	N

Table 12. Risk assessment of the active substance clothianidin (Elado). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Clothianidin (Elado)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (mg/L)			0.00000269	0.00000168	0.002214	0.002051	0.0000608	0.001256	EC 2005a	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	104.2	*O. mykiss*	39000000	62000000	47000	50800	1700000	83000	100	N
NOEC	20	*P. promelas*	7400000	12000000	9030	9800	330000	16000	10	N
EC_{50}	40	*D. magna*	15000000	24000000	18000	20000	660000	32000	100	N
NOEC	0.12	*D. magna*	45000	71000	54	58	2000	96	10	N
ErC_{50}	270	*S. capricornutum*	100400000	160000000	120000	130000	4400000	210000	10	N

Table 13. Risk assessment of the active substance cycloxydim (Focus Ultra). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Cycloxydim (Focus Ultra)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.004896	0.002608	0.000176	0.002727	0.000172	0.002167	EFSA 2010b	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	2.17	*O. mykiss*	440	830	12000	800	13000	1001	100	N
NOEC	21.5	*O. mykiss*	4400	8200	120000	7900	130000	9900	10	N
EC_{50}	1.248	*D. magna*	250	480	7090	460	7300	560	100	N
NOEC	0.079	*D. magna*	16	30	450	29	460	36	10	N
EbC_{50}	74.9	*Anabeana flos-aq.*	15000	29000	430000	27000	440000	35000	10	N

Table 14. Risk assessment of the active substance esfenvalerate (Sumi-alpha 5 FW). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Esfenvalerate (Sumi-alpha 5 FW)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 buffer zone 50 % PEC_{SW} (µg/L)			0.00273	0.00328	0.000431	0.00306	0.000431	0.00242	KEMI 2010	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.1	*O. mykiss*	**37**	**30**	230	**33**	230	**41**	100	**Y**
NOEC	0.25*	*O. mykiss*	92	76	580	82	580	103	10	N
EC_{50}	0.9	*D. magna*	330	270	2090	290	2090	370	100	N
NOEC	0.052	*D. magna*	19	16	120	17	120	21	10	N
ErC_{50}	10	Green algae	3700	3050	23000	3300	230000	4100	10	N

*Value from DG SANCO review report 6846/VI/97-final

Table 15. Risk assessment of the active substance indoxacarb (Steward 30 WG). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Indoxacarb (Steward 30 WG)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.000161	0.000128	0.0000055	0.000133	0.0000055	0.000106	EC 2005b	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.65	Fish	4040	5080	120000	4900	120000	6100	100	N
NOEC	0.15	Fish	930	1200	27000	1100	27000	1400	10	N
EC_{50}	0.6	Crustacean	3700	4700	109000	4500	109000	5700	100	N
NOEC	0.042	Crustacean	260	330	7600	320	7600	400	10	N
ErC_{50}	0.6	*P. subcapitata*	3700	4700	109000	4500	109000	5700	10	N

Table 16. Risk assessment of the active substance *lambda*-cyhalothrin (Karate 2.5 WG). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

***lambda*-Cyhalothrin (Karate 2.5 WG)**			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 m buffer zone PEC_{SW} (µg/L)			0.0317	0.0269	0.00113	0.0258	0.00102	0.0199	dRAR 2013	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
EC_{50}	0.078	*L. idus*	**2.5**	**24**	160	**26**	160	**33**	100	**Y**
NOEC	0.031	*P. promelas*	12	**9.7**	63	10	63	13	10	**Y**
EC_{50}	0.0019	*H. Azteca*	**0.74**	**0.60**	**3.9**	**0.62**	**3.9**	**0.80**	100	**Y**
NOEC	0.00022	*M. bahia*	**0.09**	**0.07**	**0.45**	**0.07**	**0.45**	**0.09**	10	**Y**
ErC_{50}	5	*P. subcapitata*	1900	1600	10200	1600	10100	2100	10	N

Table 17. Risk assessment of the active substance metazachlor (Nimbus). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Metazachlor (Nimbus CS)			**D1. ditch**	**D1. stream**	**D4. Pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 10 m buffer zone PEC_{sw} (mg/L)			0.000756	0.000808	0.000103	0.000717	0.000111	0.00098	EFSA 2008	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	8.5	*O. mykiss*	11000	11000	83000	12000	77000	8700	100	N
NOEC	2.15	*O. mykiss*	2800	2700	21000	3000	19000	2200	10	N
EC_{50}	33	*D. magna*	44000	40800	320000	46000	300000	34000	100	N
NOEC	0.1	*D. magna*	130	120	970	140	900	102	10	N
ErC_{50}	0.031	*S. subcapitatus*	41	38	300	43	280	32	10	N

Table 18. Risk assessment of the active substance metazachlor (Butisan Top). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Metazachlor (Butisan Top)			**D1. ditch**	**D1. stream**	**D4. Pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (mg/L)			0.004866	0.003995	0.000165	0.003695	0.000213	0.003131	EFSA 2008	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	8.5	*O. mykiss*	1700	2100	52000	2300	40000	2700	100	N
NOEC	2.15	*O. mykiss*	440	540	13000	580	10090	690	10	N
EC_{50}	33	*D. magna*	6800	8300	200000	8900	150000	11000	100	N
NOEC	0.1	*D. magna*	21	25	606	27	470	32	10	N
ErC_{50}	0.031	*S. subcapitatus*	**6.4**	**7.8**	190	**8.4**	150	**9.9**	10	**Y**

Table 19. Risk assessment of the active substance picloram (Galera). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Picloram (Galera)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.000921	0.00058	0.000353	0.000208	0.00000511	0.0000839	EFSA 2009	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	8.8	*O. mykiss*	9600	15000	25000	42000	1700000	105000	10	N
NOEC	0.55	*O. mykiss*	600	950	1600	2600	108000	6600	100	N
EC_{50}	44.2	*D. magna*	48000	76000	130000	210000	8600000	530000	100	N
NOEC	6.79	*D. magna*	7400	12000	19000	33000	1300000	81000	10	N
ErC_{50}	98	*P. subcapitata*	106000	170000	280000	470000	19000000	1200000	10	N

Table 20. Risk assessment of the active substance pymetrozine (Plenum 50 WG). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Pymetrozine (Plenum 50 WG)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.000478	0.000418	0.000016	0.000408	0.000016	0.000312	KEMI 2012b	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	100	*O. mykiss*	210000	240000	6300000	250000	6300000	320000	100	N
NOEC	11.7	*O. mykiss*	25000	28000	730000	29000	730000	38000	10	N
EC_{50}	87	*D. magna*	182000	208000	5400000	210000	5400000	290000	100	N
NOEC	0.0251	*D. magna*	52	60	1600	61	1600	80	10	N
ErC_{50}	21.7	Green algae	45000	52000	1400000	53000	1400000	70000	10	N

Table 21. Risk assessment of the active substance prochloraz (Sportak EW). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Prochloraz (Sportak EW)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.00293	0.002521	0.0000985	0.002461	0.000562	0.002676	EFSA 2011a	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	1.2	*C. variegatus*	409	480	12000	490	2100	450	100	N
NOEC	0.0249	*P. promelas*	**8.5**	**9.9**	250	10	44	**9.3**	10	**Y**
EC_{50}	0.77	*M. bahia*	260	305	7800	310	1400	290	100	N
NOEC	0.0222	*D. magna*	**7.6**	**8.8**	230	**9.02**	40	**8.3**	10	**Y**
ErC_{50}	0.032	*S. subspicatus*	11	13	320	13	57	12	10	N

Table 22. Risk assessment of the active substance quinmerac (Butisan Top). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Quinmerac (Butisan Top)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{SW} (mg/L)			0.001745	0.001349	0.000056	0.001272	0.000055	0.001044	EFSA 2010c	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
EC_{50}	86.8	*O. mykiss*	50000	64000	1600000	68000	1600000	83000	100	N
NOEC	3.16	*O. mykiss*	1800	2300	56000	2500	57000	3030	10	N
EC_{50}	100	*D. magna*	57000	74000	1800000	79000	1800000	96000	100	N
NOEC	100	*D. magna*	57000	74000	1800000	79000	1800000	96000	10	N
ErC_{50}	100	*A. flos-aqua*	57000	74000	1900000	79000	1800000	96000	10	N

Table 23. Risk assessment of the active substance tau-fluvalinate (Mavrik 2F). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Tau-fluvalinate (Mavrik 2F)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
PEC_{sw} (µg/L)			0.142	0.0883	0.00282	0.0874	0.00471	0.0687	EFSA 2010d	
Tox measure	**µg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	0.794	*O. mykiss*	**5.6**	**9.0**	280	**9.08**	170	**12**	100	**Y**
NOEC	0.064	*P. promelas*	**0.45**	**0.72**	23	**0.73**	14	**0.93**	10	**Y**
EC_{50}	0.021	*D. magna*	**0.15**	**0.24**	23	**0.24**	**4.5**	**0.30**	100	**Y**
NOEC	0.021	*D. magna*	**0.15**	**0.24**	23	**0.24**	**4.5**	**0.30**	10	**Y**
ErC_{50}	42000	*S. subspicatus*	300000	480000	15000000	480000	8900000	610000	10	N

Table 24. Risk assessment of the active substance thiacloprid (Biscaya OD 240). Toxicity exposure ratio (TER) is given for each organism and each scenario. N = no risk and Y = risk.

Thiacloprid (Biscaya OD 240)			**D1. ditch**	**D1. stream**	**D4. pond**	**D4. stream**	**R1. pond**	**R1. stream**	**Reference**	
Step 4. 15 m buffer zone PEC_{sw} (mg/L)			0.000462	0.000404	0.0000157	0.000394	0.0000257	0.000302	EBSF 2005	
Tox measure	**mg/L**	**Species**	**TER**	**TER**	**TER**	**TER**	**TER**	**TER**	**Trigger**	**Risk**
LC_{50}	25.2	*L. macrochirus*	56000	470000	3200000	480000	2400000	530000	100	N
NOEC	0.24	*O. mykiss*	5300	4500	31000	4600	23000	5000	10	N
EC_{50}	0.0245	*H. Azteca*	540	460	3100	470	2400	510	100	N
NOEC	0.58	*D. magna*	13000	11000	75000	11000	56000	12000	10	N
ErC_{50}	96.7	*S. subspicatus*	2100000	1800000	12000000	1800000	9400000	2020000	10	N

Appendix IV. Cumulative risk assessment of PPPs used in spring rape

- The risk assessments are listed in alphabetical order on the ***products.***
- The active substances are written in brackets.

Table 1. Cumulative risk assessment of **Biscaya OD 240** (thiacloprid) and **Cantus** (boscalid). TER_{mix} is given for each
organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 7 and 24 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	1100	52	440	530	1600
D1. Stream	1900	87	410	850	2600
D4. Pond	11000	520	2800	5200	16000
D4. Stream	2000	90	420	880	2700
R1. Pond	5900	270	2000	2700	8200
R1. Stream	1030	47	409	480	1400
Risk	N	N	N	N	N

Table 2. Cumulative risk assessment of **Butisan Top** (metazachlor and quinmerac) and **Fastac 50** (alphacypermethrin). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 2, 18 and 22 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	300	28	290	12	**6.4**
D1. Stream	340	28	290	14	**7.8**
D4. Pond	870	30	300	29	188
D4. Stream	360	28	290	14	**8.4**
R1. Pond	850	30	300	28	150
R1. Stream	390	28	290	15	**9.9**
Risk	N	N	N	N	**Y**

Table 3. Cumulative risk assessment of **Butisan Top** (metazachlor and quinmerac) and **Focus Ultra** (cycloxydim). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 13, 18 and 22 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	350	330	240	9.04*	**6.4**
D1. Stream	590	420	450	14	**7.8**
D4. Pond	9900	9700	6800	260	190
D4. Stream	590	440	430	14	**8.4**
R1. Pond	9500	8030	108000	230	150
R1. Stream	730	530	540	17	**9.9**
Risk	N	N	N	**Y**	**Y**

***No risk for the single substances applied alone.**

Table 4. Cumulative risk assessment of **Butisan Top** (metazachlor and quinmerac) and **Select** (clethodim). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 8, 18 and 22 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	1300	340	3500	**1.3**	**6.4**
D1. Stream	1600	420	4400	**1.6**	**7.8**
D4. Pond	38000	10010	103000	38	190
D4. Stream	1700	450	4600	**1.7**	**8.4**
R1. Pond	31000	8200	90000	37	150
R1. Stream	2007	530	5400	**2.0**	**9.9**
Risk	N	N	N	**Y**	**Y**

Table 5. Cumulative risk assessment of **Matrigon 72 SG** (clopyralid) and **Mospilan SG** (acetamiprid). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 1 and 10 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	18000	3600	-	4500	10400
D1. Stream	28000	5600	-	6600	17000
D4. Pond	1700000	350000	-	303000	1100000
D4. Stream	71000	14000	-	12000	47000
R1. Pond	230000	46000	-	56000	140000
R1. Stream	12000	2500	-	600	7200
Risk	N	N	-	N	N

Table 6. Cumulative risk assessment of **Select** (clethodim) and **Mavrik 2F** (tau-fluvalinate). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 8 and 23 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	**5.6**	**0.45**	**0.15**	**0.13**	62000
D1. Stream	**9.0**	**0.72**	**0.24**	**0.21**	82000
D4. Pond	280	23	**7.4**	14	2000000
D4. Stream	**9.0**	**0.73**	**0.24**	**0.21**	84000
R1. Pond	**32**	14	**4.5**	**4.01**	1800000
R1. Stream	**12**	**0.93**	**0.31**	**0.27**	100100
Risk	Y	Y	Y	Y	N

Table 7. Cumulative risk assessment of a worst-case scenario with all pesticides normally used in a growing season; **Elado** (clothianidin and beta-cyfluthrin*),* **Butisan Top** (metazachlor and quinmerac), **Biscaya OD 240** (thiacloprid), **Mavrik 2F** (tau-fluvalinate) and **Cantus** (boscalid). TER_{mix} is given for each organism and each scenario. N = no risk and Y = risk. For the individual risk assessments see Tables 6, 7, 12, 18, 22, 23 and 24 (Appendix III).

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	**5.5**	**0.45**	**0.15**	**0.15**	**6.3**
D1. Stream	**8.9**	**0.72**	**0.24**	**0.24**	**7.7**
D4. Pond	270	22	**7.4**	15	190
D4. Stream	**9.00**	**0.73**	**0.24**	**0.24**	**8.4**
R1. Pond	**14**	**1.9**	**4.2**	**2.2**	140
R1. Stream	**0.72**	**0.10**	**0.28**	**0.13**	**9.8**
Risk	Y	Y	Y	Y	Y

Table 8. Cumulative risk assessment of the same worst-case scenario as table 7 but with all the substances that are considered a risk by themselves removed, *i.e.* **Elado** (beta-cyfluthrin) and **Mavrik 2F** (tau-fluvalinate) for all organisms and **Butisan Top** (metazachlor) for algae.

TER_{mix}	Fish (acute) Trigger: 100	Fish (chronic) Trigger: 10	Crustacean (acute) Trigger: 100	Crustacean (chronic) Trigger: 10	Green algae Trigger: 10
D1. Ditch	680	46	407	20	1500
D1. Stream	990	72	390	24	2600
D4. Pond	7800	470	2400	49	14000
D4. Stream	1020	75	390	18	2600
R1. Pond	5100	260	1900	330	8200
R1. Stream	730	44	390	23	1400
Risk	N	N	N	N	N