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COMPARISON AND EVALUATION OF PESTICIDES MONITORING PROGRAMMES USING A PROCESS BASED MIXTURE MODEL

Running Title: Mixture effects of pesticides in the environment

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Abstract: A number of European countries run large scale pesticide monitoring schemes in watersheds aimed at identifying and evaluating the presence of pesticide residues in the environment. These schemes provide national and regional scale assessments of pesticide concentrations within the context of environmental quality assessment, aiming to ensure some degree of ecological protection. This study is aimed at evaluating the joint effects of the pesticide mixtures detected in monitoring programs, using a process based mixture modelthat was parameterised for *Daphnia magna*. In total over 15,000 samples containing over 1 million individual measurements were evaluated for effects.

It was found that there are only a small number of places where we can expect to have effects on daphnids, based on measured concentrations. The most polluted samples would cause extinction of a daphnid population within only 30 hrs. The results show that effects are mostly triggered by a limited nr of pesticide residues at locations with high emissions. It was also shown that the analytical detection limits are basically too high to exclude mixture effects. So despite all the effort that is put into chemical monitoring programmes it remains a challenge to make statements on whether or not the environment is protected. The paper ends with recommendations for a different setup of monitoring programs to improve on this.

Keywords: Pesticides, Environmental Monitoring Programme, Dose Response Modelling, Water Quality, Mixtures

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INTRODUCTION

The typical procedure in any environmental monitoring scheme is that measured concentrations of individual compound are compared with their respective Environmental Quality Standard (EQS). If each of the measured concentrations is below its EQS, then no chemical impact on the environmental compartment under consideration is anticipated[1, 2]. If EQS are exceeded, this suggests an effect of some severity for a certain number of unknown species. This approach to environmental protection is in itself a chemically driven approach based on individual chemicals; concentrations are measured and compared to some fixed concentration (the EQS). The biological component of the assessment is ‘hidden’ in the derivation of the EQS.

In general terms, linking environmental concentrations to toxicological effects is not a straightforward process.Relations between observed effects and exposure concentrations are non-linear and real life exposure for organisms is characterised by time-varying concentrations, short term peak concentrations, sequential exposures, simultaneous exposure to multiple compounds and changing environmental conditions. Generally uncertainties resulting from these factors are (assumed to be) accounted for when deriving an EQS by placing safety factors on a metric derived from the toxicity values available for the chemical. Especially effects of simultaneous exposureto multiple chemicals can be severe[3] but effects of combined exposureare not generally considered[4] with some exceptions, e.g. the evaluation of surface water quality if it is used to obtain drinking water: the sum of all pesticides is not allowed to exceed 0.5 µg/L[5]. However this is not a true mixture approach. Rather it derives an EQS for the combined pesticide exposure,without taking aspects such as potency into consideration.

Within the current philosophy based on comparing measured concentrations with their EQS, the consequences for populations of any species, or indeed species assemblages, of exceedancesof the EQS are not identified. Further, even when no EQS is exceeded, it is still not possible to be sure that species in the environment considered are indeed protected. This is particularly true when low concentrations of multiple chemicals are present. We have shown before that surface waters can comply with EQS for all individual compounds but the mixture effect can be such that a population of daphnids will go extinct within 24 hrs of an exposure [3]. Therefore predicting the joint effects of the pesticides mixtures is important.Developing an approach that takes mixture effects in account is possibleif relevant exposure and toxicological data is available.Exposure data is typically available for pesticides as many (European) countries do have more or less elaborate pesticide monitoring schemes operational, which are typically costly.With such investments made, it is important that we can interpret the resultswithin their appropriate protection context for indicator populations, such as *D. magna*, and communities[6]. Typically for pesticides EQS are low as pesticides in general are developed with the intention to exert serious effects in very low concentrations and they are directly released into the environment as a result of the normal utilisation. In this research we have compared monitoring data from a focussed regional pesticide sampling programme in the UK with the more standard large scale monitoring programme in the Netherlands. The UK Catchment Sensitive Farming (CSF) programme[7] is specifically targeted at monitoring pesticide concentrations and loads in the outlet of 7 catchments containing drinking water protected areas and surface water protected areas. In contrast, the Dutch monitoring programme contains all areas of the Netherlands[8] and is more comparable to the normal UK nation-wide monitoring programme. Our aim is to go beyond EQS comparison to evaluate the detailed predicted effects of the pesticides detected in

monitoring programs for sentinel populations *Daphnia magn*a - a species providing a toxicologically rich data resource. This modelling approach is used to establish the potential (mixture) effects of pesticides measured in these environmental monitoring programs and how this relates to possible population consequences.

APPROACH

Available monitoring data

The UK Catchment Sensitive Farming scheme is aregional program with a river catchment focus. In contrast, the monitoring scheme used in the Netherlands includes measurements for sites located across virtually the entire country, with emphasis given to regions with the highest agricultural intensity [8].

In the Netherlands a large very dense network of small water-bodies surrounding (nearly) every individual field with agricultural activities can be found, so direct emissions during spraying (including drift) are believed to be the major cause of contamination [9]. In the UK, the major pathway for surface water contamination with pesticides is run-off. The most important factor that determines emission due to run-off events is rainfall, especially the timing and intensity of the first substantial rain after pesticide application [10]. The extent to which releases through these different pathways are true drivers of measured concentrationsin surface water is disputed[11]. Thus including concentrations measured from both schemes allows investigation of different exposure routes and subsequent concentrations and effects. A detailed description of the CSF and Dutch measurement schemes is given below.

Catchment Sensitive Farming monitoring data

Seven river catchments containing drinking water protected areas and surface water protected areas in England have been monitored by the Environment Agency for pesticide levels since 2006 as part of the CSF programme[7], for maps see supplemental data, section 3. The aim

of the associated monitoring is to detect changes in pesticide concentrations / loads arising from the CSF activity and is run as a special project complementary to the nation-wide pesticide monitoring programme; the latter being more comparable to the nationwide Dutch programme. The core of the CSF programme consists of 7 sampling points. But over the years samples were taken at 25 different locations in the area, the additional samples were taken on an ad hoc basis and were usually analyzed for a limited nr of pesticides.

Each core site is sampled on a regular basis for 3 standard pesticides suites (Phenoxy Herbicides, Urons and Nitrogen or Phosphorous containing Pesticides); a list that together covers 88 pesticides (see Supplemental data, section 2). The selection of pesticides to be measured was based on land-use and earlier pesticide surveys. Some pesticides within the suites are classed as historical, banned or in the process of being phased out. These are, however, still measured because they are part of the analytical package and not necessarily because they are known to be used in the different catchment areas. The current assessment used data from samples taken from March 1st 2006 to October 29th 2014, spanning a period of some 8.5 years. Samples were taken throughout the year.

Typically a sampling frequency of 2 samples per week is used, however, on occasionssamples can be taken on consecutive days at the core sampling locations linked to major run-off events. Samples are collected, transported and analysed by the Environment Agency's national laboratory service within 24 hrs. In total 5400 samples have been taken and analysed. Out of this total, 3585 have been analysed for more than 10 different pesticides. In the vast majority of the measurements the analytical detection limits (DL) for individual compounds are not exceeded. The DL of a specific compound can vary in different samples, even for the same compound, because laboratory methods can change over time and also because some

samples show analytical interference with an effect on DLs. In extreme cases this can increase the DL by more than 2 orders of magnitude. An overview of the basic statistics of the CSF pesticide monitoring scheme is given in Table 1.

Dutch monitoring data

In the Netherlands the responsibility for surface water quality and quantity lies with the regional water boards, with the exception of the main rivers and large lakes which fall under the responsibility of the ministry of 'Rijkswaterstaat'. The Dutch water boards have a well-established program for monitoring pesticide contamination of surface waters. These monitoring data have been processed into a graphic format the pesticide atlas, which is accessible online (http://www.bestrijdingsmiddelenatlas.nl) [6]. This pesticide atlas is designed to provide insight into pesticide presence in surface waters and trends over time. The data are evaluated in terms of exceeding environmental quality standards (EQS) and regression based analyses are carried out to evaluate the long-term behaviour of measured concentrations or to evaluate national pesticide policies [6, 8]. Within the pesticidesatlas there is also the functionality to visualise the geospatial relationships between land use and pesticides residue EQS exceedances. Some regions of the Netherlands like 'het Westland' (greenhouses) or 'de bollenstreek' Lisse and surroundings (flower bulbs), 'Flevoland' (arable farming), 'Friesland' (cattle farming), all have local highly intensive agricultural activities with their own characteristic set of pesticide use that can lead to specific water quality concerns. Therefore different regions under different land management can have a specific measurement setup within the monitoring program, with dedicated measurement frequencies and specific analytical packages.

The number of different sampling points in the Dutch Monitoring Program differs slightlybetween years, but has been in the order of 600in recent years. Each year a total of 600-

700 different active ingredients of pesticides are monitored. The vast majority of assessed pesticides are never found above the analytical detection limits at any location. This almost certainly often reflects their lack of use, although in addition some of the pesticides are difficult to quantify at the required very low environmental levels. As we will show results below the detection limit may still be important from a regulatory point of view, but to get a clear picture on the actual occurring effects we decided to give all pesticides that were never measured above their analytical detection limit at any location in the year for which the data were analysed a default concentration of zero. This choice might underestimate the real effect however any (arbitrary) chosen value other than 0 would present a bigger possible problem, since this would by necessity increase the predicted effect without knowledge on whether or not the compound is actually present. A complete overview of the pesticides taken up in this analysis is given in Supplemental data I.

The underlying raw, but checked, data off the pesticide atlas were kindly made available for this study. We evaluated the years 2013; 2009 and 2005, which spans more or less the same period as the CSF monitoring data. In Table 2 some general statistics of the Dutch monitoring schemes are presented.

Modelling the effects

The effect analysis was carried out using a process-based mixture model that relates environmental concentrations to effects on survival on*Daphnia magna*. We chose daphnids because this taxon is commonly used for aquatic chemical hazard assessment and daphnids are considered to be representative for the invertebrates that along with fish and algae should be considered in the initial pesticide registration process in Europe [12].And we have shown in the past that themixture model can make excellent predictions on mortality of *in situ* exposed

daphnid populations exposed to actual environmental concentrations of complex mixtures [3]. We will come back to this choice, including its value and limitation for assessing predicted impacts, in the discussion.

Short description of the model

The model builds up the effect of the mixture from the individual compounds assuming no interactions in the mixture [13]. For the individual compounds it consists of a scaled one-compartment model to describe the kinetics and a hazard model to link exposure to effects on survival. This allows calculating the whole time-course of toxic effects. For single compounds a description of the model can be found in [14], or in the OECD-guidelines [15]. An excellent description of allcurrently available different single compound survival models and how the different models relate can be found in [16].

The model can be readily applied to single compound data and can be extended to summarise the effects of chemicals in combination. A mathematical and conceptual description of the model application for mixtures assessment can be found in [17]. Since the mixture effect is based on the known individual compounds,their toxicological parameters must be known for the species of interest and typically these parameters are derived from the development of toxic effects over time.In this respect the choice of *Daphnia* for model parameterisation is supported because of the extensive toxicological data available for this taxon. For each component in the mixture, three parameters relevant for determining the time-course of toxicity have to be determined:

- the elimination rate (k_e), a measure for how fast the equilibrium between internal and external concentrations is set (expressed in d^{-1});
- the No Effect Concentration (*NEC*), a toxicological threshold below which no effects on survival will occur even after prolonged exposure times (expressed in µmol/L);

- the killing rate (k_r), a measure for the toxic potency of a compound (once the NEC is exceeded) (expressed in $(\mu mol/L)^{-1}.(d)^{-1}$).

The approach to mixture assessment provides the possibility to calculate the *NEC* of the mixture. This represents the threshold for a toxic effect for the mixture. When this mixture threshold exceeds 1, the survival probability of the daphnids is affected by the toxic pressure of the mixture and the whole time course of the toxic effects can be calculated.

There is a similarity in how the behaviour of the *NEC*of the mixture is treated in this model with the more widely used Concentration Addition and Independent Action models [18, 19]. Compounds with a similar mode of action share their *NEC*. In this contextdifferent thresholdsexist for the different modes of action that are represented by chemicals found in a mixture. If the mixture *NEC*for at least one mode of action is exceeded there will be an effect on survival for the exposed species. Thisapproachalso allows calculating the contribution of each compound in the mixture to the overall toxic effect.

As a starting point for assessment, the most parsimonious approach to chemical assessment and categorisation for effects on the target taxon is to take the insecticides as a group sharing the *NEC* based on neurotoxicity.The herbicides and fungicides are considered as a second group that share effects through baseline toxicity, because relevant biochemical targets are absent in *Daphnia*. This is of course a simplified approach that can ultimately be refined as specific mode of action data becomes available for the target species. Nonetheless it has already shown good predictive power in the assessment of effects of mixtures e.g. [3, 20, 21] and so represents a tractable starting point. We will come back to this approach for categorisation in the discussion.

Taking this simplified initial approach, the first step in applying the model is to calculate the mixture*NEC*for each of the shared modes of action. This is a simple step and it involves the environmental concentration and the *NEC* of the compounds in the mixture, according to:

$$NEC_{mix} = \sum_{i=1}^{n} {}^{c_i}/_{NEC_i}$$

With

NEC_{mix} = the mixture threshold forthe available compounds

c_i = the measured concentration of compound i

NEC_i = the No Effect concentration of compound i

n = the number of compounds in the mixture, sharing the NEC_{mix}

If a mixture threshold exceeds 1 the survival probability of the exposed organisms will be affected by the exposure to the combined exposure to the pesticides. How much the survival probability is affected depends on how far the mixture threshold is exceeded and the properties of the compounds in the mixture. So the most important parameter to estimate is the *NEC* of the mixture and therefore the *NEC* of the individual compounds.

The model was previously successfully deployed to link effects of a mixture to the survival of in-situ exposed daphnids in an agricultural part of the Netherlands [3]. These mixtures contained almost 100 different compounds including pesticides, metals, nutrients and PAHs. The model proved able to make reliable predictions on both survival (if the mixture threshold was below 1) and mortality (if the mixture threshold exceeded 1) of exposed organisms. The more frequently used Concentration Addition and Independent Action models could not make reliable predictions on both survival and mortality in this real life situation [3].The problem is that these models are conceptuallyinappropriate to be used in an approach like this. First of all, it is not clear how to derive an actual effect level from exposure to toxic

units. If we have a mixture exposure of 0.2 toxic units, this cannot be translated to an actual survival probability (for any point in time). Only if all toxic units are derived from say 48 hr *LC50*(the concentration that leads to a 50% effect on survival) values, a toxic unit of 1 would imply a 50% survival probability at 48 hrs. For any other survival probability new toxic units need to be derived. In addition toxic units are only valid for a fixed single exposure time, so for each exposure time new toxic units need to be derived to allow effect predictions.

Model parameters

The optimum approach for modelling would be to estimate all relevant parameter values for all chemicals from raw survival data for organisms exposed to multiple concentrations and monitored in time. However, even in a well-studied taxon like *Daphnia*, full dose-response data are usually not available and only the 48 hr *LC50*value is reported. This is despite the fact that the OECD test guidelines for *Daphnia* toxicity tests also prescribes monitoring survival at 24 hrs exposure[22]. Since raw survival data for effects on *Daphnia* survival were not available for any of the compounds, a different approach to the derivation of model parameters was taken. Because the*NEC* is the most important parameter as it defines the concentration below which survival will not be affected,independent of the exposure time, our initial focus was to provide an estimate for this value for exposed *Daphnia*. We made a distinction between parameter estimates for the insecticides and the herbicides/fungicides.

Model parameters for the insecticides

Insecticides are designed generally to be biologically targeted to arthropods. This means that they have a high potential toxicity to this group of organisms. For daphnids the toxicity of insecticides typically exceeds that for herbicides and fungicides by some 5-6 orders of magnitude.

For the estimate of the toxicity parameters we can make use of the temporal aspects of toxicity. For survival it has been long established that *LC50*s calculated from temporal experimental survival data tend to decrease over time, approaching an asymptote represented by the incipient *LC50*[23] (Figure 1). This clearly indicates that a single time-point *LC50* value merely gives a snapshot of the actual toxicity of a compound. From an observation at only one point in time it is impossible to know whether the (e.g. 48 hr) *LC50* was measured in the steep section or tail of this time dependent curve. Further, over long-term exposure, the asymptotic *LC50* can be shown to be numerically equal to the *NEC*[24]. Hence by knowing how *LC50* changes with time, an estimate of the toxicity parameters becomes possible.

If we have at least three *LC50* values at different points in time, then the shape of the *LC50*time curve can be established and the relevant toxicity parameters (*NEC*, k_e, k_r) can be estimated. Finding time dependent *LC50*data, thus, provides a solution for toxicity parameter derivation. As a first step the USEnvironmental Protection Agency (USEPA) ECOTOX [25] database was used as a starting point to look for toxicity data at different points in time. If such information was present, then the parameters could be derived directly. If suitable time *LC50*s are not reported, but there is a 10 day (or longer) *LC50* values present, then this value is taken as the incipient *LC50* and therefore as the *NEC*, however with the loss of kinetic information (since single time-point toxicity information by definition does not contain kinetic information). If the ECOTOX database did not have *LC50* values at different points in time or long-term *LC50*s, then the Pesticide Property Data Base [26] was used to obtain a 48 hr *LC50* for daphnids. From this 48 hr *LC50*, a simple but tractable approach was then used to derive a *NEC* from the observed 48 hr *LC50*value. The 48 hr *LC50* value was then divided by 2.7, this is the median value of the fraction of the 48 hr *LC50*s and the *NEC* for the compounds where both the 48 hr *LC50* and the

NEC could be derived, see Table 3(data for this research are extracted from the ECOTOX database [25]).

Some pesticides were neither in the ECOTOX database nor in the PPDB, in these cases a further literature search, using peer-reviewed articles found via the Web of Science database, on *LC50*s for the specific compound was conducted (see Supplemental data 1).

Herbicides and fungicides

For derivation of the toxicity parameters for the herbicides and fungicides, we adopted a simple and parsimonious approach. Our assumption was that in the absence of relevant biochemical targets, herbicides and fungicides will show only baseline toxicity as a first estimate of their toxic effects. The *NEC* is plotted against log K_{ow} for the compounds where a *NEC* could be derived (Figure 2) (see Supplemental data 1). The *NEC* of most herbicides and fungicides correlates well with their octanol/water partition coefficients or log K_{ow} values, giving a slope of approximately -0.82. This relationship provides confirmation that despite the simplicity of our assumption the largemajority of compounds do have a toxicity to *Daphnia* which approximates to baseline toxicity [27-29]. Two notable exceptions to this are Linuron and Monolinuron, which are both about an order of magnitude more toxic than baseline toxicity for daphnids. This higher potency, suggests there may be an additional mode of action for this group of chemicals with respect to effects on survival. Excluding these compounds from the analysis, gives a better fit with a slope of -0.92, so very close to the theoretical value of -1 for narcotic acting compounds [30](Figure 3), and in close accordancewith the experimentally observed value of -0.90 found in a dedicated QSAR study for narcotics[29].In Figure 4 we plotted the observed *NEC* against the predicted *NEC*s based on the log K_{ow} values (for compounds where the time course of effects was available) including 95% confidence limits. The figure shows that experimentally

determined *NEC*s are within 0.8 log units of predictions, so actual values are within a factor of 6 of the theoretical numbers. Since comparable differences can be found in direct measured *LC50* values even for the same compound in literature, this level of agreement supports the general validity of the approach.

While showing an overall validity, typically compounds with a high log K_{ow} value tend to not fit with the overall approach. Fenpropimorph (fungicide) for example, was found to be far less toxic than its predicted baseline toxicity. Since baseline toxicity is a general property, this does not appear to be valid[31, 32]. This result can, however, easily be explained when temporal aspects are regarded. Fenpropimorph has a log K_{ow} of 5.5, therefore it can be expected to have an elimination rate less than 0.01 h^{-1}[33]. With such an elimination rate 95% of steady state internal concentration can be expected to be achieved only after approximately 12 days of exposure [27]. In addition, these compounds have a *NEC*in the order of 1 µmol.L^{-1}[29]. Between log K_{ow} of 5 and 6 the water solubility decreases from approximately 90 to 0.6 µmol.L^{-1}[34], therefore exposure to a concentration well above the *NEC* within 2 days needed to derive a 48 hr *LC50* valuecannot be achieved resulting in an unrealistic value for the 48 hr *LC50*. As a result of this issue, this compound was excluded from our analysis.

To summarize, we used the log K_{ow} values for the herbicides and fungicides in a first approximation in estimating the toxicity parameters.The urea based pesticides showed toxicity higher than baseline toxicity, so for this group of pesticidesthe *NEC* was derived in the same way as for the insecticides, based on *LC50* values in time or on 48 hr *LC50* values.This general approach gives a first estimate of toxicity parameters and avoids problems with the kinetic aspects of toxic effects such as with fenpropimorph. The approach is evaluated in the discussion.

Evaluation of the monitoring data

One of the difficulties for any mixture analysis for a complex environmental sample is how to deal with measurementsbelow the analytical detection-limit (DL). One approach is to assign below DL results a concentration of zero so treating them as if they were truly absent from the sample. This approach is problematic because it is recognised that even low concentration can contribute to a mixture effect[35]. The Water Framework Directive states that below DL results still have to be accounted for by using half the DL as the actual concentration[5]. Following this, it was shown from the CSF data-analysis, that compounds that were not measured above their DLs at any location during the entire 8.5 years for which we analysed the data would still have a major contribution to the predicted mixture effect. Some compounds are in a monitoring scheme because they are part of the analytical package, notbecause they are priority substances known to be used in a certain area. Recognising this in our approach, mixture effects estimates for the Dutch monitoring data were made by discounting the compoundsthat were never detected in a certain year at any location (i.e. they were assigned a default concentration of zero). Bias caused by an increase in compounds that were likely not present was, thus, avoided.

For the remaining detected chemicals, the monitoring data-sets were analysed for effects on daphnids in two different ways,accounting for both single compound and mixture effects anddetected and non-detected concentrations.

1) Establishing if the mixture threshold is exceeded, discarding all measurements below the detection limit

In this approach we evaluated if the mixture thresholds were exceeded.Only actually measured concentrations were taken into account, so below detection limit results are set to zero for this analysis.

2) Taking all measurements below the detection limit as fixed different proportions of the DL detection limit and check if the mixture threshold is exceeded

Setting the below DL measurements at different proportions (0.1, 0.25, 0.5, 0.75 and 0.9) illustrates how this choice influences the results of the analysis. Note that the detection limit of a specific compound can be different in different samples. First of all because laboratory methods can change over time but it is also possible that some samples show interference with an effect on detection limits.

RESULTS

Evaluation of CSF monitoring data

In two of the 5400 analysed samples, the mixture thresholdexceeded 1, implying that a daphnid population at these locations would go extinct if sufficiently long exposed to the measured concentrations. In both cases the predicted mixture effect is related almost exclusively to the presence of diflubenzuron. In the supplemental data, section 3, the measured concentrations and their contributions to the mixture threshold of all the individual pesticides measured above the DL for these two samples are summarised.

There are 16 compounds (typically insecticides with two exceptions) that are part of the measurement program, which were never observed above their detection limits (Table 4).

Almost all of these pesticides have been subject to use restriction in Europe meaning that for general applications their use is no longer permitted. The results of the different ways to interpret the data for mixtures are summarised on a per sample basis (Table 5).

In general terms setting below DL measurements to fixed levels of the DL greatly increases the number of sites where an extinction risk for daphnid population may be present for fractions > 0.25 DL. Applying the WFD prescribed guideline to set below DL measurements at 0.5 DL gives a total of 168 samples where the mixture threshold exceeds 1, instead of the 2 samples if below DL limit measurements are set to 0. The table also clearly shows that the vast majority of the cases where the mixture threshold is exceeded with concentrations set at a proportion of 0.5 or higher of their DL are caused by the insecticides listed in table 4 that were never observed above the DL simply because they are banned and therefore no longer used.

The possibility for effectsis driven by a limited number of compounds: diflurobenzuron, diazinon, coumaphos, ethion and pirimiphos-methyl. Diflurobenzuron has a detection limit between 0.01and 0.04 µg/L, a concentration of 0.02 µg/L already represents a contribution of 29% to the mixture NEC threshold.

Dutch Pesticide Monitoring Program

The summarized results of the data analysis are shown in Table 6.

Specific data on when and where the mixture threshold was exceeded for the samples where below detection limit results were set to 0 and which compounds were involved can be found in the Supplemental data, section 3.The overall pattern of the results reflects those observed in the CSF data-set with a somewhat higher number of samples where the mixture threshold exceeds 1. Because of the greater overall size and complexity of the Dutch data-set the trends are amplified. When measurement below DL are assigned a zero concentration then a small number of samples show a mixture thresholdhigher than 1. The majority of themeasured concentrations are below DL, including these below DL compounds at different fractions of their DL has a profound effect on the number of samples where the mixture threshold exceeds 1, with

a sharp increase at 0.25 DL. If the WFD guidelines are followed and below DL results are included at half the DL the mixture threshold would be exceeded in 20 to 35% of all samples.

DISCUSSION

Modelling approach

For the majority of the insecticides we only had a 48 hr *LC50* value available and had to use this as a basis for calculating *NEC*s. The incipient *LC50* was not reached for any of the pesticides during the 48 hr of a standardised test, where we had data available and therefore the 48 hr *LC50* value underestimates the actual toxicity of the different compounds. We are aware that the use of the median factor between the 48 hr *LC50* and the incipient *LC50* (Table 3) is only a rough approximation and there is no kinetic information available to refine these estimates at a chemical specific level. Hence in some cases, the *NEC* will be slightly over- or underestimated with a tendency for the *NEC* to be overestimated,so some effects might be missed. When multiple chemical effects are considered, this approach is, however, pragmatic and more likely to produce a realistic set of values than a worst case scenario of dividing all *LC50*s by a the maximum value listed in Table 3 or just take the 48 hr *LC50*as we know the incipient *LC50* is likely to be lower than the 48 hr *LC50*.

For mixture effect prediction, an approach was needed in which the possible effects of insecticides, herbicides and fungicides could be combined in a systematic way. The key for an approach was considering whether the *NEC* is shared by different compounds and hence whether the compounds contribute together or separately to threshold exceedance. We took a practical approach in modelling the effects based on specific mode of action considerations. The insecticides were classed as one group which were considered to have a similar mode of action (neurotoxicity) and so to be able to jointly contribute to mixture effect. The herbicides and

fungicides were then classed as a second group, also sharing a mode of action (narcosis) and so share a*NEC*. Treating the fungicides and herbicides as a single group of toxicants exerting base-line toxicity to the daphnids seems a rather crude generalisation. However plotting the *NEC*s against log K_{ow} does give a relation that is completely in agreement with the expectation for baseline toxicity (with the exception of the uron herbicides and fenpropimorph). This finding is supported by the absence of key receptor of specific herbicide and fungicide effects in *Daphnia*. Further, it has also been noted for other chemical classes. For example, Cleuvers used a similar approach as applied here to show that different pharmaceuticals exhibited largely base-line toxicity to daphnids [36].Should specific information emerge to suggest an alternative mode of action for any herbicide or fungicide group to *Daphnia*, then there is no reason that such chemical could not be considered as an addition group in the mixture. This would, however, only be important if the additional mode of action class of compounds showed toxicity substantially above that expected for baseline toxicity.

There will always be some uncertainty about how a specific compound affects some parameter, starting with the level inside the organism at which the interaction takes place on a molecular level or on a higher level inside an organism [20, 37, 38].The insecticides typically target the nerve function. Even if the target is not mediated by the same molecular initiating event (e.g. acetyl cholinesterase binding, nicotinic receptor binding or sodium channel binding). We used this approach before and exceedence of 1 for the NEC_{mix} proved to be an excellent proxy for predicting daphnid survival after exposure to complex mixtures [3].This general approach is in line with other research with both the CA and IA model to predict mixture effects for different pesticides [21, 39], though with some tendency for moderate synergistic effects in

binary mixtures. Further, it also accords with the work of De Zwart and Posthuma [20] who modelled effects of complex mixtures on single and multiple species.

Since most of the pesticides are used at comparable application rates[40], herbicides, fungicides and insecticides are typically measured at comparable concentrations. For daphnids, our analysis and modelling of the available effects data indicated that insecticides are generally some 5 – 6 orders of magnitude more toxic to this taxon than herbicides and fungicides. Hence, when assessing effects of environmental exposure to daphnids, the insecticides cause the effect. In all cases where the mixture threshold exceeded 1 the contribution of the herbicides and fungicides to the mixture effects is below 0.01%.

The analysis shows that organophosphates, carbamates and pyrethroidshave the dominant contribution to effects. In line with what could be expected, based on their sensitivity to different groups of pesticides [41]. In contrast, neonicotinoid compounds that frequently exceed water quality standards rarely appear as problem compounds in this analysis. This is because of their comparatively low toxicity to *Daphnia*. Hence including their contribution to the NEC_{mix} with the other insecticides or treating these compounds separately from the other insecticides does not greatly influence the effect estimate for survival in daphnids.A similar analysis for a species that is sensitive to these compounds would, however,come to a different conclusion.Such species may include for example insect larval species, but not groups such as commonly tested fish or algae species, which also have a relatively low sensitivity to neonicotinoids.

Daphnia have a historyof being recognised as a valuable model species for ecotoxicological research[42, 43] and based on toxicity data availability, they are probably the only taxon for which a mixture effect analysis such as the one conducted here is feasible. Some of the main principals that arise from this analysis for daphnids would be expected to apply to

other taxa such as fish. Though there will be some divergence of *NEC*s, the compounds that govern the effects and to some extent the comparable risk predicted between different samples and sites will be similar for fish and daphnids. A recent study by Bundschuh et al. [44] where the CA model was used for algae, invertebrates (characterised by D. magna) and fish for samples from the Swedish monitoring system showed that the toxic pressure for fish and invertebrates is comparable with slightly higher toxic pressure for the invertebrates. Risks for algae were found to be higher because of high herbicide exposure. Hence evaluation of monitoring schemes on effective protection across multiple taxa, requires multiple mixture based risk assessments.

Data quality

Any approach to chemical monitoring that aims to predict the frequency and/or magnitude of possible toxic effects, is dependent on both the quality of the available analytical chemistry and also importantly on the totality of available toxicity data. The analytical data that describe pesticide concentration measurements are typical for national monitoring programmes. Study design may be randomised or untargeted, while analyses are conducted by approved analytical laboratories. Over time method and pesticides included may vary, although the demand to maintain consistency and a regulatory inertia limits the rate of change. For effect estimation, the data in the PPDB [26] are considered reliable, as these are usually both recent and come from GLP studies conducted in order to bring a pesticide to market. However for some compounds large differences in reported 48 hr *LC50* values remain; a factor of 10 is not uncommon. However, even despite the difference, when taking information for multiple chemical to effect prediction an accurate predictions of the mixture effects on the survival of daphnids as an indicator taxon can be made [3].

Exceedence of the mixture threshold in CSF and Dutch monitoring data

In total well over 15,000 samples and well over 1,100,000 individual concentration measurements were evaluated. The measurements clearly show that a limited number of actually measured compounds (some 10-15 in the Dutch data and around 5 in the CSF data) contribute to the mixture effect for daphnidsandso present a toxicological hazard to individuals and probably topopulations. Half ofthe cases (only measured concentrations above DL are included) whereNEC_{mix}>1 are caused by a single compound, with the other cases being due to contributions from multiple compounds.

In the CSF programthe NEC_{mix} exceeds 1 on onlytwo occasions out of 5400 samples. In both casesdiflubenzuron alone was responsible.In the Dutch monitoring program, the number of cases whereNEC_{mix}>1seems to increase over time (19 in 2005, 18 in 2009 and 49 in 2013), though this time series is too limited to make a firm statement on this. A high proportion (~ 43 %) of the increase in the number of samples where the NEC_{mix} exceeds 1 (based only on concentration above DLs) are for mixtures containing the pyrethroids fenvalerate, esfenvalerate, alpha-cypermethrin and lambda-cyhalothrin. These compounds were not included in2005, but were in 2009 although they did not substantially contribute to mixture effect in that year. In 2013, fenvalerate and esfenvalerate are usually found in the same ratios (a fenvalerate formulation contains esfenvalerate). This observation suggests a high release of these two compounds, although usage data on these compounds over time is not available making confirmation of this difficult. In addition to the above compounds, other pesticides causing individual *NEC* and NEC_{mix} exceedance includedpirimiphos-methyl, for which the individual*NEC*was exceeded by up to a factor of 20. Such concentrations would have a profound effect on the survival of daphnids (e.g. 90% mortality within 30 h, Figure 5). Pirimicarb was also

a major contributor to mixture effects and in 2005 coumaphos was an importantcontributor, although this latter compound did not contribute significantly to mixture effects 2009 or 2013.

Both datasets clearly show issues associated with the inclusion of measurements below DL for understanding whether there is a mixture hazard. If below DL measurements are taken as increasing fractions of the DL, the number of cases when NEC_{mix} exceeds 1.0 increased from 2 to 538 (0.04 –9.8% of all cases)in the CSF dataset. Including the below DL measurements at 0.5DL, leads to 168 (3.1%) cases where NEC_{mix}> 1.A similar but amplified trend due to the higher nr of compounds taken up in the measurements is visible in the Dutch data. Including below DL measurements as half the detection limit would then lead to 350 -1200 (or 20 – 35%) cases where NEC_{mix}> 1 depending on the year, or up to 50% off all samples if below DL measurements would be included as 0.9DL.

In both approaches exceedence of the mixture threshold is completely governedby the additive effect of below DL concentrations. If compounds that were never observed above their DL were to be included, effect prediction for the Dutch data-set would suggest risk for the vast majority of samples. This is, not a realistic scenario as even pesticides that are banned are included, which leads to overestimation of effects[45]. The current structure of the WFD approach, however, does not include a specific provision within its focus for single chemicals.

Comparison of Dutch and CSF monitoring data

InTable 7 the results for measurements above the detection limit for the CSF and the Dutch data are summarised.

Reichenberger et al. [11] stated that total emissions to water from run-off are driven by rain-fall and are likely to be considerably higher than direct emissions.However, despite the higher frequency of the CSF measurements with the focus on sampling capturing high

concentrations following rain events, the average concentrations, the highest concentrations and nr of measurements >10 µg/L are substantially higher in the Dutch dataset than in the UK dataset. The percentage of measurements above the DL is comparable for both datasets.So the results suggest that direct emission routes overall lead to higher exposure concentrations than indirect emission routes.

Significance of results for environmental management

The aim of monitoring schemes for pesticides is to provide both an overview of the current range of active ingredients present in water courses and to assess whether these pose any risk to human health and ecosystems. Such an aim encompasses protection of all trophic levels from primary producers to higher tier consumers. With multiple chemical monitoring the potential for mixture effect assessment is also feasible as we show here.

Low concentration levels of compounds can make a contribution to mixture effects and so need to be considered, this requires DLs around 0.001**NEC* for individual compounds. At present DL are typically are 0.1-0.01**NEC*. In the standard evaluation (i.e. comparing monitoring data with EQS) of the Dutch data within the framework of the 'Bestrijdingsmiddelenatlas' (see section 2), compounds with a low EQS cannot be assessed because to DLs that are too high. Our data analysis indicates how despite the physical and financial effort put in to each monitoring programmes, current DLs in the monitoring programmes are insufficient to rule out mixture effects even for a relatively simple case as the survival (!) of daphnids. The reality of course is different the measurements indicate limited exposure to only a few chemicals where mixture effects are unlikely to occur, though it cannot be ruled out. Scheme designs do not provide either enough information or a way to deal with data below DL that supports decision making on the likelihood that the environment is being protected.

While current practice may be satisfactory for assessing single chemical effects, for mixtures it needs more guiding information. An effect analysis like the one described here can be used to get the monitoring programme focus on the compounds that are closest to exceed the *NEC* (with detection limits that are low enough, for the three trophic levels algae, daphnids and fish). Further, sites that have the highest possibility for mixture effects, especially those where measured concentration contribute, can be identified for focussed direct toxicity assessment.

With the current practise elaborate monitoring schemesbecome penalised in their ability to truly exclude the potential for adverse effects when chemical below DL are included at even a fraction of the DL. In the Dutch monitoring data, some samples are analysed for more than 400 different individual pesticides of which only a handful are actually found above their DL. Under the WFD guidelines for such a sample it is very likely that NEC_{mix} exceeds1.

In Table 1 of supplemental data section 4, we have summarised the *NEC* and Dutch EQS for the 25 compounds with the lowest *NEC* for daphnids. This shows a very striking mismatch between the EQS and the *NEC* for fenvalerate; this compound has an EQS of 9.7E-03 µM and a *NEC* of 2.65E-05 µM[1]. If this compound is left out of further analysis, the concentration levels of the EQS of the 24 remaining insecticides fills 47.6% of the toxic threshold for survival of daphnids of the mixture. So the average contribution of an individual insecticide at its EQS is approximately 2%or in other words this implies an average safety factor of app. 50. In general terms this is enough to rule out lethal effects for daphnids, though sub-lethal effects of course cannot be ruled out especially as for two individual compounds (fenpropidin and diazinone) the difference between the *NEC* for survival of daphnids and the EQS is less than a factor 10.

[1] Even the 48 hr LC_{50} value for daphnids for fenvalerate is lower than the EQS, it appears that this EQS is not appropriate and is currently under investigation due to this finding.

Ultimately, if monitoring data are to be used to evaluate if the environment is actually protected or not, it is important that the right chemicals are included in the suites of analysis. To better achieve this, monitoring schemes would need to be reviewed regularly, based on what is known from standard measurements and probably also market surveillance. Such practice is commonly used in human health protection (e.g. annual choice of strains of flu to include in the flu vaccine) so it is not beyond the realms of feasibility to do so considering we can/should have a good idea what pesticides are sold and in use at least for countries like the UK and The Netherlands. Including compounds not actually used adds unnecessarily to the number of samples with analytes below DL which according to strict WFD practice still contribute to the effect prediction through inclusion at 0.5 times DL. However, missing out commonly used pesticides or having high DLs for these compounds raises the risk of missing something that is actually having an effect. A more focussed monitoring programme is also likely to be cheaper, despite the higher standards needed for the detection limits and ultimately will deliver a better true picture of actual concentrations.

CONCLUSIONS

Pesticide concentrations measured in a relatively small scale monitoring program in the UK and the nation-wide pesticide monitoring program in the Netherlands were analysed with a process-based mixture model parameterised for effects on the survival of daphnids. In total well over 15,000 samples, containing well over 1 million individual pesticide concentration measurements were evaluated. This led to the following conclusions:

- In the Netherlands higher average and peak concentrations are measured, most likely caused by the more direct emissions to surface waters during application;

- Of all the different pesticides taken up in the monitoring schemes (almost 600 in the Dutch monitoring scheme and 88 in the CSF monitoring scheme) only a handful are predicted to cause possible acute effects for daphnids. If only measurements above the detection limits are regarded some 0.5 – 1.5 % of the samples in the Dutch dataset would cause acute effects. In the CSF dataset from the UK some 0.1 % of the samples would cause acute effects;
- The most polluted samples would cause a daphnid population to go extinct within 30 hrs.
- If below detection limit measurements are included in the analysis (in the way the Water Framework Directive ascribes), in up to 35% of the Dutch samples the effect of the simultaneous exposure to all pesticides is such that a direct threat to the survival of daphnids exists.
- Detection limits for the insecticides are generally too high to make reliable predictions on effects, based on the monitoring schemes when effects of multiple exposures are included. So despite all the effort that is put in the monitoring programmes the current detection limits in the monitoring programmes are insufficient to make firm statements on whether or not the overall aquatic community is affected at the sampling sites. The improvement of detection of targeted effects based analysis in combination with predictive modelling could provide a solution to this issue.

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

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Disclaimer—The opinions expressed here are those of the authors and not their respective employers.

Data Availability—Data are available upon request from authors J Rambohul at rambohul.justin@environment-agency.gov.uk (UK Data) or M Vijver at Vijver@cml.leidenuniv.nl (Dutch Data). The aggregated data are publicly reported on an annual basis and can be found on the internet, using keywords CSF monitoring programme for the UK data and Bestrijdingsmiddelenatlas for the Dutch data.

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Figure Captions:

Figure 1. Example of the development of LC_{50} values over time (example for *Daphnia magna* exposed to Pirimiphos-methyl).

Figure 2. Toxicity (NEC) for all the herbicides and fungicides plotted against their log K_{ow} values.

Figure 3. Toxicity (NEC) for the herbicides and fungicides plotted against their log K_{ow} values, with the urons monolinuron and linuron left out.

Figure 4. Predicted vs. observed NEC for the fungicides and herbicides, with 95% conf. intervals.

Figure 5. Predicted survival of *Daphnia magna* after exposure to different concentrations of Pirimiphos-methyl, from left to right 20; 6; 8; 4 and 2 times the No Effect Concentration.

Table 1 Some general characteristics of the results of the Catchment Sensitive Farming Monitoring Scheme, with the nr of samples, reported concentrations and concentrations above the Detection Limit (DL)

CSF pesticide monitoring data 2006 – 2014	
Nr samples, total	5400
Nr of individual reported concentrations	311152
Nr of individual reported concentrations > DL	20039
Nr samples with at least 10 compounds	3585
Nr Sampling points	25
Nr samples >= 10 compounds measured above DL	149
Nr of compounds, analysed total	88
Nr of compounds found above DL	72

Table 2. Some general characteristics of the results of the Dutch Monitoring Scheme, with the nr of samples, reported concentrations and concentrations above the Detection Limit (DL)

	Base-year 2005	Base-year 2009	Base-year 2013
Nr samples	2985	3822	3565
Nr individual reported concentrations	159493	299058	391242
Nr individual reported concentrations > DL	10888	18708	18588
Nr Sampling points	495	608	462
Nr samples > 10 compounds	2985	3818	3537
Nr samples with at least 10 compounds > DL	159	408	516
Nr of compounds sampled	294	596	532
Nr of compounds measured > DL	181	283	232

Table 3 Overview of 48 hr and incipient LC_{50} data for the different insecticides (see text)

Compound	48 hr LC_{50} µmol/L	Incipient LC_{50} µmol/L	48 hr LC_{50}/incipient LC_{50}	Reference
Diazinone	1.6 E-04	1.1 E-03	1.5	[3]
Dichlorphos	4.7 E-01	2.8 E-01	1.7	[3]
Dieldrin	4.6 E-01	2.7 E-01	1.7	[3]
Diflubenzuron	1.93 E-02	2.2 E-04	87	This research
Disulphotone	4.7 E-02	3.4 E-02	1.4	[3]
Endosulphansulphate	1.23	1.0 E-01	12	[3]
Endrin	1.1 E-02	1.5 E-03	1.7	This research
Methoxychlor	4.6 E-02	1.7 E-02	2.7	This research
Mevinphos	5.8 E-03	4.5 E-03	1.5	[3]
Parathion-ethyl	4.6 E-03	9.3 E-04	4.9	[3]
Pentachlorophenole	1.5	5.3	3.5	This research
Pirimiphos-methyl	6.7 E-04	1.5 E-04	4.5	[3]
Propoxur	4.3	14.1	3.3	This research
Thiomethon	3.1 E+1	2.8 E-02	15	This research
Tolclophos-methyl	1.6 E+1	1.0 E+1	1.6	[3]

Table 4 List of pesticides that were never detected above the detection limits of the analytical method used in all of 5,400 samples taken

Compound	Type of pesticide	Allowed to be used in the UK?
Chlorfenvinphos	Insecticide	No
Chloroxuron	Herbicide	No
Dichlorvos	Insecticide	No
Ethion	Insecticide	No
Fenchlorphos	Insecticide	No
Fenthion	Insecticide	No
Ioxynil	Herbicide	Yes
Methabenzthiazuron	Herbicide	No
Methiocarb	Insecticide	Yes
Metoxuron	Herbicide	No
Mevinphos	Insecticide	No
Neburon	Herbicide	No
Parathion-ethyl	Insecticide	No
Parathion-methyl	Insecticide	No
Pirimiphos-ethyl	Insecticide	No
Triazophos	Insecticide	No

Table 5 Number of samples where the mixture NEC exceeds 1 for the different ways to analyse the CSF pesticide monitoring data, with detection limits (DL) concentrations set to 0, 0.1, 0.25, 0.5, 0.75 and 0.9 times the detection limit, including all compounds or only the compounds that were present in at least one sample

	All compounds included	Excluding the pesticides listed in table 4
< DL set to 0	2	2
< DL set to 0.1 DL	4	4
< DL set to 0.25 DL	4	4
< DL set to 0.5 DL	168	5
< DL set to 0.75 DL	176	170
< DL set to 0.9 DL	538	170

Table 6 Number of samples where the mixture NEC exceeds 1 for the different ways to analyse the Dutch monitoring data, with below detection limits (DL) concentrations set to 0, 0.1, 0.25, 0.5, 0.75 and 0.9 times the detection limit, including all compounds or only the compounds that were present in at least one sample

	2005	2009	2013
< DL set to 0	19	18	49
< DL set to 0.1 DL	22	28	227
< DL set to 0.25 DL	29	235	486
< DL set to 0.5 DL	335	739	1284
< DL set to 0.75 DL	718	1239	1833
< DL set to 0.9 DL	726	1290	1835

Table 7 Comparison of Dutch and CSF concentration measurement characteristics

Dataset	% measured concentrations > DL	Average concentration above DL µg/L	Measurements > 10 µg/L	Measurements > 0.1 µg/L	Highest measured concentration µg/L
CSF	6.4	0.072	0	3159 (1.0 %)	6.1 (Carbetamide)
Dutch 2005	6.8	0.25	26 (1.6 E-02 %)	3355 (2.1 %)	128 (MCPA)
Dutch 2009	6.3	0.17	15 (5.0 E-03 %)	4372 (1.5 %)	120 (Ethofumesaat)
Dutch 2013	4.8	0.16	13 (3.3 E-03 %)	4033 (1.0 %)	76 (Propamocarb)

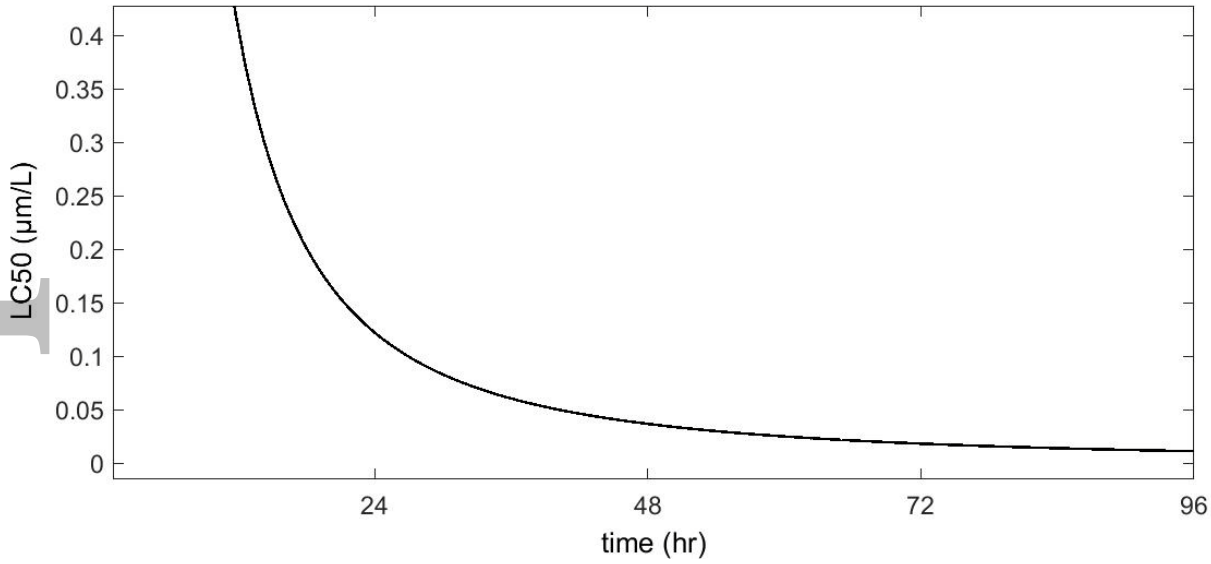

Figure 1

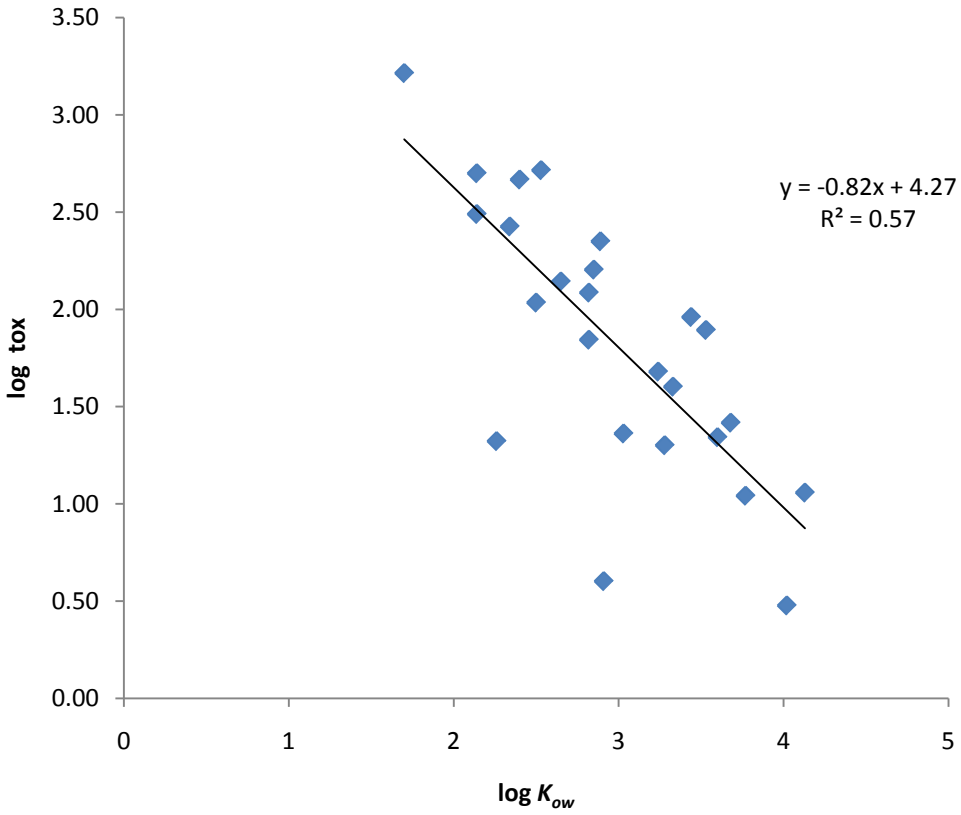

Figure 2

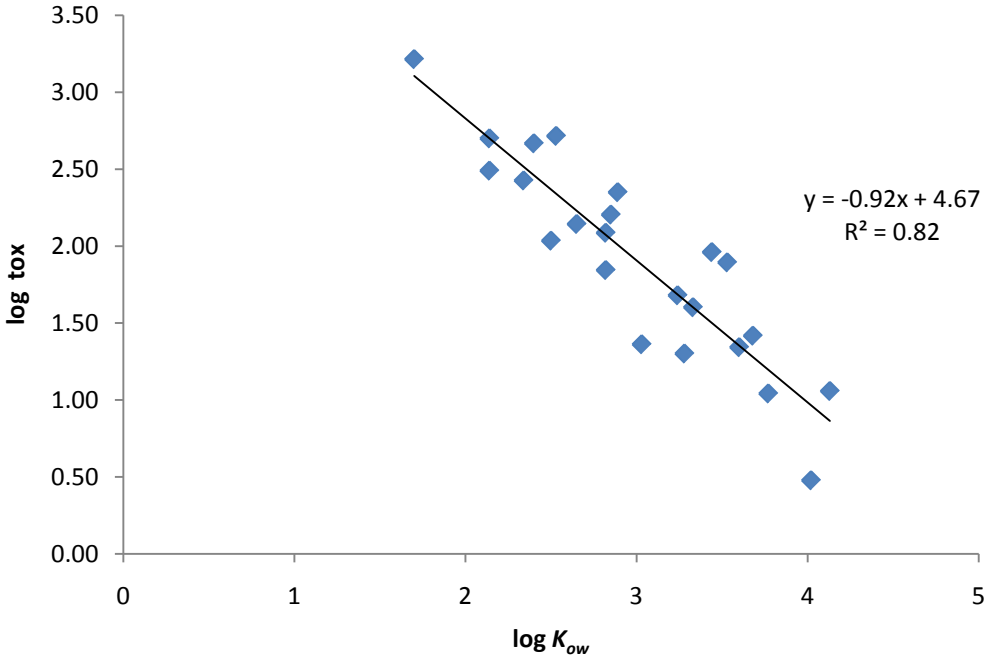

Figure 3

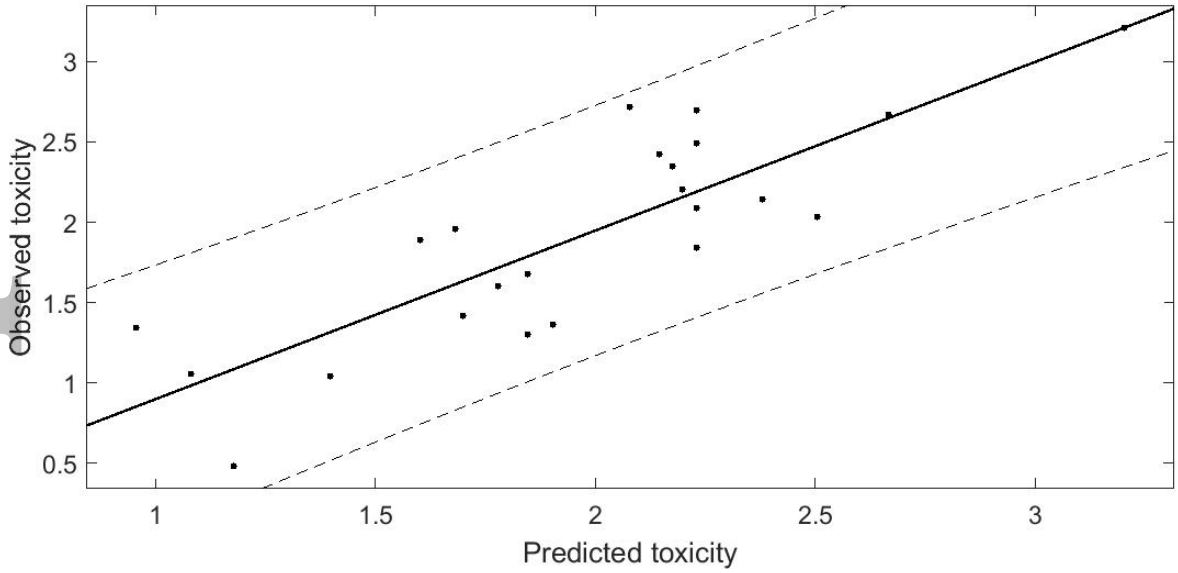

Figure 4

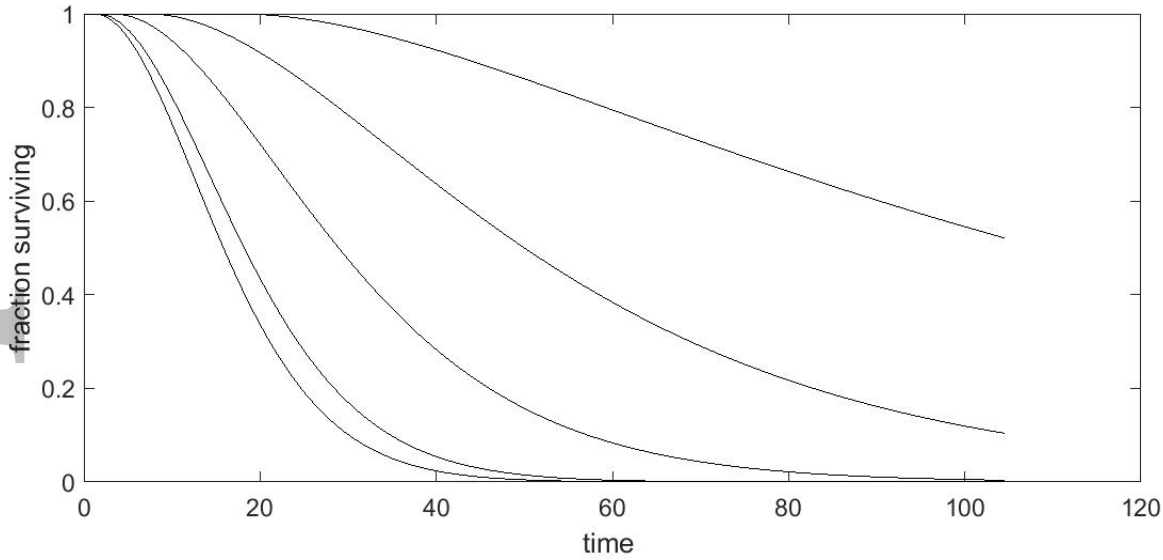

Figure 5