PROSPECTIVE AQUATIC RISK ASSESSMENT FOR CHEMICAL MIXTURES IN AGRICULTURAL LANDSCAPES

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RUNNING HEAD:

Aquatic mixture risk assessment for agricultural landscapes

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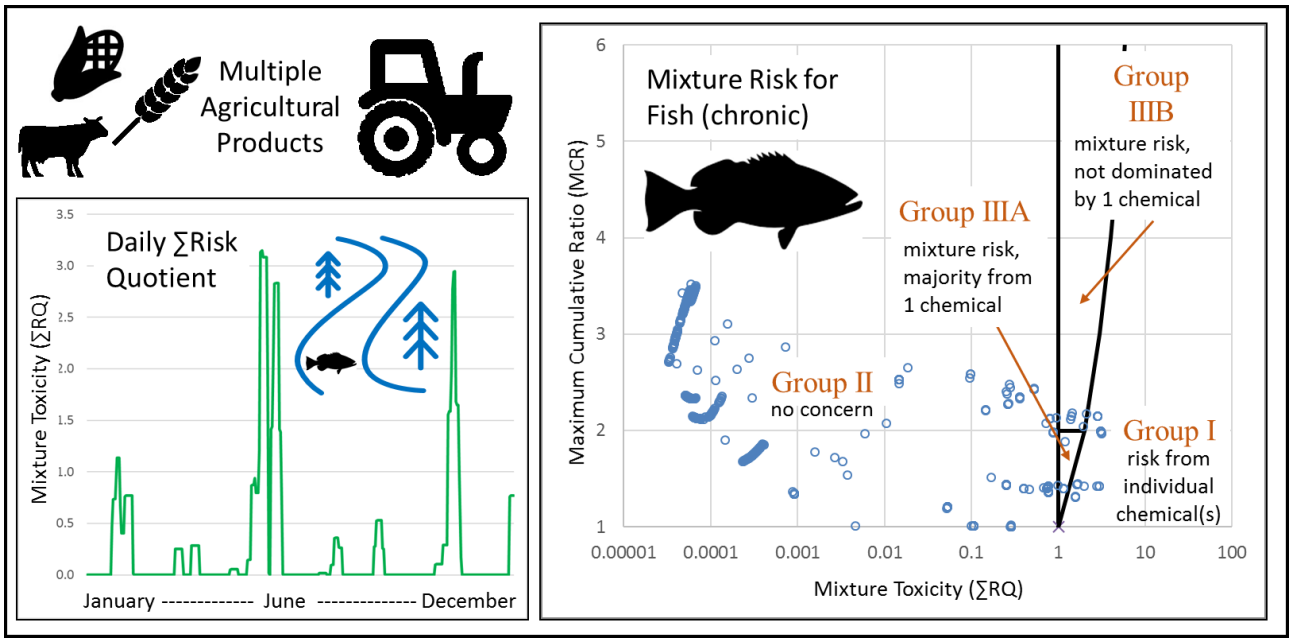
ABSTRACT

Environmental risk assessment of chemical mixtures is challenging due to the multitude of possible combinations that may occur. Aquatic risk from chemical mixtures in an agricultural landscape was evaluated prospectively in two exposure scenario case studies: at field scale for a program of 13 plant protection products applied annually for 20 years, and at a watershed scale for a mixed land use scenario over 30 years with 12 plant protection products and two veterinary pharmaceuticals used for beef cattle. Risk quotients were calculated from regulatory exposure models with typical real-world use patterns and regulatory acceptable concentrations for individual chemicals. Results could differentiate situations when there was concern associated with single chemicals from those when concern was associated with a mixture (based on concentration addition) with no single chemical triggering concern. Potential mixture risk was identified on 0.02% to 7.07% of the total days modeled, depending on the scenario, the taxa and whether considering acute or chronic risk. Taxa at risk were influenced by receiving water body characteristics along with chemical use profiles and associated properties. This study demonstrates that a scenario-based approach can be used to determine whether mixtures of chemicals pose risks over and above any identified using existing approaches for single chemicals, how often and to what magnitude, and ultimately which mixtures (and dominant chemicals) cause greatest concern.

Keywords: Risk assessment, chemical mixtures, landscape, agriculture, exposure scenarios

Graphical Abstract



Acute and chronic mixture risk was assessed for aquatic organisms using field- and catchment-scale scenarios for multiple agricultural products applied annually up to 30 years (single year shown for clarity). Results identified potential risks, and determined whether mixtures of chemicals pose risks beyond any identified using existing approaches for single chemicals, how often and to what magnitude

INTRODUCTION

Many agricultural landscapes contain a mixture of crop types and/or livestock and their management often involves the use of multiple chemicals. Many of these agrochemicals and veterinary products have the potential to move into and impact aquatic environments, resulting in a potential risk due to exposure to mixtures (Boxall et al. 2003; Smith et al. 2012; Schreiner et al. 2016). The detection of multiple chemicals in the environment has raised concern that current regulatory processes may be insufficient to assess the environmental risks of mixtures resulting from the use of different chemicals within agricultural landscapes (Kienzler et al. 2016).

Chemicals used in crop protection and veterinary products are highly regulated in most developed economies and undergo a standardized environmental risk assessment (ERA) prior to authorization. Environmental risk assessments are always conducted on single active ingredients and may also be conducted using formulated products (e.g. EU Regulation 1107/2009, US Federal Insecticide, Fungicide and Rodenticide Act), which can include more than one active substance, as well as other chemicals such as solvents or surfactants. In addition, some countries may request the assessment of pesticide tank mixes containing more than one formulated product. Beyond these intentional mixtures, applied concurrently in time and space, there is the potential for combined exposure of aquatic environments to multiple chemicals resulting from the combination of land uses, crop types and management practices within catchments (i.e. coincidental mixtures). A recent review of European and US regulations (Kienzler et al. 2016) concluded that intentional mixtures were well addressed through a prospective ERA prior to approval. It also concluded that, although the potential importance of effects coincidental mixtures is recognized, no specific details are provided on how to assess environmental mixture effects.

Regulatory prospective ERAs calculate the risk of single compounds to aquatic organisms, generally in small edge-of-field water bodies with limited potential for dilution. This is a realistic worst case for single plant protection or veterinary medicine products, but does not assess whether there is any additional risk associated with exposure to mixtures that arise from the suite of products applied to crops and/or livestock. There have been a limited number of experimental studies that have investigated the effects of a crop-specific plant protection program (Van Winjngaarden et al. 2004; Arts et al. 2006). Both of these studies concluded that risk assessments based on individual compounds were sufficiently protective for these crop protection programs. However, environmental mixtures may also arise due to different chemicals applied to different targets (crops or animals) entering the water simultaneously. Other researchers have used GIS tools that integrate information on land use, crops, pesticide use and other environmental data with exposure models to predict environmental exposure concentrations (Verro et al. 2002) and combined them with ecological and ecotoxicological information to assess potential risks (Sala et al. 2008; Solomon et al. 2013; Kapo et al. 2014). De Zwart (2005) evaluated the spatio-temporally variable net risks posed by all pesticides used in the Netherlands. Exposure was predicted using GIS to identify crop types and areas and then actual pesticide use data and models were used to predict drift, deposition, run-off and drainage. The spatio-temporally variable concentrations were transformed into risk estimates using Species Sensitivity Distributions (SSDs) and mixture toxicity modeling.

One of the key findings by De Zwart (2005) was that the ecotoxicity of environmental mixtures is generally driven by only a few compounds. A conclusion that has since been supported by empirical evidence (Belden et al. 2007; Vallotton and Price 2016). Schreiner et

al. (2016) analyzed routine monitoring results for pesticides from 4,532 monitoring sites across Europe and the USA. They found that mixtures were dominated by herbicides and that the most frequently detected mixtures contained 2 to 5 pesticides. These observations are highly relevant for prioritizing chemicals for management and, combined with the results of the landscape mapping and modelling studies discussed above, suggest that the assessment of environmental mixtures can be undertaken with a simplifying assumption that variations in land use can be used to estimate mixture exposure types and effects. This assumption is explored in the current study for agricultural landscapes and evaluated in more detail for multiple land uses in Posthuma et al. (2017).

Here we consider a mixed agricultural landscape where both plant protection and veterinary pharmaceutical products are used, to determine whether mixtures of chemicals pose a risk greater than that identified using existing single chemical or product based approaches. Standard agricultural scenarios, informed by case studies using real application regimes, are used to model daily exposures, which are then coupled with available effects data to assess the potential aquatic risk using a risk quotient approach for three taxonomic groups (i.e., fish, invertebrates and primary producers). The magnitude and temporal pattern of potential risks are investigated and characteristics of mixtures of greatest concern are identified.

Spatial scale is an important consideration in mixture risk assessment. The worst-case assumption for judging single chemicals or products is edge of field, as this is where exposure from spray-drift, run-off and drainage will be highest. Movement away from edge of field generally results in dissipation of the chemical in the water column through dilution, degradation, volatilization and adsorption. However, when considering mixtures of chemicals, edge of field may not be worst-case in terms of aggregate risk, thus a catchment-

scale (watershed) assessment should also be considered. Consideration of spatial scale should not be restricted to exposure. Protection goals may be set at the meta-population level and thus may require a larger scale than edge of field, up to and including catchments, to include the range of potential non-target species.

This paper is an output of a SETAC Pellston® workshop "Simplifying environmental mixtures - an aquatic exposure-based approach via exposure scenarios" held in March 2015 looking at: (1) whether a simplified scenario-based approach could be used to help determine whether mixtures of chemicals posed a risk greater than that identified using single chemical based approaches, and (2), if so, what might be the magnitude and temporal aspects of the exceedances, so as (3) to determine whether the application of the approach provides insights into mixtures of greatest concern, and the compounds dominating those mixtures (prioritization). The aims of this paper were to investigate these questions using standard agricultural aquatic exposure models and scenarios. Associated papers adopted the same working hypothesis to evaluate the risk of chemical mixtures from two other land use types (De Zwart et al. 2017; Diamond et al. 2017), whilst a combination of the three land use scenarios was generated to investigate these questions for catchments with different combinations of land use (Posthuma et al. 2017).

METHODS

There are well-established procedures for undertaking field-scale risk assessments for plant protection products, and to a lesser extent veterinary medicines. Regulatory risk assessments need to be internally consistent, so the mixture-oriented exposure estimates should be generated as much as possible using existing regulatory tools. Output from the exposure models is the daily loading of chemical to surface water summed for all relevant pathways.

Agricultural chemicals are applied at discrete points in time, then are dissipated in the environment, so understanding the potential for temporal co-occurrence of contaminants in water is a central requirement for an effective mixture risk assessment.

Two exposure scenarios were developed to examine edge-of-field (a single unit scenario) and catchment-scale (a multi-unit scenario) assessments. Examples of the single unit scenarios are feedlots, fields, pasture, aquaculture production areas, and potentially other inputs from non-agricultural point discharges (De Zwart et al. 2017; Diamond et al. 2017), as is Case Study 1.

The multi-unit exposure scenario is the combination of several single unit scenarios, including chemical and water outputs from each of the single unit scenarios discharging into a water body. There are two approaches to conducting a multi-unit exposure scenario assessment. The most complex is the combination of multiple fields discharging to different locations within a catchment. This method requires hydrological characterization to appropriately model the timing of the discharges into the water body, with one or more assessment points located downstream within the catchment. A less complex method of multi-unit scenario assessment assumes the simultaneous discharge of multiple field units to a water body. This latter, more conservative approach avoids the need to consider hydrology, but the estimated peaks will be higher because all discharges are to the same point in the water body and hydrological travel time of chemicals is ignored. This paper applies this second, more conservative approach to a multi-unit exposure scenario in Case Study 2. A more detailed discussion on field-scale and catchment-scale assessment and exposure scenarios is provided in the Supporting Information (S.I.).

Case study 1: Assessment at the unit of a single field - winter wheat in the UK

Problem formulation.

This case study addresses the question: *Is there any additional risk associated with exposure of the aquatic environment to mixtures that arise from the suite of plant protection products applied to a crop that would not be identified using single chemical assessments*?

The risk for a single crop is expected to be greatest at edge-of-field scale where there is limited potential for dilution and degradation within the receiving water body. A single-field unit was modeled assuming a single crop comprising winter wheat in the United Kingdom (UK). The case study is intended as proof of concept and not as a regulatory risk assessment, although exposure estimates are generated using an existing regulatory modelling framework for consistency with current practice. Furthermore, regulatory risk assessment at an EU level is based on single substances, whereas at member state level it is on a product basis. Products can contain more than one active substance and there is often some assessment of combined risk. Whilst in these case studies some active substances would have been applied together as a single product, the assumption is that the assessments were done at a single substance level for any comparisons with the mixture.

Approach to exposure assessment.

Pesticide risk assessments are based on either individual active substances or co-formulated mixtures of active substances applied to the crop. Pesticide usage data for the UK are collected on a biannual basis (Garthwaite et al. 2013). Data for a single agricultural season (2009/10) were obtained for a large arable farm in eastern England. There were 16 fields cultivated with winter wheat and all fields were treated with the same suite of 13 active

substances. Dates of application and actual rates were available (Table S2), so the risk assessment pertains to real conditions of use rather than the maximum label usage normally considered in prospective regulatory assessments.

The FOCUS Surface Water Scenarios (FOCUS 2001) provide a consistent framework for assessing risks to the aquatic environment from pesticides in European regulatory procedures. Ten scenarios cover the broad conditions of agriculture across Europe in terms of soils, weather, cropping and field-edge surface water bodies. Spray drift inputs to water are based on an analysis of a large database of drift experiments (Rautmann et al. 2001). The models PRZM (Suárez 2005) and MACRO (Larsbo and Jarvis 2003) simulate fate of pesticides in soil and generate estimates of water and pesticide emissions via surface runoff and drainage, respectively. Outputs from these models and the spray drift calculator are inputs to TOXSWA (Beltman 2006), which simulates the fate of pesticides in surface water, generating aquatic predicted environmental concentrations (PECs). While the FOCUS exposure models give PECs for water column, pore water and sediment, we focused on water column for this case study.

One FOCUS scenario (i.e., R1 runoff) that is directly applicable to UK agricultural conditions was used to generate exposure estimates. This scenario includes a range of crop types including winter cereals and has been identified as having primary relevance to the UK agricultural situation, particularly in south-eastern England (FOCUS 2001). Standard regulatory modelling procedures set out by FOCUS (2001) were followed except for three deviations. First, actual dates and rates of application were used as input. Secondly, FOCUS modeling normally relies on pre-assessment of pesticide application date against a 20-year weather dataset to select a worst-case 100-day profile (i.e., rainfall occurring soon after

application). This means that pesticides with different application dates will often be assessed with different sections of the long-term weather data set. To overcome this, all simulations were run with the full 20-year series of daily weather data and inputs to the stream were integrated using the STEPS1234 model (Klein 2007) to generate a long-term profile of exposure concentrations. It was assumed the same set of substances were applied in each of the 20 years. This ensures the assessment of exposure was conducted under a range of weather conditions whilst ensuring that simulations for different pesticides are consistent. Finally, only standard, laboratory studies to generate environmental fate parameters for modeling were used to ensure consistency between the different chemicals. No use was made of higher tier data, such as the generation of soil degradation half-lives from field dissipation studies. Additional details are provided in the S.I.

Risk characterization.

For each of the 13 active substances (Table 1), aquatic ecotoxicology data were taken from their respective EU review report or EFSA conclusion to calculate a Regulatory Acceptable Concentration (RAC). The RAC is the effects assessment endpoint expressed in terms of a permissible concentration in the environment that is directly used in the risk assessment by comparing it to the appropriate field exposure estimate (Brock et al. 2010; EFSA 2013). If the RAC is not exceeded, the environmental effects of a chemical are assumed to be acceptable and low risk is concluded. RACs were calculated using the methodology according to the EFSA Aquatic Guidance (EFSA 2013). Risk to primary producers (algae and macrophytes) and acute and chronic risk to fish and aquatic invertebrates were calculated separately. If higher tier ecotoxicity data were available they were also used, using the endpoints generally as presented in the respective EU assessments and following current guidance (EFSA 2013). These higher tier data included additional species tests and aquatic

micro/mesocosm studies for primary producers and invertebrates. The ecotoxicity data for the different taxonomic groups are presented in Table 2.

RACs for primary producers and acute and chronic for fish and aquatic invertebrates were compared to the PECs produced by the model to give a Risk Quotient (RQ = PEC/RAC) for each predicted daily chemical concentration, with RQ < 1 indicating acceptable risk on a per-chemical basis. RQ values for mixtures were calculated by summing the derived RQs of the 13 individual compounds for each day. This approach assumes concentration addition and estimates the daily total aquatic risk from all the pesticides applied in the wheat field. Following the guidance, chronic fish and chronic invertebrate risk assessments were refined using 7-day time weighted average concentrations rather than the daily concentrations (EFSA 2013).

It is often observed in risk assessments of defined chemical mixtures that the risk is driven by one, two or only a few chemicals (e.g., De Zwart 2005; Backhouse and Karlsson 2014). A useful method of expressing how mixture risk is characterized is the Maximum Cumulative Ratio (MCR) approach of Price and Han (2011). The MCR is given by the sum of individual RQ values for each chemical (ΣRQ) in the mixture divided by the maximum RQ within that mixture.

The MCR was calculated for each time step (i.e. daily). Following the methods of Price et al. (2012), combined exposures were grouped into categories to facilitate risk assessment and risk management. The categories are described below.

Group I contains combined exposures where one or more chemicals are of concern because they have an individual RQ >1

Group II contains combined exposures where the ΣRQ < 1 and consequently these exposures are of low concern

Group III contains combined exposures where ΣRQ is > 1 only by summing the chemicals, no individual chemical has a RQ >1

Group IIIA, the MCR is < 2 i.e., the majority of the toxicity is from one chemical

Group IIIB, MCR is > 2 i.e., the toxicity is not dominated by a single chemical

Group IIIB is where the model used for mixture toxicity is most important and where further refinement based on mode of action may be important.

Results for case study 1.

Table 2 gives the number of days when the RQ exceeded 1 for individual chemicals for primary producers and acute and chronic risk to aquatic invertebrates and fish, together with the number of days where ∑RQ across all the chemicals exceeded 1 for each group. Table 3 translates these results into MCR categories. Table 2 also includes information on the duration of ∑RQ exceedances expressed as the number of times the ∑RQs exceeded 1 for a consecutive sequence of days (e.g., for 2, 3, 4, or 5 days consecutively), as well as the longest duration of ∑RQ exceedance.

For primary producers, only mesosulfuron-methyl and flufenacet individually had RQs which exceeded 1 on 14 and 2 days, respectively, with maximum values of 5.46 and 1.07. Only 16 days were in MCR group I, where an RQ of 1 was exceeded by individual chemicals, out of a total of 63 days where ∑RQ was >1. Whilst not exceeding an RQ of 1, epoxiconazole, iodosulfuron-methyl and pendimethalin, in particular, contributed to occasions where ∑RQ exceeded 1 in MCR group III.

For acute risk to invertebrates, cypermethrin was the only chemical where the individual RQ exceeded 1 (maximum 1.67), which was the case for 17 days out of a total ∑RQ exceedance of 1 for 111 days. Of the 94 days in group III, indicating a mixture risk, the majority were in group IIIA indicating the dominance of cypermethrin as the risk driver (Table 3), however significant contributions to ∑RQ also came from pendimethalin, fluoxastrobin, and chlorothalonil.

For chronic risk to aquatic invertebrates only fluoxastrobin and cypermethrin exceeded RQs of 1 on 47 and 17 days, respectively, and with maxima of 3.16 and 1.67, respectively. Unlike some of the other chemicals, which had refined effects assessment information, there were no higher tier data available for fluoxastrobin. There was a total of 159 days in group III indicating a potential mixture risk, with the majority of those days in group IIIB. Pendimethalin and to some extent chlorothalonil, epoxiconazole and prochloraz made significant contributions to ∑RQ. When refined using a 7d TWA exposure, the number of exceedances was reduced and there were no days where single chemical RQs exceeded 1 and only 13 days (0.17% of total days) where ∑RQ exceeded 1.

There were very few exceedances of an RQ of 1 for single chemicals for acute risk to fish. Only chlorothalonil and cypermethrin RQs exceeded 1 for 9 and 1 days, respectively, at maxima of 1.59 and 1.02, respectively. Pendimethalin made a significant contribution to ∑RQ, resulting in a total of 43 days where ∑RQ was >1, with 33 days in group III, split 12 days in IIIA and 21 days in IIIB.

RQs for chronic risk to fish exceeded 1 for cypermethrin, chlorothalonil, pendimethalin and epoxiconazole, on 263, 39, 123 and 1 days, respectively, with maximum values of 9.48, 9.0, 2.18 and 1.05, respectively. Only 71 days were in group III, with 56 in group IIIB, and with the majority of the contribution to the RQ coming from the afore mentioned compounds. When refined with a 7 d TWA the magnitude of the RQs was significantly reduced and for pendimethalin, all the RQs became <1. For cypermethrin and chlorothalonil, there was some reduction in the number of days where RQs exceeded 1, but the change was not as large, which is explained by the magnitude of the RQs for those compounds. The concentration is effectively spread across a number of days when using a TWA concentration, resulting in some days exceeding an RQ of 1 using a 7d TWA where they previously did not when based on the modeled concentration for just that day. This is illustrated by the large increase in the number of times the ∑RQ exceeded 1 for a set of consecutive days, and by the increase in longest duration of ∑RQ > 1 (Table 2). In these run-off scenarios, exposures are typically short and thus probably warrant further investigation of the potential for chronic effects on fish from short-term exposures.

The longest duration of exceedances (∑RQ > 1) were 3 or 4 days across all taxa other than refined chronic fish, and the number of days where ∑RQ > 1 consecutively for more than 2 days ranged from 2 to 8 occurrences across taxa. For refined chronic fish (using the 7-day

TWA), the longest duration of ∑RQ > 1 was 14 days, with 240 days when ∑RQ > 1 consecutively for more than 2 days. Full results are presented in Table 2.

Figure 1 graphically presents the daily predicted mixture toxicity values over 20 years for each of the taxonomic groups assessed. The topmost chart for primary producers contains the labeled MCR groups using the categories of Price and Han (2011).

Case study 2: Assessment at the small catchment unit scale – USA corn together with cattle grazing and feedlot operations

Problem formulation.

Agricultural fields do not exist in isolation within the agricultural landscape. The landscape consists of fields with different uses, both for crops, pasture and animal husbandry. All have potential chemical inputs into the aquatic environment. This case study addresses the question:

Is there any additional risk associated with exposure of the aquatic environment to mixtures that arise from a suite of plant protection products and veterinary medicines within the same catchment (watershed) that would not be identified using a single chemical assessment?

The risk assessment represents multiple sources of chemical inputs associated with a scenario of corn production in Iowa, USA. It considers input from crop protection activities, together with veterinary pharmaceutical inputs from use in beef cattle, from three runoff sources: pastures, manure-applied fields, and directly from feedlots.

Approach to exposure assessment.

Plant protection products

The agency responsible for pesticide risk assessment in the USA is the US Environmental Protection Agency (USEPA). They use a tiered risk assessment system for ERAs in which conservative assumptions are used as inputs for simplistic models in a screening level risk assessment at Tier I. In a Tier II assessment, there are several environmental scenarios encompassing a multitude of crops and their growing regions. These scenarios define the soil characteristics and daily weather inputs for the exposure models, which are used along with the product label information and the environmental fate properties of the active substances for the crop and chemical specific inputs. The study reported here used a standard Tier II scenario for modeling exposure. Environmental exposure estimates were modeled using the Surface Water Concentration Calculator (SWCC) (Fry et al. 2014). While the USEPA exposure models give concentrations for water column, pore water and sediment, as with Case Study 1, we are focusing on the water column.

Over 38 million ha of land was put in corn production in the US in 2012, accounting for 30% of the harvest cropland area (USDA-NASS 2014). For this case study, the EPA standard Tier II Iowa corn scenario (USEPA 2017) was selected as representative of intense US corn production.

The standard USEPA ecological exposure assessment is based on a single 10 ha field in which all runoff and erosion drains to a single 1 ha, 2 m deep pond. However, for our exposure scenario in which multiple fields within a catchment drain to a common water body, the USEPA Index Reservoir (USEPA 2010) was implemented because this allows for a

mixed-use watershed. The Index Reservoir is based on an actual watershed, the Shipman City Lake located in Illinois, which is a 172 ha catchment that drains to a surface water body of 5.26 ha surface area and a depth of 2.74 m. The exposure modeling uses the conservative assumption that chemicals from all areas in the catchment reach the waterbody at the same time.

A typical crop protection treatment regime was defined using most common practices in that area. The program consists of 12 active ingredient applications, including the most widely (by area treated) applied seed treatment, corn root worm treatment, herbicide program and fungicide. All applications were made at the standard application rate, implementing the label buffer specified on the most conservative label (200 ft (61 m) around natural or impounded lakes and reservoirs as specified for atrazine (Syngenta 2015)). Substances were applied to the corn fields as pre- and post- emergence herbicides, fungicidal and insecticidal seed treatments, a soil insecticide and foliar fungicides (Table S4). Critical crop dates include emergence (25 May), maturation (24 July) and harvest (19 October) as specified in the standard Iowa corn scenario.

Veterinary pharmaceuticals

Veterinary pharmaceuticals were considered in addition to crop protection products, using beef cattle as the animal receiving treatment. Analysis of USDA Census of Agriculture National Agricultural Statistics Service data in Zoetis (2014) indicated that western Iowa contains a high density of beef feedlot cattle as well as cropland receiving manure applications. An analysis was conducted to identify highly vulnerable watersheds based on beef cattle feedlot density, manured cropland, and climate (Zoetis 2014). This analysis

identified two counties in western Iowa (Lyon and Sioux) that are representative of highly vulnerable landscapes, within which a single watershed was selected based on high exposure potential, and characterized by land use. The total watershed was 9016 ha, consisting of 56.6% corn, 2.3% pasture, 0.94% feedlot, with the remainder composed primarily of other agriculture and developed land. More details are in the S.I., and full details in Zoetis (2014).

Land use area percentages for this watershed were used within the USEPA Index Reservoir scenario to calculate predicted environmental concentrations. These percentages for manured land, pasture and feedlot were used to scale the daily PRZM runoff and erosion chemical mass loadings (which assumes cropland, pasture and feedlot are each 100% of the watershed) simulated by an individual PRZM model run before the mass enters the water body.

To model potential transport of veterinary medicines to surface water for this case study, it was assumed beef cattle were treated annually with an injection of tilmicosin, a macrolide antibiotic. Subsequent excretion of the active ingredient was modeled for 14 days after treatment, assuming a 50% metabolism rate, with no degradation in the manure. Cattle were also treated annually with moxidectin as a 'pour on' application, used for parasite control. Subsequent excretion of the active ingredient was modeled for 20 days (feedlot) or 26 days (pasture) after treatment, assuming a 61% metabolism rate, with no degradation in the manure. Runoff from manure containing moxidectin and tilmicosin was modeled from pasture, as manure applied to corn fields (Table S5), and from feedlots using the inputs listed in S.I.. Collection water from feedlot lagoons was assumed to have 10% of the chemical mass, and was applied to the corn fields as irrigation four times annually.

Risk characterization.

A RAC was determined for each of the 12 pesticide active substances in a manner comparable to the UK wheat scenario in Case Study 1. Because this was a US scenario, the pesticide RAC values were typically the US EPA aquatic life benchmarks (USEPA 2016), except where stated otherwise in Table 4. For the veterinary pharmaceuticals, the tilmicosin RAC was based on the assessment factors (AFs) in the relevant guidance (EMEA 2005) and for moxidectin the RAC value was taken from an environmental risk assessment report (Fort Dodge Animal Health 1997) submitted for regulatory decision making. One aspect highlighted was the difference in the amount of available effects data between plant protection products and veterinary medicines, where the former have more comprehensive data requirements and typically smaller AFs. This is likely a reflection of the relative route of exposure and ecological concern where veterinary products are often fed, poured on the hide, or administered by injection to animals and residues enter the environment through excreta after metabolism *in vivo* versus being sprayed or directly applied to the field or crop as for pesticides.

It was assumed the same set of substances were applied in each year over a 30-year period. For calculation of chronic risk, TWAs of 21 and 60 days were used for aquatic invertebrates and fish, respectively (USEPA 2017). The methodology for summing daily RQs to indicate risk were the same as for Case Study 1, as was the use of the MCR and grouping into the categories I, II, IIIA and IIB to facilitate communication of the risk.

Results case study-2.

Table 5 gives the number of days when the RQ exceeded 1 for individual chemicals for primary producers, and acute and chronic risk to aquatic invertebrates and fish, together with

the number of days where the ∑RQ across all the chemicals exceeded 1 for each group. Table 3 translates these results into MCR categories. Table 5 also includes information on the duration of ∑RQ exceedances expressed as the number of times the ∑RQs exceeded 1 for a consecutive sequence of days (e.g., for 4, 21, or 60 days consecutively), as well as the longest duration of ∑RQ exceedance.

The exposure profiles for the individual chemicals which drove the risk assessments were very different in this case study compared to the UK case study. The UK water body is flowing, and convective transport out of the considered portion of water body is important when characterizing exposure. In contrast, turnover of water (i.e., water entering and leaving) is much slower in the reservoir used in the US case study, so there is limited loss of chemicals under conditions where degradation is slow. As a consequence, chemicals showed much slower dissipation after an initial pulse and compared to the UK study there was generally a higher proportion of the total days which showed RQs exceeding 1 both for single substances and for a mixture. This is also illustrated by the larger number of times the ∑RQs exceeded 1 for a consecutive set of days (e.g., for 4, 21, or 60 days consecutively), as well as the increase in the longest duration of ΣRQ exceedances for the US case study.

For primary producers, the ΣRQs exceeded 1 on 1,100 (10.04%) of the 10,957 days modeled (1 January 1961 to 31 December 1990), indicating potential further refinement, mitigation or risk management is required. The herbicides acetochlor and atrazine were the main drivers; their individual RQs reached 18.19 and 2.21, respectively, and exceeded 1 on 575 and 361 days, respectively. All other chemicals made minor contributions to the overall RQ, with only 285 days in MCR group III (no single chemical exceeding a RQ of 1) and only 17 days in group IIIB (Table 3).

For acute risk to aquatic invertebrates, the ΣRQ exceeded 1 on only 113 days (1.03% of the total), dominated by tefluthrin and moxidectin with individual maximum RQs of 9.89 and 3.18, respectively and exceeding 1 on 41 and 48 days, respectively. There were only 44 days in MCR group III and of these only 3 days in IIIB, indicating the dominance of the two chemicals driving the risk. For chronic risk to invertebrates (using a 21-day TWA) the RQ of 1 was exceeded on 824 days, yet the only chemical which exceeded an RQ of 1 was tefluthrin with a maximum RQ of just 1.45 and for only 49 days. Group IIIA and IIIB contained 307 and 468 days, respectively, indicating less dominance of one or two chemicals. Acetochlor, flumetsulam, atrazine and clothianidin all contributed to the ∑RQ, resulting in exceedance of 1.

∑RQs for acute risk to fish was exceeded on 49 days, driven largely by a single chemical, tefluthrin, with a maximum RQ of 11.54 and exceedance of 1 on 47 days. There were only 2 days when there was a mixture risk and again it was largely driven by tefluthrin, with minor contributions from acetochlor and pyraclostrobin being sufficient to take the ∑RQ above 1. For chronic risk to fish (using a 60-day TWA) ∑RQ exceeded 1 on 1980 days, 18.07% of the total, with a maximum ∑RQ of 5.92. Only two chemicals were driving this, tefluthrin and atrazine, resulting in the majority of days being in group I and 416 in group IIIA, with only 8 days in group IIIB.

For acute exposures, the longest duration of exceedances (∑RQ > 1) was 3 and 5 days for invertebrates and fish, respectively, and 177 days for primary producers (driven by the 60-day TWA for atrazine, see footnote in Table 4). The longest duration of exceedances for chronic exposures were higher due to the use of a TWA, with 115 days for invertebrates (21-

day TWA) and 279 days for fish (60-day TWA). The number of days where ∑RQ > 1 consecutively for more than 21 days was 0 for acute exposures to invertebrates and fish, and ranged from 510 for chronic invertebrates to 1602 days for chronic fish exposures. Full results are presented in Table 5.

Figure 2 graphically presents the daily predicted mixture toxicity values over 30 years for each of the taxonomic groups assessed. The topmost chart for primary producers contains the labeled MCR groups using the categories of Price and Han (2011).

DISCUSSION

We have demonstrated the value in applying simplified, scenario-based approaches to assessing the risks from chemical mixtures. Our case studies address agriculture in two continents and at the scale of a single unit and a multi-unit system and the approach allowed the consistent analysis of chemicals used for different purposes and currently assessed under different regulatory schemes (i.e. plant protection products and veterinary medicines). Apart from the mixture assessment step, the models we applied are those used for single chemical registration. Regulatory scenarios are developed to provide a pre-specified vulnerability for exposure due to single chemicals (e.g. FOCUS 2001; Fry et al. 2014) that is associated with the stated protection goal (e.g. EFSA 2013). Applying these scenarios in the context of chemical mixtures reframes the problem formulation and will require reappraisal of the environmental context to deliver an appropriate level of vulnerability/protectiveness. There were some constraints in our direct application of modelling approaches aimed at single chemicals. For example, the EU surface water assessment is a short-term (100-day) calculation (FOCUS 2001) where the time window of assessment is selected according to

timing of use from a total range of possibilities spanning 20 years. It was necessary to develop a custom approach with a full 20 years of assessment to put the analysis onto a consistent time basis for all mixture components and to investigate the range of mixtures possible as a function of variation in weather. It is notable that work is currently planned to move single chemical exposure assessment onto this longer-term basis (EFSA 2017). Current guidance on exposure modeling of veterinary medicines does not provide specific time series exposure scenarios, so the models and scenarios used for pesticides were adapted following Zoetis (2014). The SWPP model used for EPA Tier II exposure modeling in the US directly links the model for off-site transport of chemical to the receiving water body model. As multiple routes of runoff entry were modeled for veterinary medicines (pasture, manured fields, feedlot), a custom step was needed to aggregate the daily mass entering the reservoir from all three sources before receiving water modeling was performed.

We applied a default approach of concentration addition to the effects assessment, investigating whether exposure to multiple chemicals would significantly alter the risk compared to separate assessments for each individual component of the mixture. Both case studies (edge of field and catchment scale) delivered some evidence to support considering mixtures in addition to single compounds, as there were instances triggering concern for the predicted mixtures when the individual compounds would not have raised concerns in the current assessment approach. This occurred for primary producers, aquatic invertebrates and fish in both the UK and US case studies. However, in common with other mixture toxicity studies (Belden 2007), we found that a small number of chemicals were the primary drivers of instances where $\sum RQ > 1$ and generally these key components of mixture toxicity were chemicals where individual risk was indicated on occasions. However, we also identified chemicals where individual RQ did not approach 1, but that made a significant contribution

to mixture toxicity through frequent presence at concentrations with RQs <1 but > 0.1. The signature of an individual chemical in terms of whether and how it contributes to mixture toxicity will be a function of extent of use, persistence, pathway(s) into the environment, and toxicity profile; the implication of our results is that future work could combine these factors to categorize chemicals into different characteristic contributions to mixture toxicity.

Characteristics of the receiving water body had a significant influence on assessment results both in terms of level of risk and type of risk identified. In the UK case study fish were the taxa identified most often at potential risk, driven by RQs derived from chronic RACs compared with 7-d TWA exposures. This case study used an EU scenario with a flowing water body where advective losses of pesticide from the system were a dominant route of dissipation. The use of a TWA reduces the RQs and may often be sufficient to demonstrate acceptable risk; failing this, a long-term toxicity test in which the predicted, modelled exposure profile is mimicked could be conducted to link the exposure to effects. Further effects refinement could examine whether application of the concentration addition assumption is appropriate, particularly for the chronic effect endpoints i.e. do the chemicals studied have the same mode of action or have common adverse outcomes.

The water body considered within the US exposure scenario was a reservoir with long hydraulic residence times; modeled chronic exposures were thus much more common, as were the resulting risks from single chemicals and mixtures. A generalized finding from this research is that the risk consequences of the combination of chemical use profiles and scenario characteristics can be studied in relevant detail by considering the inherent vulnerability of different taxa and the nature of potential impacts on those taxa of specific chemicals (e.g., insecticides affecting arthropods), so helping to prioritize management decisions.

The scenario-based approach made it possible to place the exposure assessment for two chemical groups with different regulatory paradigms onto a consistent basis, as illustrated for plant protection products and veterinary medicines in the US case study. Consistency in effects assessment was more difficult to achieve because of the different demands on data generation for different chemical types. Plant protection products are data rich with respect to ecotoxicology when compared to most animal health products. Consequently, to derive a RAC for this exercise, the AFs applied to the animal health products were large in comparison to the plant protection products, which could have led to the animal health products being given undue weight in the mixture risk assessment. There were instances of mixture toxicity across plant protection products and veterinary medicines, implying the need for better sharing of risk methodologies and risk outcomes across types of chemical. This theme is explored further in Posthuma et al. (2017) in consideration of more complex mixtures in larger catchment systems.

Our compilation of effects data highlighted a number of issues pertinent to risk assessment of chemicals and in particular mixtures. The effects data can be limiting, with the most obvious example being the disparity between the data-rich agrochemicals and the more data-sparse veterinary medicines in the US scenario. This resulted in different AFs being applied and potentially more precaution for the veterinary medicines. However, amongst the pesticides there are differences in the availability of data for refinement. For example, the UK scenario indicated fluoxastrobin as the major contributor to ∑RQ for chronic risk to aquatic invertebrates; unlike some of the other chemicals this was not based on a higher tier effects evaluation and so again was likely to be more precautionary. For chronic risk to fish in the US, atrazine was a major contributor to the ∑RQ, however the current US EPA benchmark of

5 µg/L is based on a study classified as supplemental and where the LOAEC is 50 µg/L. This is a much larger range between NOAEC and LOAEC than is typical, indicating the benchmark of 5 µg/L may be conservative and that further refinement of the effects value is a possibility.

Ecological risk assessment is geared towards protecting populations, communities and ecosystems, rather than the individual, although an exception to this is vertebrates where no visible mortality of individuals is often the protection goal (EFSA 2013). At lower tiers, an assessment factor is added to single species laboratory acute (LC/EC50) and, if available for the EU, chronic (NOEC, ECx) values, to extrapolate to a concentration at which no effects on the community are expected. Higher tiers can involve extrapolation from laboratory toxicity data for additional species (e.g. Species Sensitivity Distributions – SSDs) or community level studies (microcosms / mesocosms) to give concentrations at which no effects or no adverse/unacceptable effects on exposed communities would be expected. The concentration addition concept, which is widely accepted as being a conservative, default assumption for assessing the impact of chemical mixtures (EFSA 2017) is based on single species approaches. Community level effects may depend not only on direct toxicological based effects, but indirect ecological effects and ecological interactions (SHER 2012) and it is uncertain as to how, or indeed whether, these should be combined using concentration addition. Many agrochemicals require higher tier tests, such as community level studies, to establish safe use. Without the use of higher tier data, therefore, a mixture assessment would likely indicate unacceptable risk, as the risk from these single chemicals would already be considered unacceptable. In order to avoid this situation, a pragmatic approach has been adopted in the EU (EFSA 2013) whereby data from both lower and higher tiers are combined in an additive risk assessment using the RACs. Comparison of risk assessment outcomes

executed in this way with thresholds of effects in multi-species (field) tests or field ecosystems can elucidate the level of protection for this approach.

Retrospective assessment of chemical mixtures yields important information that can be used to validate modelling steps, calibrate the outcomes of prospective assessments, and determine whether any environmental impairment can be expected from, or attributed to, combinations of chemicals present in the environment. Use of monitoring data for retrospective analyses may be challenging, because data exist only for sampling locations that are specifically located in space and time, and only for chemicals that are specifically analyzed. Two approaches may be used for monitoring strategies of chemicals and mixtures. The first of these is targeted monitoring at a specific site or sites using prior knowledge of chemical use to indicate what to look for, such as monitoring for pesticide residues in watersheds draining from sugar-cane growing areas in Australia (O'Brien et al. 2014). The second approach is to search monitoring databases retrospectively and determine whether there was likely to be any potential risk due to individual chemicals and/or mixtures. This can be done to analyze for any trends of increasing or decreasing risk (when data are available over time) and it may help to quantify the effectiveness of past mitigation measures, such as changes in the authorization of specific pesticides in reducing single-chemical or mixture risks. Vallotton and Price (2016) illustrated this approach for pesticides in surface waters from across the US, using results from the National-Water-Quality Assessment (NAWQA) program of the US Geological Survey (USGS) from 1992 to 2001. Using a total of 4,380 samples across the US, pesticide residues were found in 3,099 and a total of 81 different pesticides were detected (average of 9 per sample, minimum of 5, maximum of 29). HQs, equivalent to the RQs discussed herein, and MCRs were calculated and refined based on different organism groups: fish, invertebrates, vascular (macrophytes) and non-vascular plants (i.e. algae). Like the case

studies in this paper, the retrospective analyses of Vallotton and Price (2016) allowed identification of the dominant contributors to mixtures, which were the insecticides diazinon and chlorpyrifos and the herbicides atrazine and acetochlor; interestingly, these are the same two herbicides giving the most concern in our US simulation, case study 2.

CONCLUSIONS

While the two case studies presented are illustrative and have limitations, the results encompass some clear patterns which relate to the study goals. First, both case studies (edge of field and catchment scale) generated evidence to support prospectively considering mixtures in addition to single compounds, as there were instances across all taxa examined triggering concern for the predicted mixtures when the individual compounds would not have raised concerns in the current assessment approach. For the UK edge of field study, this only occurred between 0.18 to 2.67% of the days modeled for primary producers, invertebrates (acute and chronic), and fish (acute and chronic). This accounted for 20 to 100% of the total days when the ∑RQ exceeded 1. For the US catchment-scale case study, mixture concerns in the absence of single chemical concerns occurred between 0.02 and 7.07% of the days modeled across the same taxonomic groups. This accounted for 4 to 94% of the total days when the ∑RQ exceeded 1. Second, the case studies provide insights into how often and by how much chemical exposures exceeded levels of concern either singly or in combination. Third, the case studies indicated that the relative importance of chemicals in mixtures differs, and identified the chemicals that most often have a RQ >1 individually, and those that may often contribute to the overall toxicity without ever exceeding a RQ of 1.

The characteristics of the receiving water body used in the exposure assessment play a key role in determining which types of substances contribute to ecological risk. Our case studies examined two different types of surface water; a flowing water body with significant dissipation (UK case study), and a predominantly static reservoir where aquatic degradation was the primary mechanism (US case study). Results showed that the physical-chemical properties of the substances modeled helped to define which chemicals contributed to the mixture risk in each case study.

The amount and types of data available for different components of a mixture can greatly affect the AFs used and thus the resulting RACs and RQs. This can have a major effect on the outcome of the assessment and indicates the difficulty in assessing risks for mixtures which contain chemicals where effects profiles have been categorized to different extents. This may result in mixture risk being driven by the compounds with the greatest uncertainty (least data) rather than greatest toxicity.

The approach presented here, based on regulatory models currently used on individual chemicals, allows for the prioritization of mixtures for further investigation or management. Use of taxa-specific effects data, appropriate TWA concentrations or pulsed exposure studies, refinement of many of the worst-case assumptions used in the exposure modeling, and/or inclusion of more refined catchment-scale processes, would further support drawing meaningful conclusions on the risks identified in the case studies. Further considerations could include investigation of mode of action and/or common adverse outcome groups to evaluate whether concentration or response addition is appropriate, or indeed whether synergy or antagonism is a potential outcome.

Supplemental data

The Supplemental data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Disclaimer

The authors do not have any conflict of interest with the study or results. The opinions expressed in the present study are those of the authors and not their respective employers.

Data Availability

Data pertaining to this manuscript can be obtained from the corresponding author.

REFERENCE LIST

Arts GHP, Buijse-Bogdan LL, Belgers JDM, Van Rhenen-Kersten CH, Van Wijngaarden RPA, Roessink I, Maund SJ, Van den Brink PJ and Brock TCM, 2006. Ecological impact in ditch mesocosms of simulated spray drift from a crop protection programme for potatoes. *Integrated Environmental Assessment and Management*, 2, 105–125.

Backhouse and Karlsson 2014. Screening level mixture risk assessment of pharmaceuticals in STP effluents. *Water Research*, 49, 157 – 165.

Belden JB, Gilliom RJ, Martin JD, Lydy MJ. 2007. Relative toxicity and occurrence patterns of pesticide mixture in streams draining agricultural watersheds dominated by corn and soybean production. *Integrated Environmental Assessment and Management*, 3(1): 90–100.

Beltman WHJ, Ter Horst MMS, Adriaanse PI, De Jong A. 2006. Manual of FOCUS_TOXSWA v2.2.1. Alterra-rapport 586. Wageningen, the Netherlands.

Boxall ABA, Kolpin DW, Halling-Sorensen B, Tolls J. 2003. Peer reviewed: are veterinary medicines causing environmental risks? *Environ. Sci. Technol*., 2003, 37(15), pp286A-294A.

Brock TCM, Alix A, Brown CD, Capri E, Gottesbüren BFF, Heimbach F, Lythgo CM, Schulz R and Streloke M (Eds). 2010. *Linking aquatic exposure and effects: risk assessment of pesticides*. SETAC Press & CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 398 pp.

De Zwart D. 2005. Ecological effects of pesticide use in The Netherlands: Modeled and observed effects in the field ditch. *Integrated Environmental Assessment and Management*, 1(2):123–34.

De Zwart D, Adams W, Galay-Burgos M, Hollender J, Junghans M, Merrington G, Muir D, Parkerton T, de Schamphelaere K, Williams R, and Whale G. Submitted for the ET&C-Pellston mixture workshop series. Aquatic exposures of chemical mixtures in urban environments: approaches to impact assessment. *Environ Toxicol Chem*

Diamond J, Dyer S, Tolls J, Snape J, Coors A, Altenburger R, Leung K, Zhang X, Koelmans A. Submitted for the ET&C-Pellston mixture workshop series. Use of Prospective and Retrospective Risk Assessment Methods that Simplify Chemical Mixtures Associated with Treated Domestic Wastewater Discharges. *Environ Toxicol Chem*

European Food Safety Authority (EFSA). 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290

European Food Safety Authority (EFSA). 2017. https://ess.efsa.europa.eu/doi/doiweb/wg/683430 Accessed May 2017.

European Medicines Agency (EMEA). 2005. VICH-GL38 Guideline on Environmental Impact Assessment for Veterinary Medicinal Products - Phase II, CVMP/VICH/790/03-FINAL, London, October 2005. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004393.pdf

FOCUS, 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2, 245 pp.

Fort Dodge Animal Health. 1997. Fort Dodge Animal Health Environmental Assessment – CYDECTIN moxidectin 0.5%, Pour-On for Cattle. http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-av-gen/documents/document/ucm072383.pdf

Fry M, Milians K, Young D, and Zhong H. 2014. Surface Water Concentration Calculator User Manual. Environmental Fate and Effects Division, Office of Pesticides, United States Environmental Protection Agency, Washington, DC

Garthwaite DG, Hudson S, Barker I, Parrish G, Smith L, Pietravalle S. 2013. Pesticide Usage Survey Report 250 - Arable Farm Crops in the United Kingdom, 2012. London: Defra

Kapo KE, Holmes CM, Dyer SD, De Zwart D, Posthuma L. 2014. Developing a foundation for eco-epidemiological assessment of aquatic ecological status over large geographic regions utilizing existing data resources and models. Environmental Toxicology and Chemistry, 33: 1665–1677.

Kienzler A, Bopp SK, van der Linden S, Berggren E, Worth A. 2016. Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives. Regulatory Toxicology and Pharmacology, 80: 321e334

Klein M. 2007. Long term surface water simulations with STEPS-1-2-3-4. In Environmental Fate and Ecological Effects of Pesticides. ed. by DelRe AAM, Capri E, Fragoulis, G Trevisan M. Proc. XIII Symposium Pesticide Chemistry, Piacenza, Italy, p. 950-957

Larsbo M, Jarvis NJ. 2003. MACRO 5.0. A model of water flow and solute transport in macroporous soil. Technical description. Rep Emergo 2003:6, Swedish University of Agricultural Sciences, Uppsala, Sweden, 49 pp.

O'Brien D, Lewis S, Davis A, Gallen C, Paxman C, Smith R, Turner R, Warne MStJ, Mueller J, Brodie J. 2014. Pesticide exposure within Barratta Creek Catchment. Reef Rescue Research & Development Technical Report RRRD037. Available from: http://reefrescueresearch.com.au/resources/research-outcomes-reports/23-final-reports/174-rrrd037-final-report-2.html Accessed 07/01/2017

Posthuma L, Brown C, de Zwart D, Diamond J, Dyer S, Holmes C, Marshall S and Burton G. Submitted for the ET&C-Pellston mixture workshop series. Prospective mixture risk assessment and management prioritizations for river catchments with diverse land uses. *Environ Toxicol Chem*

Price PS, Han X. 2011. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. Int. J. Environ. Res. Public Health, 8: 2212–2225.

Price PS, Han X, Junghans M, Kunz P, Watts C and Leverett D. 2012. An application of a decision tree for assessing effects from exposure to multiple substances to the assessment of human and ecological effects from combined exposures to chemicals observed in surface waters and waste water effluents. Environ. Sci. Eur., 24: 34.

Rautmann D, Streloke M, Winkler R. 2001. New basic drift values in the authorisation procedure for plant protection products. In Forster, R.; Streloke, M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM). Mitt.Biol.Bundesanst.Land-Forstwirtsch. Berlin-Dahlem, Heft 381.

Sala S, Vighi M. 2008. GIS-based procedure for site-specific risk assessment of pesticides for aquatic ecosystems. Ecotoxicology and Environmental Safety, 69: 1–12.

Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Consumer Safety (SCCS), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), 2012. Opinion on the Toxicity and Assessment of Chemical Mixtures, 55 pp.

Schreiner VC, Szöcs, Bhowmik AV, Vijver MG, Schäfer, RB. 2016. Pesticide mixtures in streams of several European countries and the USA. Science of the Total Environment, 573: 680–689.

Smith R, Middlebrook R, Turner R, Huggins R, Vardy S; Warne MStJ. 2012. Large-scale pesticide monitoring across Great Barrier Reef catchments – Paddock to Reef Integrated Monitoring, Modelling and Reporting Program. Marine Pollution Bulletin, 65: 117–127.

Solomon KR, Giesy JP, Lapoint TW, Giddings JM, Richards RP. 2013. Ecological risk assessment of atrazine in North American surface waters. Environmental Toxicology and Chemistry, 32: 10–11.

Suárez LA. 2005. PRZM-3, A Model for Predicting Pesticide and Nitrogen Fate in the Crop Root and Unsaturated Soil Zones: User's Manual for Release 3.12.2. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-05/111.

Syngenta, 2015. AAtrex 4L label. http://www.syngenta-us.com/current-label/aatrex-4l. Accessed May 2015

United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). 2014. 2012 Census of Agriculture, 2012 Census Volume 1, Chapter 1: U.S. National Level Data. USDA, NASS: Washington, DC. https://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_US/ Accessed September 2016.

United States Environmental Protection Agency (USEPA). 2010. Development and Use of the Index Reservoir in Drinking Water Exposure Assessments. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/development-and-use-index-reservoir-drinking-water.

United States Environmental Protection Agency (USEPA). 2016. The Office of Pesticide Programs. Aquatic life benchmarks. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration

United States Environmental Protection Agency (USEPA). 2017a. Models for Pesticide Risk Assessment. Pesticides in Water Calculator. PWC zip file. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#aquatic

United States Environmental Protection Agency (USEPA). 2017b. Technical overview of Ecological Risk Assessment: Risk Characterization. (Updated Sept 19 2016). https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-risk

Vallotton, V, Price PS. 2016. Use of the maximum cumulative ratio as an approach for prioritizing aquatic coexposure to plant protection products: A case study of a large surface water monitoring database. Environmental Science and Technology, 50: 5286−5293.

Van Wijngaarden RPA, Cuppen JGM, Arts GHP, Crum SHJ, Van den Hoorn MW, Van den Brink PJ and Brock TCM, 2004. Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. Environmental Toxicology and Chemistry, 23: 1479–1498.

Verro R, Calliera M, Maffioli G, Auteri D, Sala S, Finizio A, Vighi M., 2002. GIS based system for surface water risk assessment of agricultural chemicals. 1. Methodological approach. Environmental Science and Technology, 36: 1532–1538.

Zoetis, 2014. Environmental Assessment for Synovex® ONE (Estradiol Benzoate and Trenbolone Acetate Extended Release Implant) Feedlot and Grass for Beef Steers and Heifers. http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/EnvironmentalAssessments/UCM409353.pdf. Accessed May 2015.

List of Figures

Table 1. Effects data and Regulatory Acceptable Concentrations (RAC) in µg/L for UK wheat case study

		Primary producers					Invertebrates										Fish										
Active Ingredient	Group	Tier 1	AF	Higher tier	AF	RAC	Acute tier 1	AF	Acute higher tier	AF	Acute RAC	Chronic tier 1	AF	Chronic higher tier	AF	Chronic RAC	Acute tier 1	AF	Acute higher tier	AF	Acute RAC	Chronic tier 1	AF	Chronic higher tier	AF	Chronic RAC	Reference
Boscalid	F	1340	10			134	5330	100			53.3	1310	10			131	2700	100			27	125	10			12.5	1
Chlorothalonil	F	9.6	10	30[b]	3	10	84	100	30[b]	3	10	8.5	10	30[b]	3	10	38	100	15 (HC5)[c]	9	1.7	3	10			0.3	2
Cypermethrin	I	>100	10			10	0.3	100	0.05[b]	3	0.017	0.04	10	0.05[b]	3	0.017	2.8	100			0.028	0.03	10			0.003	3
Epoxiconazole	F	13.8[a]	10			1.38	8690	100			86.9	62.5	10			6.25	3140	100			31.4	10	10	30[f]	10	1.0	4
Flufenacet	H	2.43	10	12[b]	3	4	30900	100			309	3260	10			326	2130	100			21.3	200	10			20	5
Fluoxastrobin	F	350	10			35	60.4	100			0.64	0.61	10			0.061	435	100			4.35	28.6	10			2.86	6
Iodosulfuron-methyl-sodium	H	0.83[a]	10			0.083	$>10^5$	100			1000	10^4	10			1000	$>10^5$	100			1000	10^4	10			1000	7
Mesosulfuron-methyl	H	0.62[a]	10			0.062	$>10^5$	100			1000	1800	10			180	$>10^5$	100			1000	32000	10			3200	8
Pendimethalin	H	6	10	5[b]	3	1.67	147	100			1.47	14.5	10			1.45	196	100			1.96	6.3	10	32[ef]	10	0.63	9
Prochloraz	F	> 32	10			3.2	770	100	1820[e]	10 0	18.2	22.2	10			2.22	1200	100	1340	10 0	13.4	24.9	10			2.49	10
Proquinazid	F	250	10			25	287	100			2.87	1.8	10			0.18	349	100			3.49	3	10			0.3	11
Prothioconazole	F	2180	10			218	1300	100			13	560	10			56	1830	100	3870	10 0	38.7	308	10			30.8	12
Pyraclostrobin	F	>843	10			84.3	16	100	8[b]	3	2.7	4	10	8[b]	3	2.7	6	100	4.6 (HC5)[d]	3	1.53	2	10			0.2	13

1 Boscalid SANCO/3919/2007-rev.5 21 January 2006
2 Chlorothalonil SANCO/4343/2000 final (revised) 28 September 2006
3 Cypermethrin SANCO/4333/2000 final 15 February 2005
4 EFSA Scientific Report (2008) 138, 1-80
5 Flufenacet 7469/VI/98-Final 3 July 2003
6 Fluoxastrobin EFSA Scientific Report (2007) 102, 1-84
7 Iodosulfuron SANCO/10166/2003-Final 3 July 2003

[a] *Lemna,* others based on green algae
[b] Mesocosm
[c] Acute 96h LC50 HC5 from SSD of 11 species
[d] Acute 96h NOEC HC5 from SSD of 7 species
[e] Geometric mean
[f] Higher tier NOEC for use against PEC max only

8 Mesosulfuron-methyl PPDB Univ Hertfordshire
9 EFSA Journal 2016; 14(3), 4420
10 EFSA Journal 2011; 9(7):2323
11 EFSA Journal 2009; 7(10):1350
12 EFSA Scientific Report (2007) 106
13 Pyraclostrobin SANCO/1420/2001-Final 8 September 2004, DAR 2001
AF = Assessment Factor

Table 2. The number and percentage of total days when individual chemicals RQ and ΣRQ were greater than one in the UK edge of field scale case study, together with the maximum RQ and consecutive days exceeding 1

	Primary Producers			Invertebrate Acute			Invertebrate Chronic			Inv. Chronic - refined			Fish Acute			Fish Chronic			Fish Chronic - refined		
	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ
	No.	% total		No.	% total		No.	% total		No.	% total		No.	% total		No.	% total		No.	% total	
Boscalid	0	0.00	0.01	0	0.00	0.04	0	0.00	0.01	0	0.00	0.00	0	0.00	0.07	0	0.00	0.16	0	0.00	0.03
Chlorothalonil	0	0.00	0.27	0	0.00	0.27	0	0.00	0.27	0	0.00	0.10	9	0.12	1.59	39	0.52	9.00	37	0.49	3.49
Cypermethrin	0	0.00	0.00	17	0.23	1.67	17	0.23	1.67	0	0.00	0.55	1	0.01	1.02	263	3.50	9.48	148	1.97	3.14
Epoxiconazole	0	0.00	0.76	0	0.00	0.01	0	0.00	0.17	0	0.00	0.05	0	0.00	0.03	1	0.01	1.05	0	0.00	0.29
Flufenacet	2	0.03	1.07	0	0.00	0.01	0	0.00	0.01	0	0.00	0.00	0	0.00	0.20	0	0.00	0.21	0	0.00	0.06
Fluoxastrobin	0	0.00	0.01	0	0.00	0.30	47	0.63	3.16	0	0.00	0.95	0	0.00	0.04	0	0.00	0.07	0	0.00	0.02
Iodosulfuron-methyl	0	0.00	0.80	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Mesosulfuron-methyl	14	0.19	5.46	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Pendimethalin	0	0.00	0.82	0	0.00	0.93	0	0.00	0.95	0	0.00	0.37	0	0.00	0.70	123	1.64	2.18	0	0.00	0.85
Prochloraz	0	0.00	0.15	0	0.00	0.03	0	0.00	0.22	0	0.00	0.05	0	0.00	0.04	0	0.00	0.19	0	0.00	0.05
Proquinazid	0	0.00	0.00	0	0.00	0.00	0	0.00	0.07	0	0.00	0.02	0	0.00	0.00	0	0.00	0.04	0	0.00	0.01
Prothioconazole	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Pyraclostrobin	0	0.00	0.00	0	0.00	0.01	0	0.00	0.01	0	0.00	0.00	0	0.00	0.01	0	0.00	0.11	0	0.00	0.04
ΣRQ	63	0.84	7.00	111	1.48	2.45	223	2.97	5.06	13	0.17	1.46	43	0.57	2.94	353	4.69	18.86	364	4.84	6.15
Max. duration ΣRQ>1 (days)	3			3			3			4			3			4			14		
Days ΣRQ >1 for > 1 day	13			15			29			7			8			47			300		
Days ΣRQ >1 for > 2 days	2			3			3			3			2			8			240		
Days ΣRQ >1 for >3 days	0			0			0			1			0			1			187		
Days ΣRQ >1 for >4 days	0			0			0			0			0			0			140		

RQ = Risk Quotient

Table 3. The number and percentage of days that mixture toxicity was classed as groups based on MCR categories

Taxonomic Group	Group I	Group II	IIIA	IIIB
	(single chemicals have RQ >1)	(∑RQ<1)	(∑RQ >1, no single chemical RQ >1)	
			MCR <2	MCR >2
UK Case Study – Edge of Field Scale Wheat				
Primary producers	16 (0.21%)	7456 (99.16%)	20 (0.27%)	27 (0.36%)
Invertebrates acute	17 (0.23%)	7408 (98.52%)	76 (1.01%)	18 (0.24%)
Invertebrates chronic	64 (0.85%)	7296 (97.03%)	41 (0.55%)	118 (1.57%)
Inv. chronic refined	0 (0.00%)	7506 (99.83%)	8 (0.11%)	5 (0.07%)
Fish acute	10 (0.13%)	7476 (99.43%)	12 (0.16%)	21 (0.28%)
Fish chronic	282 (3.75%)	7166 (0.95%)	15 (0.20%)	56 (0.74%)
Fish chronic refined	163 (2.17%)	7155 (95.16%)	137 (1.82%)	64 (0.85%)
US Case Study – Catchment Scale Corn and Beef				
Primary producers	815 (7.44%)	9857 (89.96%)	268 (2.45%)	17 (0.16%)
Invertebrates acute	113 (1.03%)	10844 (98.97%)	41 (0.37%)	3 (0.03%)
Invertebrates chronic	49 (0.45%)	10133 (9.25%)	307 (2.80%)	468 (4.27%)
Fish acute	47 (0.43%)	10908 (9.96%)	2 (0.02%)	0 (0.00%)
Fish chronic	1556 (14.2%)	8977 (81.93%)	416 (3.80%)	8 (0.07%)

Table 4. Effects data and Regulatory Acceptable Concentrations (RAC) in µg/L for US corn case study

		Primary Producers					Invertebrates						Fish					
Active Ingredient	Group	EC50	AF	Higher tier	AF	RAC	Acute	AF	RAC	Chronic	AF	RAC	Acute	AF	RAC	Chronic	AF	RAC
Acetochlor	H	1.34	1			1.34	8200	2	4100	22.1	1	22.1	380	2	190	130	1	130
Atrazine	H	<1	1	10	1	10[a]	720	2	360	60	1	60	5300	2	2650	5	5	5
Clopyralid	H	6900	1			6900	113000	2	56500	17000	1	17000	1968000	2	984000	10800[b]	1	10800
Clothianidin	I	64000	1			64000	22	2	11	1.1	1	1.1	>101500	2	>50750	9700	1	9700
Flumetsulam	H	3.1	1			3.1	254000	2	127000	111000	1	111000	293000	2	146500	197000	1	197000
Glyphosate	H	11900	1			11900	53200	2	26600	49900	1	49900	43000	2	21500	25700	1	1800
Ipconazole	F	2200[c]	1			2200	1700	2	850	10.9[c]	1	10.9	1530	2	765	0.18	1	0.18
Metalaxyl	F	6250	1			6250	28000	2	14000	100	1	100	130000	2	65000	9100	1	9100
Metconazole[d]	F	1700	1			1700	4200	2	2100	78	1	78	2100	2	1050	2.91	1	2.91
Moxidectin	VM	87	100			0.87	0.03	100	0.0003			0.0003	160	100	1.6			1.6
Pyraclostrobin	F	1.5	1			1.5	15.70	2	7.85	4.00	1	4	6.20	2	3.1	2.35	1	2.35
Tefluthrin[e]	I	>1050	1			1050	0.070	2	0.035	0.008	1	0.008	0.06	2	0.03	0.004	1	0.004
Trifloxystrobin	F	37.1	1			37.1	25.30	2	12.65	2.76	1	2.76		2.76	14.3	2	7.15	4.30
Tilmicosin	VM	84	100	41	10	4.1	57300	1000	57			57	716000	1000	716			716

[a] Current regulatory Concentration Equivalent Level of Concern (CE-LOC) for aquatic plants as a 60-day average https://www.epa.gov/ingredients-used-pesticide-products/atrazine-background-and-updates
[b] EFSA 2005 50, 1-65. Conclusion on the peer review of clopyralid
[c] No value in US EPA aq benchmark or associated document, taken from ipconazole EFSA conclusion 2013
[d] Data from US EPA 2005 https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/125619/125619-2005-07-28a.pdf
[e] No reference given for benchmark values, taken from tefluthrin EFSA conclusion 2013
AF = Assessment Factor

Table 5. The number and percentage of total days when individual chemicals RQ and ΣRQ were greater than one in the US corn catchment, together with the maximum RQ and consecutive days exceeding 1

	Primary Producers			Invertebrate Acute			Invertebrate Chronic			Fish Acute			Fish Chronic		
	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ	Days RQ >1		Max. RQ
	No.	% total		No.	% total		No.	% total		No.	% total		No.	% total	
Acetochlor	575	5.25	18.19	0	0.00	0.01	0	0.00	0.81	0	0.00	0.13	0	0.00	0.08
Atrazine	361	3.29	2.21	0	0.00	0.08	0	0.00	0.42	0	0.00	0.01	1188	10.84	4.42
Clopyralid	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Clothianidin	0	0.00	0.00	0	0.00	0.05	0	0.00	0.41	0	0.00	0.00	0	0.00	0.00
Flumetsulam	0	0.00	0.26	0	0.00	0.00	0	0.00	0.72	0	0.00	0.00	0	0.00	0.00
Glyphosate	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Ipconazole	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.01
Metalaxyl	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Metconazole	0	0.00	0.00	0	0.00	0.00	0	0.00	0.01	0	0.00	0.00	0	0.00	0.14
Moxidectin	0	0.00	0.00	48	0.44	3.18	0	0.00	0.84	0	0.00	0.00	0	0.00	0.00
Pyraclostrobin	0	0.00	0.38	0	0.00	0.07	0	0.00	0.06	0	0.00	0.18	0	0.00	0.06
Tefluthrin	0	0.00	0.00	41	0.37	9.89	49	0.45	1.25	47	0.43	11.54	599	5.47	2.49
Tilmicosin	0	0.00	0.13	0	0.00	0.01	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Trifloxystrobin	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
ΣRQ	1100	10.04	18.57	113	1.03	11.44	824	7,52	3.47	49	0.45	11.63	1980	18.07	5.92
Max. duration ΣRQ>1 (days)	177			5			115			3			279		
Days ΣRQ >1 for > 1 day	1080			53			806			15			1962		
Days ΣRQ >1 for >4 days	1023			2			752			1			1908		
Days ΣRQ >1 for >21 days	754			0			510			0			1602		
Days ΣRQ >1 for 60 days	387			0			142			0			937		

RQ = Risk Quotient

Fig. 1

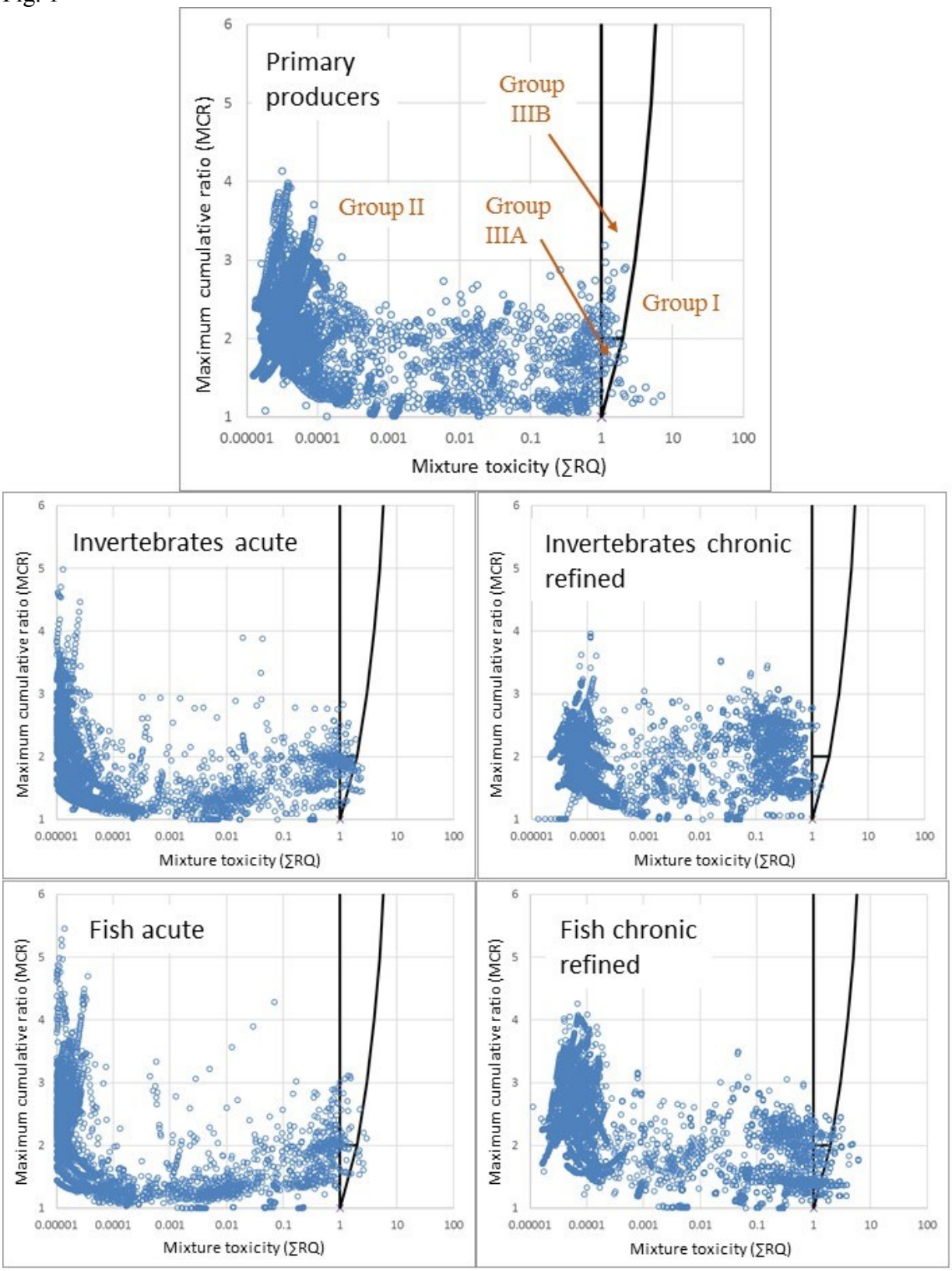
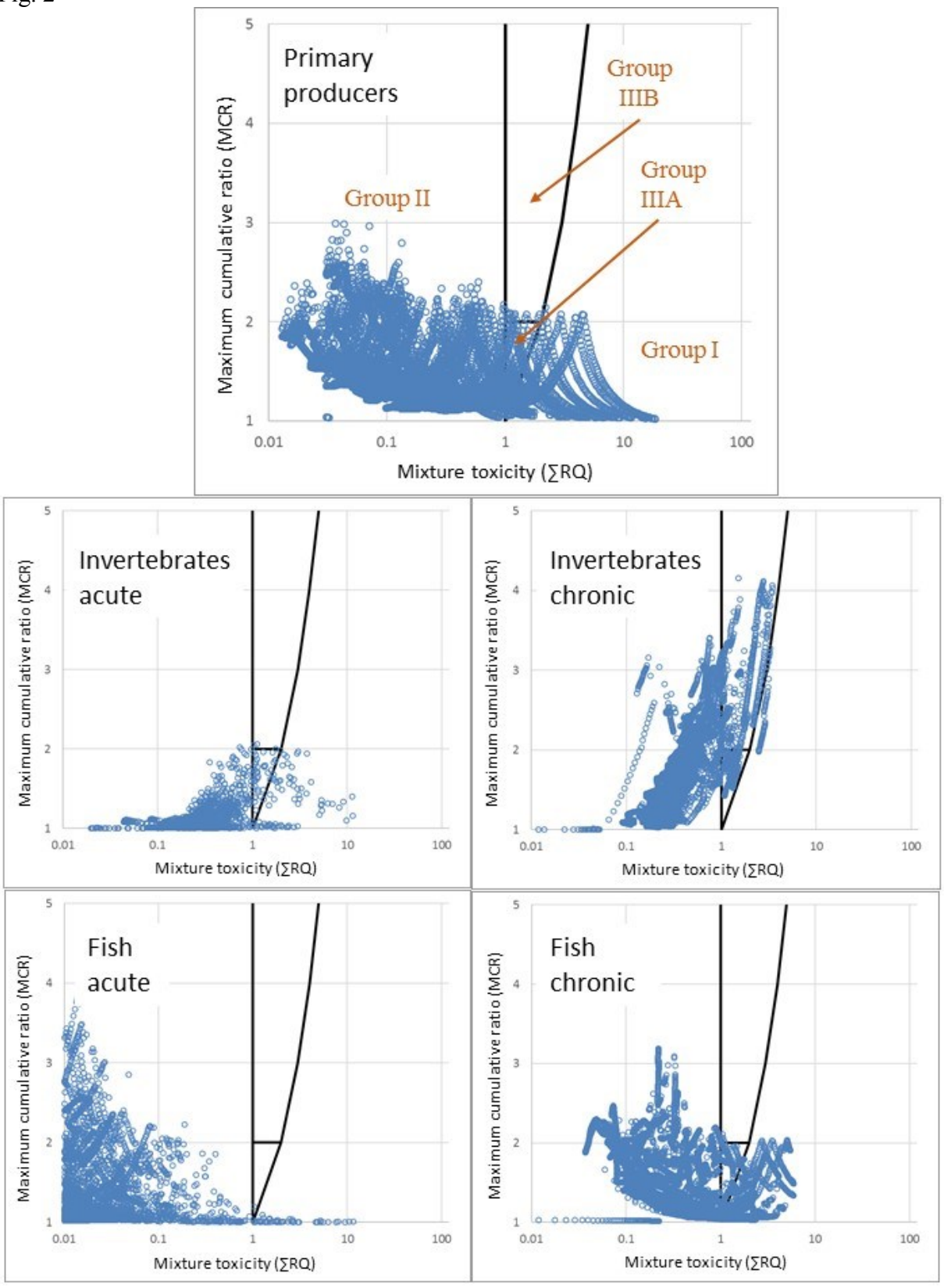

Primary producers
Group IIIB
Group II
Group IIIA
Group I
Maximum cumulative ratio (MCR)
Mixture toxicity (∑RQ)
Invertebrates acute
Invertebrates chronic refined
Fish acute
Fish chronic refined
0.00001
0.0001
0.001
0.01
0.1
1
10
100

Fig. 2


Primary producers
Group IIIB
Group IIIA
Group II
Group I
Maximum cumulative ratio (MCR)
Mixture toxicity (∑RQ)
Invertebrates acute
Invertebrates chronic
Fish acute
Fish chronic
0.01
0.1
1
10
100