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Simulating spray series of pesticides in agricultural practice reveals evidence for accumulation of environmental risk in soil

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1. Introduction

In 2016, 51.1% of the total territory of Germany was used agriculturally but only 7.5% of this agricultural land was farmed organically, i.e. without the use of synthetic organic pesticides (BMEL, 2016; Statistisches Bundesamt, 2017). In 2016, 753 different pesticides (PPPs) with 270 different active substances were registered in Germany (BMEL, 2017; BVL, 2017). It was demonstrated previously that the increased use of pesticides acts amongst many other adverse factors towards a steep decrease in biodiversity in the agricultural landscape (Geiger et al., 2010; Hole et al., 2005; Robinson and Sutherland, 2002; Schäffer et al., 2018). In the following, the term pesticide describes the entire product, while the active substance only describes the pesticidal substance in the product.

Pesticide applications lead to contamination of soil not only in the agricultural fields, but also in adjacent surface waters, off crop areas, and biotopes by several transport processes, such as spray drift, evaporation, deposition, runoff, erosion or drainage (Knauer, 2016). Both in soils and rivers pesticides occur typically in mixtures due to the use of different pesticides and spray series within the same catchment area or landscape (Chiaia-Hernandez et al., 2017; Moschet et al., 2014; Schreiner et al., 2016). Mitigation measures like buffer zones or drift-reducing nozzles help to minimize the surface water contamination are already integrated within the assessment and registration processes (Carter, 2000; Gartiser and Jäger, 2011). Nevertheless in agricultural soil, organisms like earthworms which are exclusively located in the treated fields, cannot be protected by special exposure mitigation measures (Felsot et al., 2011).

Tank mixtures (i.e. more than one pesticide mixed by the farmer in the tank before spraying) as well as combination products (i.e. pesticides with more than one active substance) and single pesticides are usually applied in the course of the growing season in a so called spray series. The average number of applications in apple cultures in Germany in 2013 was about 21 per season. Up to 20 spray events of one or several pesticides in apple cultures were recorded in the PAPA-survey (panel crop protection applications), which quantifies the annual application of pesticides in survey farms (Roßberg and Harzer, 2015). The PAPA-survey is a national survey on the use of chemical pesticides in relevant crops. The treatment frequencies for herbicides and insecticides in apple cultures in Germany have not changed significantly in the last 10 years. The frequency of fungicide application has increased since 2011, due to an increasing resistance problem with scab fungi (Roßberg and Harzer, 2015).

Unlike registered combination products, tank mixtures and spray series lead to multiple exposure of non-target organisms and to mixture toxicity for soil organisms, which is not systematically considered in risk assessment today (Frische et al., 2014). The current risk assessment takes already into account the risk for combination products but only for those tank mixtures that are explicitly defined as “recommended” or “obligatory” by the applicant seeking an authorization (BVL, 2015). The risk for an entire spray series is not considered at present, however. The consideration of mixtures is important because it can elicit significant effects even if single compounds of the mixture are contained in concentrations below individual effect levels (Kunz et al., 2011).

Some active substances remain in the soil for a long time depending on their specific degradation or dissipation time (Rafique et al., 2016). Recent investigation detected pesticide residues remaining in soil from previous applications (Aktar et al., 2009; Chiaia-Hernandez et al., 2017; Jablonowski et al., 2012).

There are different ways to predict mixture toxicity of pesticides based on the toxicity of individual mixture components, such as concentration addition (CA) or independent action (IA). Concentration addition assumes mixtures to consist of non-interacting compounds with same mode of action, i.e., the individual components contribute to the mixture toxicity according to their individual toxicity (Loewe and Muischnek, 1926). The risk indicator, consisting of exposure and an ecotoxicological endpoint, is added for each component of the mixture. Conceptually, CA assumes that one component can be fully or partially replaced by an equi-effective concentration of another component without changing

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the toxicity of the mixture (Faust et al., 2001). De Zwart and Posthuma (2005) stated that about 70% of the mixtures act in conformity with the prediction of concentration addition. This analysis is in line with the funnel hypothesis, which predicates an increasing number of components in a mixture leads to an increasing tendency to act similar to concentration addition (Warne and Hawker, 1995). In contrast, the concept of independent action described by Bliss (Bliss, 1939) predicts the mixture toxicity of toxicants with dissimilar mode of action (De Zwart and Posthuma, 2005). IA is based on the assumption that one component affects only those parts of a population that were not previously affected by another mixture component (Backhaus et al., 2004b; Kunz et al., 2011).

The number and composition of actually applied pesticides underlie frequent modifications to meet the needs in agricultural practice. Experimental testing of every possible combination of pesticides or every potentially resulting mixture is not feasible. Instead, models predicting mixture risk for different pesticide combinations would enable different pesticide applications to be compared. The aim of this study is to apply a recently developed model that is able to predict the mixture risk of multiple pesticide applications for earthworms (Sybertz et al., 2019). The new model called MITAS (Mixture toxicity of application spray series) considers applications of substances during a spray series not as independent events, but as part of the entire spray series. Tank mixtures and combination products are considered as well as applications of different pesticides in the growing season including their degradation over time. It is therefore possible to track the exposure and risk of the single substances as well as for the complete spray series within the simulated time period.

2. Material and methods

The model MITAS developed by some of the authors was used to investigate a spray series and its time dependent (accumulated) mixture risk. A pesticide spray series was modeled for up to three years, to visualize the exposure and risk over time, respectively. In MITAS, a variable soil mixing depth (migration depth of the substances in the soil) was used depending on the K_{foc}-value (Freundlich coefficient normalized to soil organic carbon content) of the pesticide. For substances with K_{foc} below 500 L/kg, the mixing depth was 2.5 cm, and above this trigger value 1 cm, the latter leading to a 2.5 fold initial exposure concentrations (Fent et al., 1999). K_{foc}-values above 500 L/kg indicate high adsorption of the substance to the solid matrix and, thus, a low leaching potential. Applied substances remain in the top soil immediately after application, which is why the soil depths of 2.5 and 1 cm respectively were selected (EFSA PPR Panel et al., 2017). The bulk density value selected for this simulation was 1.5 g/cm^3 based on the value for the top 30 cm soil in the FOCUS scenario Hamburg (FOCUS, 2014). Crop interception is considered on the basis of the BBCH stage (phenological growth stage) of the crop and the FOCUS interception for FOCUS scenario Hamburg (FOCUS, 2014; Hack et al., 1992). To consider substance degradation, first order reaction kinetics is used. All DT50 values and K_{foc}-values were provided by the UBA as well as NOEC values (OECD222) (dithianon, trifloxystrobin, flusilazole, fluquinconazole) and others from EFSA reports (EFSA, accessed 15.03.2019; UBA, 2017). For the inorganic fungicide sulfur data from the PPDB database were used (Lewis et al., 2016). NOEC values could not be generated for the substances fenoxycarb and spirodiclofen and therefore these substances were not included in the calculation. Earthworm (*Eisenia fetida*) was chosen for this simulation because it is an important soil organism and is used as standard test organism in pesticide assessment. Thus the data availability for the different substances is relatively large. Other soil organisms, such as Collembolan, could also be used. For these organisms the ecotoxicological information is usually lower. Exposure-calculation (PEC [Predicted Environmental Concentration]) in MITAS is based on FOCUS soil persistence models (Boesten et al., 1997). The risk indicator ETR (Exposure Toxicity Ratio) is calculated as quotient of PEC and an ecotoxicological endpoint (here: NOEC *OECD222*). All simulations were calculated with the variable soil mixing depth described previously. MITAS simulations provide the following results on a time-dependent (daily) scale: (1) A continuous time series of PEC-values, considering first order degradation kinetics of the individual compounds, (2) the acute and chronic risk and PECs of each individual component of a mixture, and (3) the acute and chronic risk of the whole mixture assuming concentration addition (Loewe and Muischnek, 1926; Berenbaum, 1985). For detailed information about the individual parameters and the structure of MITAS see Sybertz et al. (2019).

We chose a spray series for apple orchards from 2007 based on information provided by the Private Institute for Sustainable Agriculture GmbH (INL, *Halle/Saale*). The selected spray series represents a still current and realistic application regime. Data on the spray series comprise the size of the treated area, the dates of application, crop types, pesticides and concentrations applied, and the growth stage of the crops. The selected spray series consisted of 15 different active substances and 26 different application dates during the growing season (Fig. 1).

The model MITAS considers the influence of temperature on degradation speed of the active substances (Boesten et al., 1997). For our simulations, monthly average temperatures for Germany in 2007 were received from Germany's National Meteorological Service (DWD, 2019) (Table 1).

3. Results

As MITAS allows simulating the accumulated mixture risk over time, the simulation of the chosen spray series for apple orchards can be used to investigate different aspects of the spray series as explained in the following.

3.1. Exposure and degradation

Exposure (PEC) was calculated time-dependently for each applied active substance of the spray series (Fig. 2). Single substances were considered by the date of application, the initial exposure concentration and the degradation development. For each day of the simulation (1 year) the respective PEC value is calculated for each applied substance. All applications are initially considered independently of each other.

In the chosen spray series for apple orchards various substances were applied repeatedly, such as mancozeb (4 times) and captan (14 times). Some of the applied substances degrade rapidly and others slowly. The fungicide mancozeb with a fast degradation (DT50: 0.13 days) reaches a maximum PEC value of 4.26 mg/kg soil (UBA, 2017). Sulfur is often applied with a high application rate of 3.192 kg/ha (Fig. 1) which is also visible in the exposure (light blue line, Fig. 2). Other substances like methoxyfenozide or penconazole show much lower PECs in comparison to the substances mentioned above.

To obtain an overview of the total soil exposure, the daily exposure (PEC) of all substances was added for each simulation day (Fig. 3). Due to the high application quantities of sulfur, the cumulative exposure without sulfur was additionally calculated (Fig. 3b). High application rates of sulfur lead to a very high accumulated PEC value which starts with 0 mg/kg soil at day 1 and reaches a maximum of 32.12 mg/kg soil at day 143 (Fig. 3a). The integrated graph, excluding the consideration of sulfur, illustrates

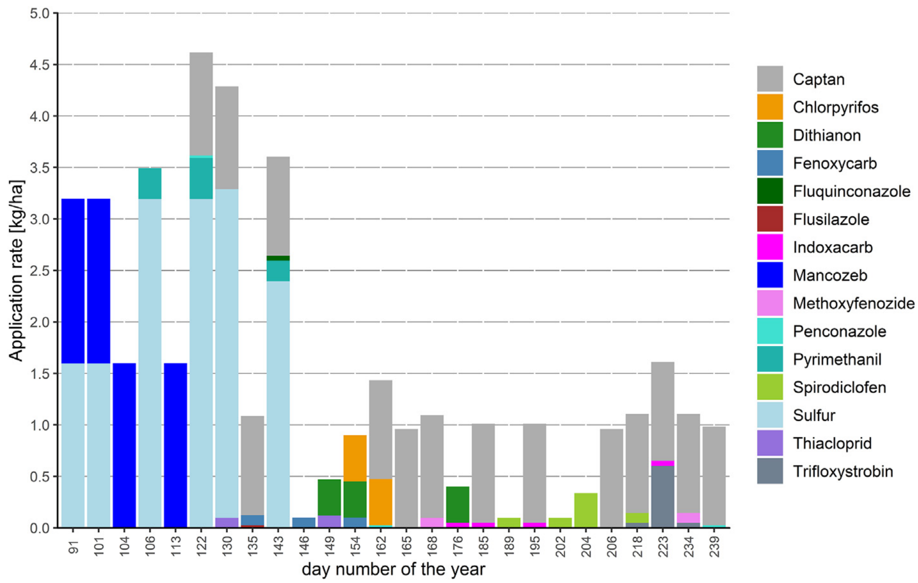

Fig. 1. Applied substances of the spray series for apple orchards. The x-axis displays the days in the course of the year when substances were applied. The y-axis represents the application rate [kg/ha]. The different colors highlight the various applied substances. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Monthly average temperature applied for the spray series from January to December 2007 (DWD, 2019).

Monthly average temperature [°C]:											
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
4.8	3.9	6.2	11.5	14.2	17.4	17.2	16.9	12.6	8.4	3.7	1.6

Year: 2007

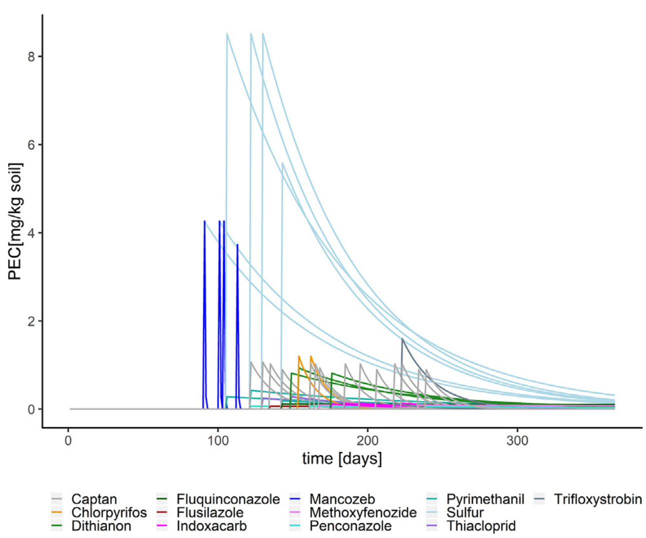

Fig. 2. Single soil exposure values for each applied substance. The x-axis represents the days of a year, the y-axis the Predicted Environmental Concentrations (PECs) in mg/kg soil. The single graphs represent the maximal PEC-value of the substances at the day of application and their degradation in soil after application. The different colors correspond to the various applied substances. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

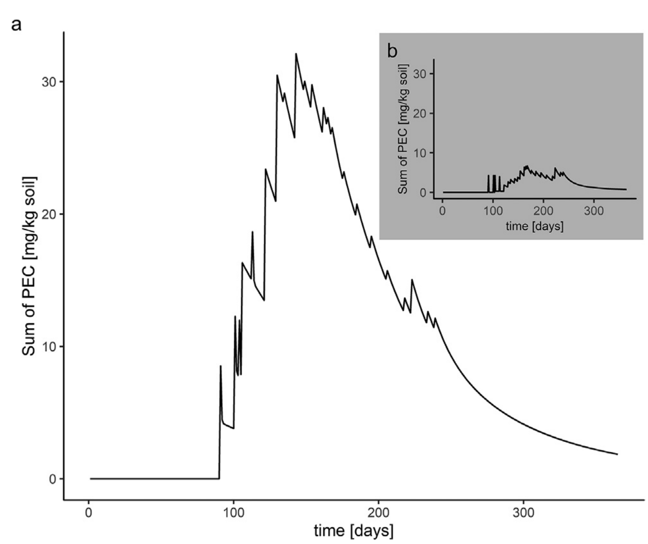

Fig. 3. Cumulated soil exposure of all pesticides used in the spray series for apple orchards for one year. The x-axis represents the days of the years, the y-axis the cumulated soil exposure (sum of PEC) over time. The integrated graphic (b) in the grey box shows the cumulated soil exposure excluding sulfur.

that the consideration of sulfur in this spray sequence has a considerable influence on the cumulated PEC values (Fig. 3b, grey box).

3.2. Mixture risk

The chronic risk of the mixture is calculated by considering the exposure and the toxicity of the substances based on the concept of concentration addition (Loewe and Muischnek, 1926). Up to now MITAS calculates the in-crop mixture risk of a pesticide spray series for earthworms in soil. It is intended, however, to include more soil organisms in the model in the future.

3.2.1. Chronic mixture risk

Acute risk to earthworms is no longer considered in the current regulation, so we focused on chronic risk (EU, 2009). For this reason, the exposure of soil organisms over long time periods has to be modelled. Fig. 4 visualizes the according chronic mixture risk of the spray series for apple orchards. For each day of the simulation (1 year) the respective chronic risk indicator (ETR) is calculated for each applied substance. In addition, the chronic mixture risk (ETRmix) is calculated from the individual risks for each day of the simulation. Mixture risk is calculated based on the concept of concentrations addition (CA).

A high ETR-value (Exposure Toxicity Ratio) represents high risk of the substance for exposed organisms. Thiacloprid (purple graph, Fig. 4) shows a chronic risk with a maximum of 1.51 (ETR). The initial chronic risk of fluquinconazole (dark green graph, Fig. 4) is 0.46 (ETR), but until the end of the simulation the substance is hardly degraded. In particular, the high risk of thiacloprid and the persistence of fluquinconazole influence the chronic mixture risk of the spray series, as shown in Fig. 4. Sulfur has no considerable influence on the mixture risk of the spray series. Calculating the mixture risk without considering sulfur hardly changes the mixture risk, due to the very low chronic toxicity of sulfur. The chronic mixture risk (red graph) has a maximum ETR-value of 3.46 meaning that the multiple exposures exceed about 3.5 times the predicted mixture-NOEC. After the day of the maximum mixture risk, ETRmix does not drop below a value of 0.54 until the end of the year.

The European Commission (EC) defines threshold-values when assessing the environmental risk of pesticides, in case of the chronic risk for earthworms ETR is 0.2 (EFSA, 2009; European Comission, 2002). We compared the chronic mixture risk of the spray series with the threshold for earthworms. As seen in Fig. 5, after 122 days the chronic mixture risk exceeds the critical threshold of 0.2 and remains clearly above this regulatory threshold during the rest of the season. For substances exceeding such thresholds, adverse effects cannot be excluded and further testing has to be conducted (European Comission, 2002).

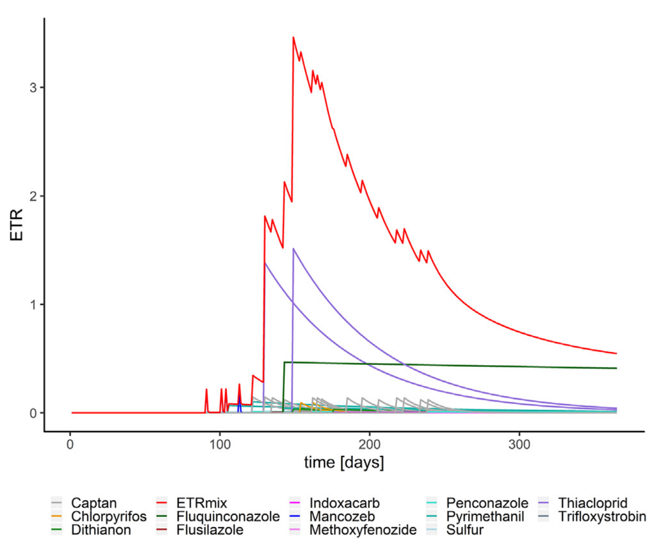

Fig. 4. Time-dependent chronic risk for the individual substances and the resulting chronic mixture risk. The x-axis represents the days of a year, the y-axis the exposure toxicity ratio (ETR). Single graphs show the individual chronic risk-development of the applied substances in consideration of their degradation. The different colors represent the various applied substances. The red graph represents the chronic mixture risk assuming concentration addition (ETRmix). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

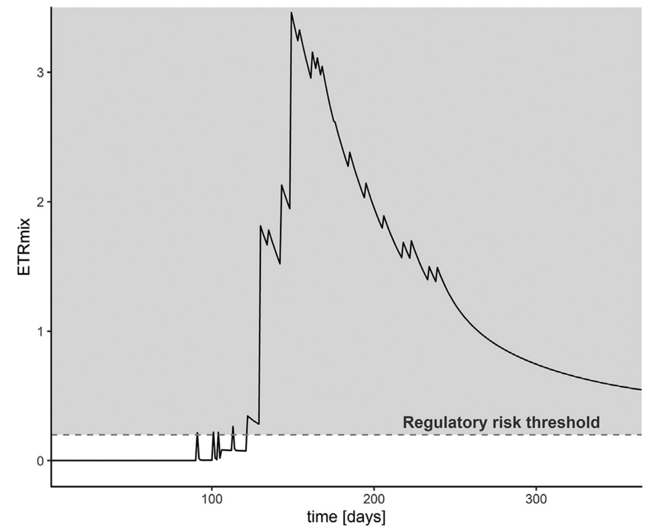

Fig. 5. Chronic mixture risk using ETR-values as indicator. The x-axis represents the days of a year, the y-axis the chronic mixture risk as ETRmix. The graph shows the chronic mixture risk of the spray series for apple orchards predicted with concentration addition. Visualization of the threshold value for chronic risk (ETR = 0.2) is done through the horizontal dotted line in the plot.

3.3. Simulation for three years

We modelled the total exposure (sum of PEC) and the chronic mixture risk (ETRmix) for the spray series for apple orchards for three subsequent years, assuming an identical annual treatment which is a realistic scenario for permanent crop cultures like apples.

Fig. 6 shows the cumulative exposure (sum of PEC) of all applied pesticides of the spray series under the same MITAS-simulation assumptions as for the single-year simulation. The daily cumulative soil exposure for three subsequent years was calculated by addition of the individual daily exposure (PEC) of all substances. After the first application at day 91 the maximum annual cumulative exposure increases only marginally during the simulated two and a half years.

To illustrate the development over several years, the chronic mixture risk for earthworms was compared with the threshold

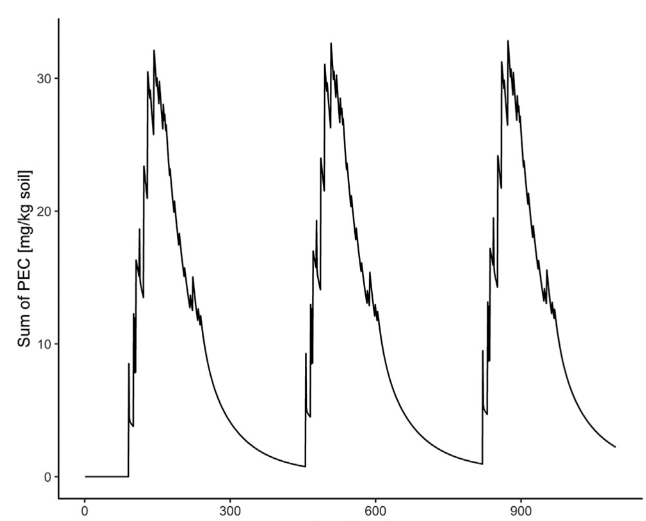

Fig. 6. Cumulated soil exposure of all pesticides used in the spray series for apple orchards over three years. The x-axis represents the days of the three years, the y-axis the cumulated exposure (sum of PEC) over time.

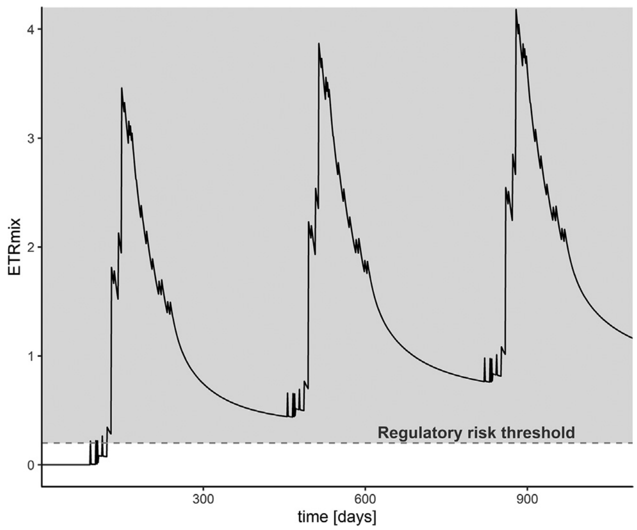

Fig. 7. Chronic mixture risk upon annual application of the spray series for apple orchards over a period of three years. The x-axes represent the days of three years, the y-axis the chronic mixture risk as ETRmix. The dotted line visualizes the threshold value for the chronic risk for earthworms (ETR = 0.2).

for earthworms (ETR = 0.2) for three subsequent years (Fig. 7). However, the chronic mixture risk (ETRmix) increases from a maximum of 3.46 in the first year up to a maximum value of 4.18 in the third year. Fig. 7 shows that the ETRmix remains above the threshold value after day 122 until the end of the simulated three years. Already at the beginning of the second simulation year, ETRmix is above the risk threshold.

4. Discussion

Pesticide application as spray series is common practice in the agricultural landscape and leads to the entry of pesticide mixtures in the environment. The corresponding mixture risk for exposed organisms can be addressed by our model MITAS.

4.1. Prediction of mixture risk

Sulfur as an inorganic substance has to be considered differently compared to organic substances with regard to exposure, since the substance is not degraded but transformed to other sulfur species (Hinckley et al., 2010). Thus, a value for dissipation instead of degradation was used for sulfur. In the spray series investigated here, sulfur is used very frequently and with high application rates, therefore it is important to consider this substance. Nevertheless, the influence of sulfur on the considered mixture risk proved to be very small due to its low toxicity.

The ETRmix (chronic mixture risk) of our selected spray series for apple orchards exceeded the threshold of an environmentally acceptable ETR (0.2) obviously starting at day 122 and does not drop below the trigger until the end of the simulation (Fig. 5). When considering subsequent years with identical application patterns, the threshold is already exceeded at the beginning of a new season. The study of Yasmin and D'Souza (2007) showed a significant reduction in the amount of young earthworms after 8 weeks when a mixture of three pesticides was applied. In a pesticide spray series, as simulated here, pesticide application would have been repeated after 8 weeks or earlier. This means that the organisms are exposed to renewed stress even though the recovery from the first application is not yet complete. The fact that ETR surpasses the threshold for long periods questions the concept of recovery in environmental risk assessment and identifies a gap that should be addressed in the future (EFSA PPR Panel et al., 2017; EFSA Scientific Committee, 2016).

However in MITAS, the exposure from the spray series is represented dynamically over time but the mixture risk calculation is based on NOECs: mainly NOEC 56d reproduction data are available. The NOEC values used here compares the exposure (PEC) of organisms to a single concentration (NOEC). This quotient (ETR) first indicates the extent to which the exposure exceeds the existing NOEC value. There is still a need for research on the validation of the calculated exposure and effects. Another shortcoming of our simulation is the lack of considering indirect effects at the population level or between populations although there is a strong need to take such interdependencies into account. As a first attempt we focused in our model on the pesticide impact on earthworms, but other soil organisms should be implemented by use of corresponding toxicity data (EFSA PPR Panel et al., 2017). Additionally the organisms in the treated area do not occur as single organisms, but an ecological community. It is important to protect these communities against the effects of multiple applications of pesticides. Within a community indirect effects and interactions can influence the effects caused by pesticides. Therefore, indirect effects should be considered in the evaluation of pesticides in the future.

We assessed the risk of the spray series assuming concentration addition. The funnel hypothesis of Warne and Hawker (1995) supports the assumption that the toxicity of mixtures with substances of variable modes of action, like in our selected spray series for apple orchards with 15 pesticides, can be assessed with the CA model. Using this model for mixtures with similarly and dissimilarly acting compounds, reliable predictions of observed mixture toxicity have been described (Backhaus et al., 2004a; Junghans et al., 2003). Thus, the CA model is broadly accepted as reasonable approximation of the mixture risk of pesticides (Frische et al., 2014).

4.2. Application simulation three years in a row

Jablonowski et al. (2012) and others demonstrated that pesticides may persist in soil at low concentrations and the accumulated mixtures might lead to long-term effects on exposed organisms. Based on this statement we simulated the long-term exposure and mixture risk of the spray series for apple orchards for earthworms. Permanent crops like apples require annually periodic pesticide applications. This was simulated with the annual application of the same spray series over three subsequent years. Our results show that in the second and third year residues from the first year of application are still present in the soil, i.e., the concentrations of all pesticide residues (sum of PEC) after one year amounts up to 1.85 mg/kg soil and after three years to 2. 23 mg/kg soil. Pesticide residues from the previous pesticide spray series remain in soil and cause the threshold value to be exceeded already at the beginning of the second and third year. Chiaia-Hernandez et al. (2017) was able to detect 45% of the pesticides applied in the soil, although sampling often took place more than one year after application.

In fact, the annual maximum chronic mixture risk (ETRmix) increases within the simulated three years and the threshold for chronic risk for earthworms was surpassed for about 2.5 years reaching a maximum of about 4 (ETRmix), which is considerably above the threshold of 0.2. This indicates that previous applications may contribute to the mixture risk of subsequent applications. Multi-year spray series, as used in permanent crops or crop rotations, cannot be considered as independent events. To evaluate the simulated data over time, additional data on the exact application of pesticides would be required to compare simulated PEC values with monitored, i.e. measured, residue analyses in the field. We are aware that the simulated PECs refer to the total concentrations of pesticides accumulating in soil with no differentiation between bioavailable and non-bioavailable residues, an experimental challenge which may be addressed by using passive sampling methods (Schmidt et al., 2013). Also non-extractable residues (NER) may have formed in such soils by aging, from which only type I NER (sequestered residues) are considered as remobilizable, whereas type II NER (covalently bound residues) and type III NER (biogenic residues) we assumed to pose no risk (Kastner et al., 2014; Schaffer et al., 2018). As a consequence, the calculated risk in MITAS may partially overestimate the actual risk as soil bioavailability is not taken into account.

5. Conclusion

The simulation of a real pesticide spray series indicates that the threshold value defined in the risk assessment is exceeded when several substances are considered for a long period of time. Due to the high frequency of pesticide applications and the different degradation behavior of the substances, soil organisms are exposed to mixtures of pesticides even over longer periods of time. The annual repetition of pesticide spray series may result in high exposure of soil organisms to pesticides. This high exposure shows that the intensive use of pesticides must be considered more closely in the future. From our findings we suggest including a modelling of the risk of spray series with multiple pesticide exposure for protection of soil organisms in the long-term risk assessment. Prior to this, additional data are required to confirm the predicted exposure and effect. So far, we applied the model to assess the risk for earthworms but it can be extended to other soil organisms and environmental compartments (such as field margins) as well, depending on data availability. The effect prediction of the model will be further expanded in future. To obtain a good prediction model regarding direct and indirect effects of pesticides in the agricultural landscape, it is necessary to couple pesticide exposure over time with effect models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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