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Final report

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Environmental risks of pesticides between forecast and reality: How reliable are results of the environmental risk assessment for individual products in the light of agricultural practice (tank mixtures, spray series)?

Final report

by

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S.K. and M.L. coordinated the project and are responsible regarding the evaluation and analysis of aquatic data (chapter 2, 3, 4), spray series (chapter 3) and the risk assessment of the selected spray series (chapter 5). Under coordination of M.R-N. B.S.S., B.D., R.O. A.S. and A.Sch. are responsible regarding the evaluation and analysis of terrestrial data (chapter 2, 3, 4), spray series (chapter 3) and the risk assessment of the selected spray series (chapter 5). These authors also compared the existing model approaches and developed the MITAS tool.

Abstract: Environmental risks of pesticides between forecast and reality: How reliable are results of the environmental risk assessment for individual products in the light of agricultural practice (tank-mixes, spray series)?

Many plant protection products (PPPs) that are authorized in the European Union for agricultural and private use consist of more than one active substance. Combination products with several active ingredients are frequently used with other PPPs in tank mixtures in a spraying sequence of field applications. Therefore, the assessment of mixture toxicity effects under realistic treatment regimes presents an essential part for the environmental risk assessment of PPPs. Many studies have addressed effects of toxicant mixtures on various organisms and endpoints. The model of concentration addition (CA) seems in many cases able to predict the joint effect of toxicant mixtures, both for aquatic and terrestrial organisms. Nevertheless, there is still a lack of understanding regarding the predictability of mixture toxicity for chronic effects and effects at higher levels of biological organization in different environmental compartments. For this, we evaluated in the present COMBITOX project existing literature and available data. We also critically reviewed available models and approaches (i.e. HAIR 2014, SYNOPS-WEB, PRIME-beta etc.) regarding their usefulness for predicting the combined risk of treatment regimes for terrestrial and aquatic ecosystems. Furthermore, we analyzed an unique and large data set of actual PPP spray series from twelve different agricultural crops in Germany to determine crop specific treatment regimes. Four spray series were selected to quantify the additional environmental risk of treatment regimes compared to single applications of active substances. The additional risk was determined using the concept of the maximum cumulative ratio (ratio of the toxicity-exposure ratio, TER, of the most toxic substance in a mixture to the cumulative TER of all components in a mixture). Our analyses show that CA can predict chronic mixture toxicity at the individual level in many cases when toxicity data are based on chronic EC_x values than less precise NOEC-values. In contrast, existing scientific literature and studies from regulatory databases hamper a clear statement on the applicability of concepts such as CA or independent action for the prediction of mixture effects on community endpoints. We further identified crop specific patterns especially in terms of applied PPP classes and treatment frequency. The risk assessment of the four spray series revealed an additional risk of treatment regimes with a median factor of 2.18 (50^{th} percentile) to 5.26 (90^{th} percentile) considering all investigated risk indicators and spray series. Finally, we developed a new approach, MITAS, to assess the time-dependent mixture exposure and toxicity in a treatment regime. We conclude that the additional risk of PPP applications in treatment regimes is ecotoxicologically relevant and must be considered for a protective risk assessment of PPPs. However, uncertainties remain regarding the influence of synergistic effects, indirect effects, seed coating, repeated exposure or environmental stressors on the impact of PPP treatment regimes.

Kurzbeschreibung: Umweltrisiken von PSM zwischen Prognose und Realität: Wie belastbar sind Ergebnisse der ökotoxikologischen Risikobewertung zum einzelnen Mittel vor dem Hintergrund der üblichen Anwendungspraxis (Tankmischungen, Spritzfolgen)?

Viele Pflanzenschutzmittel (PSM), die in der Europäischen Union für den landwirtschaftlichen und privaten Gebrauch zugelassen sind, enthalten mehr als einen Wirkstoff. Solche Kombinationsprodukte und auch Monoformulierungen mit einem Wirkstoff werden häufig mit anderen Pflanzenschutzmitteln in Tankmischungen in einer Sequenz von Feldanwendungen eingesetzt. Daher stellt die Bewertung von realistischen Behandlungsregimes einen wesentlichen Bestandteil der Umweltrisikobewertung von PSM dar. Seit Jahrzehnten beschäftigen sich viele Studien mit den Effekten von Schadstoffmischungen auf verschiedene Organismen und Endpunkte. Das Modell der Konzentrationsadditivität (CA) gilt in vielen Fällen

als geeignet die Wirkung von Schadstoffmischungen vorherzusagen. Dennoch fehlt es an Kenntnissen zur Vorhersagbarkeit von chronischen Mischungseffekten und von Effekten auf aquatische oder terrestrische Gemeinschaften. Zur Beantwortung dieser Fragestellung haben wir im COMBITOX-Projekt vorhandenes Wissen und Daten zu chronischen Effekten sowie für aquatische und terrestrische Gemeinschaften ausgewertet. Zudem haben wir verfügbare Modelle und Ansätze (z.B. HAIR 2014, SYNOPS-WEB, PRIME-beta etc.) kritisch evaluiert, um deren Nutzen für eine Risikovorhersage von Behandlungsregimes in terrestrischen und aquatischen Ökosystemen zu bewerten. Weiterhin wurde ein einzigartiger und großer Datensatz von agrarwirtschaftlichen Spritzfolgen für verschiedene Agrarkulturen in Deutschland analysiert. Aus diesem Datensatz haben wir vier Spritzfolgen ausgewählt, die verschiedene Behandlungsszenarien (Worst-Case- und Typical-Case-Spritzfolgen) für Apfel- und Winterraps darstellen. Für diese Spritzfolgen wurde das zusätzliche Umweltrisiko im Vergleich zur Einzelanwendung von PSM quantifiziert. Das zusätzliche Risiko wurde mit dem maximalen kumulativen Verhältnis (MCR) berechnet (Verhältnis des geringsten *toxicity exposure ratio*, TER, für die toxischste Substanz zum kumulativen TER einer Mischung oder Spritzfolge). Unsere Analysen ergaben, dass CA chronische Mischungseffekte auf Individuenebene in vielen Fällen vorhersagen kann, wenn die Toxizitätsdaten auf EC_X-Werten im Vergleich zu weniger präzisen NOEC-Werten basieren. Aus der verfügbaren wissenschaftlichen Literatur und regulatorischen Studien konnten keine klaren Aussagen zur Anwendbarkeit von CA oder Effektaddition für die Vorhersage von Mischungseffekten auf Gemeinschaftsebene getroffen werden. Anhand der Spritzfolgendaten haben wir kulturspezifische Muster bezüglich der angewandten PSM-Klassen und der Behandlungshäufigkeit identifiziert. Für die vier Spritzfolgen wurde ein zusätzliches Risiko der Behandlungsregime mit einem Faktor von 2,18 (50. Perzentil) bis 5,26 (90. Perzentil) über alle untersuchten Risikoindikatoren und Spritzfolgen ermittelt. Darüber hinaus wurde ein neuer Ansatz, MITAS, entwickelt, um die zeitabhängige Mischungstoxizität in einem Behandlungsregime bewerten zu können. Es wird geschlussfolgert, dass das zusätzliche Risiko von PSM-Anwendungen in Spritzfolgen ökotoxikologisch relevant ist und für eine protektive Risikobewertung von PSM berücksichtigt werden muss. Unsicherheiten bestehen noch hinsichtlich des Einflusses von Synergismen, indirekten Effekten, Saatgutbehandlung, wiederholter Exposition oder Umweltstressoren auf die Effekte von PSM-Spritzfolgen.

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List of abbreviations

a.i.	Active ingredient
a.s.	Active substance
BBCH-code	German code for developmental stages of plants
BMEL	Ministry of food and agriculture
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
CA	Concentration addition
CAS	Chemical Abstracts Service
DT_{50}	Time required for the concentration to decline to half of the initial value
EFSA	European Food Safety Authority
(E)RA	(Environmental) risk assessment
ETR	Exposure toxicity ratio
IA	Independent action
Full set	Set of studies for one mixture and corresponding single active ingredient
HQ	Hazard Quotient
INL	Institute für nachhaltige Landwirtschaft
JKI	Julius-Kühn Institute
LC_{50}	Lethal concentration resulting in 50% mortality compared to control
LD_{50}	Lethal dosis resulting in 50% mortality compared to control
LOEC	Lowest observed effect concentration
MAF	Multiple application factor
MCR	Maximum cumulative ratio
MDR	Model deviation ratio
MITAS	Mixture Toxicity of Application Series
MOA	Mode of action
NTA	Non-target arthropod
NTTP	Non-target terrestrial plant
NOAL	No observed effect level
NOEC	No observed effect concentration
OECD	Organization for Economic Cooperation and Development
PAPA	Panel Pflanzenschutzmittel-Anwendungen
PEC	Predicted environmental concentration
PER	Predicted environmental rate
PPDB	Pesticides properties database
PPP	Plant protection product

RAC	Regulatory acceptable concentration
TER	Toxicity exposure ratio
TER_{min}	Lowest toxicity exposure ratio
TER_{single}	Toxicity exposure ratio for one compound and application
TER_{mix}	Toxicity exposure ratio determined for a mixture
TF	Treatment frequency
TI	Treatment index
TU_{max}	Highest toxic unit
TU_{sum}	Sum of all toxic units
UBA	Umweltbundesamt, German Environment Agency

Summary

The relevance and prediction of effects of realistic treatment regimes in the environmental risk assessment of plant protection products

Authors: S. Knillmann[1], Björn Scholz-Starke[2], Susanne Bär[3], Benjamin Daniels[2], Tobias Frische[3], Richard Ottermanns[2], Andreas Schäffer[2], Alexandra Sybertz[2], Christian Ullrich[3], Martina Roß-Nickoll[2], Matthias Liess[1]

Introduction

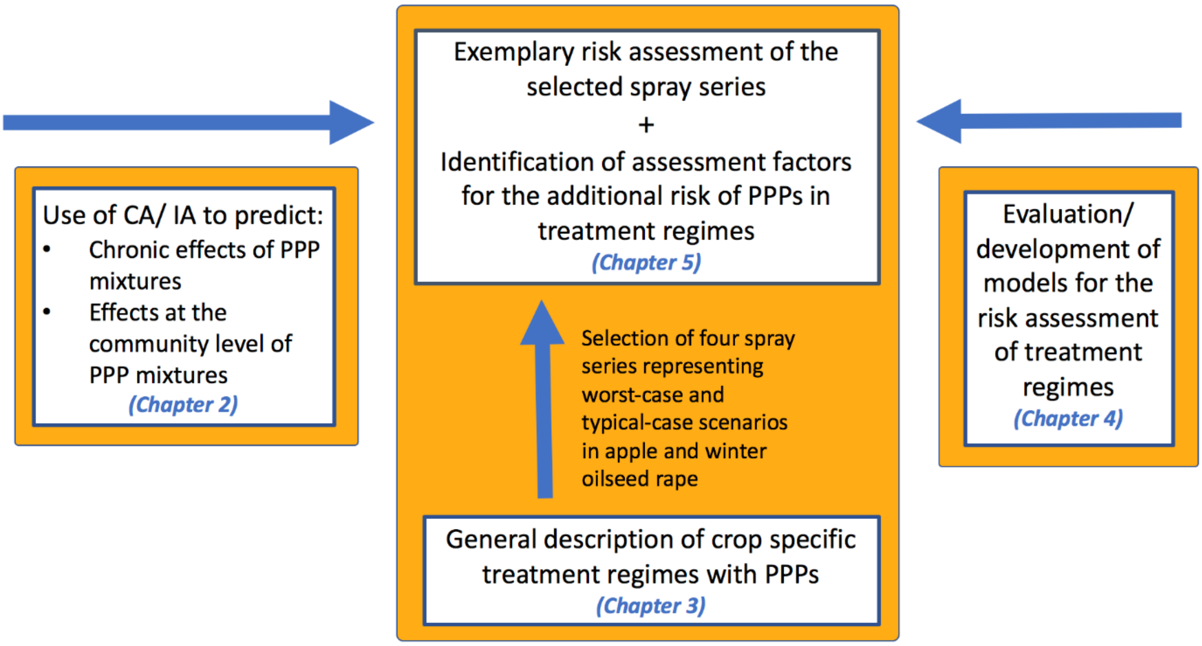
In view of the current agricultural practice, the protectivity of the environmental risk assessment (ERA) for non-target organisms conducted for individual plant protection products (PPPs) is questioned for different reasons. One issue: PPPs are commonly applied in tank mixtures and spray series that are often not predictable for regulators and formally not considered in legal procedures or a regular risk assessment. For this reason, it is necessary to know the type of tank mixtures, the frequency of its use in spray series, and the composition including the exact chronological sequence of the spraying sequence within a growing period. This information is crucial for realistic risk predictions of complex PPP mixtures in treatment regimes (Frische et al. 2016). However, such detailed data on PPPs use in Germany are usually not available, mostly only in highly aggregated form.

It is well-described that mixture toxicities of PPPs are in many cases adequately predictable for acute, mainly aquatic endpoints by applying the concept of concentration addition (CA, Loewe and Muischnek 1926, Cedergreen et al. 2014, Kortenkamp et al. 2009). One of the questions remaining in this area is whether such predictions are also possible for communities and chronic effects on individuals. This knowledge is crucial to understand and predict the ecological impact of pesticide mixtures in the field. Chronic studies include long-term reproduction studies with earthworms or aquatic invertebrates; single individuals or populations. Community studies comprise aquatic and terrestrial natural (e.g. field studies) as well as experimental systems (e.g. freshwater enclosures, mesocosms or microcosm studies, multi-species assemblages).

Within the present COMBITOX project, we aimed to answer the following questions (see also Summary Figure 1):

- How suitable are existing approaches, such as concentration addition (CA) and independent action (IA), to predict chronic PPP mixture effects and mixture effects at the community level in aquatic and terrestrial ecosystems?
- What are typical treatment regimes of main crop types in Germany in terms of applied PPPs, frequency of application and common tank mixtures?
- What approaches are available to predict the combined effects of toxicant mixtures? Do common assessment models consider the risk due to treatment regimes?
- How protective is the current environmental risk assessment of PPPs considering realistic spray series instead of single PPP applications? What is the additional risk from tank mixtures and spray series compared to single PPP applications?

Summary Figure 1: Graphical overview of COMBITOX



Methods

Analysis of scientific literature, regulatory study reports and assessment models

We reviewed the scientific literature regarding mixture effects of PPPs and the applicability of CA or IA. This review comprised laboratory experiments, semi-field or field studies including single populations and communities. We also evaluated approaches to predict mixture effects that are based on species sensitivity distributions (SSDs). In addition, we reviewed the state of art of the prediction of mixture effects and human health endpoints.

With regard to available regulatory studies, we selected mixtures for which reports were available both on single compounds and mixtures (full sets). The ecotoxicological information of PPPs related to regulatory studies was retrieved from the Information System on Chemical Safety (ICS) of the German Environment Agency.

To improve the prediction of environmental risks of PPPs and realistic PPP applications, we looked into several assessment models. The review encompassed the evaluation of mathematical approaches and mechanistic approaches with well-known tools for the risk assessment of PPPs. These tools are PRIME-beta (Pesticide Risk Mitigation Engine) (ipmPRIME), SYNOPS-WEB (*Synoptische Bewertung des Risikopotentials chemischer Pflanzenschutzmittel*, v.1.0) (JKI) and HAIR 2014 ("Harmonised environmental Indicators for pesticide Risk") (HAIR). In a subsequent step, members of the consortium (RWTH Aachen) developed an own tool, MITAS, to quantify the toxic risk of a given treatment regime over time.

Data on real use patterns of PPPs

Extensive data on real use patterns for twelve representative crops comprising a total of 873 single spray series in Germany were provided by the *Institut für Nachhaltige Landbewirtschaftung* (INL) and one additional agricultural farm. We queried ecotoxicity data of PPP from different databases (PPDB, ECOTOX, ICS) to harmonise application rate units and chemical names as well as to assess toxicities resulting from the application rates. We received registration data containing allowed substances and application rates from German authorities. The treatment data contained information on the date of application, size of the treated field, applied product, area treated, crop, farm, active substances and their contents in the applied products, applied rates and occasionally the pest that triggered application. We analyzed spray series for crop specific application patterns of PPPs. We further defined typical- and worst-case crop protection scenarios per crop by ranking toxic pressures (expressed as toxic units derived from available endpoint measures and the actual application rates). We checked the rankings for validity consulting the number of PPP applications and the regulatorily accepted indicator treatment index (TI).

Maximum cumulative ratio (MCR) and MITAS

We quantified the additional risk of tank mixtures and sequential exposure using the dataset of actual agricultural treatment regimes. For this, we used the four spray series from apple and winter oilseed rape representing one worst-case and one typical-case spray series per crop type. The respective predicted environmental concentrations (PECs) were modelled applying the tool “risk profiler” together with the German Environment Agency. We determined 28 risk indicators for each application of active substance as toxicity exposure ratios (TER). Regarding the additional risk due to tank mixtures and sequential exposure, we applied the concept of the maximum cumulative ratio (MCR). The MCR represents the ratio between accumulated risk TER_{mix} based on the concept of concentration addition (CA) and TER_{min}, the active substance with the highest estimated risk per tank mixture or spray series.

The developed model MITAS allows to estimate the time-dependent risk of pesticide mixtures, which are applied during one spray series. The model can assess the maximum mixture risk for soil organisms and the duration, how long a certain risk threshold may be exceeded based on the simulated pesticide degradation processes.

In a final step, we determined the share of spray series per crop type with potentially synergistic tank mixtures and compared the MCRs of the modelled TER-values with MCRs for aquatic monitoring data. The monitoring data comprised the assessment of PPP peak exposure and effects on macroinvertebrates in small streams. The monitoring data was recorded in Central Germany in the period 1998-2000 and 2013.

Results and discussion

Use of CA or IA to predict mixture effects of PPPs at the community level

Regarding aquatic communities, CA could predict mixtures of similar acting compounds, with results generally limited to algal communities. The predictive power of CA/IA for other aquatic communities and mixtures of dissimilar acting compounds was less clear and only a few studies were available. For the terrestrial compartments (i.e. non-target arthropods, non-target plants, soil organisms), we could not evaluate the predictability of community effects as we did not find scientific literature aiming at the description of combined effects of PPPs.

The analysis of existing field studies in the scientific literature showed that effects of pesticide mixtures on aquatic invertebrate communities can be described with the most toxic pesticide (TU_{max}) and with a very similar correlation with the sum of all toxic pesticides (TU_{sum} based on the concept of CA) in a mixture. However, TU_{max} and also TU_{sum} underestimate the observed effects on aquatic community composition two to three orders of magnitude below the acute toxicity of test organisms under laboratory conditions (Schäfer et al. 2012, Knillmann et al. 2013, Liess and von der Ohe 2005). The underestimation of pesticide effects in the field implies that all factors acting in the field (i.e. mixture effects, environmental stress, sequential exposure) are cumulating to a ”lab to field sensitivity enhancement factor” for every PPP substance.

Aquatic reports from the ICS-database comprised only three full sets of PPP mixtures. The evaluation of mixture toxicity related to the CA/IA approach at the community level was affected by variations in the study design and non-comparable communities. The analysis of regulatory earthworm field tests for the assessment of mixture toxicity revealed that only in very rare cases significant effects on biomass and abundance were detected by standard statistical procedures. Therefore, ICS-report data of earthworm field tests were not suitable for the analysis of mixture toxicity. Even the results of the novel statistical approach CPCAT showed that the significance of the identified effects observed throughout the field test only indicated a trend towards initial effects within the field tests of the full set.

Regarding the use of SSDs for the prediction of mixture effects at the community level, only few studies validated the use of SSD-based approaches for the prediction of PPP mixture effects. Examples showed that mixture predictions based on species sensitivity distributions (SSDs) provided good links to PPP effects on aquatic organisms in the field, as for example shown for the bioindicator SPEAR. However, the use of msPAF could lead to strong underestimations of effect thresholds in the field, which would require a "lab to field sensitivity enhancement factor" for each PPP substance. In addition, SSD based approaches are generally associated with a lack of toxicity data and uncertainties concerning for example taxa number or taxa selection.

Use of CA or IA to predict chronic mixture effects of PPPs

For the prediction of chronic effects of mixtures on aquatic endpoints, the use of CA was more precise by a factor of two to three for herbicide and fungicide mixtures when predictions were based on EC_X – values compared to NOEC values (Coors and Frische 2011). Further studies indicated similar findings for insecticide mixtures. Studies on chronic mixture effects with PPPs from different classes of pesticides are underrepresented according to our search. However, we identified few examples that focused on mixtures leading to synergistic effects (fungicides + insecticides) showing that such effects can persist or even increase over time.

In comparison to aquatic endpoints, CA proved to predict mixture toxicity relatively well using chronic NOEC values for earthworms Overall, the results of the MDR calculation for earthworm reproduction tests showed high compliance between predicted and observed mixture toxicity. Averaged over all pesticide groups, the MDR median of 0.67 indicated that mixture toxicity of combination products tended to be slightly more overestimated. The calculated NOECs of the single-component-studies and for mixture studies were strongly biased by the applied test concentrations. We have observed that when a significant effect can be detected, the resulting MDR is highly dependent on the single test concentration that was often chosen in the study design. This outcome did not correspond in any way to a threshold effect concentration so that reliable conclusions towards mixture toxicity mechanisms could not be drawn from these data.

We conclude for aquatic and terrestrial organisms that generally the application of effect level values (EC_x) may reduce uncertainty in the prediction of mixture toxicity. Otherwise, we could not find indications that the predictive power of CA for chronic mixture effects is significantly different from the prediction of acute mixture effects.

Prediction of mixture effects on human health endpoints

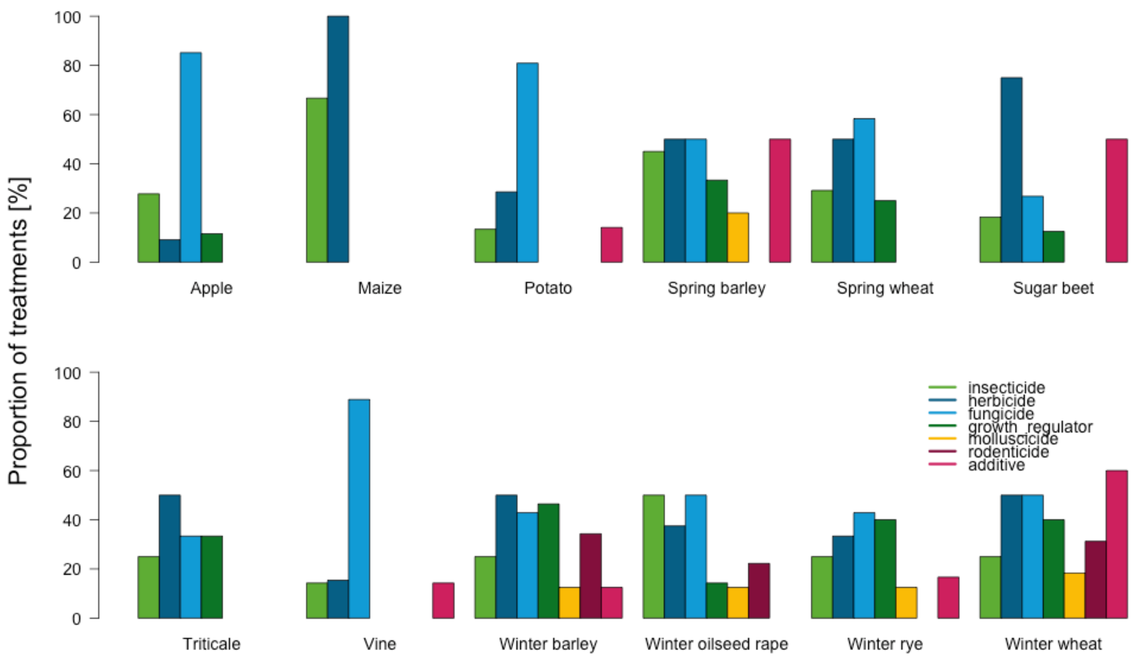
According to the state of knowledge on the prediction of mixture effects and human health assessment criteria, CA often provides a good prediction of mixture effects for different endpoints. However, non-additive effects of mixtures were also observed in some cases and there was a lack of data especially regarding information on exposure and compound-specific effects of single compounds in a mixture. Scientific opinions by the EFSA for the cumulative risk assessment of PPP residues in food assume the use of dose addition (equivalent to CA) as a pragmatic approach independent of the mode of action (MOA, EFSA-PPR-Panel 2013a) and the grouping of PPP substances to common assessment groups (CAGs) to overcome existing data gaps.

Crop specific patterns of PPP use and selection of four representative spray series

The analysis of available data on 873 spray series revealed clear crop specific patterns. The total number of active substances within maize applications was characterized by few substances. Apple was treated with the most PPP substances. Apple, vine and potato were dominated by fungicide use, cereals are sprayed by a wide variety of PPP classes (Summary Figure 2). Cereals underwent two to six spray events with 50% probability per spray series. With five to nine and four to ten spray events per season, the application frequency for winter oilseed rape and potato is slightly higher than for cereals. With a median of 20 spray events, the most frequently treated crop was the permanent culture apple. The other permanent crop, vine, ranked with potato and winter oilseed rape at medium levels of treatment frequencies and experienced eight spray events per season in the median. 63% of spray events, across all spray series and crop types, are realized through tank mixtures of several PPPs. In apple particularly, critical fungicide-insecticide mixtures are applied at 14% of all spray events. However, most tank mixtures contained PPP-classes of the same PPP class (i.e. herbicide-herbicide-combinations).

Summary Figure 2: Mean proportion of pesticide classes that were applied in spray series for twelve relevant crop types. Crops shown: apple, maize, potato, spring barley, spring wheat, sugar beet, triticale, vine, winter barley, winter oilseed rape, winter rye and winter wheat

A fungicide bar proportion of 80% means that one or more fungicide a.s. was applied in an average of 80% of all spray events within a spray series and per crop type. We averaged the proportions for all spray series within each of the twelve crop types.



The two priority criteria “toxic unit soil” and “toxic unit aquatic” enabled a valid description of toxic pressures and thus a plausible ranking of the spray series. Further indices such as the total number of spray events and the treatment index provided additional information for the selection of surrogate spray series for typical- as well as worst-case scenarios. The classification of the different spray series proved to be in good accordance with the treatment indices of JKI for the focal crop winter oilseed rape, as well as for cereals, potatoes, maize, and sugar beet crops. In apple, the treatment indices of both, the worst-case spray series (TI = 34) and the typical-case spray series (TI = 28) were close to the range of the treatment index reported by the JKI for the period between the years 2011 and 2013 (TI = 28-33). This finding suggests that the data at hand reflects more typical situation for apple and does not include realistic extremes. For the crop winter oilseed rape, worst- and typical case spray series were in closer agreement with the corresponding treatment indices.

Summary Table 1: General comparison of the tools PRIME-beta, HAIR2014, SYNOPS-WEB and MITAS

The table displays the names of the various tools in the heading. The first column lists the different aspects of the tools. Among others, the symbols ✓ (true), ~ (partly true) and ✗ (does not apply) are used to compare the tools.

	PRIME-beta	HAIR	SYNOPS-WEB (v.1.0)	MITAS
Substance degradation	~	✓	✓	✓
Multiple application	✗	✓	✓	✓
Risk classes / risk thresholds	✓	✗	✓	✓
Calculation of mixture risk	✓	✗	✓	✓
Time-dependent mixture risk (ETR mix)	✗	✗	✗	✓
Period exceeding a risk threshold (ETR mix)	✗	✗	✗	✓
Simulation time		more than 1 year	1 year	more than 1 year
Risk indicator		ETR	ETR	ETR / TER
Compartments	Soil, surface waters	Soil, surface waters, field margin biotopes	Soil, surface waters, field margin biotopes	Soil

Assessment models

Most of the investigated models are not able to predict the mixture toxicity from individual a.s. of PPPs within a treatment regime and do not consider synergistically interacting substances. The heuristic model with k-functions is one of the few models predicting mixture toxicity for interacting substances (e.g. synergism). However, this requires a lot of experimental information. None of the three models examined so far is able to reflect the complexity of a spray series (multiple applications, mixture toxicity, sequences, degradation, see also Summary Table 1). Apart from synergistic effects MITAS does integrate these aspects and the model is still expandable.

Risk assessment of four spray series regarding protectivity and the additional risk due to tank mixtures and spraying sequences

Regarding the protectivity of the risk assessment in the selected spray series of apple and winter oilseed rape, we frequently observed unacceptable risks at the Tier 1-level. We determined these risks even after the consideration of risk management measures (drift reduction, distance regulation). From a cross-sectional point of view through the assessment areas only few single spray events of PPP did not cause any exceedances of the risk triggers. Regarding Higher Tier-risk indicators (i.e. regulatory acceptable concentration – RAC), we determined unacceptable risks for the application of thiacloprid in apple and winter oilseed rape. The identified unacceptable risks of single PPP applications can be explained with (i) outdated risk assessment, (ii) not considered risk refinements for Tier 1-risk indicators, (iii) emergency authorizations or (iv) the results of a risk-benefit-analysis.

Tank mixtures of the investigated spray series consisted of two to four active substances and mixture toxicity was often dominated by the substance with the highest toxicity, i.e., the substance with the lowest TER value (TER_{min}). We quantified the additional risk of tank mixtures as the MCR (ratio of TER_{min}/TER_{mix}) with TER_{mix} based on concentration addition. MCR per tank mixture was relatively similar for all 28 risk indicators and only showed little differences between the investigated spray series. The MCR indicated an increase in risk by a median factor for all risk indicators and tank mixtures between 1.2 and 1.4 for each spray series. Nevertheless, the true increase in the risk of mixture compared to single a.s. applications might be slightly underestimated, because ecotoxicological data were partly not available for all a.s. and endpoints in the present study.

We also applied the MCR to account for the additional risk of the spraying sequence compared to the single a.s. application with the highest toxic pressure (TER_{min}) of the complete spray series. For pragmatic reasons, we did not consider recovery, adaptation or an increase in sensitivity between the single exposure events. We detected major differences regarding the MCRs of the single risk indicators. Independent of the spray series, we observed the highest MCRs mainly for mammals, birds and fish. The median MCR across all risk indicators ranged between 3.40 (apple, worst-case spray series) and 1.17 (winter oilseed rape, typical-case spray series). The worst-case spray series in apple had the highest number of spray events (26) and also showed the highest additional risk due to the spraying sequence. Considering the MCRs across all 28 risk indicators and the four spray series, we determined an overall increase in risk with a median factor of 2.18 (50th percentile) to 5.26 (90th percentile). As for tank mixtures, the true MCR might be slightly higher due to missing data regarding selected a.s. and endpoints.

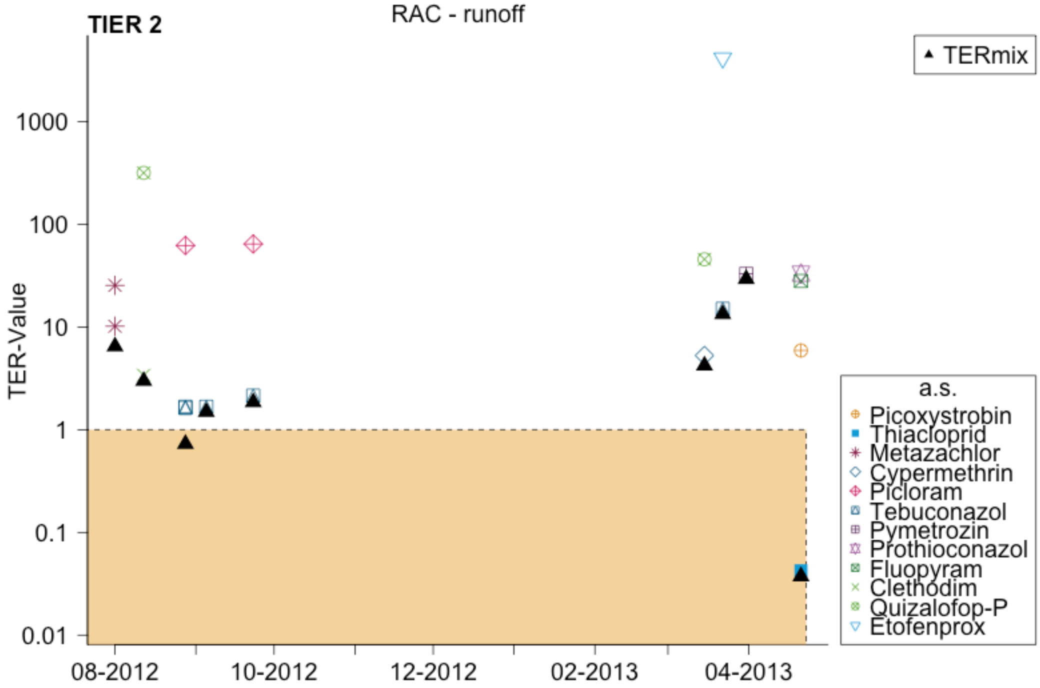
MITAS enables the mixture risk (ETR) of a spray sequence in apple cultivation to be observed over time. In this case, the threshold value for the chronic risk (earthworm, ETR = 0.2) for the spray sequence is exceeded from approximately half of the simulation time until the end. Adverse effects on the organism cannot be excluded for about half of the simulation period.

Maximum cumulative ratio (MCR) for aquatic monitoring data in Central Germany

MCRs for a set of aquatic monitoring data of PPP exposure in small streams in Germany in 2013 are in a similar range to modelled MCR-values for the selected spray series. We assessed the MCR for the monitoring data per measured exposure events (median factor of increase 1.35-1.53 for invertebrates and algae, respectively) as the equivalent to tank mixtures and over the main monitoring season (median factor of increase 1.58-1.74 for invertebrates and algae, respectively) as the equivalent to spray series.

Summary Figure 3: TER_{single} and TER_{mix} per tank mixture for one exemplary TER-risk indicator (regulatory acceptable concentration - RAC) in winter oilseed rape - worst case spray series

Each colored point displays the application of an active substance (a.s.) with available TER_{single} values. No TER_{single} values were available for the applied a.s. clopyralid, dimethenamid-P (RAC runoff). TER_{mix} was determined based on all available TER_{single} values per application date. The dashed horizontal line at TER = 1 represents the threshold of acceptable risk. The area below this threshold is colored orange to highlight TER-values that present a risk.



Conclusions

CA predicts chronic mixture toxicity at the individual level in many cases when toxicity data are based on more precise chronic EC_x values. In contrast, existing data in scientific literature and regulatory databases on community endpoints do not allow a clear statement on the applicability of additive concepts (i.e. CA or IA) for the prediction of mixture effects.

We identified that realistic treatment regimes of PPPs lead to a relevant additional risk for the environment. The analyzed tank mixtures and treatment regimes were often ecotoxicologically dominated by one or few PPP substances. However, with the MCR approach and without considering synergistic effects, we are able to show that the total risk of PPP mixtures and spray series can exceed the risk of the most toxic individual substances. This is especially important, since single PPPs are often regulated very close to the threshold of acceptable risk.

The surrogate spray series can be further used to assess the protectivity of realistic treatment regimes with different application intensities. However, application patterns identified here will change due to registration and bans of substances as well as, climate and land use driven changes in treatment regimes. Accordingly, a realistic ERA needs regularly updated information on treatment regimes.

Uncertainties remain related to potential synergistic effects of PPPs from treatment regimes. Further, the relevance of seed coating, repeated exposure and the presence of additional environmental stressors might increase the risk of treatment regimes on non-target organisms.

All these aspects of uncertainty should be taken into consideration in future projects to provide a protective and realistic extrapolation of PPP effects from the lab to the field.

Zusammenfassung

Umweltrisiken von PSM zwischen Prognose und Realität: Wie belastbar sind Ergebnisse der ökotoxikologischen Risikobewertung zum einzelnen Mittel vor dem Hintergrund der üblichen Anwendungspraxis (Tankmischungen, Spritzfolgen)?

Einführung

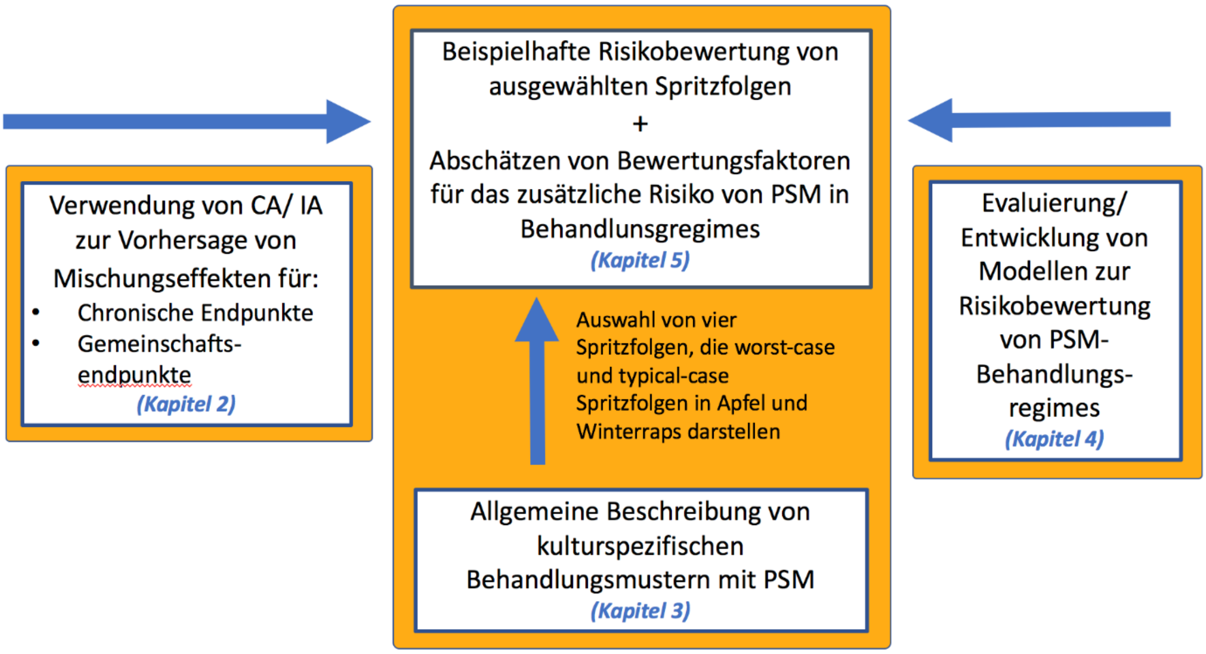
Vor dem Hintergrund der aktuellen landwirtschaftlichen Praxis wird die Protektivität der Risikobewertung von einzelnen Pflanzenschutzmitteln (PSM) für Nichtzielorganismen aus verschiedenen Gründen in Frage gestellt. PSM werden häufig in Tankmischungen und Spritzfolgen eingesetzt, die für die Regulatorik oft nicht vorhersehbar sind und in einer regulären Risikobewertung nicht berücksichtigt werden. Aus diesem Grund ist es notwendig, die Art der Tankmischungen, die Häufigkeit ihrer Verwendung in Spritzfolgen und den genauen zeitlichen Ablauf der PSM-Anwendungen in einer Spritzfolge zu kennen. Diese Informationen sind ausschlaggebend für eine realistische Risikovorhersage komplexer PSM-Mischungen in Behandlungsregimes (Frische et al. 2016). Solche Daten über den Einsatz von PSM sind in Deutschland jedoch in der Regel nicht verfügbar oder liegen meist nur in hochaggregierter Form vor.

Auf der Basis zahlreicher Untersuchungen gilt das Konzept der Konzentrationsadditivität (CA, Loewe und Muischnek 1926) als geeignet um die Mischungstoxizität von PSM für akute und vor allem aquatische Endpunkte ausreichend vorherzusagen (Cedergreen et al. 2014, Kortenkamp et al. 2009). Offene Fragen in diesem Bereich sind jedoch, ob solche Vorhersagen auch für chronische Mischungseffekte auf Individuenebene und Gemeinschaften möglich sind. Dieses Wissen ist entscheidend, um die ökologischen Auswirkungen von Pestizidmischungen auf die Umwelt zu verstehen und abzuschätzen. Chronische Studien umfassen zum Beispiel Langzeitreproduktionsstudien mit Regenwürmern oder aquatischen Wirbellosen auf Individuen- oder Populationsebene. Gemeinschaftsstudien umfassen aquatische und terrestrische Freilanduntersuchungen sowie experimentelle Studien (z.B. Mesokosmen- oder Mikrokosmenstudien, artübergreifende Gemeinschaften).

Im Rahmen des vorliegenden COMBITOX-Projekts haben wir uns mit den folgenden Fragen beschäftigt (Abbildung 1):

- Wie geeignet sind die bestehenden Ansätze Konzentrationsadditivität (CA) und Effektaddition (IA), um chronische PSM-Mischungseffekte und Effekte auf aquatische und terrestrische Gemeinschaften vorherzusagen?
- Welche typischen Behandlungsregimes gibt es für die wichtigsten Kulturpflanzen in Deutschland in Bezug auf angewandte PSM, Anwendungshäufigkeit und häufige Tankmischungen?
- Welche Ansätze gibt es, um die kombinierte Wirkung von Schadstoffmischungen vorherzusagen? Berücksichtigen gängige Bewertungsmodelle das Risiko von Behandlungsregimen?
- Wie protektiv ist die aktuelle Risikobewertung, wenn realistische Spritzfolgen statt einzelne PSM und deren Anwendungen berücksichtigt werden? Wie hoch ist das zusätzliche Risiko von Tankmischungen und Spritzfolgen im Vergleich zu einzelnen PSM?

Abbildung 1: Graphische Übersicht von COMBITOX



Methoden

Analyse der wissenschaftlichen Literatur, regulatorischen Studienberichte und Bewertungsmodelle

Wir haben verfügbare wissenschaftliche Literatur auf die Anwendbarkeit von CA oder IA zur Vorhersage von PSM-Mischungseffekten analysiert. Die Recherche umfasste Studien mit Laborexperimenten, naturnahen Experimenten oder Feldstudien mit Individuen, Populationen und Gemeinschaften für terrestrische und aquatische Ökosysteme. Dabei haben wir haben auch Ansätze berücksichtigt, die auf der Empfindlichkeitsverteilung von Arten basieren (*species sensitivity distribution* - SSDs) und den aktuellen Wissensstand zur Vorhersage von Mischungseffekten für Endpunkte der menschlichen Gesundheit zusammengetragen.

In einem weiteren Schritt haben wir Daten aus bestehenden Zulassungsstudien analysiert. Wir haben hierfür PSM-Mischungen ausgewählt, für die sowohl für die einzelnen Wirkstoffe als auch für die Mischung Berichte vorliegen (*Full sets*). Die ökotoxikologischen Informationen für die PSM in den regulatorischen Studien wurden aus dem Informationssystem zur Chemikaliensicherheit (ICS) des Umweltbundesamtes abgerufen.

Zur Verbesserung der Vorhersage von realistischen PSM-Anwendungen und deren Umweltrisiken, haben wir mehrere Bewertungsmodelle evaluiert. Die Auswertung beinhaltete die Bewertung von mathematischen und mechanistischen Ansätzen mit bekannten Modellen für die Risikobewertung von PSM. Diese Modelle sind PRIME-beta (*Pesticide Risk Mitigation Engine*) (ipmPRIME), SYNOPS-WEB (Synoptische Bewertung des Risikopotenzials chemischer Pflanzenschutzmittel, v.1.0) (JKI) und HAIR 2014 (*Harmonised Environmental Indicators for Pesticide Risk*) (HAIR). Darüber hinaus entwickelten die Mitglieder des Konsortiums (RWTH Aachen) einen eigenen Modellansatz, MITAS, um das ökotoxikologische Risiko einer PSM-Spritzfolge über die Zeit zu quantifizieren.

PSM-Anwendungsdaten

Über das Institut für Nachhaltige Landbewirtschaftung (INL) und einem Einzelbetrieb wurden umfangreiche Spritzfolgendaten für zwölf repräsentative Kulturen mit insgesamt 873 Einzelspritzfolgen in Deutschland zur Verfügung gestellt. Zudem haben wir Daten zur PSM-Ökotoxizität und zur Zulassung aus verschiedenen Datenbanken (PPDB, ECOTOX, ICS) abgefragt (bzw. von den entsprechenden Behörden erhalten), um die Anwendungseinheiten und chemischen Namen zu harmonisieren sowie die daraus resultierende Toxizität zu charakterisieren. Die Spritzfolgendaten enthielten Informationen über das Datum der Anwendung, Feldgröße, Produktname, Behandlungsfläche, Kultur, Betrieb, Wirkstoffe und deren Konzentration in den Produkten, Anwendungsmengen und zum Teil die Indikation. Auf dieser Basis haben wir Spritzfolgen für kulturspezifische Anwendungsmuster von PSM analysiert. Darüber hinaus haben wir typische und worst-case Pflanzenschutzszenarien pro Kulturpflanze definiert, indem wir die toxischen Belastungen (ausgedrückt als toxische Einheiten, die sich aus verfügbaren Endpunktmessungen und den tatsächlichen Ausbringungsmengen ergeben) klassifiziert und in eine Rangfolge gebracht haben. Am Ende haben wir die Rangfolgen und Szenarien mit dem Behandlungsindex (*treatment index* – TI) und der Anzahl an Anwendungen auf die entsprechende Validität überprüft.

Maximum cumulative ratio (MCR) und MITAS

Das zusätzliche Risiko von Tankmischungen und sequentieller Exposition quantifizierten wir auf Basis der tatsächlichen Spritzfolgendaten. Dazu haben wir die vier repräsentative Spritzfolgen aus Apfel- und Winterraps verwendet, die jeweils den Worst-Case und Typical-Case pro Kulturart darstellen. Die erwarteten Umweltkonzentrationen wurden hierfür gemeinsam mit dem Umweltbundesamt und dem Modell *Risk Profiler* berechnet. Insgesamt haben wir 28 Risikoindikatoren für jede Wirkstoffanwendung auf Basis des *toxicity exposure ratio* (TER) ermittelt. Im Hinblick auf das zusätzliche Risiko durch Tankmischungen und sequentielle Exposition haben wir das Konzept des *maximum cumulative ratio* (MCR) angewendet. Der MCR stellt das Verhältnis zwischen dem kumulierten Risiko TER_{mix} (basierend auf dem Konzept der Konzentrationsaddition) und TER_{min} dar, dem Wirkstoff mit dem höchsten geschätzten Risiko pro Tankmischung oder der gesamten Spritzfolge.

Neben dem MCR-Ansatz ermöglichte das im Projekt entwickelte Modell MITAS die Abschätzung des zeitabhängigen Risikos von PSM-Applikationen, die während einer Spritzfolge angewendet werden. Mit diesem Modell kann nicht nur das maximale Mischungsrisiko für Bodenorganismen abgeschätzt werden, sondern auch, wie lange aufgrund der simulierten Abbauprozesse ein bestimmter Risikoschwellenwert überschritten werden kann.

Abschließend haben wir den Anteil an potenziell synergistischen Tankmischungen für alle Spritzfolgen pro Kulturart ermittelt und die MCR-Faktoren der TER-Werte aus den Spritzfolgen mit MCRs aus aquatischen Monitoringdaten verglichen. Die Monitoringdaten umfassten die Messung der PSM-Spitzenbelastung und der Auswirkungen auf Makroinvertebraten in kleinen Fließgewässern. Die Monitoringdaten wurden in Mitteldeutschland im Zeitraum 1998-2000 und 2013 erhoben.

Ergebnisse und Diskussion

Verwendung von CA oder IA zur Vorhersage von Mischungseffekten von PSM auf Gemeinschaftsebene

In Bezug auf aquatische Gemeinschaften und auf Basis der verfügbaren Literatur kann CA Mischungen ähnlich wirkender Substanzen vorhersagen. Diese Aussage beschränkt sich jedoch im Wesentlichen auf Algengemeinschaften. Die Vohersagefähigkeit von CA/IA für andere aquatische Gemeinschaften und Mischungen von ungleich wirkenden Substanzen war weniger klar und es lagen zudem nur wenige Studien vor. Für die terrestrischen Bewertungsbereiche (d.h. Nicht-Ziel-Arthropoden, Nicht-Ziel-Pflanzen, Bodenorganismen) konnten wir die Verwendung von CA/IA zur Vorhersage von Gemeinschaftseffekten nicht bewerten, da keine Studien vorlagen, welche kombinierte Wirkungen von PSM beschrieben haben.

Die Analyse von Freilandstudien zeigt, dass die Auswirkungen von Pestizidmischungen auf aquatische Wirbellose mit dem toxischsten Pestizid (TU_{max}) sowie der Summe aller toxischen Pestizide (TU_{sum} nach dem Konzept der CA) in einer Mischung beschrieben werden können. TU_{max} und auch TU_{sum} unterschätzen jedoch die beobachteten Effekte auf die aquatische Gemeinschaft um zwei bis drei Größenordnungen im Vergleich zur akuten Toxizität von Testorganismen unter Laborbedingungen. Die Unterschätzung der Wirkschwellen im Feld deutet an, dass für jeden PSM-Wirkstoff alle Einflussfaktoren (d.h. Mischungseffekte, Umweltstress, sequentielle Exposition) zu einem „*lab to field sensitivity enhancement factor*" aufsummiert werden müssten.

Studienberichte aus der ICS-Datenbank beinhalteten drei *full sets* mit PSM-Mischungen und deren Wirkung auf aquatische Gemeinschaften. Die Bewertung der Mischungstoxizität dieser Datensätze mit CA/IA wurde jedoch durch Unterschiede im Studiendesign und wenig vergleichbaren Gemeinschaften beeinträchtigt. Die Auswertung von Regenwurmfeldversuchen zur Beurteilung der Mischungstoxizität ergab, dass signifikante Effekte auf Biomasse und Abundanz nur in seltenen Fällen durch statistische Standardverfahren festgestellt werden können. Daher waren die ICS-Studien zu Regenwurmfeldversuchen nicht für die Analyse der Mischungstoxizität geeignet. Auch die Ergebnisse der neuartigen Statistik CPCAT bewiesen, dass die Signifikanz der im Feldversuch beobachteten Effekte nur einen Trend zu ersten Wirkungen innerhalb der Feldversuche des gesamten *full sets* anzeigt.

Bezüglich der Verwendung von SSDs zur Vorhersage von Mischungseffekten auf Gemeinschaftsebene konnten wir nur wenige Studien identifizieren, die den Einsatz von SSD-basierten Ansätzen zur Vorhersage von PSM-Mischungseffekten validiert haben. Beispiele zeigen, dass Mischungsvorhersagen mit SSDs gut mit Freilandeffekten von PSM auf aquatische Wirbellose zusammenhängen (z. B. Bioindikator SPEAR). Es wurde aber auch beschrieben, dass die Verwendung von SSD-basierten Ansätzen zu einer starken Unterschätzung der Effektschwellen im Freiland führen können. Eine solche Unterschätzung würde ebenfalls einen „*lab to field sensitivity enhancement factor*" für jeden PSM-Wirkstoff erfordern. Zudem sind SSDs generell mit einem Fehlen an Toxizitätsdaten und Unsicherheiten, z.B. bezüglich Artanzahl oder Artauswahl, verbunden.

Verwendung von CA/IA zur Vorhersage von chronischen Mischungseffekten durch PSM

Die Literaturrecherche ergab, dass die Vorhersage chronischer Mischungseffekte für aquatische Endpunkte mit CA um den Faktor zwei bis drei genauer war, wenn die Vorhersagen auf EC_X-Werten im Vergleich zu NOEC-Werten basierten (Herbizid-und Fungizidmischungen, Coors und Frische 2011). Wenige weitere Publikationen beschrieben ähnliche Ergebnisse für Insektizidmischungen. Studien über die Vorhersage chronischer Mischungseffekte mit verschiedenen PSM-Typen (z. B. Herbizide + Insektizide) sind laut unserer Auswertung unterrepräsentiert. Es gab jedoch Beispiele, die sich auf Mischungen mit synergistischen Effekten konzentriert haben (Fungizide + Insektizide). Für diese Mischungen wurde gezeigt, dass die synergistischen Mischungseffekte über mehrere Wochen andauern oder sogar zunehmen können.

Im Vergleich zu den aquatischen Endpunkten konnte der CA-Ansatz die Mischungstoxizität für Regenwürmer unter Verwendung chronischer NOEC-Werte gut vorhersagen. Insgesamt ergaben die Ergebnisse der MDR-Berechnung für Regenwurmreproduktionstests eine hohe Übereinstimmung zwischen vorhergesagter und beobachteter Mischungstoxizität. Der MDR-Median war 0,67 (gemittelt über alle PSM-Gruppen) und weist darauf hin, dass die Mischungstoxizität tendenziell etwas stärker überschätzt als unterschätzt wurde. Die berechneten NOECs für einzelne Substanzen und für die Mischung sind stark durch die angewandten Testkonzentrationen beeinflusst. Wir haben beobachtet, dass, wenn ein signifikanter Effekt festgestellt werden kann, der resultierende MDR stark von der einzelnen Testkonzentration im Studiendesign abhängt. Dieses Ergebnis entsprach keiner Schwellenwert-Effektkonzentration, so dass aus diesen Daten keine zuverlässigen Rückschlüsse auf die Mechanismen der Mischungstoxizität gezogen werden konnten.

Zusammenfassend kann die Anwendung von Effektkonzentrationen (EC_x) die Unsicherheit bei der Berechnung der Mischungstoxizität verringern. Davon abgesehen konnten wir keine Hinweise darauf finden, dass sich die Vorhersagekraft von CA bei chronischen Mischungseffekten signifikant von der Vorhersage akuter Mischungseffekte unterscheidet.

Vorhersage von Mischungseffekten auf Endpunkte der menschlichen Gesundheit

Nach dem Wissensstand über die Vorhersage von Mischungseffekten liefert CA oft eine robuste Vorhersage von Mischungseffekten auf verschiedene Endpunkte der menschlichen Gesundheit. Allerdings wurden in einigen Fällen auch nicht-additive Effekte von Mischungen beobachtet. Zudem fehlen generell Daten zur Schadstoffexposition und zu den spezifischen Wirkmechanismen einzelner Substanzen in einer Mischung. Wissenschaftliche Stellungnahmen der EFSA zur kumulativen Risikobewertung von PSM-Rückständen in Lebensmitteln schlagen die Verwendung der Dosisaddition (äquivalent zu CA) unabhängig von der Wirkungsweise der einzelnen Mischungskomponenten vor (MOA, EFSA 2013a). Des Weiteren wurden Ansätze entwickelt, um PSM-Substanzen in gemeinsame Bewertungsgruppen (*common assessment groups* - CAGs) zu klassifizieren und somit bestehende Datenlücken zu überwinden.

Kulturspezifische Anwendungsmuster von PSM und Auswahl von vier repräsentativen Spritzfolgen

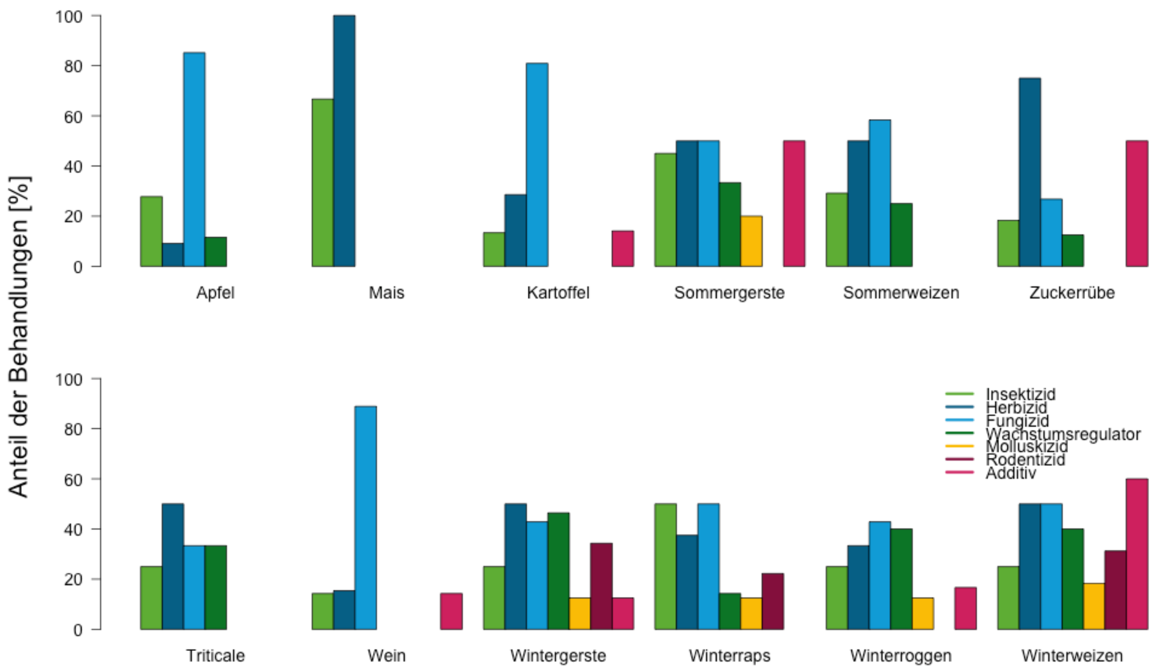
Die Analyse der verfügbaren Daten von 873 Spritzfolgen ergab klare kulturspezifische Muster. Die Gesamtzahl der Wirkstoffe in Maisspritzfolgen war durch wenige Substanzen gekennzeichnet. Die Dauerkultur Apfel wurde mit den meisten unterschiedlichen PSM-Substanzen behandelt. Bei Apfel, Wein und Kartoffel dominierte der Einsatz von Fungiziden, wobei Getreide mit von einer Vielzahl von PSM-Klassen behandelt wurde (Abbildung 2). Im Getreide gab zwei bis sechs Behandlungen mit einer Wahrscheinlichkeit von 50 % pro Spritzfolge. Mit fünf bis neun und vier bis zehn Behandlungen pro Saison war die Anwendungshäufigkeit bei Winterraps und Kartoffel etwas höher als bei Getreide. Apfel war mit einem Median von 20 Behandlungen die am häufigsten behandelte Kulturart. Die andere Dauerkultur, Wein, war vergleichbar mit Kartoffel- und Winterraps und wurde acht mal pro Saison im Median behandelt. 63 % der Behandlungen aller Spritzfolgen und Kulturarten wurden mit Tankmischungen aus mehreren PSM realisiert. Insbesondere bei Apfel wurden bei 14 % aller Behandlungen kritische Fungizid-Insektizid-Mischungen eingesetzt. Die meisten

Tankmischungen enthielten jedoch PSM-Klassen der gleichen Art (z. B. Herbizid-Herbizid-Kombinationen).

Die beiden Kriterien zur Priorisierung " toxische Einheit – Boden (*toxic unit soil*)" und "toxische Einheit – aquatisch (*toxic unit aquatic*)" ermöglichten eine gute Beschreibung der toxischen Belastungen und damit eine plausible Klassifizierung der Spritzfolgen. Weitere Indizes wie die Gesamtzahl der PSM-Behandlungen und der Behandlungsindex (TI) lieferten zusätzliche Informationen für die Auswahl der repräsentativen Spritzfolgen (typische und worst-case Szenarien). Die Klassifizierung der verschiedenen Szenarien stimmte gut mit den Behandlungsindizes vom Julius-Kühn-Institut (JKI) für die Kulturen Winterraps, Getreide, Kartoffeln, Mais und Zuckerrüben überein. Für die Kultur Apfel lagen die Behandlungsindizes für die Worst-Case-Spritzfolge (TI = 34) und auch die Typical-Case-Spritzfolge (TI = 28) im Bereich Behandlungsindexes (TI = 28-33), welcher vom JKI für den Zeitraum zwischen 2011 und 2013 ermittelt wurde. Dieses Ergebnis deutet darauf hin, dass die vorliegenden Daten eine typische Situation für Apfel widerspiegeln und keine realistischen Extreme beinhalten.

Abbildung 2: Mittlerer Anteil der PSM-Klassen, die in den Spritzfolgen der zwölf relevanten Kulturarten angewendet wurden. Dargestellte Kulturen: Apfel, Mais, Kartoffel, Sommergerste, Sommerweizen, Zuckerrüben, Triticale, Wein, Wintergerste, Winterraps, Winterroggen und Winterweizen

Ein Fungizidanteil von 80 % bedeutet, dass ein oder mehrere Fungizidwirkstoffe bei durchschnittlich 80 % aller Behandlungen innerhalb einer Spritzfolge und pro Kulturart eingesetzt wurden. Wir haben die Anteile für alle Spritzfolgen innerhalb der zwölf Kulturen gemittelt.



Bewertungsmodelle

Die meisten der untersuchten Modelle sind nicht in der Lage, die Mischungstoxizität von PSM-Wirkstoffen innerhalb eines Behandlungsregimes vorherzusagen und berücksichtigen keine synergistischen Effekte. Das heuristische Modell mit k-Funktionen ist eines der wenigen Modelle, welches die Mischungstoxizität für interagierende Substanzen (z.B. Synergismus) vorhersagt. Dies erfordert jedoch eine Menge experimenteller Informationen. Keines der drei bisher untersuchten Modelle (HAIR2014, PRIME-beta, SYNOPS-WEB) ist in der Lage, die Komplexität einer Spritzfolge widerzuspiegeln (Mehrfachanwendung, Mischungstoxizität, Sequenzen, Abbau, siehe auch Tabelle 1). Außer der synergistischen Effekte integriert MITAS diese Aspekte und das Modell ist zudem erweiterbar.

Tabelle 1: Allgemeiner Vergleich der Modelle PRIME-beta, HAIR2014, SYNOPS-WEB und MITAS

Die Tabelle zeigt die Namen der verschiedenen Modelle in der Überschrift an. Die erste Spalte listet die verschiedenen Aspekte der Werkzeuge auf. Zum Vergleich der Modelle werden unter anderem die Symbole ✓ (trifft zu), ~ (trifft teilweise zu) und × (trifft nicht zu) verwendet.

	PRIME beta	HAIR	SYNOPS-WEB (v.1.0)	MITAS
Wirkstoffabbau	~	✓	✓	✓
Mehrfachanwendung	×	✓	✓	✓
Risikoklassen/ Risikoschwellenwerte	✓	×	✓	✓
Berechnung des Mischungsrisikos	✓	×	✓	✓
Zeitabhängiges Mischungsrisiko (ETR_{mix})	×	×	×	✓
Zeitintervall, wo ein Risikoschwellenwert überschritten wird (ETR_{mix})	×	×	×	✓
Simulationszeitraum		Mehr als 1 Jahr	1 Jahr	Mehr als 1 Jahr
Risikoindikator		ETR	ETR	ETR / TER
Bereiche	Boden, Oberflächen-wasser	Boden, Oberflächen-wasser, Saumbiotope	Boden, Oberflächen-wasser, Saumbiotope	Boden

Risikobewertung von vier Spritzfolgen hinsichtlich der Protektivität für Nicht-Zielorganismen und dem zusätzlichen Risiko durch Tankmischungen und Spritzfolgen

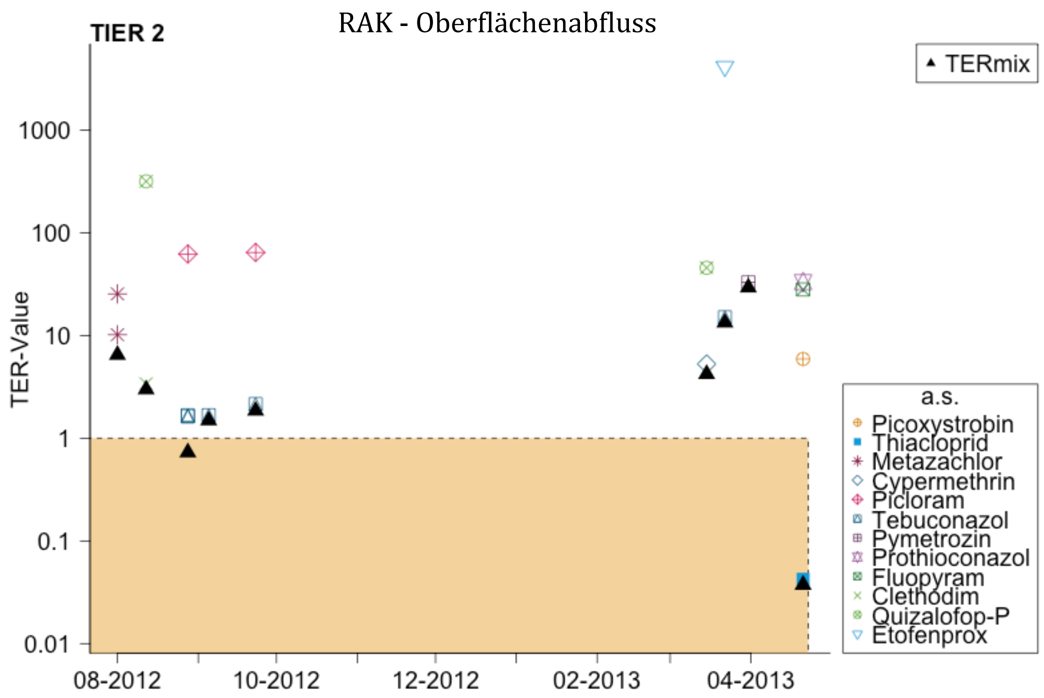
Bezüglich der Protektivität der PSM-Risikobewertung haben wir häufig nicht-akzeptable Risiken für die ausgewählten Spritzfolgen, Apfel und Winterraps, bei Tier 1-Risikoindikatoren festgestellt. Diese Risiken wurden nach Berücksichtigung von Risikomanagementmaßnahmen (Abstand, driftmindernde Technik) beobachtet. Bei Betrachtung aller Bewertungsbereiche führten nur wenige einzelne PSM-Wirkstoffanwendungen zu keiner Überschreitung der Risikoschwellen. Mit Blick auf Higher Tier-Risikoindikatoren (d.h. regulatorische akzeptable Konzentrationen - RAK) haben wir eine Risikoüberschreitung für die Anwendung von Thiacloprid in den Kulturen Apfel und Winterraps ermittelt (siehe auch Abbildung 3). Die beobachteten Risiken der Wirkstoffanwendungen lassen sich mit (i) veralteter Risikobewertung,

(ii) nicht berücksichtigten Risikoverfeinerungen für Tier-1-Risikoindikatoren, (iii) Notfallzulassungen oder (iv) den Ergebnissen einer Risiko-Nutzen-Analyse erklären.

Die Tankmischungen der untersuchten Spritzfolgen umfassten zwei bis vier Wirkstoffe (Beispiel Winterraps, Abbildung 3). Die Mischungstoxizität wurde häufig von der Substanz mit der höchsten Toxizität dominiert, d.h. der Substanz mit dem niedrigsten TER-Wert (TER_{min}). Wir quantifizierten das zusätzliche Risiko von Tankmischungen mit dem *maximum cumulative ratio* (MCR). Der MCR-Faktor pro Tankmischung war für alle 28 Risikoindikatoren relativ ähnlich und zeigte nur geringe Unterschiede zwischen den vier Spritzfolgen. Die MCR-Faktoren zeigten eine mittlere Risikoerhöhung von Faktor 1,2 und 1,4 pro Tankmischung in den untersuchten Spritzfolgen. Die tatsächliche Risikoerhöhung von Tankmischungen im Vergleich zu einzelnen Wirkstoffanwendungen kann jedoch leicht höher sein, da ökotoxikologische Daten bei manchen PSM und Endpunkten für den vorliegenden Bericht gefehlt haben.

Abbildung 3: TER_{single} und TER_{mix} pro Tankmischung für einen exemplarischen TER-Risikoindikator (regulatorisch akzeptable Konzentration – RAK, Eintragspfad Oberflächenabfluss) im Winterraps, Worst-Case Spritzfolge.

Jeder farbige Punkt zeigt eine Wirkstoffanwendung mit verfügbaren TER_{single}. Für die Wirkstoffe Clopyralid und Dimethenamid-P lagen keine TER_{single} vor. TER_{mix} wurde auf Basis aller verfügbaren TER_{single} pro Anwendungszeitpunkt ermittelt. Die gestrichelte horizontale Linie bei TER = 1 stellt den Grenzwert für ein akzeptables Risiko. Der Bereich unterhalb dieses Schwellenwerts ist orange gefärbt, um TER-Werte im Risikobereich hervorzuheben.



Wir haben ebenfalls das MCR-Konzept angewendet, um das zusätzliche Risiko der Spritzfolge gegenüber der einzelnen Wirkstoffanwendung mit der höchsten toxischen Belastung (TER_{min}) zu ermitteln. Dabei wurden aus pragmatischen Gründen keine Wiedererholung, Anpassung oder Zunahme der Sensitivität zwischen den einzelnen Behandlungsereignissen angenommen. Bei der Analyse haben wir große Unterschiede in Bezug auf die MCRs der einzelnen 28 Risikoindikatoren festgestellt. Unabhängig von der Spritzfolge beobachteten wir die höchsten zusätzlichen Risiken bei Säugetieren, Vögeln und Fischen. Der Median der MCR-Werte über alle Risikoindikatoren lag zwischen 3,40 (Apfel, Worst-Case-Spritzfolge) und 1,17 (Winterraps, Typical-Case-Spritzfolge). Die Worst-Case-Spritzfolge Apfel hatte die höchste Anzahl Spritzereignissen (26) und wies das höchste zusätzliche Risiko auf. Unter Berücksichtigung der MCR-Werte über alle 28 Risikoindikatoren und die vier Spritzfolgen hinweg haben wir einen Gesamtrisikoanstieg mit einem Medianfaktor von 2,18 (50. Perzentil) bis 5,26 (90. Perzentil) erfasst. Ähnlich wie bei den Tankmischungen kann der tatsächliche MCR-Faktor für die Spritzfolgen leicht höher sein, da nicht für alle Wirkstoffe und Endpunkte ökotoxikologische Daten verfügbar waren.

Das Modell MITAS ermöglichte die Analyse des Mischunsgrisikos (ETR) über die Zeit für eine Apfelspritzfolge. Dabei wurde der Schwellenwert für das chronische Risiko (Regenwurm, ETR = 0,2) von etwa der Hälfte der Simulationszeit bis zum Ende der Spritzfolge überschritten. Somit können nachteilige Auswirkungen für den Bodenorganismus für etwa die Hälfte der Simulationszeit nicht ausgeschlossen werden.

Maximum cumulative ratio (MCR) für aquatische Monitoringdaten in Mitteldeutschland

Das zusätzliche Risiko durch PSM-Mischungen und PSM-Sequenzen wurde für einen Datensatz an aquatischen Monitoringdaten bestimmt. Die MCR-Faktoren lagen in einem vergleichbaren Bereich wie die MCR-Faktoren für die ausgewählten Spritzfolgen. Das zusätzliche Risiko wurde für die Freilanddaten pro gemessenem Expositionsereignis (Medianfaktor der Zunahme 1,35-1,53 für Invertebraten bzw. Algen) als Äquivalent zu Tankmischungen und über die Hauptanwendungszeit der PSM (Medianfaktor der Zunahme 1,58-1,74 für Wirbellose bzw. Algen) als Äquivalent zu den Spritzfolgen bestimmt.

Diskussion und Schlussfolgerungen

Das Konzept der Konzentrationsadditivität ergibt in vielen Fällen robuste Vorhersagen für chronische Mischungseffekte auf Individuenebene, wenn die Toxizitätsdaten auf genaueren chronischen EC_x-Werten basieren. Im Gegensatz dazu erlauben der aktuelle Stand der wissenschaftlichen Literatur und die regulatorischen Studien auf Gemeinschaftsebene keine klare Aussage über die Anwendbarkeit von CA oder IA zur Vorhersagbarkeit von Mischungseffekten.

Wir haben festgestellt, dass realistische PSM-Behandlungsregime zu einem relevanten zusätzlichen Risiko für die Umwelt führen. Das Risiko der analysierten Tankmischungen und Spritzfolgen wurde häufig von einem oder wenigen Wirkstoffen dominiert. Mit dem MCR-Ansatz und ohne Berücksichtigung von Synergieeffekten sowie Wiedererholung/ Anpassung können wir jedoch zeigen, dass das Gesamtrisiko von Tankmischungen und Spritzfolgen das Risiko der toxischsten Einzelanwendung übertreffen kann. Dies ist besonders wichtig, da einzelne PSM oft sehr nahe der akzeptablen Risikoschwelle reguliert werden.

Die repräsentativen Spritzfolgen aus dem Projekt können weiterverwendet werden, um die Protektivität realistischer Behandlungsregimes mit unterschiedlichen Anwendungsintensitäten zu beurteilen. Die hier identifizierten Szenarien werden sich aber durch die PSM-Zulassung und das Verbot von Stoffen sowie durch klima- und landnutzungsbedingte Veränderungen der PSM-Anwendungen ändern. Dementsprechend benötigt eine realistische Risikobewertung von PSM regelmäßig aktualisierte Informationen zu den Spritzfolgen.

Unsicherheiten bestehen weiterhin in Bezug auf potenzielle synergistische Effekte von PSM in Spritzfolgen. Darüber hinaus kann unter anderem die Saatgutbehandlung, die wiederholte Exposition und das Vorhandensein zusätzlicher Umweltstressoren das Risiko von Spritzfolgen für Nichtzielorganismen erhöhen. Diese Unsicherheiten sollten bei zukünftigen Projekten berücksichtigt werden, um eine protektive und realistische Extrapolation von PSM-Effekten aus dem Labor ins Freiland Feld zu gewährleisten.

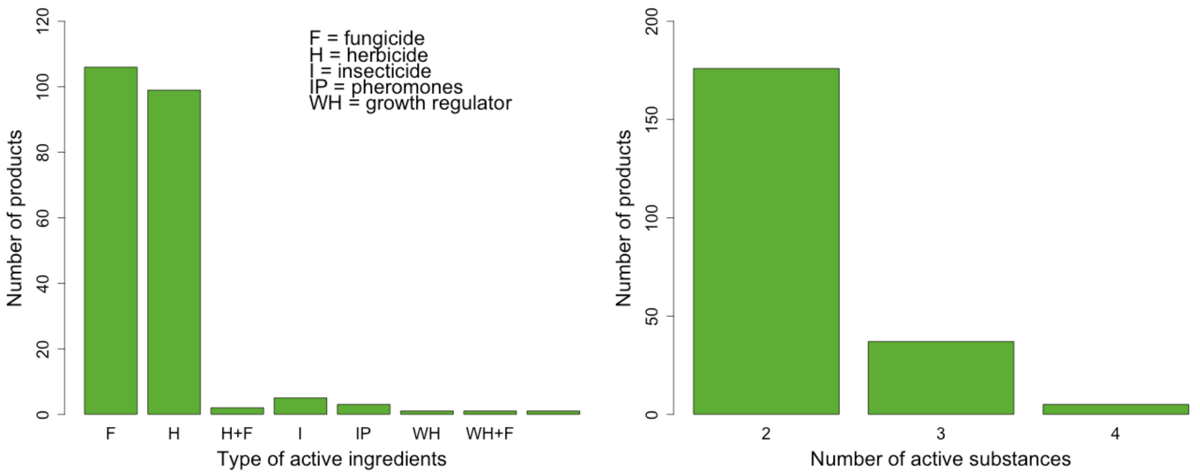
1 Introduction

In 2015, 218 combination products of PPP were authorized for professional and amateur use in Germany (BVL 2015). Combination products included 99 herbicide products, 106 fungicide products and five insecticide products. Only four combination products presented a mixture of different pesticide types, such as the combination of herbicides and fungicides (2) or growth regulator and fungicides/ herbicides (one each) (Fig. 1). In terms of ingredients, most combination products contained two active substances, but to a lower percentage also three and four active substances.

Single combination products are in turn applied with other PPP in tank mixtures for field applications in agriculture. Applications of single PPP and tank mixtures during one period of crop cultivation again sum up to a so-called spray series that can contain many different types of PPP. Hence, the risk assessment of PPP applications under realistic exposure scenarios presents an essential part for the environmental risk assessment off PPP.

Since decades, many studies have addressed mixture effects on various organisms and endpoints. The model of concentration addition (CA, Loewe 1926) has become the most relevant and accepted concept to predict mixture toxicity of similar acting compounds in ecotoxicology (e.g. Cedergreen 2014, Altenburger et al. 2013, Frische 2014). Nevertheless, there is still a lack of data and uncertainty with regard to the predictability of mixture effects for non-additive compound combinations, long-term effects and effects at higher levels of biological organization. This understanding is needed for a protective risk assessment of PPP and therefore constitutes the focus of the present report.

Figure 1: Overview on authorized PPPs with more than one active including type and number of active substances (a.s., herbicide, fungicide, insecticide, growth regulator)*



* Data are based on BVL 2015

1.1 Previous reports – German Environment Agency

1.1.1 Effect assessment of pesticide mixtures

Previous reports and expert opinions on mixture toxicity have mainly focused on pesticide and biocide mixture predictions for effects on aquatic organisms. Regarding the assessment of chronic mixture effects, the expert opinion: "*Die Eignung von Konzentrations-Additivität zur Vorhersage der toxischen Kombinationswirkung technischer Stoffgemische für Wasserorganismen*

– *analysiert am Beispiel von Pflanzenschutzmittel-Kombinationspräparaten*" by Coors (2009) and also the publication Coors and Frische (2011) present a relevant starting point for the current project. All available study reports on combination products and corresponding active substances within the frame of pesticide authorization were reviewed. Studies included the acute and chronic toxicity of pesticides on algae, macrophytes, daphnids and fish until 2009. The main aim was to assess the predictive power of concentration addition (CA) for the present pesticide combination products. The study described a total of 448 comparisons between predicted and observed mixture toxicity. The authors observed for 35% of all comparisons a difference of less than factor two and for 56% a difference of less than factor five between predicted and observed effect concentration. An underestimation of mixture toxicity with CA was significantly more often the case than the overestimation. Comparing acute and chronic effects, the distribution of model deviation ratios was much flatter for chronic than for acute effects. However, no clear under- or overestimations of model deviations could be identified for chronic effects. Coors (2009) further analyzed and discussed the presence of formulation additives in the mixtures as one reason for the observed deviations. However, the assessment of additives for the effect prediction of combination products is generally challenging due to the high number of different additives and the confidentiality of applied additives.

The subsequent report "Ecotoxicological combined effects from chemical mixtures - Part 1: Relevance and adequate consideration in environmental risk assessment of plant protection products and biocides" by Altenburger et al. (2013) presented a literature overview to the current state of assessing mixture toxicity and suggestions on how to assess pesticide/ biocide mixtures better in the existing risk assessment. The authors concluded from the literature review and own analyses that (i) only non-interactive effects (= non synergistic or non-antagonistic mixture effects) of mixtures can be predicted systematically, (ii) CA and independent action (IA) represent relevant approaches to perform component-based mixture effect prediction that can be applied for various endpoints and most toxicants, (iii) mode of action is the driving factor for predicting mixture toxicity, (iv) CA mainly predicts higher effects of mixture toxicities compared to IA and can be seen as the "worst-case-scenario" and (v) a knowledge gap exists for predicting the effect of additives in PPPs and biocides, effects at higher levels of biological organization and synergistic interactions. Furthermore, according to Altenburger et al. (2013) the main limit for the use of component-based approaches (CA, IA) is the insufficient knowledge on (i) mixture components (e.g. data on all active substances in mixtures are often not available) and (ii) the modes of action of active substances within a mixture.

The second part of the report was authored by Coors et al. (2013). Within this report three biocides (wood preservatives) and their eluates were investigated alone and in mixture. The assessed endpoints included growth inhibition of algae and acute/chronic effects on *Daphnia magna*. The authors observed that acute and chronic effects could be predicted using CA with a deviation of less than the factor two if all mixture components including additives are known. It was further outlined that effects of sub-lethal endpoints at low effect levels were generally overestimated using CA.

1.1.2 Estimation of realistic exposure scenarios with plant protection products

The expert opinion "*Pestizid-Mischungen in der Umwelt: Beschreibung der Relevanz von Kombinationswirkungen für die ökotoxikologische Risikobewertung vor dem Hintergrund der üblichen Anwendungspraxis von Pflanzenschutzmitteln*" by Coors et al. (2008) identified 15 simultaneous PPP applications as typical tank mixtures in German agricultural practices. The authors selected frequent tank mixtures in wheat, winter oilseed rape and potatoes using PPP recommendations from regional authorities for plant protection. The analysis showed that mixtures of herbicides and mixtures of fungicides presented the most common tank mixtures within one PPP. The toxicant exposure ratio of these tank mixtures (TER_{mix}) was calculated using the approach of CA. TER_{mix} could often not be assessed for the most sensitive endpoint due to missing ecotoxicological data of single active substances. It was further observed that TER_{mix} for the investigated tank mixtures was below the acceptable threshold for fish and daphnids.

In the more recent report by Altenburger et al. (2013, see also above) three frequent exemplary tank mixtures in winter wheat and winter oilseed rape were evaluated based on agricultural application data. The tank mixtures comprised a total of eight active substances including one herbicide mixture, one fungicide mixture and one fungicide-insecticide mixture. For these mixtures no risk was observed for the available standard endpoints. It was assumed that the missing risk was due to mitigation measures. Nevertheless, the authors discussed that due to missing data a risk of tank mixtures could not be excluded for bees or earthworms.

In summary, the previous reports focused mainly on tank mixtures. In comparison, the aim of the present report is to identify and assess the risk of realistic treatment regimes for relevant German crops that contain a series of single PPP applications and tank mixtures.

2 Prediction of mixture effects for community-level endpoints and chronic endpoints

The aim of this chapter is to assess PPP mixtures with regard to chronic effects and effects at the community level within the environmental risk assessment (ERA). In respect to community endpoints we consider natural (e.g. freshwater enclosures, field studies for later stages of the project) and artificial communities (e.g. mesocosms or microcosm studies, multi species assemblages) in aquatic and terrestrial ecosystems.

In comparison to the community endpoints, the second part of this chapter addresses chronic effects at the individual or population level separated from a community context and are mainly identified under laboratory conditions. Definitions off chronic effects at the population level are often blurred and mainly refer to the duration of exposure (Newman 2010). We consider chronic effects as long-term or delayed effects that may develop over time and do not appear directly after the start of a given exposure. With respect to endpoints we consider not only survival, but also sub-lethal endpoints such as reproduction, emergence, life history traits or functional responses. With respect to previous reports (Coors 2009) toxicant mixtures and chronic effects have been partly evaluated, but only for aquatic standard test taxa including algae (*Desmodesmus subspicatus, Pseudokirchneriella subcapitata*), duckweed (*Lemna* sp.), daphnids (*Daphnia magna*) and fish (mainly *Oncorhynchus mykiss*). Hence, the relevance of toxicant mixtures for chronic effects on terrestrial organisms and further aquatic organisms present a further core aspect in chapter 2. The evaluation of chronic effects without the community context will be essential to identify effect ranges and ecological mechanisms at the individual/population level.

In order to address existing knowledge gaps in the assessment of PPP mixtures for ERA we included the following endpoint domains in the literature research and the identification of relevant studies from the pool of reports conducted within the frame of pesticide authorization.

- Chronic *Daphnia* sp. (additional studies to Coors (2009))
- Sediment dwellers (*Chironomus* spec.)
- Non-target plants (terrestrial and aquatic)
- Non-target arthropods
- Earthworms and other soil organisms
- Aquatic mesocosms / multispecies tests (mainly zooplankton, phytoplankton, macro-invertebrates, periphyton, macrophytes, bacterial communities)
- Terrestrial mesocosms / multispecies tests
- Birds and mammals

Regarding earthworm data from the ICS-database of the UBA we searched for the attribute “REGENWURM” and filtered for data on plant protection products, metabolites, pharmaceuticals and the active substances of plant protection products. The data compilation comprised 6718 observations from 3205 ecotoxicity tests and 103 attributes (i.e. variables). The query was last updated at 2015-06-20. Several revisions and harmonization of the attributes and recalculations of attribute values were necessary in order to use the database. A detailed description was provided by an expert opinion on the influence of study design variants on ecotoxicity test results with earthworms (Scholz-Starke 2015).

2.1 Assessing mixture toxicity for communities and aggregated endpoints

2.1.1 Scientific Literature research on mixture effects on communities

The literature research for mixture effects on the community level was conducted using ISI- web of science database similar to the literature research as described in Table 1. The query was adapted for the search for community endpoints according to the following set of keywords 2,3,6 in Table 25 (Appendix A.1) within the time period 1987-2016.

2.1.1.1 Aquatic communities

We applied the combination of constraints and keyword sets as outlined in Table 1. More specifically we selected the keyword sets: TS1, TS2, TS3, TS6. Additionally. We added the keyword set TS = ("aquatic" OR "freshwater" OR "*plankton" OR "algae" OR "periphyton" OR "macro invertebrate"). In a first step we screened titles and abstracts to select studies that investigated toxicant mixtures on aquatic communities including community endpoints. Finally, we also screened existing reviews (e.g. Verbruggen and van den Brink, 2010) and general reports (e.g. Kortenkamp et al. 2009).

Summary of the selected community studies

The range of previously selected studies includes reviews, original research studies and one meta-analysis of field studies. Approximately half of the original studies that considered artificial pesticide mixtures alone and in combination are part of the review from Verbruggen and van den Brink (2010, see also Table 26). The identified studies investigate a range of macroinvertebrate, zooplankton, phytoplankton, periphyton, macrophytes and microbial communities. Several studies included more than one of the above listed communities (e.g. zooplankton and phytoplankton). If available, we extracted the method for assessing mixture effects (when addressed with a specific approach) such as for example CA or IA.

With respect to exposure we also identified studies that simulated pesticide applications for a specific crop (e.g. Arts et al. 2006, Wijngaarden et al. 2004, or Wendt-Rasch et al. 2004). The studies regarding the effects of simulated PPP application patterns are described into more detail in chapter 4. Finally, we also evaluated studies on the effect prediction of pesticide mixtures in the field (i.e. Schäfer et al. 2013).

In addition to the direct effects of mixtures, several studies described indirect mixture effects at the community level. Indirect effects mainly due to trophic interactions can cause effects, which are difficult to be predicted with the approaches CA or IA. Such indirect effects were partly observable if pesticides were targeted to different biological groups. For example, Relyea (2009) detected indirect negative, but also positive effects of a herbicide-insecticide mixture on the survival and growth of amphibians. Another study by Grünwald (2003) observed that shortly after the contamination to an insecticide and herbicide, direct toxic effects played the most important role. Secondary or indirect effects due to food web interactions developed only on the long-term after the pesticide exposure. More specifically, the author reported that zooplankton richness was stronger affected under the joined exposure to the herbicide and insecticide due to the presence of biotic interactions.

In summary, indirect effects are ecologically important, but also very complex for the assessment of mixture toxicity within the given project frame. Furthermore, it is not possible to completely exclude the role of other factors related to the specific action of the substance. Such characteristics include the presence of delayed and more than additive effects in the given studies (e.g. effects due to a slow uptake and bioaccumulation). Therefore, we focused in the current report on the analysis of short-term or direct toxic effects at the community level including community level endpoints and effects on single populations within the community context.

Studies combining single and mixture exposure with pesticides

To assess the type and relevance of combined effects at the community level we only considered selected experimental studies that investigated single and mixture exposure within the same study. By doing so, we ensured the use of comparable communities, study designs and statistical analyses. The details of these 14 studies are presented in Table 26 (Appendix A.2). Some of the described studies are part of the review by Verbruggen and van den Brink (2010), but we also identified and evaluated more recent semi-field studies. The combined effect could not be interpreted for all studies, because of mixtures that were dominated by one substance or inconsistent outcomes of the combined effects. For example, studies like Choung et al. (2013) or Relyea at al. (2009) observed pesticide concentrations that caused especially high effects in single exposure treatments also dominated the effect observed in the mixture exposure treatment.

For the remaining studies, it became clear that mixtures of similar acting compounds in equi-toxic mixtures caused direct additive combined effects that could be mostly predicted with the CA-approach (e.g. Knauert et al. 2009, Knauert et al. 2008, Arrhenius et al. 2004, Porsbring et al. 2010). It has to be noted here that studies investigating similar acting compounds could be only identified for algae communities. Only few studies allowed to draw conclusions for the prediction of dissimilar acting substances with CA or IA and their direct effects were less consistent. Examples include a study by Backhaus et al. (2011) testing the mixture toxicity of pharmaceuticals and personal care products on marine periphyton communities. According to the authors CA and IA worked well to predict mixture effects at higher effect concentrations. In contrast, low effect concentrations of mixture caused hermetic effects that could not be predicted with CA or IA. Arrhenius et al. (2006) investigated the mixture toxicity of three antifoulants on algal communities and observed different outcomes. CA and IA underestimated mixture toxicity at low effect concentrations and overestimated mixture toxicity and higher effect concentrations.

Field studies on pesticide mixtures

All ecotoxicological test systems, including mesocosms, do not reflect the real impact of PPPs because they are based on artificial communities and do often not include factors that are typical under field conditions (i.e. interspecific competition, abiotic stressors). Hence, field investigations are the only possibility to validate and calibrate the prediction of mixture effects. According to our literature research the substance with the highest toxic pressure in a pesticide mixture correlated with the main effect on non-target organisms for invertebrate communities in agricultural streams in Europe and Australia. Examples include field studies by Liess and von der Ohe (2005), Münze et al. (2017), Orlinskiy et al. (2015) and also the meta-analysis by Schäfer et al. (2013) or Knillmann et al. (2018) that identified similar significant correlations between the observed effects and the maximum toxic unit per sampling season (TU_{max} as well as with the summation of toxic units (TU_{sum}, based on the approach of CA). TU_{max} and TU_{sum} were determined using the measured field concentration of each pesticide and the LC_{50}-value of *Daphnia magna* or the most sensitive standard test organism (e.g. *D. magna* or *Chironomus riparius*). The similarity of both relationships can be explained by the fact that TU_{sum} and TU_{max}

per season and site are positively correlated and show only minor differences in terms of absolute values (see also chapter 5.3.3.2). Hence, the relatively small differences between TU_{sum} and TU_{max} implies that TU_{max} substantially describes the variance of effects on the community composition (i.e. SPEAR). However, observed negative effects are present already three orders of magnitude below the acute toxicity of standard test organisms (*D. magna*, *C. riparius*). The increase in sensitivity is due to several reasons. It has been shown for aquatic organisms that the presence of environmental stressors increases individual sensitivity to toxicants (pesticides, trace metals) by a factor of up to 100 (Liess et al. 2016). Additionally, it was shown that repeated toxicant pulse of populations that are challenged with interspecific competition may result in a multigenerational culmination of low-dose effects (Liess et al. 2013, Dolciotti et al. 2014). Hence, all factors acting in the field (i.e. mixture effects, environmental stress, sequential exposure) are cumulating to a "lab to field sensitivity enhancement factor" for every PPP substance.

Moreover, the ecotoxicological dominance of one or few PPP substances does not mean that only few selected substances pose a risk for non-target organisms. Any PPP can represent the most toxic compound depending on the exposure context and assessment area under consideration. As outlined by Vallotton and Price (2016), there are of course also mixture toxicities driven by multiple PPPs in the field, which need to be considered in a prospective assessment (for more details, see also chapter 5.3.3.2).

2.1.1.2 Terrestrial communities

The available scientific literature on mixture effects of toxicants on terrestrial communities from the relevant risk assessment areas, i.e. birds, mammals, soil organisms, non-target plants and non-target arthropods yielded 17 hits (Table 27, Appendix A.2). Out of these 17 publications, only eight studies dealt with terrestrial communities in the above-defined sense and were thus considered relevant. The extracted attributes of the literature were aligned with the aquatic lists (Table 27, Appendix A.2).

Mismatches due to inadequate literature tagging

One major problem using generic literature search phrases was that abstracts and keywords often contained phrases from external fields, e.g. aquatic risk assessment. This was as authors tended to use general phrases "…for aquatic and terrestrial risk assessments reliable endpoints are used…", on the other hand, they drew comparisons to the related areas "…as already defined in the aquatic guidance document…". This led to a considerable number of false positives that had to be eliminated manually. The systematic approach produced further non-productive results. Two examples from Frampton and Wratten (2000) and Vaj et al. (2011) illustrate the mechanisms of keyword searches due to very broad definitions of literature tagging. This is regardless of the quality of the research reported.

Frampton and Wratten (2000) investigated the effects of several fungicides on collembolan communities in the field. However, they neither reported genuine community endpoints (instead responses of single populations), nor was the design of their study aligned to study mixture effects of the tested PPP. The publication was found by the systematic query merely because mixture issues were raised in the discussion section. The authors broached generically difficulties in transferring their own study results into general conclusions due to the fact that realistic application patterns contain tank-mixes consisting of up to five different fungicidal substances.

Vaj et al. (2011) described an integrated methodological approach for assessing and mapping pesticide risks in different ecosystems, both aquatic and terrestrial. Predicted environmental concentrations in aquatic and terrestrial compartments were used in connection with adequate endpoints to calculate sums of toxic units for the characterization of the risk, applying the concentration addition concept. While the evaluation was complete for the aquatic risk assessment, applying multivariate analyses of the communities of freshwater invertebrates, data analysis and validation for the terrestrial risk assessment was discontinued.

Statistical approaches describe mixture effects

None of the above identified 17 publications investigated the prevalent concepts of concentration addition (CA) or independent action (IA). In contrast, the authors of the studies applied statistical approaches to describe the exclusive and interactive effects of the chemicals. Two studies systematically addressed mixture toxicity questions in designed experiments. Schnug et al. (2015) investigated bactericides, insecticides and fungicides alone and in mixture. The authors used Generalized Linear Models for analyses and concluded that specific response patterns for earthworm communities were not predictable by results from laboratory standard tests with *Eisenia fetida*. They applied Generalized Linear Models (GLM)[1].

Schmitz et al. (2014) combined insecticides, herbicides and fertilizer in a plot-design and investigated the effects on non-target plant communities in comparison to plots with single exposure patterns of the substances. Herbicide-fertilizer interaction effects exceeded individual effects and treatments led to long-term shifts of the plant communities. The authors justify this result with the possibility of direct synergistic effects between herbicides and fertilizer but do not exclude that indirect effects may also change competitiveness within the plant community and lead to synergistic responses. It is discussed that type and degree of interaction effects may also vary with different products of fertilizer and herbicides and for different types of vegetation. Schmitz et al. concluded, that based on the measured data a prediction of mixture toxicity from individual effects was not feasible.

2.1.2 Further databases (ICS-database, Regulatory reports, own data, further regulatory databases- e.g. earthworms)

Potential studies from the authorization process of pesticides were screened and pre-selected together with the contracting authority. Corresponding studies on aquatic and terrestrial communities were provided. One key aspect was to identify studies at the community level that describe mixture effects and additional studies for the single pesticide substances. These studies were summarized to a potential full set consisting of studies with the individual active substances and the corresponding mixtures, which can be used for subsequent analyses. The selected studies include experimental reports with technical standards and commercial products. The comparability of the studies within one full set is an important condition to apply different approaches, such as CA or IA, to predict the effects of mixture toxicity.

2.1.2.1 Aquatic community studies and limitations

Most of the studies from the regulatory database that were provided by the UBA investigated toxicant effects on plankton and periphyton communities. We identified three full sets (study reports for mixtures and all single compounds available). However, the studies on mixtures and

[1] A GLM originates from a family of statistical regression models that try to explain the quantities of the dependent variable by environmental factors. In the referred case, the dependent variable was the abundance of earthworms and a factor was the concentration of various substances in soil. A GLM can be regarded as an extension of an ANOVA approach. Therefore, statistical approaches using a GLM can help to estimate the impact caused by different experimental design variables, e.g. the influence of each substance for an observed mixture effect. Nevertheless, the application of GLM for mixture toxicity cannot be used to quantify and predict possible additive, synergistic or antagonistic effects of mixtures. GLM may be useful to indicate such patterns retrospectively and to identify the most toxic components of mixtures for existing data sets. They cannot supersede currently used prediction models for mixture toxicity.

single substances correspond to independent studies with different community compositions and study designs regarding toxicant exposures (application type, product or technical substance), study duration or investigated endpoints. Furthermore, not all available and necessary reports for the prediction of combined effects identified significant effects on some or all analyzed communities. Hence, this variability between regulatory reports requires further approaches of evaluation and standardization to enable a reliable assessment of mixture effects at the community level. Such approaches should address (i) a more unified design of community studies (i.e. test duration, assessment criteria), (ii) a checklist for the validity of community studies (i.e. presence of sensitive taxa, development of control community, presence of other stressors) or (iii) a general tool to evaluate PPP studies despite existing variations between studies (i.e. application of toxic units as a common unit).

2.1.2.2 Terrestrial data and limitations

Description of the data analyses

Based on the ICS-database ("Information System Chemical Safety", see Scholz-Starke 2015) query and pre-processing, data of field-studies for earthworm communities were subsequently analyzed.

The ICS-database contains 10 full sets of field studies with earthworms (Table 1). Nine full sets consist of two single substances and the corresponding mixture, one identified full set is a three-component-set (boscalid, epoxiconazole, pyraclostrobin). A number of 34 entries (=reports) were found containing data of test substances which belong to at least one of the full sets. An overview of the identified full sets for earthworm field- test procedures is given in Table 1.

Characteristics of the data

The majority of the field studies (31 out of 34) were carried out according to the ISO test guideline 11268-3 (1999) "Soil quality - Effects of pollutants on earthworms - Part 3: Guidance on the determination of effects in field situations" or in consideration of the BBA (Biologische Bundesanstalt) guideline part VI, 2-3. (1994) "Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, Nr. VI, 2-3, Auswirkungen von Pflanzenschutzmitteln auf Regenwürmer im Freiland". Therefore, the analyzed test procedures follow a common approach:

All reports contain earthworm species, numbers, and biomass collected by sampling plots treated with a test substance in randomized complete blocks (four replicates per treatment) and compared with those collected from control and reference plots (e.g. carbendazim or benomyl). The sampling dates are usually chosen about 1-3 months, 4-6 months and 12 months after application. Tests start in April/May; therefore, the sampling dates lie within the periods of activity of the earthworms. The calculations of effects within the test procedures are mainly limited to the evaluation of total abundance and biomass on species level and of all earthworms. Beyond, juvenile earthworms were summarized and evaluated on genus level (morphological groups: *Tanylobous* and *Epilobous*). Univariate statistical analyses for multiple (tests with more than one treatment) or pairwise comparisons (control vs. treatment) were applied. For multiple comparisons Dunnett/Williams tests for normally distributed and homogeneous data were used and otherwise Bonferroni U-test or Jonckheere-Terpstra Step-down-test. For pairwise comparisons Student's t-test and otherwise Mann-Whitney U-test were used. For 32 out of the 34 reports raw data are available and can be looked up in the pdfs that were provided by the UBA (no raw data documented for reports of prochloraz). The composition of the earthworm test community is included in the report, the community generally consists of a number of different identified species (e.g. *Aporrectodea caliginosa, Aporrectodea longa, Aporrectodea rosea, Dendrodrilus rubidus, Lumbricus rubellus, Lumbricus terrestris, Octolasion tyrtaeum*). The field studies have been conducted with different amounts of application numbers and rates, as well as heterogeneous sampling points after application. Four of the reports only contain supplementary material to other reports, such as another sampling date or another test substance application. This leads to a slight reduction of the available raw data. Furthermore, only data for fungicides were found in the ICS-database (Table 1), except for one herbicide set (s-metolachlor and terbuthylazine). Therefore, other plant protection product types cannot be considered within the analysis of field tests of the ICS database.

Table 1: Full sets, field-studies for single substances and mixtures available in the ICS database

The table contains an overview of all identified full sets for earthworm field studies from the ICS database. Ten full sets, the respective single compounds and the product class of the tested compounds are listed.

Fullset_no*	Class	Component 1	Component 2	Component 3
015	fungicide	boscalid	dimoxystrobin	-
016	fungicide	boscalid	epoxiconazole	-
017	fungicide	boscalid	epoxiconazole	pyraclostrobin
018	fungicide	chlorothalonil	tebuconazole	-
019	fungicide	dimoxystrobin	epoxiconazole	-
020	fungicide	prochloraz	epoxiconazole	-
021	fungicide	thiophanat-methyl	epoxiconazole	-
022	fungicide	prochloraz	tebuconazole	-
057	fungicide	boscalid	pyraclostrobin	-
155	herbicide	s-metolachlor	terbuthylazine	-

* the attribute "Fullset_no" of the database contains all identified studies of both field and chronic test reports

Regarding the aim of analyzing field data endpoints by applying mixture toxicity models and comparing their significance, the screening of all identified reports from the ICS database showed that no adverse effects in standard statistical procedure could be detected throughout the field studies. Therefore, no mixture effects in the comparison of single substance-tests and their combined counterparts for single endpoints can be derived.

Advanced statistical analyses: CPCAT and the Model Deviation Ratio (MDR)

Raw data of the ICS database had to be digitalized manually. This was done for raw data (biomass and abundance, all species and functional groups) of three field studies belonging to the herbicide-full set (#155, s-metolachlor and terbuthylazine).

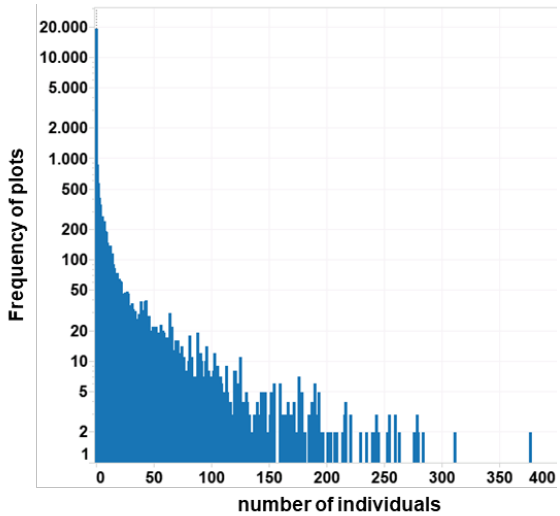
The statistical calculation to determine significant effects of the treatments compared to control groups for all time points, treatments and tested species or functional groups was initially carried out with current standard Guideline test approaches (multiple Williams and Dunnett test, 5% significance level, one-sided testing). For these kinds of testing no significant effects could be observed over all three tests of a full set in both test procedures (Dunnett and Williams). The use of statistical tests from t-test family for this type of data has been further criticized because normal distribution and homogenous variances of the data are often not fulfilled, the procedure implies the drawback of α-inflation and thus effects are often veiled.

Therefore, a new statistical procedure to detect effects in field test data was conducted by using a Poisson model to describe the test data in combination with a Closure Principle Computational Approach Test (CPCAT, Lehmann et al. 2016).

The illustration of the frequency of plots for all digitized field test data against the respective number of sampled earthworm individuals (Fig. 2) shows that Poisson distribution ("the law of rare events") can be assumed for the statistical calculation of this data set.

The performance of the CPCAT analysis for full set #151 (s-metolachlor, terbuthylazine and the combination product) shows that significant effects can be found for significantly more species and exposure times (total number of performed tests: 204, Table 3) in comparison to parametric t-test procedures. For s-metolachlor (two treatments) 60 (29.4%) and 50 (24.5%) significant test results were identified, for terbuthylazine (one treatment) 61 and for the combination product (two treatments) 44 significant differences in each treatment were found. In total, a number of 30 common time- and endpoints with significant effects in all three tests of the full set (s-metolachlor, terbuthylazine and the combination product) were found. This corresponds to a proportion of 14.7% of the total tests performed on this full set.

Figure 2: Frequency of individual numbers per plot for each taxa/sampling day/ replicate/treatment for earthworm field test (ICS database) indicates a Poisson distribution of data, which is a prerequisite to apply CPCAT for multiple testing instead of parametric t-test-procedures (Williams/Dunnett)



The derived lowest- or no-observed effect concentrations (NOEC) of the CPCAT approach was subsequently used to compare observed mixture toxicity of the combination product with predicted mixture toxicity (according to CA) by calculating the model deviation ratio (MDR). The model deviation ratio (MDR) was calculated using the following Equation 1.

Table 2: Percentages of observed significant test results (treatment vs. control) for full set #151 (s-metolachlor, terbuthylazine and the combination product) with CPCAT analysis

The table shows the percentages of significant test results using the CPCAT approach for earthworm field tests of the substance s-metolachlor, terbuthylazine and the respective combination product. The percentages of significant results are displayed in total for every substance and treatment (first row), and differentiated according to measured endpoints, times of exposure and tested species.

Percentage of significant differences for [factor]		S-Metolachlor		Terbuthyl-azine	Combination product		Overlap within full set
		Treat-ment 1	Treat-ment 2		Treat-ment 1	Treat-ment 2	
Total		29.4%	24.5%	30.0%	21.6%	21.6%	14.7%
Endpoint	Abundance	40.6%	22.8%	30.7%	24.5%	18.6%	18.6%
	Biomass	18.4%	26.2%	29.4%	18.6%	24.5%	10.7%
Exposure time (extract)	35 days	23.5%	47.1%	35,3%	41.2%	26.5%	26.5%
	170 days	15.6%	9.4%	12.5%	15.6%	18,8%	6.25%
	371 days	35.3%	11,8%	35.3%	29.4%	20.6%	8.8%
Species (extract)	*Apporectodea caliginosa* (epilobous)	16.7%	25.0%	33.3%	16.7%	33.3%	25.0%
	Lumbricus terrestris (tanylobous)	-	25.0%	33.3%	8.3%	16.7%	8.3%
	Total earthworms	66.6%	33.3%	41.7%	41.7%	25.0%	16.7%

Equation 1: Calculation of the Model Deviation ratio (MDR), the predicted NOEC of the mixture ($NOEC_{pred}$) based on single substance testing and the observed NOEC ($NOEC_{obs}$) of the combination product

The calculation of the Model Deviation Ratio equation:

$$MDR = \frac{NOEC_{pred}\,[\frac{mg}{kg}]}{NOEC_{obs}\,[\frac{mg}{kg}]} \quad (1.)$$

with

$$NOEC_{pred}\,[\frac{\mathrm{mg}}{\mathrm{kg}}] = \frac{1}{\sum_{i=1}^{n} \frac{p_i}{NOEC_i}} \quad (2.)$$

(calculation based on concentration addition)

$$NOEC_{obs}\,[\frac{\mathrm{mg}}{\mathrm{kg}}] = \mathrm{NOEC}\,\left[\frac{\mathrm{mg}}{kg}\right] * \frac{\sum a.i.[\frac{g}{l}]}{density\,[\frac{g}{l}]} \quad (3.)$$

Due to the use of a limit-design within the terbuthylazine-field test it was not possible to derive a NOEC for these data. Therefore, a MDR_{NOEC} can only be calculated in the case of significant effects of the single treatment concentration compared to the control or if the respective test concentration (=LOEC) is used in the MDR calculation (in this case the actual NOEC is lower than the used LOEC). With this limitation, the MDR_{NOEC} of all calculations from this full set is 0.56. However, a MDR_{LOEC}, can be calculated for significant deviations between treatments and control group for all of the 30 time points, ranging between 0.50 and 0.74 (mean: 0.57, st.dev.: 0.06).

The CA prediction and measured mixture toxicity differed in these cases by a factor of 2 and less, which indicates compliance between prediction and mixture toxicity observation. Nevertheless, the data and underlying test design on which the MDR-calculations for earthworm field studies are based does not seem reliable for general statements on the applicability of the CA-model: The earthworm field studies in a regulatory context were mostly performed using a limit design (or NOEC design with generally two treatment concentrations). Consequently, the MDR can only be calculated using censored data (NOEC smaller than the lowest tested concentration) and/or is limited to one possible value. This calculated MDR will be the same for every tested species, endpoint and sampling day within a full set, the test design does not allow for a more sensitive differentiation. Moreover, the tested substance concentrations within these studies are often the intended maximum application rates of the registration procedures (plus twice the application rate in some studies). Therefore, the calculated NOECs of the single-component-studies and for mixture studies are strongly biased by the applied test concentrations. If a significant effect can be detected, the resulting MDR is highly dependent on the one chosen test concentration per test. However, this does not correspond in any way to a threshold effect concentration, so that reliable conclusions towards mixture toxicity mechanisms cannot be drawn from these data.

Regarding the analysis of earthworm field tests for the assessment of mixture toxicity reveals that only in very rare cases significant effects (of tested substances on biomass / abundance against control) can be detected by standard statistical procedures. Therefore, ICS-report data of earthworm field tests are not suitable for the analysis of mixture toxicity. Even the results of the CPCAT procedure point out that the significance of the identified effects observed throughout the field test should be interpreted critically. Effects observed with CPCAT are rather randomly distributed throughout tested endpoints, species and functional groups, e.g. no concentration-dependent effects and no certain sensitive species or functional groups for all tests of a full set can be identified. Results of the CPCAT analysis indicate a trend towards initial effects within the field tests of the full set.

Furthermore, the predominant limit design of field studies hampers a meaningful calculation of MDR and mixture assessment (see above). The limit- or NOEC design of the field studies with a maximum of 3 treatments (usually only 1-2) does not allow sufficient accuracy to distinguish between different endpoints per test. As a result, a calculation procedure would always lead to same MDR values for every species per test, exposure time and endpoint (depending on the few chosen treatment test concentrations). Results of the advanced statistical procedure by using CPCAT show that the ICS earthworm field test data do not lead to conclusions on mixture toxicity due to the shortcomings of the test design and data.

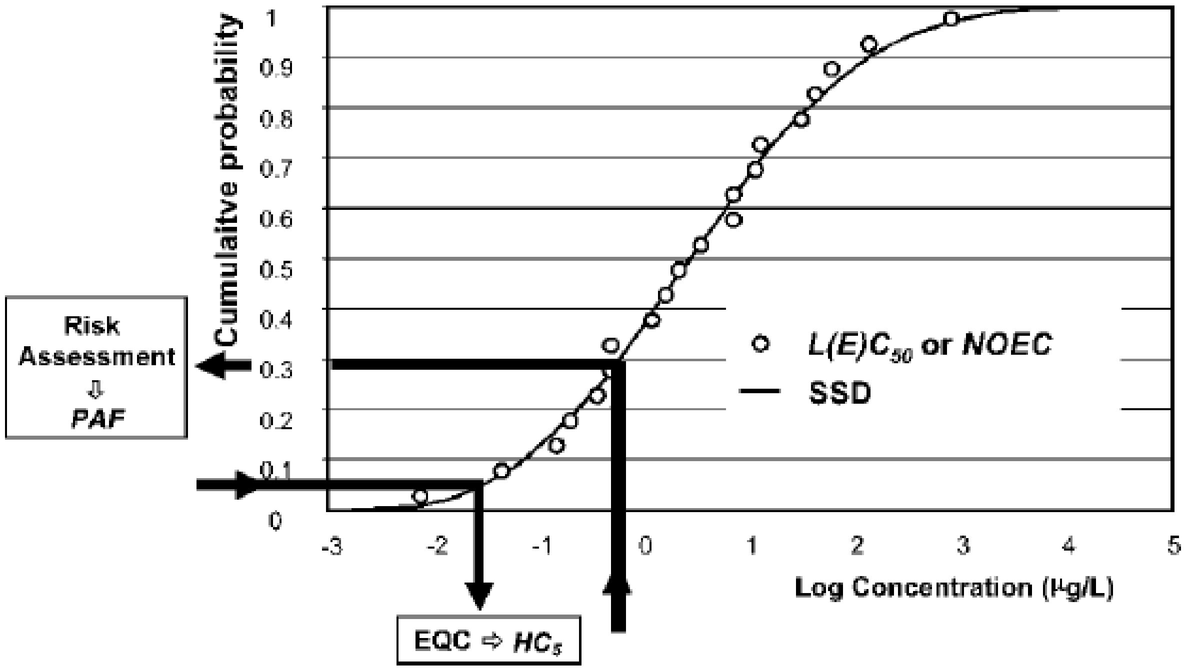
2.1.3 Species Sensitivity Distribution (SSD) for the prediction of mixture toxicity at the community level

2.1.3.1 Use of SSD for effect prediction of toxicant mixtures

Here we describe the use of Species Sensitivity Distributions (SSD) and corresponding potentially affected fractions (PAFs) for the prediction of mixture toxicity at the community level. This description also includes the discussion of uncertainties, such as for example regarding underlying database and empirical evidences that support the realism and use of SSD-based predictions of effective concentration in the regulatory context.

SSD-based predictions have been increasingly applied as a decision tool in the risk assessment of toxicants since the 1980s (Belanger et al. 2017). The SSD approach follows the principle to extrapolate from single-species toxicity tests to effects at the community level. In order to obtain SSDs, laboratory-derived toxicity data (i.e. NOECs or EC_{50}) towards a specific compound are compiled for a set of species and assumed to follow a log-normal or log-logistic statistical model (see Fig. 3). Based on SSD-curves the PAF of corresponding field communities can be predicted (i.e. hazardous concentration for 5% of all species in the community under consideration-HC5). Regarding the assessment of several toxicants, Klepper et al. (1998) suggested the PAF approach for separate compounds assuming no correlation in sensitivities and additive effects. Further studies were published that developed a multi-substance PAF (msPAF) to assess the combined effect of toxicant mixtures (Posthuma et al. 2002, Traas et al. 2002, De Zwart and Posthuma 2005). De Zwart and Posthuma (2005) suggested for compounds with similar modes of action that the slopes of single dose response curves must be equal. Extrapolated to communities and ecosystems this approach implies that also the slopes of SSD-curves are equal or should deviate by a maximum of 10% for substances with the same mode of action (MOA). Subsequently, concentration addition (CA) is then applied to assess the combined effect of compounds with similar MOA using hazard or toxic units (HU, TU) and the approach of independent action (IA or also response addition) to aggregate different MOAs (De Zwart and Posthuma 2005).

Figure 3: Conceptual SSD curve for one compound based on a set of LC_{50} or NOEC-values from different species*



* Posthuma and De Zwart (2006)

SSD-curves and the corresponding msPAF approach present one possibility to predict combined effects at the community level based on single laboratory toxicity tests. However, the approach contains several severe limitations and uncertainties that will be discussed in the following.

A recent publication by Del Signore et al. (2016) critically reviewed SSD-related studies from 2002-2013 and aimed to answer various questions including for example the influence of data availability, data selection, endpoints and statistical methods for the derivation of SSD-based predictions. Most of the reviewed studies by Del Signore et al. (2016) applied the SSD approach on freshwater ecosystems, followed by soil and marine ecosystems. Nevertheless, the aspects discussed are expected to be transferrable to other ecosystem types. Del Signore and colleagues (2016) emphasize that underlying toxicity data used for the construction of SSDs present an essential factor for the prediction of combined effects. For example, ecotoxicological data availability is a common problem and affects the number of species that can be used for the construction of SSD-curves. Schäfer et al. (2013) compared different exposure metrics including toxic units based on HC_5 (TU_{HC5}) and msPAF approach to predict the combined effect of pesticides and organic toxicants on macroinvertebrate communities in the field. The authors could not determine SSD curves for 70% of the investigated compounds and had to overcome this problem by assigning a mean slope for the different groups of MOAs. Nevertheless, the Schäfer et al. (2013) identified TU_{HC5} as the most reliable exposure metric to assess the mixture effect on stream invertebrates. An additional concept to deal with lack of available data is to model toxicity values with interspecies correlation estimations (ICEs; Dyer et al. 2006, Dyer et al. 2008) and quantitative structure–activity relationships (QSARs, e.g. Aldenberg and Rorije 2013). Regarding data quantity different regulations address the problem by suggesting a minimum sample size. Minimum sample sizes differ from 5 (e.g. European Union, Australia and New Zealand) to 8 (US EPA and also European Union) species that are requested (Del Signore et al.

2016). Scientific studies even propose a minimum sample size of 10 to 15 taxa in order to obtain reliable SSD curves (Wheeler et al. 2002). For lower sample sizes, several studies observed that the inclusion of at least four data points reduced the uncertainty to SSD curves and HC5 values (Del Signore 2016).

Other factors that influence the SSD-based predictions are the selection of taxa used to derive SSD-curves (i.e. Smetanova et al. 2014), which is a very crucial aspect in terms of ecological realism. Verbruggen and van den Brink (2010) discussed that SSDs do not consider different sensitivities among the various taxonomic groups to one specific compound within one ecosystem (e.g. insects and plants towards herbicides). Therefore, it has been already proposed by Posthuma et al. (2002) to determine PAFs for taxonomic groups separately. Another important aspect is that the selected taxa, which are used to construct SSD-curves and predictions, rarely reflect the typical taxa composition in the field (e.g. many sensitive insects are hard to culture and to test for sensitivities). This aspect is by far not well considered and should be addressed in future works as also outlined in the eLINK workshop (Brock et al. 2009).

The selection of endpoints can also affect SSD-curves, but according to the review by Del Signore et al. (2016) the selection of endpoints does affect the corresponding HC_5 values, but not the shape of the SSD curve itself. Nevertheless, SSDs based on different endpoints (e.g. acute and chronic data) will yield different outcomes and need to be evaluated with caution. Finally, also the applied statistics (model fitting) might affect the outcome of SSD-based predictions, but to a lesser extent than the data selection.

2.1.3.2 SSD - predictions and empirical evidences

Over the last decades SSD-based predictions have been applied and validated with field and experiment effects in several studies. For the present report we exemplarily reviewed selected studies that validated SSD-based predictions for toxicant mixtures with ecological effects in the field. We could only identify studies that focused on aquatic ecosystems. Nevertheless, the review will help to identify evidences and general limitations of SSD-based predictions of mixture effects.

De Zwart (2005) investigated Dutch ditches regarding the presence of pesticides and the effects on diversity and overall abundance of aquatic organisms. The author modelled pesticide exposure and derived SSD-based risk predictions for aquatic communities. However, De Zwart (2005) detected weak correlations between predicted msPAF and macrofauna community in the ditches (number of species, total abundance of individuals), when very abundant and scarce species were removed from the analysis. In comparison, no correlation between msPAF and community endpoints was found for macrophytes. Hence, no reliable link between predicted pesticide risk and effects on the diversity and overall abundance of aquatic biota could be shown. Another field study by Posthuma and De Zwart (2006) validated risk estimates of toxicant mixtures on North-American fish assemblages. Correlation analyses showed that predicted risks could be associated to species level response patterns. Effects at the community level (e.g. diversity or total abundance) could not be related due to increasing and decreasing taxa in abundance following the exposure to toxicants. In addition to pesticide effects, the authors detected significant, non-linear correlations between the chronic msPAFs for household product chemicals, metals and ammonia and the local abundance of fish taxa. More recent studies by Posthuma and De Zwart (2012) suggest that risk predictions expressed as acute msPAF-EC_{50} can be related to the observed affected fraction of benthic macrofauna with a change in species abundance. The toxic pressure in this study was caused by metals and organic pollutants. They also showed that SSD-curves based on acute and chronic endpoints presented similar shapes of the curve and only differed in the effect concentrations.

A meta-analysis on field studies by Schäfer et al. (2013) applied different exposure metrics and related them to effects on aquatic macro-invertebrates expressed as the trait-based indicator SPEAR (Liess and von der Ohe 2005) in European and Australian stream sites. The exposure metrics also included TUs based on HC_5-values from SSD curves and msPAF-predictions. The authors concluded that TUs (TU_{max} and TU_{sum}) based on HC_5 correlated best with the observed effects on invertebrate communities, followed by TUs based on *Daphnia magna*. In contrast, the risk estimates based on msPAF were found to be better correlated with the effects on stream invertebrates in only one study region, where also other organic pollutants were present. The poor performance of msPAF for pesticide mixtures was explained by the authors with the consideration of specific MOAs and use of HC_{50}.- values for msPAF that contain a relatively high noise.

Another study by Smetanová et al. (2014) reported the use of msPAF for the prediction of PPP effects on stream invertebrates represented by the bioindicator SPEAR. The authors calculated msPAF based on EC_{50}-values for a set of stream sites in Germany, France and Finland according to De Zwart and Posthuma (2005) and Traas et al. (2002). The authors further derived chronic SSD-values from the acute SSD-values to compare both approaches. Smetanová et al. (2014) observed a good correlation between msPAF-values and SPEAR, but they highlighted that field effects on macroinvertebrates were strongly underestimated by the determined msPAF threshold HC_5. This was especially true for msPAF using acute SSD-values and is crucial, since acute SSD-values are often applied for msPAF predictions.

Potential deviations in the msPAF prediction of mixtures effects (HC_5 or HC_{50}) were also identified in a study by Gregorio et al. (2013). Gregorio evaluated the robustness of CA or response addition (RA) directly applied on SSD curves with theoretical data sets. For the application of CA on SSD-curves the authors concluded that this approach can lead to underestimations, especially if species' sensitivities are not correlated for all substances in a mixture. High predicted msPAF-values were also less robust than low predicted msPAF-values based on CA. In comparison, the authors described under –and overestimations for the application of RA on SSD-curves based on species with very flat or very steep dose-response curves, respectively, for the individual mixture compounds.

Main findings chapter 2 – Communities and aggregated endpoints

Community effects - aquatic

According to the literature review, CA proved to predict mixture toxicity on algae communities for mixtures with similar acting compounds. Predictive power of CA/IA for other aquatic communities and mixtures of dissimilar acting compounds was less clear and only few studies were available. Experimental studies on PPP mixtures with single effect-dominating substances masked the additional toxic effect of the other substances and impeded to draw conclusions regarding the predictive validity of CA or IA.

Studies from the ICS-database comprised only three PPP full sets. In addition, variations in the study design and tested communities between single experiments hampered a reliable assessment of CA/IA for the prediction of mixture toxicity at the community level.

Based on existing field studies, we summarize that PPP mixture effects on aquatic invertebrate communities can be described with the most toxic pesticide (TU_{max}) as well as the sum of all toxic pesticides (TU_{sum} based on the concept of CA) in a mixture. However, TU_{max} and also TU_{sum} underestimate the observed effects on aquatic community composition two to three orders of magnitude below the acute toxicity of aquatic standard test organisms. This underestimation implies that all factors acting in the field (i.e. mixture effects, environmental stress, sequential exposure) are cumulating to a "lab to field sensitivity enhancement factor" for every PPP substance.

Community effects - terrestrial

Earthworm field studies from the ICS-database are not suitable for the analysis of mixture toxicity mechanisms. They show effects only in rare cases and without an opportunity to calculate reliable effect threshold concentrations for different species (due to the applied test design). Validation of mixture toxicity models based on this data is scientifically not tenable.

Literature search on terrestrial community effects provided no further information on the applicability of the toxicity models.

Use of SSD-based approaches

Regarding empirical evidences, only few studies investigated the use of SSD-based approaches for the prediction of PPP mixture effects. Case studies proved that SSD-based approaches to predict mixture effects can be correlated with community effects in the field. One study compared different exposure metrics and observed that the approach of TU_{sum} (i.e. based on concentration addition) and TU_{max} based on SSDs even outperformed all other exposure metrics regarding the relation with effect aquatic invertebrate communities (Schäfer et al. 2013). Moreover, some studies also highlighted for the use of CA on SSD-curves that msPAF predictions can lead to significant underestimations of effects. Hence, the use of SSD based approaches for the prediction of mixture effects in the field is so far comparable to TU based approaches with LC_{50} values of reference organisms and also requires a "lab to field sensitivity enhancement factor". However, the underlying database of SSD based approaches is associated with a lack of toxicity data and uncertainties regarding for example taxa number or taxa selection for the construction of SSDs. While we only identified and evaluated studies for aquatic ecosystems, studies for the terrestrial compartment are needed to draw general conclusions for the ecological realism of SSD-based predictions of toxicant mixture effects.

2.2 Assessing mixture toxicity for chronic effects

2.2.1 Literature on mixture effects and chronic endpoints

2.2.1.1 Aquatic studies

Exemplarily, we identified studies that investigated chronic effects of pesticide mixtures and their prediction. As described before, previous reports (Coors 2009, Coors et al. 2013, Coors and Frische 2011) evaluated acute and chronic tests regarding mixture effects on aquatic organisms. Coors and Frische (2011, see also Coors 2009) investigated the predictability of combined effects from 66 herbicidal and 53 fungicidal combination products (registered PPPs with more than one active substance). Acute effects on *Daphnia magna* and fish of more than 70% of the mixtures with fungicides could be predicted by concentration addition (CA, MDR between 0.5 and 2). In contrast, the MDR for chronic effects of mixtures with fungicides on *D. magna* and fish showed more deviations from the concept of additivity. The predictions of CA were less precise (i.e. < 30% of fungicidal mixtures) and lower by a factor of two to three compared to the prediction of mixture toxicity on *Lemna* and algae. The factor of predictability was similar for the chronic effects of herbicide mixtures on fish. Only for daphnids the predictability of chronic and acute toxicity of herbicide mixtures was similarly low (< 30%). The difference in predictability of chronic effects compared to acute effects using CA was explained with the fact that predictions for daphnids and fish were based on NOEC values, while the predictions for algae and *Lemna* were based on EC_X values. Accordingly, NOEC values seem to impair a reliable prediction of chronic mixture effects on aquatic test organisms. Despite the large number of investigated products by Coors and Frische (2011), no insecticide mixtures or mixtures with different PPP classes were investigated. This information would be highly relevant, since such mixtures are to be expected in tank mixtures and treatment regimes (refer also to chapter 3 and chapter 5). In addition, chronic mixture effects were not investigated for insects, which represent a large share of vulnerable organisms in aquatic and terrestrial ecosystems.

Regarding the chronic mixture effects of insecticides, we identified few examples from scientific literature that support the findings by Coors and Frische (2011). Trimble et al. (2009) evaluated the combined effect of pyrethroid insecticides on *Hyalella azteca* (LC_{50} after 10 days) and summarized that the tested binary mixtures could be predicted by CA (MDR < 2). Two further studies by Maloney (2018a, 2018b) described the chronic mixture toxicity of three neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) on chironomids under laboratory and semi-field conditions. Under laboratory conditions Maloney et al. (2018a) observed that most binary and ternary mixture toxicities could be predicted with CA using EC_{50}-values. Only the cumulative mixture of imidacloprid and thiamethoxam showed a 10% stronger reduction on emergence than predicted by CA. In comparison, in the study under semi-field conditions no synergistic effects 28 days and 56 days after exposure could be identified (Maloney et al 2018b). Similarly, Coors et al. (2013) reported that chronic effects (NOEC and EC_X) of three wood preservatives on daphnids and algae could be mostly well predicted with CA.

Little information is available for the use of the CA and IA approach with PPP mixtures of different classes. During the literature search we only detected studies that investigated chronic effects of mixtures with fungicides and insecticides that are known to cause synergistic effects on acute endpoints. Examples include an experiment by Bjergager et al. (2017), who tested the mixture effects of azol fungicides and a pyrethroid insecticide on *D. magna* at different time points (2 days, 14 days). The authors determined that the threshold concentrations for synergistic effects based on IA and CA predictions even decreased over time. Another study by Bjergager et al. (2012) investigated the synergy between prochloraz (azol fungicide) and esfenvalerate (pyrethroide insecticide) on the survival of *D. magna* in microcosms. The synergy between both compounds was similar or even higher 7 days after exposure compared to the synergy 2 days after exposure under laboratory conditions (Bjergager et al. 2012). Similar findings were observed by Shahid et al. (under review) for synergistic effects of prochloraz and esfenvalerate on *D. magna* under high and low food conditions in the laboratory. Hence, the synergistic interactions of selected mixtures are still detectable for chronic endpoints and even seem to increase over time (Bjergager et al. 2017, Bjergager et al. 2012).

2.2.1.2 Terrestrial studies

243 hits from the systematic ISI-search have been checked for usefulness for the research questions of the project. 20 citations were identified as relevant and their contents were outlined in tabular form (Table 28, Appendix A.3).

It can be summarized that only few authors were interested in disentangling the predictability of effects of a toxicant mixture from the individual toxicities of its components. Sometimes non-chemical factors like temperature (Bednarska et al. 2010), radioactivity (Gault et al. 2010) or the influence of the composition of artificial soil substrates (Amorim et al, 2005, An and Lee, 2008) were investigated. The studies that focused on effects of mixture estimated synergistic or antagonistic effects of the individual substances mainly by using the concept of concentration addition (Amorim et al. 2012, Cedergreen et al. 2007, de Boer et al. 2013, Druart et al. 2010, Gomez-Eyles et al. 2009). In chronic laboratory studies earthworms (*Eisenia fetida*) (Gao et al. 2016, Gomez-Eyles et al. 2009) or collembolans (*Folsomia candida*) (Broerse and van Gestel 2010, Amorim et al. 2012, de Boer et al. 2013) were tested, however, the number of replicates for the deduction of this finding was generally low. A variety of standard endpoints (biomass) and sublethal endpoints (reproduction, enzyme activities, gene expression patterns) (Fisker et al. 2016, Gao et al. 2016, Gault et al. 2010) were reported.

The experiments show very clearly that some mixtures cause synergistic effects on the prediction from single substances (Amorim et al. 2012), some ingredients work counteractive as antagonists (e.g. cadmium and phenanthrene, de Boer et al. 2013), and not too rarely the prediction by the concentration addition concepts works well. It turned out that the responses for the different laboratory species are species specific, a fact that hampers simple interpolation between species and extrapolation to the field situation. Authors generally conclude that the underlying mechanisms of the effects of mixture are poorly understood. Accordingly, the prediction of mixture effects – highly relevant for a reliable risk assessment - needs a better database, and a better understanding of the relevant processes.

2.2.2 Further database (Regulatory reports, own data)

As described in chapter 2.2.2, mixture and single substance reports from the authorization process of pesticides were screened and pre-selected jointly with the responsible project coordinators at the UBA. Corresponding studies on chronic endpoints of aquatic and terrestrial taxa were provided to UBA and described in the following. Studies that describe mixture effects and additional studies for the single pesticide substances were checked for comparability as described into more detail below. Study endpoints include *Daphnia magna, Chironomus riparius* and earthworm taxa.

2.2.2.1 Aquatic data

We identified four full sets (study data present for the mixture and single exposure to all compounds) for long-term studies on *C. riparius* and eleven full sets for *Daphnia magna*. From chronic studies on *C. riparius* we extracted information on test substance (formulation or technical substance), exposure scenario (spiked water, spiked sediment), endpoints (emergence and development) and test duration. Data were obtained from German Environment Agency and further also from the PPDB (Pesticides Property DataBase, Lewis et al. 2016) for *D. magna*.

For comparing the different mixture predictions on *C. riparius* we screened the underlying data sets regarding the endpoint mean emergence rate in order to check if NOEC data is available for the same endpoint, tested compartment (sediment or water) for single substance and mixture exposure (data origin: ICS and further also PPDB for *D. magna*, refer to Table 4). As described before, the majority of existing chronic effect studies on *D. magna* were evaluated by the expert opinion of Coors (2009, see also Coors and Frische 2011). Therefore, it was agreed to focus only on studies published after 2009. Five out of the eleven full sets after 2009 contained PPP mixtures that were already assessed by Coors (2009).

To evaluate the mixture toxicity on chronic endpoints for *D. magna* and *C. riparius*, we calculated the predicted effects of the mixture (Equation 1). The calculations are based on toxicity data for formulations (mixture toxicity) and the a.s. as technical grade (single compounds). The predicted NOEC was then compared to the observed NOEC in order to obtain the model deviation ratio (MDR). The identified eight full sets are listed including the assessment of MDR in Table 3. The determined MDRs for chronic endpoints of *D. magna* and *C. riparius* (NOEC) show that only one out of the eight studies are in the range between 0.5 and 2 indicating an acceptable prediction of concentration addition (CA). The other MDRs indicate either an underestimation (e.g. bromuconzaole + tebuconazole) or an overestimation of the combined toxicity (dimethomorph + folpet). The low predictability of the chronic effects of mixtures may be due to the inhomogeneity of input data. We included data from technical grades, formulations and also from different data sources if necessary (German Environment Agency, PPDB - Lewis et al. 2016). In comparison to most single compound studies, the tested mixtures were always combination products that contain a.s. and additives. The disregard of additives can contribute to the deviations from the predicted mixture toxicity and has been discussed in previous works (e.g. Coors et al. 2013). In addition, we used NOEC-values to predict mixture toxicity and MDR that was also shown by Coors and Frische (2011) to cause a low robustness of predictions on mixture toxicity.

In conclusion, our results show that the prediction of chronic effects of mixtures with CA based on NOEC values frequently leads to an overestimation or underestimation of the observed effects. This finding is in line with the outcomes by Coors and Frische (2011), but results must be considered with care due to the small amount of investigated full sets.

Table 3: Chronic full sets and the estimated MDR values

Given are the analyzed full sets for the organisms D. magna and C. riparius with the corresponding chronic endpoints. $NOEC_{obs}$ refers to the observed NOEC and $NOEC_{pred}$ to the predicted NOEC of the mixture according to Equation 1.

Full set / Mixture	**Organism**	**Endpoint** (A = immobilisation 21 d, B = emergence rate after 28 d)	**$NOEC_{obs}$ [a.i. ug * L^{-1}]**	**$NOEC_{pred}$ [a.i. µg * L^{-1}]**	**Model deviation ratio (MDR)**
benalaxyl + mancozeb*	*D. magna*	A	3.175	8.036	2.531
bentazon* + terbuthylazine	*D. magna*	A	600	782.953	1.305
bromuconazole + tebuconazole*	*D. magna*	A	1175.46	14.368	0.012
dimethomorph + folpet	*D. magna*	A	20.4	590.462	28.944
glyphosate* + 2,4D	*D. magna*	A	2152	37993.421	17.655
clothianidin + beta-cyfluthrin + Imidacloprid	*C. riparius*	B	3.082	0.738	0.239
diflufenican + penoxsulam	*C. riparius*	B	7000.500	114.809	0.016

Full set / Mixture	Organism	Endpoint (A = immobilisation 21 d, B = emergence rate after 28 d)	$NOEC_{obs}$ [a.i. ug * L^{-1}]	$NOEC_{pred}$ [a.i. µg * L^{-1}]	Model deviation ratio (MDR)
tebuconazol + spiroxamine	*C. riparius*	B	1.980	3338.218	1685.969**

* Toxicity for these data were taken from the Pesticides Property DataBase (PPDB, Lewis et al. 2016).
** This value is exceptionally high and based on the lower toxicity of the single substances tested as technical grade (tebuconazole and spiroxamine). If for spiroxamine only the NOEC value for the study with spiroxamine in a mono formulation is considered (5 µg/L) the MDR is 3.85.

2.2.2.2 Terrestrial data

Description data analyses

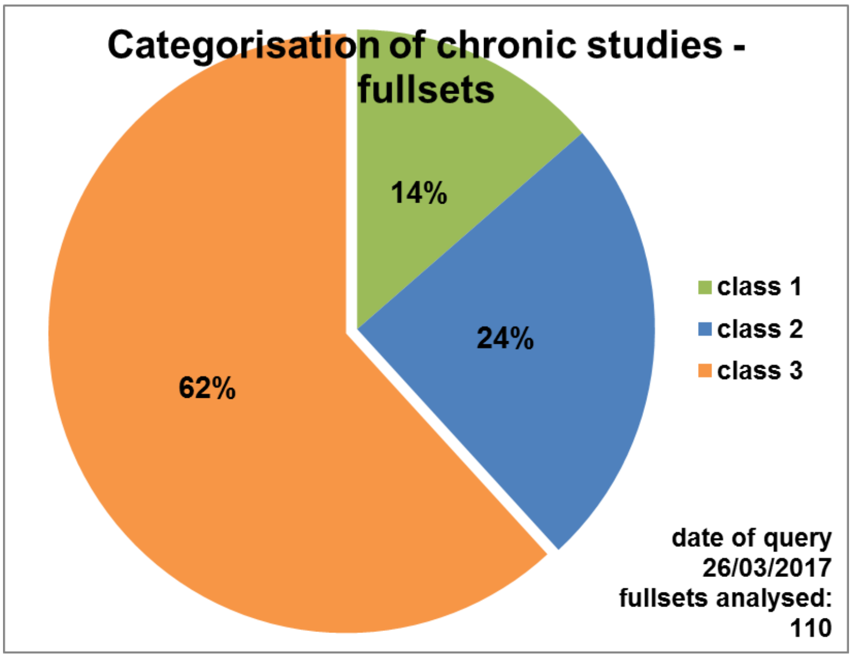
For the identification of terrestrial data with chronic effects, a similar approach was used as chosen for field data (chapter 2.2.2.2) Initially, the ICS-database was filtered to identify chronic laboratory tests. The query leads to a number of 629 database records for chronic earthworm laboratory tests. For mixture toxicity-test procedures with more than one active substance (= combinations), entries of reports for the respective single substances were searched. If tests for a mixture and all single substances are included in the database, full set-numbers for the respective entries were added. 145 full sets out of the 629 entries were identified. 116 of these full sets consist of two single substances and the corresponding mixture, 25 identified full sets are based on a three-component-mixture with data of all single substances and four full sets have four active substances.

Characteristics of the data

To assess the quality of the full set-data with regard to the comparability of test results for mixture effect analysis, the full sets were categorized into three different classes (Table 3), based on the available data from the ICS database: Class 3 contains full sets for chronic laboratory tests of earthworms with data for all active substances tested as single substances and at least one test with a mixture of theses active substances. In addition to that, full sets of class 2 should have similar database entries for the tested endpoint, calculated statistical values, test duration (exposure time) and tested earthworm species. Test procedures of reports for class 1-full sets were conducted using standardized approaches based on the same guidelines and types of application.

The applied classification system with requirements for all classes is shown in Table 4. Results of the categorization-performance for all of the 110 chronic earthworm full sets are shown in Fig. 4. The full sets should be at least classified into class 2, to be able to compare similar endpoints and sampling dates, so that the identified full sets can be analyzed according to the objectives of the project. It turns out that this is only the case for 38% (42 out of 110) of the report-sets.

Figure 4: Distribution of categorized earthworm full sets for chronic effects. The definitions of classes are illustrated in Table 5



The test procedures are based on the standardized guidelines BBA VI 2-2, ISO 11268-2 and OECD 222. It turns out, that the only comparable endpoint of the ICS database-full sets for chronic effects on earthworms might be reproduction after 56 days exposure. For these data, a NOEC was calculated. For this, the full sets with comparable endpoints (class 1 and 2) were initially converted and adapted to uniform units, not usable reports were removed (e.g. in the case of lacking effects throughout the test).

Table 4: Applied classification system to assess the quality, compatibility and comparability of identified full sets for earthworm tests with chronic effects

The table shows the applied classification system to categorize chronic earthworm study full sets into three different classes of data quality. In each column of the table the data characteristics of the respective class is summarized.

Class 1	Class 2	Class 3
single active substances and mixtures tested	single active substances and mixtures tested	single active substances and mixtures tested
Test type (laboratory, chronic)	Test type (laboratory, chronic)	Test type (laboratory, chronic)
endpoint (reproduction, biomass, mortality)	endpoint (reproduction, biomass, mortality)	
Calculated value (NOEC, LOEC, ECx etc)	Calculated value (NOEC, LOEC, ECx etc)	
Time of testing (28d, 56d, etc)	Time of testing (28d, 56d, etc)	
Test species (*Lumbricus terrestris, Eisenia fetida/ andreii* etc.)	Test species (*Lumbricus terrestris, Eisenia fetida*/ andreii etc.)	
guideline (BBA VI 2-2, ISO 11268-2, etc)		

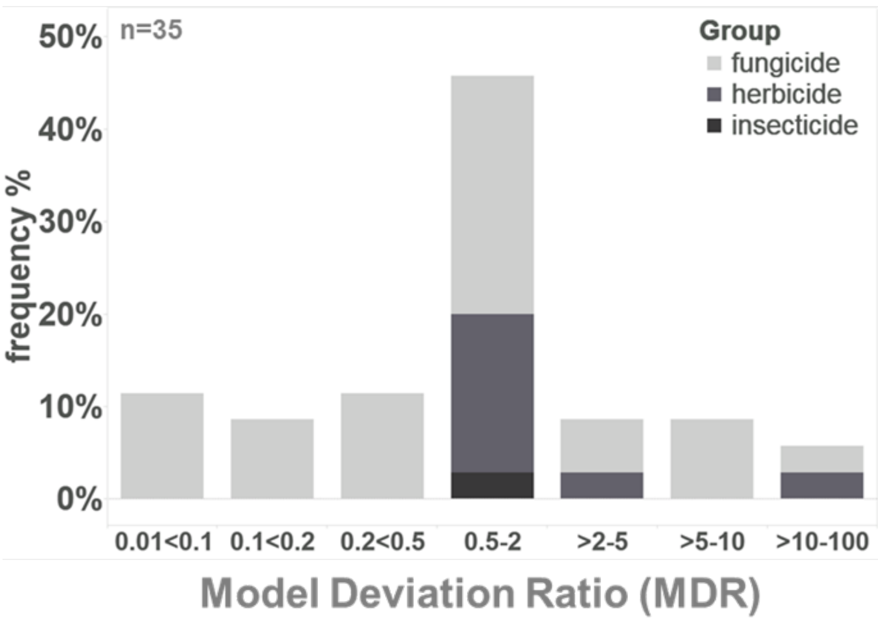
Class 1	Class 2	Class 3
Type of application (sprayed, mixed etc.)		

Calculation of the Model Deviation Ratio (MDR)

For NOEC values of the remaining full sets (n = 35) Model Deviation Ratios (MDR) were subsequently calculated to illustrate the uncertainty of the concentration-addition (CA) prediction in comparison to observed NOEC-values of the combination product. The methodological approach to calculate the MDR regarding the predicted NOEC ($NOEC_{pred}$) of the mixture (based on single substance testing) and the observed NOEC ($NOEC_{obs}$) of the combination product is given in Equation 1.

The results of the MDR-calculation for earthworm reproduction tests, plotted as a frequency distribution of all model deviation ratios of the identified full sets, are shown in Figure 5. The deviation of less than a factor 2 between prediction and observation of the NOEC was the most frequent result; about 46% of the studies were found within this range (16 out of 35). In these cases, the use of the concentration addition approach to predict mixture toxicity in chronic earthworm tests can be suggested due to reasonably small uncertainties.

Figure 5: Frequency distribution of model deviation ratios (MDR) for earthworm reproduction tests (NOEC, 56days)

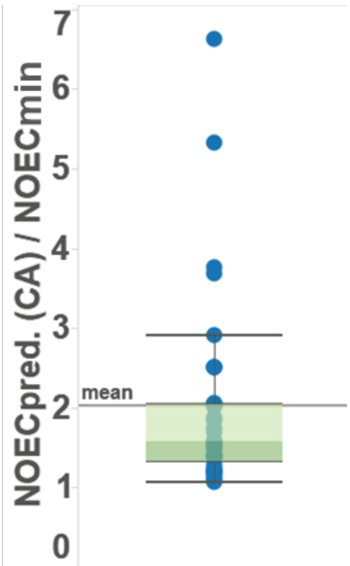


We determined for eleven of the 35 combination products MDR values of less than 0.5. This indicates that the concentration addition prediction overestimates the toxicity of the mixture, so the combination product is less toxic than predicted in these cases. All of these eleven full sets were fungicidal combination products. Only for eight combination products (six fungicides, two herbicides) a clear underestimation of the predicted toxicity of the mixture was found. The results of our calculations for earthworm reproduction tests suggest that the concept of concentration addition can be applied in almost 50% of the cases to predict the toxicity of mixtures. Overall, results of the MDR calculation for earthworm reproduction tests show a high compliance between predicted and observed mixture toxicity. Averaged over all pesticide groups, the MDR median of 0.67 indicates that effects of mixtures of combination products tended to be slightly more overestimated.

However, an increase in the uncertainty of the calculated MDR for combination products in earthworm reproduction tests is to be expected when EC_X instead of NOEC or LOEC are used to identify the MDRs. This is because the scientifically correct use of the CA concept requires defined effect level (like EC_X values), which are not provided by no-observed effect thresholds. Therefore, the MDR_{NOEC}/LOEC of earthworm reproduction studies has to be interpreted more critically than the predicted aquatic toxicity of pesticide mixtures (Coors and Frische 2011). As already stated by Coors and Frische (2011) for aquatic data, we recommend to not use the CA-based mixture prediction with terrestrial NOEC data as toxicity measures. It cannot be ruled out that a clear overestimation (31.4% of full sets) or underestimation (22.9%) of the concentration addition model is biased by highly heterogeneous input data. However, as the results of the chronic terrestrial data sets show a general applicability of the CA prediction model for terrestrial species, we suggest to confirm this pattern with data on defined effect level.

In a further calculation step, the NOEC of the single substances was compared with the $NOEC_{pred}$ to determine whether the more toxic of the two substances ($NOEC_{min}$) is a reliable predictor for $NOEC_{pred}$ (and thus also for $NOEC_{obs}$). For the analyzed data set of earthworm reproduction tests, the more toxic of the two substances corresponds to an average of 62.4% (± 21.5%, range: 15.09% -88.39%) of the concentration of $NOEC_{pred}$ in the evaluated full sets. The $NOEC_{min}$ is a median factor of 1.6 lower than the NOEC based on CA prediction (Figure 6).

Figure 6: Box-Whisker-Plot for the distribution of the ratio between predicted NOEC of the CA model ($NOEC_{pred}$) and the NOEC of the more toxic substance ($NOEC_{min}$) from a two-component product



Due to the mathematical structure of the equation for CA prediction (Equation 1) the more toxic substance of a full set has always a lower NOEC than the predicted mixture ($NOEC_{pred}$/NOEC >1). Based on these findings, $NOEC_{min}$ of the most toxic substance seems to be a protective and relatively precise estimator of mixture toxicity (median factor of 1.5, majority of 76% of all full sets with $NOEC_{pred}/NOEC_{min}$ between 1.0 and 2.1). Nevertheless, analyses also showed that in 15% of the analyzed full sets the $NOEC_{min}$ is only 15-35% of the $NOEC_{pred}$, an estimation of mixture toxicity based on single components clearly fails in these cases.

Regarding a regulatory use of the more toxic substance for the prediction of mixtures it also has to be stated, that these model calculations only focus on laboratory concentrations and not on absolute amounts of exposed products, tank mixtures or spray series in field situations. For the application in field situations in a regulatory context the toxicity measures of the more toxic substance would be assigned to total amounts of tank mixtures or spray series, regardless of the actual composition and toxic measures of the ingredients. This would increase uncertainty caused by an imprecise estimation of the mixture toxicity with using the $NOEC_{min}$ and is accordingly not advisable. Therefore, a prediction of the mixture toxicity for combination products considering only the toxicity of the more toxic substance of the product seems to lead only to vague estimations.

2.3 State of art: predict mixture toxicity with regard to human health

For an overview on the current state of art on mixture toxicity and human health we focused on recent literature reviews and reports.

2.3.1 General findings from literature

The Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and Scientific Committee on Consumer Safety (SCCS) also released a joint opinion paper in 2011 on the toxicity and assessment of chemical mixtures. The authors analyzed existing approaches and recent publications regarding the prediction of mixture effects on human health and the environment. By doing so, they concluded that chemical mixtures cause generally larger effects than the effects due to single exposure that can be commonly predicted with CA or IA. However, if the mode of actions (MOAs) of all compounds in the mixture are not known, the more conservative CA should be used rather than IA. The authors also summarized from the reviewed studies that interactions between chemical compounds generally occur at medium or high dose levels (i.e. dosis > NOALs, NOECs or benchmark dose levels). Nevertheless, it is stated that interactions between toxicants are difficult to predict and need to be assessed for each case individually. Regarding major challenges for the risk prediction of chemical mixtures, the authors outline the lack of data on exposure information and MOAs.

In 2016 the Joint Research Centre (JRC) published a technical report from Bopp and colleagues (Bopp et al. 2016) who analyzed 21 case studies to address the effects of chemical mixtures for human health and environmental risk assessment (14 studies on human health assessment, 6 studies on environmental risk assessment, 1 study on both assessments). The case studies included the exposure to different classes of compounds, such as pesticides, phthalates, parabens or polybrominated diphenyl esters in different exposure media, such as food, pharmaceuticals, breast milk, surface water or contact materials.

The reviewed studies comprised the use of different hazard data, such as for example data on mortality (i.e. aquatic organisms), anti-androgenic specific endpoints, chronic inhalation toxicity data for non-cancer effects or the Tolerable Daily Intake. Most of the investigated case studies assumed additive effects and applied the model of concentration addition (CA) for the assessment of chemical mixtures. The Hazard Index (HI) approach was the main tool to apply CA. The HI represents the sum of hazard quotients (ratio between exposure and reference endpoint). Some case studies further applied the Maximum Cumulative Ratio (MCR) in order to identify the main drivers in combined risks. Bopp et al. (2016) concluded that the relevance of interactions in mixtures is still not well understood. The authors also outlined several uncertainties and assumptions that hamper the evaluation of pesticide mixtures for human health and environmental risks (including also the underestimation of combined effects):

- limited knowledge on the simultaneous exposure (selected chemical monitoring) and which chemicals actually contribute to combined effects
- mixture assessments that only consider one compound class (e.g. pesticides) and do not consider co-exposure
- neglecting bio-accumulation and chemical metabolites
- often assumption of only additive effects
- unsuited environmental monitoring or measurement techniques (e.g. in surface water, WWTP effluents)

Additionally, a recent review by Rizzati et al (2016) presents an update on literature regarding pesticide mixtures and human health. Rizzati et al. (2016) evaluated studies between 2000 until 2014 that were conducted in mammalian models and covered various endpoints (e.g. metabolism, neurotoxicity, apoptosis, reproduction). Most of the investigated mixtures contained only insecticides (46%), only fungicides (15%) and only herbicides (4.5%). Hence, reliable conclusions can be drawn mainly for insecticide mixtures, and to a lesser extend for fungicide and herbicide mixtures. The authors classified studies to the presence of additive effects and interactions between compounds including synergic, antagonistic and potentiating effects. Synergic interactions were defined by the authors as effects generally higher than predicted assuming additivity and potentiating interactions as an increase in toxicity of one or several compounds in a mixture. Overall, 35% of the investigated studies showed interactions (71% synergic, 21% antagonistic, 8% potentiating), 48% of the studies showed additive effects and 17% of the studies showed no additive and no interaction of compounds at all (effect driven by the most effective compound). The authors also concluded that synergistic interactions were mainly observed for only insecticide mixtures, while no synergistic effects could be observed for only herbicide mixtures, fungicide-herbicide mixtures or insecticide-fungicide-herbicide mixtures. It was further determined that fungicide mixture mainly led to additive effects.

2.3.2 Cumulative risk assessment of PPP residues in food

The European Food and Safety Agency (EFSA) developed approaches for cumulative risk assessment methods for pesticide residues in food (i.e. EFSA 2008, 2009b, 2013a, 2014). The approaches also include proposals to overcome existing data gaps on the specific toxicity of PPPs on human health. It has been suggested to apply the concept of dose addition independent of the mode of action (MOA) of the single compounds in a mixture. In addition, it has been further suggested to group PPPs with common adverse outcomes on the same organ or system into common assessment groups (CAGs). The use of dose addition and CAGs is considered a pragmatic and conservative default approach given the existing data gaps. The grouping into CAGs is a tiered approach with different levels. The first level refers to the common target organ and the second level describes phenomenological effects. There is also a third and fourth level including the specific mode and/or mechanism of action. Since this information on the mode or mechanism of action is often not available, the Scientific Opinion of the PPR Panel (EFSA-PPR-Panel 2014) proposed the grouping into CAGs using phenomenological effects. Active substances with dissimilar or similar MOAs are supposed to be grouped in one CAG as long as they cause the same specific effect (i.e. CAGs with regard to the nervous or thyroid system). The grouping of

PPP into CAGs is interesting, but the scientific opinions by the EFSA do not provide clear scientific evidence that CAGs enable a more protective or robust risk assessment of mixture effects. In addition, the grouping of a.s. into CAGs may affect the resulting tolerable maximum residue levels and requires a very careful analysis of existing toxicity data. Also, specific effects are not always clear due to potential secondary (i.e. indirect effects) or unspecific effects (Colnot and Dekant 2017) and will affect the specific assignment to CAGs. Finally, a comparative assessment of the doses/ concentrations using defined reference values for the single CAGs is still not established for the risk assessment of PPP residues in food (EFSA-PPR-Panel, 2013b, 2014). Based on the difficulties regarding the grouping of single substances (i.e. secondary, unknown or unspecific effects) and the so far missing evidence for the advantage of using CAGs in a cumulative risk assessment, we do not suggest at this stage the use of CAGs in the ERA of PPPs.

Main findings chapter 2 – chronic effects

Chronic effects – aquatic

According to previous studies we summarize that the use of CA for the prediction of herbicidal and fungicidal mixtures matched better by a factor of two to three when predictions were based on chronic EC_X – values compared to chronic NOEC values (i.e. Coors and Frische 2011). Few studies indicate similar findings for insecticide mixtures. Further few studies on mixtures with PPPs from different classes focused on synergistic mixtures (fungicides + insecticides) showing that synergistic effects persist or even increase over time. Evaluation of few mixture on chronic effects on *Daphnia magna* and *Chironomus riparius* confirmed that the use of NOEC values the prediction of chronic mixture toxicities often leads to deviations from the CA model.

Chronic effects - terrestrial

The CA approach shows compliance for a majority (almost 50% MDR between 0.5 and 2) of the analyzed chronic data for terrestrial compartments (only ICS studies of earthworm reproduction considered). Major uncertainties are to be expected due to the use of NOEC values as toxicity metrics. The NOEC of the most toxic substance is a protective but mainly too imprecise estimator for mixture toxicity.

We conclude for terrestrial and aquatic organisms that NOEC-values are not recommended for the prediction of chronic mixture toxicity. The application of effect level values (EC_X) would reduce uncertainty in mixture toxicity calculation. Otherwise, we could not find indications that the predictive power of CA for chronic mixture effects is significantly different from the prediction of acute mixture effects.

Mixture toxicity and human health

CA/IA provides in some cases a robust prediction of mixture toxicity on assessment criteria of human health. However, Several studies also outline that non-additive effects (i.e. synergistic effects) are also present and difficult to predict (i.e. assessment on a case-by-case basis).In addition, there is a lack of data regarding especially information on exposure and compound specific effects of single compounds in a mixture.

Approaches by the EFSA for the cumulative risk assessment of PPP residues in food propose the use of dose addition (equivalent to CA) and the grouping of PPP substances to common assessment groups (CAGs) based on the common target organ and phenomenological effects.

However, the clear grouping of compounds is compromised by the presence of unknown, unspecific and secondary effects. In addition, there is no clear scientific evidence that CAGs enable more realistic mixture effect predictions than no grouping and only additive effects are considered.

3 Exploration of treatment regimes of main crop types in Germany

Different resources regarding the description of general use patterns of pesticide mixtures in common treatment regimes were available. We screened generic treatment patterns, official recommendations of the German federal chambers of agriculture, the data from the German Panel on Plant Protection Measures (PAPA) and a data set of actual spray series. The generic treatment patterns were extracted from companions (Landwirtschaftskammer NRW 2015). "Generic" means that agricultural advisors usually consider a PPP treatment as necessary under the typical climatic conditions and the pest pressures. The chronological sequence of the application of plant protection products is highly correlated with the developmental stages of the crops (BBCH- or EC-stages). However, due to the large data set from actual spray series available, we focused on their analyses and mainly used the generic and recommended spray series for validation.

This information served to calculate spraying frequencies and played an important role in the description of exposure duration and patterns of predicted environmental concentrations. Subsequently, we validated the actual application data with the treatment index as used by the Julius Kühn Institute (JKI) and generic treatment patterns per crop type. The treatment index was also used to identify risk scenarios (treatment regimes with high or median risk) as described under section 3.3.

We describe the different data sources (data on application patterns of spray series and on the ecotoxicity of the active substances applied) and evaluate the comprehensiveness of them in terms of a nationwide risk assessment of complex exposure patterns of PPP. The dataset of actual spray series is explored in detail, focusing on an explorative description of emerging mixture toxicity issues. We analyzed how often specific combinations of well-known critical (e.g. fungicides and insecticides) PPP-classes or active substances occurred. We developed a conceptual approach; which indicators are available and suitable for the classification of treatment regimes and the derivation of typical as well as worst-case spray series scenarios. Subsequently, we validated the actual application data with the treatment index as used by the JKI and generic treatment patterns per crop type. The treatment index was also used to identify risk scenarios (treatment regimes with high or median risk) as described under section 3.3.

Examples of classified treatment regimes are given: potatoes, winter wheat, winter oilseed rape and apples. On the one hand, the data on actual application patterns is used in chapter 3 for the classification of spray series into "typical" or "worst-case" spray series by qualitative and quantitative assessments. Using this classification, we selected spray series for a detailed risk characterization in chapter 5.

3.1 Data sources

The identity of both PPPs and active substances was important to match with other databases on PPP registration and chemical characteristics. For the deduction of risk scenarios, the actual application patterns and toxicity data for different terrestrial and aquatic endpoints were linked. In the following section, we describe the data we requested, queried, compiled and harmonized for further analysis, compared to the above-mentioned requirements.

3.1.1 Relevant crop types

Data on the intensity of the use of plant protection products in Germany was published by the JKI for the year 2011, based on the PAPA-surveys (Roßberg 2013). A treatment index provides an aggregated metric for use intensity by combining information on the frequency of application, the maximum registered application rate and the treated area. The monitored crops were chosen by the treatment indices and treated area combined with the relevance that results from the National Action Plan for sustainable use of pesticides in Germany (BMEL 2013). Nine main cultures were analyzed. Arable crops comprised maize, winter barley, summer barley, winter wheat, summer wheat, winter oilseed rape, sugar beet and potato. Vertical and permanent crops were represented by apple, vine and hop. For these crops, information on advised and actual use was aimed to be collected extensively from the literature or publicly available data sources.

3.1.2 Actual application patterns

For the analysis of the actual application patterns, data were provided by INL - Privates Institut für Nachhaltige Landbewirtschaftung GmbH and one farm near Leipzig. A treatment regime is defined as a series of plant protection and plant growth regulation measures by mainly synthetic chemical substances applied over the growth period ("growing season"). It applies for a distinct crop from sowing (for arable crops) or from leaf development (for permanent crops) to harvest in each case. The spray events within one treatment regime are characterized by the pest pressure (indicated by the pesticide class, e.g. insecticide, herbicide, fungicide), the time of application and the applied plant protection product at the recommended or realized rate (see box "terminology and definitions "actual application patterns" below). Technically, one spray event was identified from data as a single date at which one or more plant protection products containing one or more active substances were applied. For this, a treatment regime starts and finishes during the year. The term is used synonymous with "spray series", which indicates that there is a consecutive exposure pattern, acting both toxic on target and on non-target organisms. Each unique combination of crop, treatment year and farm from the dataset was considered a distinct spray series (as described above).

The INL and the single farm near Leipzig provided a large and comprehensive data set of real application patterns, and data entries were harmonized for the linkage to other databases and further analysis, e.g. product names and application rate units were aligned for unique identification.

The COMBITOX project database held 14095 datasets (observations) from twelve crops (apple, maize, potato, spring barley, spring wheat, sugar beet, triticale, vine, winter barley, winter oilseed rape, winter rye, winter wheat). Hop was previously identified as a relevant crop in Germany, but the culture was not in the portfolio of the data donor, due to its very restricted cultivation region and thus was not integrated into the project database.

Terminology and definitions "actual application patterns"

Spray series

A spray series is defined as containing all plant protection and plant growth regulation measures using chemical substances that are applied over the complete growth period ("growing season") on a distinct field. The growth period for arable crops lasts from sowing, for permanent crops from leaf development to harvest. The reference year is defined by the timing of harvest. The term "spray series" can be used synonymous to "treatment regimes". The spray series is composed of one or several spray events.

Spray event

A spray event is defined as an application of one or several plant protection products in a single tractor passage over the field at a certain date. In our dataset all applications occurring on a certain date are assumed to happen simultaneously. During a spray event single products or mixtures can be filled into the tank and being sprayed.

Tank mixture

If several plant protection products are filled in a single spray tank at a spray event, the spray liquid is called a tank mixture. This implies that the mixture in the actual configuration has never been tested ecotoxicologically nor the resulting risks have been assessed because registration was done for each product separately. The rationale of the farmer is to save passages over the field and thus time, fuel, water and minimize soil compaction. A tank mixture contains mono-formulations or even several combination products, and thus consisting of many different interacting active substances. Here, it is deduced indirectly from data that farmers do not drive twice for reasons of time and cost efficiency over their fields if more than one PPP is reported for a certain date.

A representative set of spray series from about 100 partner farms were used to develop a general workflow to define complex worst- and best-case spray series, a classification derived from indicators of toxic pressures, which were based on the actual application rates and ecotoxicological effect data on their part (see section 3.3 for the description of the methodology of spray series classification). The data from the INL-partner farms did comprise data from different German federal states and hence, different agricultural regions all over Germany and, additionally, from other European countries (Austria). The data on PPP treatment included information on the date of application, the field (identification number, size), the product applied (name, content of active substance in spray liquid), the actually treated area, the crop, the farm (ID, region), the active substances and their contents in the product, the applied rate and occasionally the pest that was the root cause of the treatment.

The database held 28 original and secondary derived variables. Application rates of the actual spray series were given as kg product per hectare. Total contents of a.s. in the respective products were given in the database.

3.1.3 Toxicity data for the calculation of toxic units (TU)

3.1.3.1 Terrestrial endpoint values

Toxicity endpoint values from different assessment areas were necessary for the calculation of toxic units. For earthworms, acute LC_{50} after 14 days of exposure and chronic NOEC data after 56 days of exposure, measuring the reproduction as number of juveniles of earthworms from standard laboratory studies, were compiled from the ICS database (methodology described in Scholz-Starke 2015, data queried in June 2015), from the Dutch Environment Agency RIVM (via the database of HAIR, Kruijne et al. 2011) and from the US EPA ECOTOX Knowledgebase (US EPA 2017), searching for lacking active substances via hyphen-eliminated lists of CAS-numbers.

3.1.3.2 Aquatic endpoint values

Aquatic endpoints included regulatory acceptable concentrations (RAC) for 108 substances (German Environment Agency). Acute toxicity values after 48 hours of exposure for *Daphnia magna*, *Chironomus riparius* (partly also *Hyalella azteca*) and chronic data after 72 or 120 hours for algae (*Pseudokirchneriella subcapitata / Raphidocelis subcapitata*) were extracted for single substances from the US EPA ECOTOX database (US EPA 2017). Further data was queried from the IUPAC - Pesticide Properties DataBase (PPDB, Lewis et al. 2016).

3.1.4 Registration data

Maximum allowed application rates were needed for the calculation of the treatment index according of the methodology developed by the Julius-Kühn-Institut – JKI (Roßberg 2013). The German Federal Office of Consumer Protection and Food Safety (BVL) provided data for all plant protection products registered on November 2016 and expired registrations of the eight years before November 2016. For technical reasons, data on expired registrations are not comprehensive. The list of product names was unified with the actual application data for best matching results joining the different databases. Additionally, a list of parallel registrations in other European countries was provided with the German data to cover the spray series from Austria. For the maximum allowed application rates as registered by the BVL and provided for the period covered by actual spray series, twenty different conversions of the original units (e.g. g product / ha, l product / ha, ml product / m^2) were necessary to obtain homogenously units of kg product/ha. Registration data has to be matched with the actual application data, e.g. for the calculation of the treatment index (Equation 3). In cases that do not allow a proper product matching (e.g. a parallel registration in Austria was not found), an “orphan” applied product remains (see Table 8).

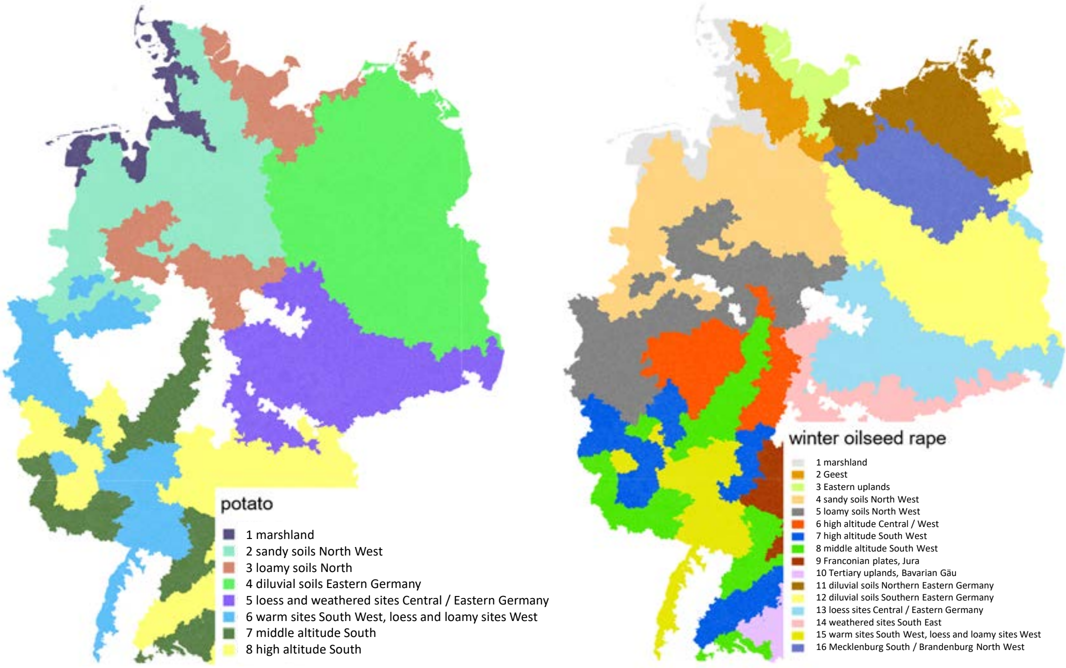
3.1.5 Regional cultivation practice

In Germany, as a territorial state of significant widespread extension, regional differences of agricultural practices and plant protection practices obviously occur. Therefore, it was intended that possibly most of the regional variability should be covered to a sufficient extent by data. As a reference for the variety of cultivation areas and for most of the relevant crops, the Julius-Kühn-Institut (JKI) defined growing regions with homogenous climatic conditions and pedogenic properties and provided data and ontology. The methodology was described by Graf et al. (2009). It was assumed that on a small-geographic scale the intensity of PPP-use can vary greatly and is thus objected to high variability.

Fig. 7 shows exemplary for winter oilseed rape and potato that due to differences in soil properties combined with climatic characteristics between the regions, cultivation areas can be distinguished specifically between crops. Large-scaled regions as the Federal States of Germany were sufficiently covered by data. However, it was not possible to ensure representativeness on the scale of soil-climate regions for each of the twelve relevant crops.

Figure 7: Soil-climate regions in Germany for winter oilseed rape (left) and potato (right)*

Maps were drawn using the open source software QGIS (Version 3.2.3 Bonn) using online data from WMS-Servers (JKI 2014, http://geoportal.julius-kuehn.de/). Maps were provided for conventional agricultural practice (https://geoservices.julius-kuehn.de/geoserver/konv/wms?) and organic farming (https://geoservices.julius-kuehn.de/geoserver/oeko/wms?) separately, based on soil-climate-regions that were allocated to each administrative unit (on the level of municipalities).



* Graf et al. (2009)

Most data were available for Germany (Table 5). The federal state of Sachsen-Anhalt held by far the most datasets. For the yellow-colored federal states of Fig. 8 at least few observations were available. For the red colored areas, no data was available. In summary, a comprehensive database was available that allowed for far-reaching descriptions of the usual (advised) agricultural plant protection practice in Germany. Data for Austria complemented the overall picture for underrepresented, nonetheless intensive cultures, namely vine and apple. For advanced analyses, we focused on spray series from Germany and Austria (only apple), because the regions were assumed comparable in terms of climate and further culture conditions.

Figure 8: Map of Germany, boundaries show the 16 German Federal States. Colors represent five non-equidistant coverage classes derived from the distribution of 873 spray series over twelve relevant crops

Abbreviation code Federal States of Germany: BE Berlin, BW Baden-Württemberg, BY Bayern, HB Bremen, HE Hessen, HH Hamburg, MV Mecklenburg-Vorpommern, NI Niedersachsen, NW Nordrhein-Westfalen, RP Rheinland-Pfalz, SH Schleswig-Holstein, SL Saarland, SN Sachsen, ST Sachsen-Anhalt, TH Thüringen. Color coding of the areas: Red No spray series data available, yellow 1-50 spray series available, light green 51-100 spray series available, medium green 101-150 spray series available, dark green 151-250 spray series available.

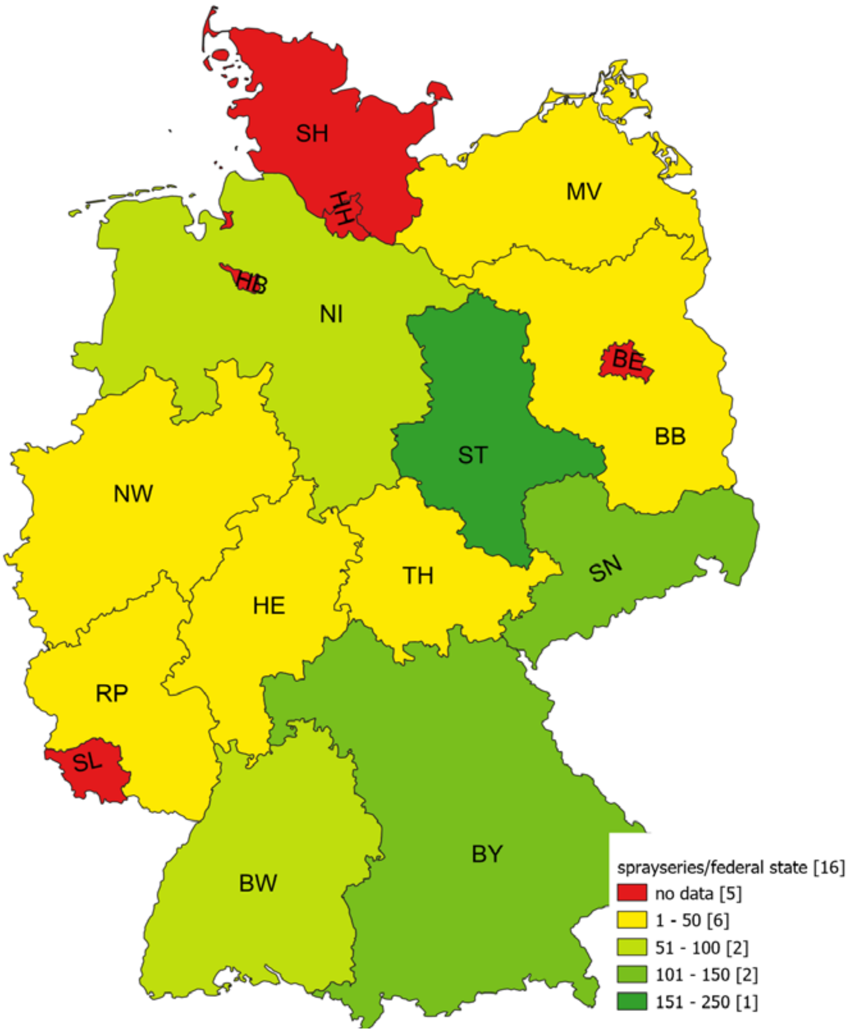


Table 5: Cross-table of the number of spray series over 16 German Federal States including Austria and twelve relevant crops

GFS: German Federal State (including Austria), all crops (ac), apple (ap), maize (ma), potato (po), spring barley (sb), spring wheat (sw), sugar beet (su), triticale (tr), vine (vi), winter barley (wb), winter oilseed rape (wo), winter rye (wr), winter wheat (ww).

GFS	ac	ap	ma	po	sb	sw	su	tr	vi	wb	wo	wr	ww
Brandenburg	17		3		1			1		3	3	3	3
Berlin													

GFS	ac	ap	ma	po	sb	sw	su	tr	vi	wb	wo	wr	ww
Baden-Württemberg	67			25	7	2	12			5	8		8
Bayern	135		20	12	8	2	30			6	10	16	31
Bremen													
Hessen	43		9		7					5	7	1	14
Hamburg													
Mecklenburg-Vorpommern	34		2			1	4			6	6		15
Niedersachsen	61		17	5	1		8	3			7	9	11
Nordrhein-Westfalen	18				2	1				2	6		7
Rheinland-Pfalz	47				6		32			2			7
Schleswig-Holstein													
Saarland													
Sachsen	116		11		7		15	6		15	10	36	16
Sachsen-Anhalt	249	17	24	17	18		29	11	12	44	20	23	34
Thüringen	24		3		3		3		6	3	3		3
Austria	62	33							29				

3.2 Crop specific patterns of PPP use

Each crop receives under the specific pest pressure characteristic numbers of active substances during the growing season. The behavior of the farmer is further triggered by the regulatory and economic situation and by the products actually registered and thus available. The products are often subjected to application restrictions related to the actual growth stage. The focus of spray series data analysis was on the selection of typical and worst-case spray series, applying a toxic unit approach for the indication of toxic pressure. The rationales of use and the computational approaches are described in chapter 3.3). A qualitative description on the use patterns of PPP introduces the data briefly.

3.2.1 A comprehensive database

The dataset at hand represents a unique collection of actual treatment data that was not publicly available. Other sources of information were not suited to assess real application patterns. The development of open data concepts for the free availability of publicly funded data on treatment regime would be highly appreciated and necessary to describe the toxic pressures and risks acting on the biodiversity in agricultural landscapes, as stipulated by the national action plan for the Open Data Charter G8 (BMI 2017).

A total of 889 different spray series were deduced from the data (Table 6). The main focus was on three years between 2012 and 2014, because 80% of spray series came from this period (Fig. 9). Fig. 10 gives an overview on the distribution of spray series data per crop type. The best represented crop was winter wheat, comprising 149 spray series. A similarly broad database was available for sugar beet (133 spray series). The least represented crops were spring wheat and triticale (6 and 21 spray series, respectively).

Table 6: Overview of spray series data

Given are the data entries of the COMBITOX dataset on actual treatment regimes.

Characteristic	Number
spray series	873
spray entries	13744
spray events	4931
active substances	220
PPP (classes)	9
crops	12
PPP (products)	515

Figure 9: Number of spray series of nine consecutive growing seasons between the years 2007 and 2015

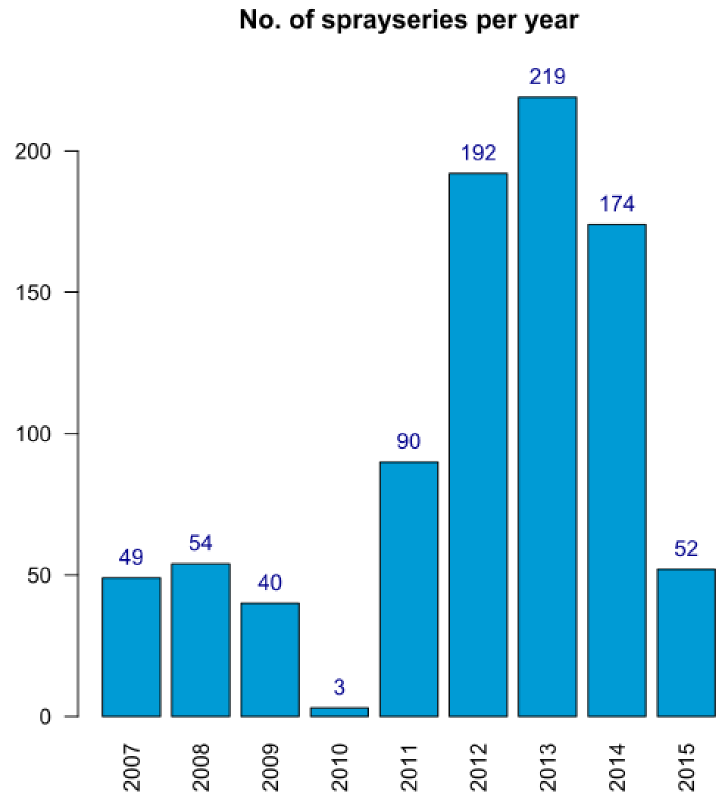
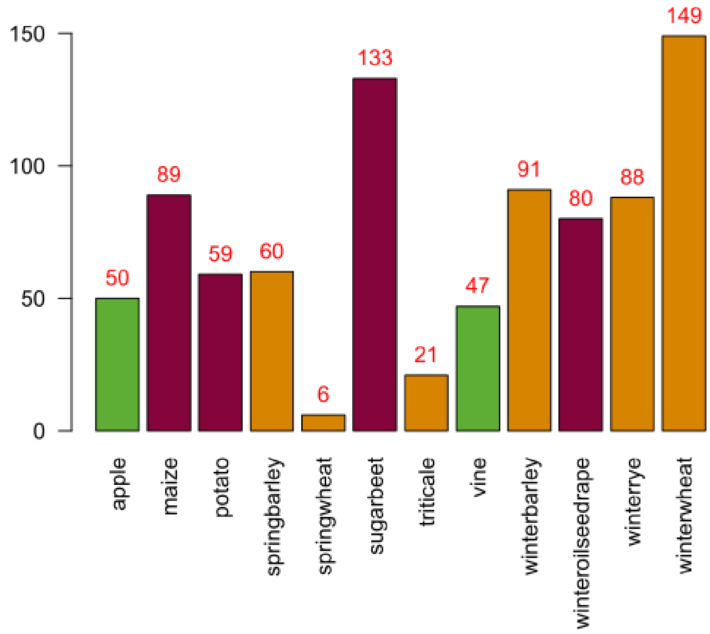


Figure 10: Number of spray series that fall into twelve crops types that were pre-identified as relevant drivers of toxic pressures



3.2.2 High frequencies of pesticide application

Fig. 11 shows that the group of cereals (brown colored boxes) appear quite homogenous and received a constant number of two to six spray events per spray series with 50% probability. The application frequency for winter oilseed rape and potato (arable crops other than cereals red colored in Fig. 11) was slightly higher than for cereals; these crops received five to nine and four to ten spray events per year, respectively. The crop treated most frequently was the permanent culture apple. Vine as the second permanent crop in our database, ranks like potato at medium levels of treatment frequencies.

Since Fig. 11 shows the intensity of spray events typical for the respective crops, Fig. 12 and Fig. 13 focuses on the variety of different substances affecting the non-target organisms within and beside the crops. The number of different active substances (a.s., Fig. 12) ranged between five and 17 with 50% probability around the median for nine arable crops and appeared to be quite consistent between them. Two groups stand out from the general picture: apples were treated with 13 to 18 different a.s. and represented the worst-case, whereas maize was the best-case in terms of the variety of a.s.. Vine showed the most extreme outliers; however, the variability of spray events per spray series (as the coefficient of variation) did not vary clearly between crops and was around 30 to 50%. The picture for the number of products (Fig. 13) was very similar to the number of a.s..

Figure 11: Distribution of total spray events per spray series compared for twelve relevant crops

The boxplots show the upper hinge at 75 % quantile, the lower hinge at 25 % quantile, the median value as black line and the upper/lower whisker as the largest/lowest observation +/- 1.5 * distance between the first and the third quartiles. Points show outliers. The number of observations, i.e. spray series available per crop, is given below the boxplots.

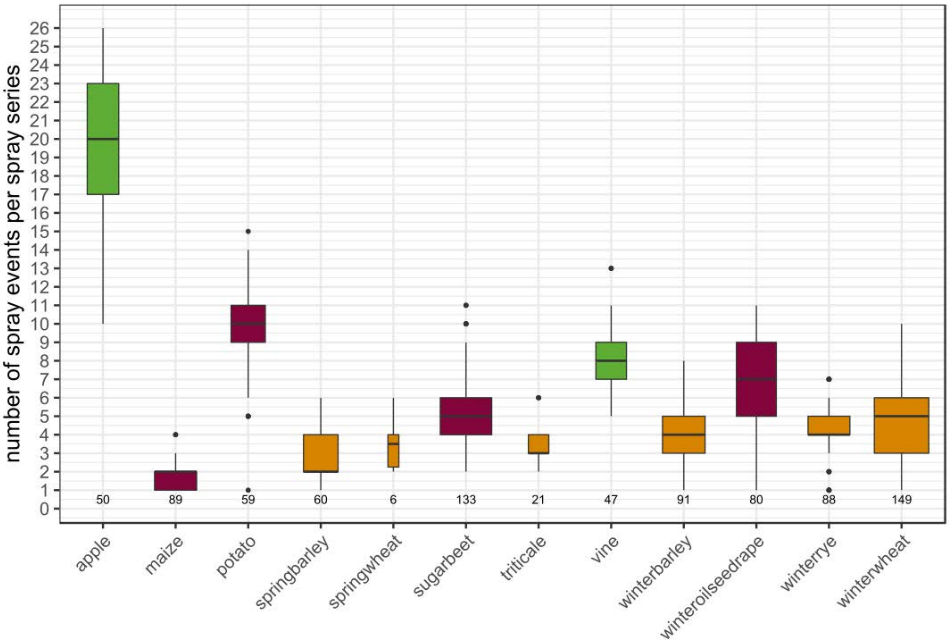


Figure 12: Distribution of total numbers of different active substances per spray series compared for twelve relevant crop types

The boxplots show the upper hinge at 75 % quantile, the lower hinge at 25 % quantile, the median value as black line and the upper/lower whisker as the largest/lowest observation +/- 1.5 * distance between the first and the third quartiles. Points show outliers. The number of observations, i.e. spray series available per crop, is given below the boxplots.

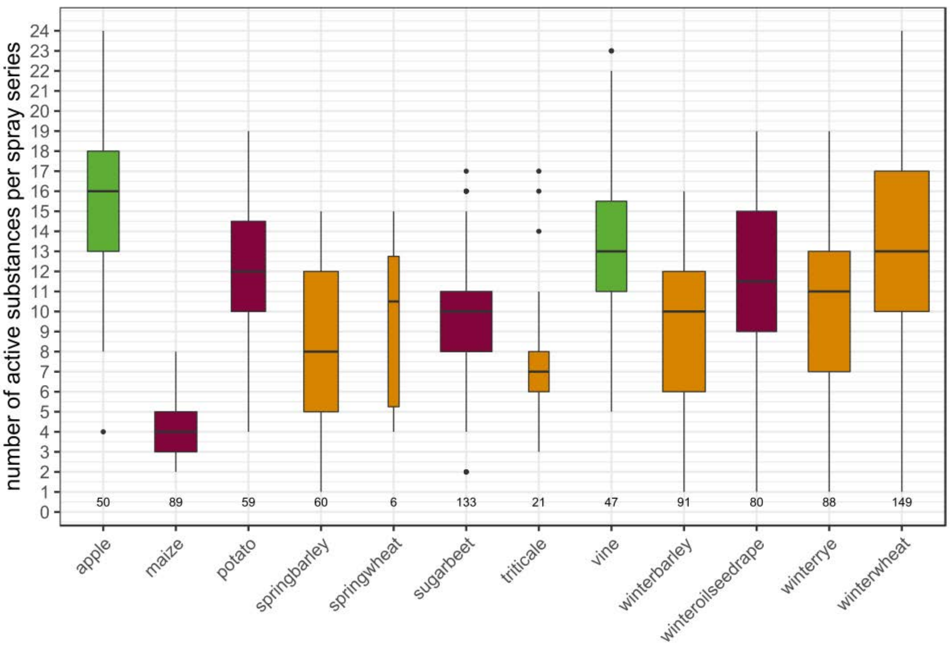
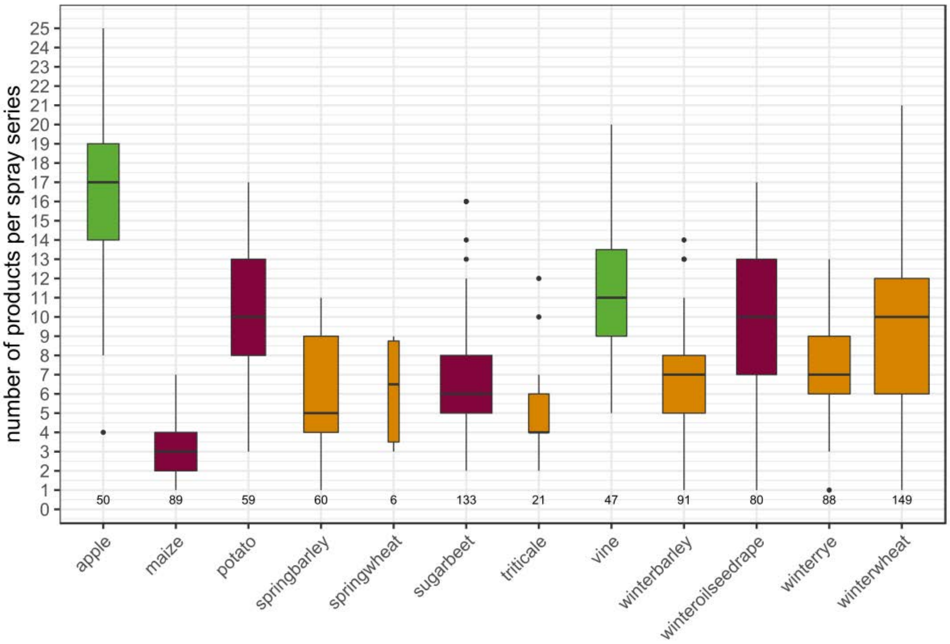


Figure 13: Distribution of total numbers of plant protection products per spray series compared for twelve relevant crop types

The boxplots show the upper hinge at 75 % quantile, the lower hinge at 25 % quantile, the median value as black line and the upper/lower whisker as the largest/lowest observation +/- 1.5 * distance between the first and the third quartiles. Points show outliers. The number of observations, i.e. spray series available per crop, is given below the boxplots.

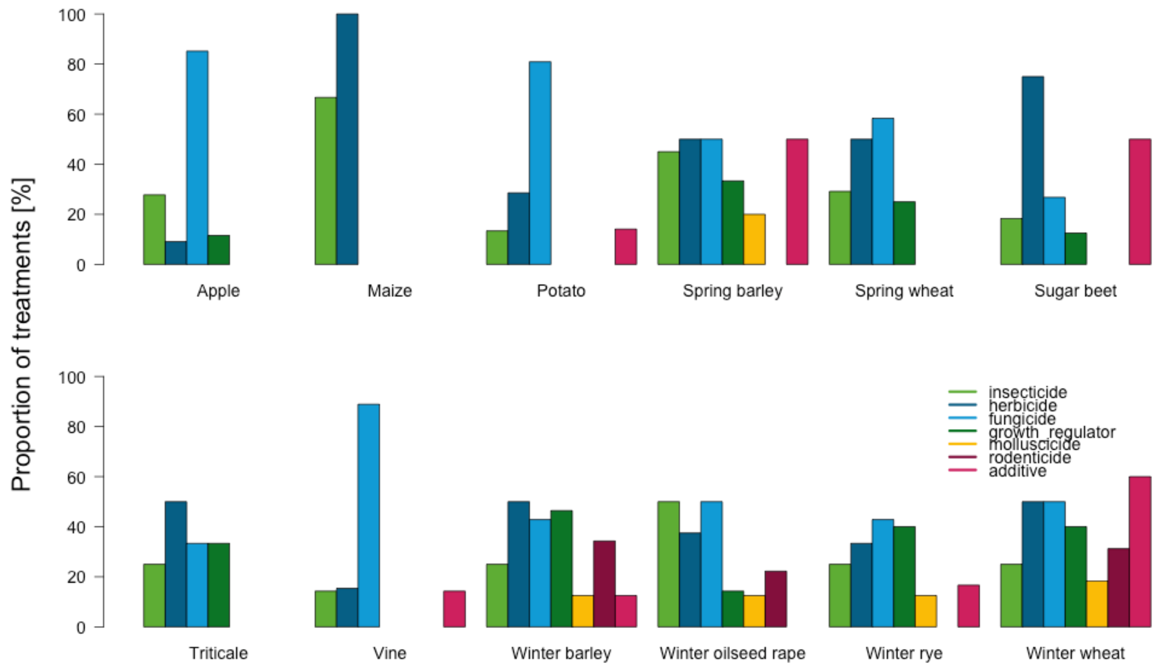


Application patterns of plant protection products showed mixtures of different active substances occurring over all crops at high frequencies. Apart from common application practice of more than one active substance at once, we also analyzed PPP-class mixtures for each crop type. We assessed general treatment patterns by the proportional presence of a chemical class across all PPP applications in a single spray series (considering the six pesticide classes insecticides, herbicides, fungicides, growth regulators, molluscicides, rodenticides and additives) for each of the twelve crop types (Fig. 14). The comparison showed that crops exhibited distinctive, typical treatment regimes. In maize or wine only two to three pesticide classes were applied per spray event, whereas other crop types regularly received much more heterogeneous treatment regimes with the application of up to seven pesticide classes, as observed for some cereal crops.

Furthermore, tank mixtures of crop types with a small number of applied pesticide classes were usually dominated by one specific class. For example, apple, potato or vine cultures were mainly treated by fungicides and maize or sugar beet cultures by herbicides. Generally, herbicidal and fungicidal substances were also the pesticide classes that were applied most frequently.

Figure 14: Mean proportion of pesticide classes that were applied in a spray series for twelve relevant crop types

Each PPP class (insecticide, herbicide, fungicide, growth regulator, molluscicides, rodenticide, additive) that was applied at least once was displayed as one bar per crop type. The y-axis displays the proportion of application events in %. A fungicide bar proportion of 80% means that one or more fungicide a.s. was applied in an average of 80% of all spray events within a spray series and per crop type.

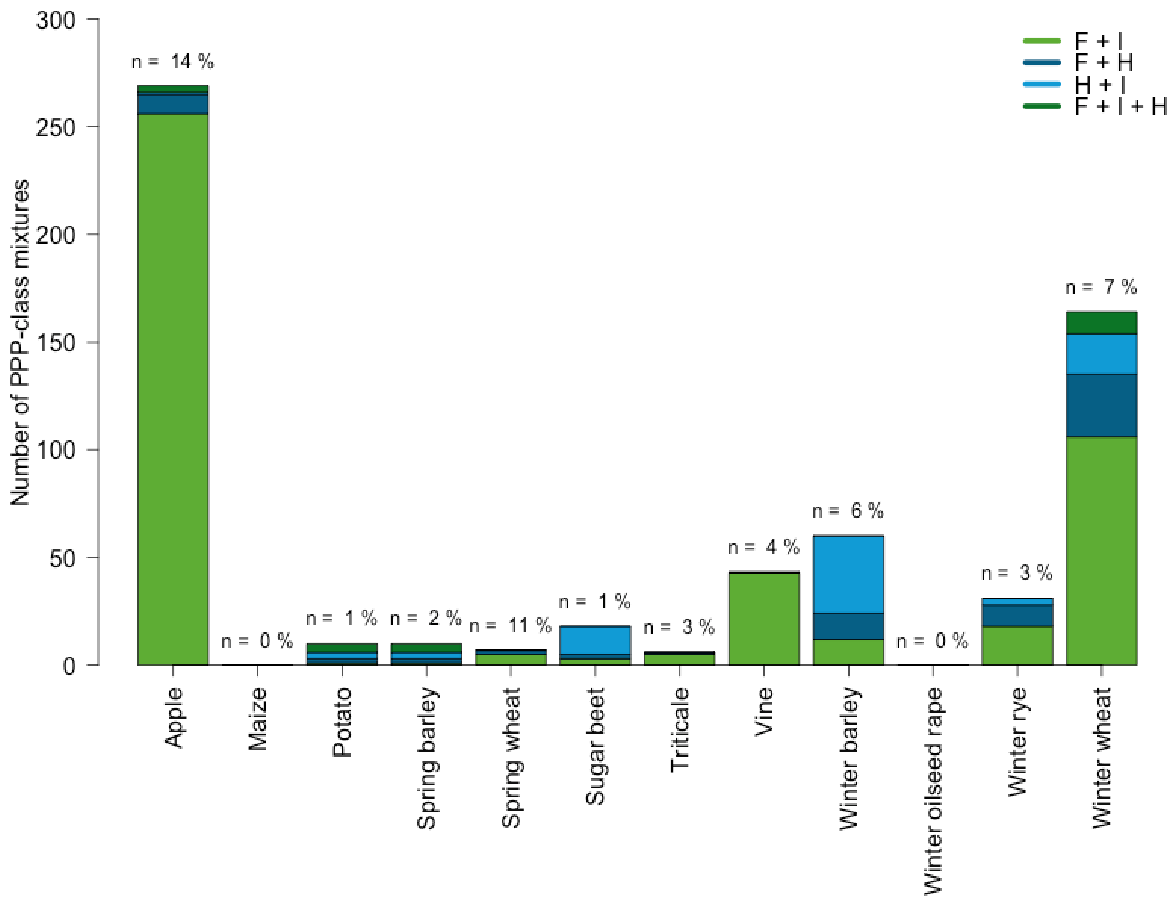


3.2.3 Predominance and frequency of mixtures

For the analysis of PPP-class mixtures, the presence of fungicide-insecticide (F + I), herbicide-fungicide (H + F), herbicide-insecticide (H + I) and triple-mixtures (F + I + H) was considered, covering the possible combinations of three main PPP-classes. From Fig. 15 can be seen that spray events containing PPP-class mixtures were observed for most crops, except of maize and winter oilseed rape. Mixtures of fungicides were regarded exceptionally critical because of synergistic effects reported from the literature (Cedergreen 2014), sometimes providing elusive information on the underlying toxic mechanism. In permanent, vertical cultures (apple and vine), mixtures of fungicides and insecticides occurred regularly in approx. 11% and 6% of all spray events, respectively. In potatoes and cereals, mixtures of fungicides and herbicides occurred additionally. However, regarding the relative number of spray events with PPP-class mixtures, a percentage greater than 10% was only detected for apple and spring wheat spray series. In sum, mixtures of different pesticide classes were seen in less than in 5 % of the possible cases. A closer look on the most frequent combinations of active substances (on the level of a single spray event that actually complies with a tank mixture, Table 7) gave indication that the most common combinations consisted of two or more a.s. of the same PPP-class.

Figure 15: Absolute and relative number of spray events containing PPP-class mixtures for twelve relevant crop types

The percentages on top of the bars indicate the number of PPP-class mixtures relative to all application events per crop. F = fungicide, I = insecticide, H = herbicide.



In the dataset at hand, the most frequent combination was a mixture of the five compounds "desmedipham and ethofumesat and lenacil and phenmedipham and metamitron", which contained herbicidal compounds exclusively (Table 7). Four out of five compounds were applied frequently as the quaternary combination product "Betanal Maxx Pro". The fifth substance metamitron came in by other products like "Goltix Gold" or "Metafol SC". Combinations of these herbicidal substances were applied via alternative "Betanal" products "Expert" or "Maxx", those were also responsible for the combination ranked third with four active substances. The combination ranked second, "dithianon and sulphur" was never sold as a combination product and thus not tested as such. The substance dithianon was contained in most cases in the product "Delan WG", sulphur in various products, mainly "Netzschwefel".

A high proportion of active substances were combined to tank mixtures without being tested as combination products during the process of registration. Over all available spray series, 73% of the spray events contained tank mixtures (based on the products as defined in the box "terminology and definitions actual application patterns" above). On the other hand, 48% of all spray events contained at least one combination product. Consequently, however you turn it, most spray events contained tank mixtures of untested risks.

Table 7: Most frequent combinations of active substances per spray event

The frequency is given as the total number of spray events containing the respective combination out of all 4931 spray events in the project database. Additionally, the name of the active substance, the table contains information on the pesticide class assigned to the a.s. (fungicide = (F), insecticide =(I), herbicide = (H)).

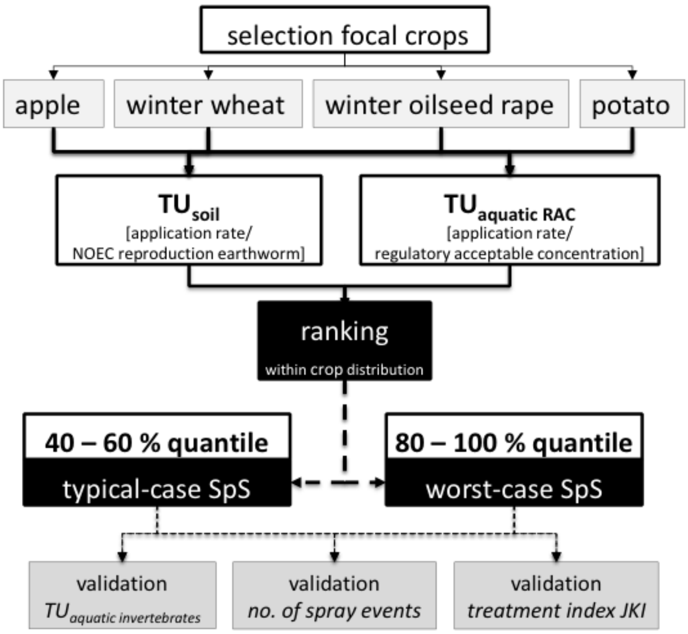
Active substances (pesticide class)	No. spray events
desmedipham (H) & ethofumesate (H) & lenacil (H) & metamitron (H) & phenmedipham (H)	94
dithianon (F) & sulphur (F)	68
desmedipham (H) & ethofumesat (H) & metamitron (H) & phenmedipham (H)	50
mancozeb (F) & sulphur (F)	39
diflufenican (H) & flufenacet (H) & flurtamone (H)	36
metribuzin (H) & prosulfocarb (H)	35
fluopicolide (F) & propamocarb (F)	31
difenoconazol (F) & fenpropidin (F)	30
diflufenican (H) & isoproturon (H)	30
bixafen (F) & fluoxastrobin (F) & prothioconazole (F)	29
cymoxanil (F) & fluazinam (F) & mancozeb (F)	28
bixafen (F) & prothioconazole (F) & tebuconazole (F)	24
deiquat (H) & fluazinam (F)	24
mancozeb (F) & metalaxyl-m (F)	23
captan (F) & granulosevirus (I)	22
captan (F) & sulphur (F)	21
carbendazim (F) & flusilazol (F)	21
cyazofamid (F) & cymoxanil (F) & mancozeb (F)	19
epoxiconazol (F) & kresoxim-methyl (F)	19
dimethomorph (F) & fluazinam (F) & mancozeb (F)	18
ethofumesate (H) & haloxyfop (H) & metamitron (H) & phenmedipham (H)	18

3.3 Classification of treatment regimes

The realistic assessment of environmental risks requires a multitude of reliable assumptions, on both the exposure of organisms and the relevant effect measures. This is different for each of the different terrestrial and aquatic areas. Due to this complexity and the huge amounts of high-quality data necessary, a workflow was developed how to select few representative spray series out of the total 873 (Fig. 16). Standard first – and higher-tier indices of toxic pressures (and after classification risk indices for important example spray series, refer to chapter 5) were calculated for the resulting reduced set of spray series. Criteria were defined to identify typical-case and worst-case spray series of application patterns. A step-wise approach was established to select spray series for further analyses. Firstly, four crops, namely apple, winter wheat, winter oilseed area and potato, were chosen as focal crops. Secondly, indicators of toxic pressure were computed, applying a toxic unit approach that uses chronic earthworm effect data for the soil compartment and regulatory acceptable concentrations for the aquatic environment with the application rates reported by the farmers. No further methods of exposure estimates were applied at this stage. Finally, further indicators (toxic units of laboratory studies with aquatic invertebrates, the number of spray events per spray series and the treatment index of the JKI) were used to check for plausibility and validity of the ranking procedure.

Figure 16: Workflow for the classification of spray series in two classes: worst-case and typical-case spray series

Focal crops (apple, winter wheat, winter oilseed rape, potato) were chosen initially. Equally important, toxic units for soil and aquatic compartments were used for ranking spray series within each crop and for the calculation of distribution of the indicator values. The 20% percentiles around the median and the 90st percentile served as base for negotiating the best compromise between soil and aquatics areas, and between indicators used for validation.



All indicators of toxic pressure were calculated for each of the four spray series. A rank according the indicator value was assigned. The highest value was ranked one and the lowest obtained the number of total spray series for the respective crop (e.g. 80 for winter oilseed rape).

3.3.1 Toxic units as indicators of toxic pressure for soil and aquatic organisms

Information on the expected effects have to be queried additionally from alternative external and internal toxicity databases chapter 3.1.3). Toxic units (TU) based on application rates should enable a relative ranking and classification of the data, so the identification of worst- and typical-case spray series could be achieved. Two endpoints were used to calculate toxic units. For the aquatic ERA, regulatory acceptable concentrations (RAC) and for the soil compartment chronic earthworm (NOEC reproduction after 56 days of exposure) were available. TU were ranked with the most toxic spray series first and the least toxic ranked last. For the computation of TU, the general form refers to the definition of Sprague (1970), Equation 2 below. For the characterization of the toxic pressure caused by the whole spray series TU were summed up and the following analyses were done with the sum of all toxic units (TU_{sum}).

Equation 2: Toxic unit calculation

TU_{sum} is the sum of all toxic units of the pesticides *1-n* in a spray series; C_i is the concentration of the pesticide *i* in soil or water; TM_{xi} is the toxic endpoint measure for the respective pesticide in soil or water. Taking the logarithm to the base 10 is only necessary if the values over scored several orders of magnitude.

$$TU_{sum} = \sum_{i=1}^{n} log \frac{C_i}{TM_{x_i}}$$

For the calculation of aquatic TU, application rates were used as amounts of active substances per area (converted to $\mu g/m^2$). These were divided by RAC [µg/l] or LC_{50} or aquatic invertebrates [µg/L] of a specific substance. Since exposure via drift or runoff was not determined for the pre-selection of spray series, TU were only considered a relative value for the potential toxic pressure to aquatic organisms. TU_{soil} were calculated using the toxicity endpoint values as described in chapter 3.1.3. For the calculation of the terrestrial toxic units, they were converted into mg a.s./kg dry weight of soil by using standard assumptions for the ease of interpretation: Soil density 1500 g/cm^3, soil depth = 2.5 cm, density of product = 1 (i.e. 1 litre of product comes up to 1 kg weight). The resulting numbers for TU_{soil} were dimensionless and directly express the proportion between the toxicity endpoint and the exposure.

For soil evaluation, comparable data were not available for all substances. This was mainly because for many substances no studies were triggered by standard risk assessment procedures as laid down in the guidance document on terrestrial ecotoxicology (European Commission 2002). These a.s. would anyway contribute little to the sum of TU due to relatively low toxicity.

3.3.2 Preliminary ranking of spray series

In a final step, percentiles of the distribution of ranked spray series were computed as characteristic descriptors. The "worst-case" class covered the upper 20% of the ranks (80st to the 100st percentile of the distribution). The "typical-case" class reached from the 40st to the 60st percentile of the distribution. Depending on the total number of spray series in the dataset, several spray series occupied each of the percentiles according to their ranks from soil and aquatic evaluation of toxic pressures. Further, the classification was checked for validity and plausibility using the indicators described in the following sections. The final ranking was done using all available indicators (section 3.3.4). Following the computation of toxic units of RACs and chronic effects on earthworms, TU for aquatic invertebrates were calculated for validation purposes. Aquatic TUs were calculated based on the toxicity endpoint values as described in chapter 3.3.1. For neonicotinoids, *Chironomus riparius* or *Hyalella azteca* was used as the reference organism, since *D. magna* is known to be insensitive towards neonicotinoids (see also Knillmann et al. 2018, Münze et al. 2017).

Surveying the actual use of chemical plant protection products is stipulated by the legislative institutions of the European Union by Directive 2009/128/EC (European Union 2009). This directive on the sustainable use of PPP lays down obligatory clauses that the member states have to follow. They are requested to monitor the use of PPP by establishing National Action Plans and report suitable indicators of the intensity of pesticide use. The measures implemented by the National Action Plans should be suitable in reducing the impacts of chemical plant protection in the long-term. The member state Germany decided to report two indicators for the assessment of pesticide use intensity, the treatment frequency (TF) and the treatment index (TI). The data on actual application rates and treated agricultural areas used by the German government is based on a network of farms cooperating within the "panel pesticide applications –PAPA" (Roßberg 2013). Here, the treatment index was used as an additional parameter for the selection of representative spray series. The TI poses a common parameter to describe the intensity of treatment regimes aggregated over larger regional scales. We used the TI to complementarily assess the general validity of the above described ranking by toxic unit approaches on the level of single spray series´. This means, we did not use the absolute values as a measure of toxic pressure, but we compared rather the relative ranks of a spray series within a subset for a specific crop. Additionally, we checked for plausibility of the processed values compared to those reported in the literature by the responsible administration.

The treatment index was calculated according the methodology described by Roßberg 2013 and adapted to the COMBITOX data at hand (Equation 3).

Equation 3: Formula to calculate the treatment index TI for single spray series

The dimensionless treatment index is calculated using the actual application patterns, i.e. the treated area compared to the total area and the actual application rates from the project database. Data is complemented by registration data provided by the BVL. The data sources are described in chapter 3.1.

$$\text{treatment index}_{\text{spray series}} = \sum_{1=1^{st}\text{spray event of the spray series}}^{n=\text{total number of spray events}} \text{areal coefficient} \times \text{application rate coefficient}$$

$$= \sum_{k}^{n} \frac{\text{treated area}}{\text{total area}} \times \frac{\text{actual application rate}}{\text{maximum application rate registered}}$$

The data on the real application patterns was available on the level of single fields, the treated and total area of a spray series relate to this unit. The treatment index has no absolute reference value and for reasons of comparison, it has to be put into relation to other TI, be that from own or external calculations. The results of computations were checked for validity (in terms of ranking results from prioritized toxic unit approaches) and plausibility (by comparing TI with those reported by the responsible authority JKI). The validation procedures and results are described below in chapter 3.3.5. The plausibility check fails for vine. In our dataset, the TI for vine was between 0.5 – 15.8, the most likely value was 5.6 and the mean was 9.1 (Table 8). The discrepancy between mode and mean was due to a clearly left-skewed distribution of TI-values. The index in vine as reported from PAPA-JKI was between 13.1 – 19.8 (Roßberg and Ipach 2015). While the indices for the actual application patterns in vine were exceptionally low, various reasons led to these findings. One obvious reason was the incompleteness of the registration data ("orphan" products, see last column of Table 8), which directly led to lower values because the total TI is the sum of TI from all single spray events within a spray series. For vine as for other crops in our dataset applies that the maximum allowed rates were not completely exhausted (i.e. the application rate coefficient was far from 1, which signifies full utilization of the maximum allowed application rate). We assume that the farmers related to our database, who voluntarily took part of a survey program, were well-advised regarding the necessary measure of PPP use. These findings and argumentations are also generally valid for apple crops.

Good accordance and well plausible results of our own calculations of the TI were found for cereals, potatoes, maize and sugar beet crops (Table 8). The fact that the exact indication, i.e. the reason for the actual plant protection measure, was unknown for the vast majority of cases did not lead towards higher or lower values of TI but to higher inherent uncertainties of the values:

in cases where more than one possible maximum application rate was found, the minimum value was taken. The rationale of doing this was not to underestimate the intensity of PPP use.

Table 8: Reported treatment indices from PAPA surveys* in different crops compared to treatment indices from actual datasets from the present project

For literature data, the range of reported values is given, as long as the yearly aggregated index has been reported for more than a single year. For the data, the range of the minimum and maximum values of the empirical cumulative density function of 90 percent of the values left and right of the median value are given. In squared brackets, the mode as the most likely value, and the arithmetic mean are given. Additionally, the proportion of plant protection products in the project-dataset without registration data ("orphans") are presented.

Crop type	JKI Range in reference	COMBITOX mode, mean of distribution of TI-values [90 % interval around median of distribution]	COMBITOX Orphan products in %
Apple	33.07 Roßberg 2013	20.6, 25.2 [6.7 – 45.5]	34
Maize	1.89 Roßberg 2013	1.5, 13.4 [0.5 – 4.6]	5
Potato	10.8 – 12.6 Roßberg 2016	13.4, 14.4 [5.4 – 24.8]	9
Sugar Beet	3.72 Roßberg 2013	9.9, 15.1 [3.6 – 18.6]	11
Vine	13.1 – 19.8 Roßberg and Ipach 2015	5.6, 9.1 [0.5 – 15.8]	69
Winter barley	4.2- 4.3 Dachbrodt-Saaydeh et al. 2016	9.2, 11 [0.8 – 9.2]	10
Winter oilseed rape	6.5 – 6.9 Dachbrodt-Saaydeh et al. 2016	9.2, 11 [0.8 – 13.9]	10
Winter wheat	5.9 - 6.4 Dachbrodt-Saaydeh et al. 2016	7.7, 7.6 [1.6 – 11.4]	8

* as published mainly by the Julius-Kühn-Institute - JKI

3.3.3 Number of spray events

For each crop, the distribution of numbers of spray events per spray series was calculated and the spray series was accordingly ranked. The highest number ranked first ("worst"), the lowest last ("best"). A spray event occurs when the farmer fills the tank of the spraying device with one or more compounds and passes over the field. The number of spray events within a spray series gives indication on the intensity of pesticide use regarding the frequency of applications. Thus, it correlated with the indicator "treatment frequency" of the JKI and serves as one criterion for validation of the given spray series data and the classification of worst- and typical case spray series. For further validation, we also aimed to check for dependencies between the treatment index, TUs and number of spray events (see 3.3.5).

3.3.4 Final ranking and selection of spray series for in-depth risk analysis

A final ranking procedure was performed to find a compromise between two major ranking criteria "toxic units based on aquatic regulatory acceptable concentrations - TURAC" and "toxic units based on reproduction effects on earthworms - TU_{soil}". Results were weighed by the validation criteria "total number of spray events - TSE", "treatment index - TI" and "toxic units based on acute aquatic invertebrate effect data - TU_{inv}". A list of fifteen candidate spray series was chosen for the final selection of the four focal spray series (Table 9). The ranking was done within each crop. For the example of 50 apple cultures, there were ten candidate spray series in the upper and median 20% percentiles. In the case of winter wheat, the corresponding number of candidates was about 30 out of a total of 149 spray series. A summarizing category to be used as a treatment scenario was assigned to each of the classified spray series:

- Spray series absolutely ranked first→ "absmax"
- Spray series absolutely ranked last → "absmin"
- Spray series ranked within the upper 20% percentile → "worst"
- Spray series ranked within the median 20% percentile → "typical"
- Spray series ranked within the lower 20% percentile → "best"

Table 9: Fifteen spray series out of the data pool from the four preselected crops apple, potato, winter oilseed rape and winter wheat shortlisted for in-depth risk analysis

CROP: one of four preselected crops apple, potato, winter oilseed rape and winter wheat; $AQRAC_{rank}$: rank of the spray series based on the aquatic TU_{RAC}; $AQRAC_{cat}$: summarizing category assigned to a spray series according the percentile of rank distribution based on TU_{RAC}; $SOIL_{rank}$: rank of the spray series based on the TU_{soil}; $SOIL_{cat}$: summarizing category assigned to a spray series according the percentile of rank distribution based on TU_{soil}; $AQINV_{rank}$: rank of the spray series based on the aquatic TU_{inv}; TSE_{rank}: rank of the spray series based on the total number of spray events within a spray series ; TI_{rank}: rank of the spray series based on the treatment index of JKI.

Crop	$AQRAC_{rank}$	$AQRAC_{cat}$	$SOIL_{rank}$	$SOIL_{cat}$	$AQINV_{rank}$	TSE_{rank}	TI_{rank}
Apple	2	worst	1	absmax	41	1	13
Apple	3	worst	2	worst	25	6	9
Apple	20	NA	22	typical	35	24	41
Apple	29	typical	25	typical	27	45	21
Potato	3	worst	6	worst	4	8	10
Potato	9	worst	8	worst	8	6	9
Potato	28	typical	30	typical	21	28	47
Potato	30	typical	28	typical	23	30	42
Winter oilseed rape	2	worst	4	worst	4	34	13
Winter oilseed rape	6	worst	2	worst	7	20	4
Winter oilseed rape	39	typical	38	typical	51	40	42
Winter oilseed rape	42	typical	36	typical	59	55	47

Crop	$AQRAC_{rank}$	$AQRAC_{cat}$	$SOIL_{rank}$	$SOIL_{cat}$	$AQINV_{rank}$	TSE_{rank}	TI_{rank}
Winter wheat	14	worst	26	worst	23	13	69
Winter wheat	59	typical	73	typical	60	101	112
Winter wheat	65	typical	65	typical	87	32	70

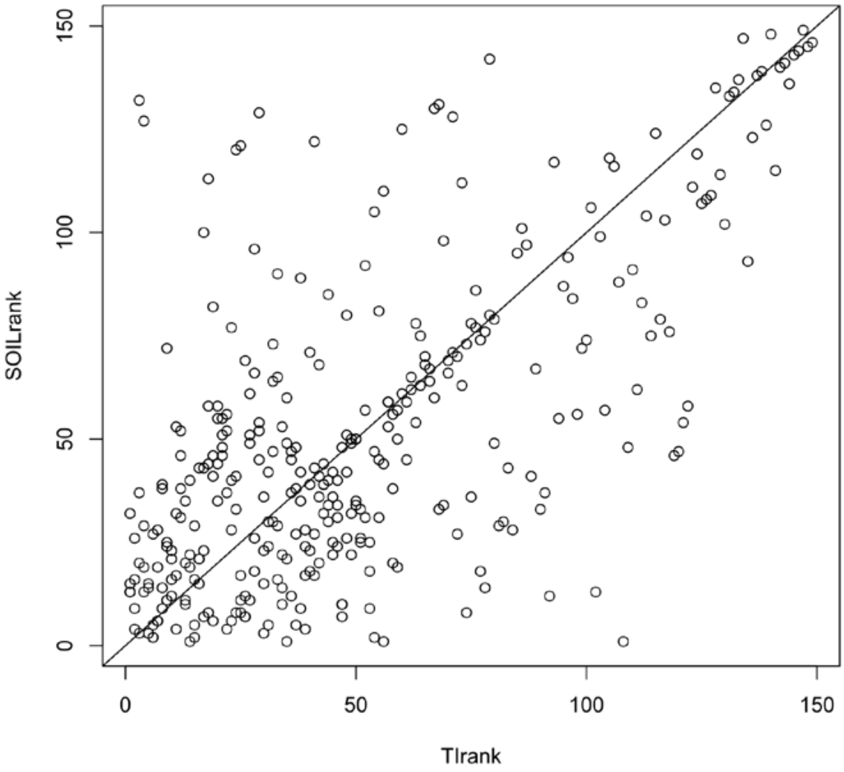
3.3.5 Correlation between main and validation indicators of toxic pressure

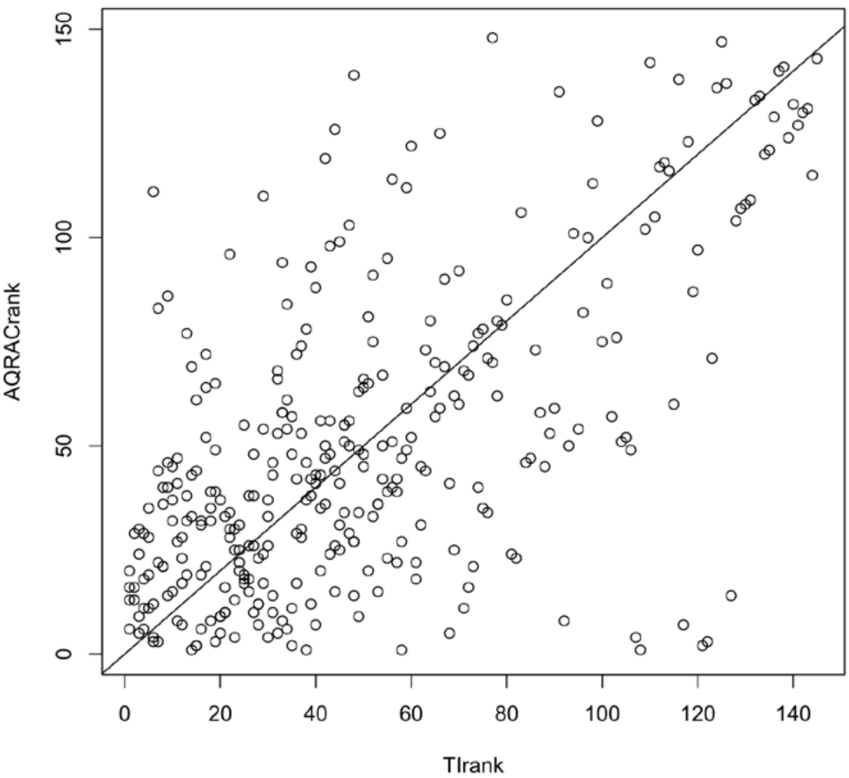
The toxic pressure of a spray series served as an indicator to identify worst-case and typical-case spray series within the great variety of use patterns in the project dataset of actual spray series. The ranking according to TU_{RAC} and TU_{soil} resulted in a selection of spray series that is quite consistent amongst the two indicators as shown above in chapter 3.3.4 and Table 9. To enhance plausibility and unambiguousness of the selection, further indicators were computed and in cases of doubt used for the final selection. All indicators were ranked, within each crop, and the ranks were plotted against each other to show qualitatively the correlation between them (Fig. 17). This gave information on the validity of the main indicators (in case of strong correlations between two indicators) or on the contrary evidence was seen that the two indicators did not hold identical but complementary information. With linearly correlated indicators, the points in Fig. 17 would lie exactly on the diagonal line. In this case, both indicators result in the same rank for the spray series.

The maximum possible rank in Fig. 17 was 149 for the lowest criterion value in winter wheat with 149 spray series in total, thus the axes reach to 149 in both the ordinate and abscissa. There were no strong correlations between the priority ranking criteria and validity criteria, except the rank $AQRAC_{rank}$ vs. rank aquatic invertebrates. This is because the RAC was (to a great extent) partly deduced from acute aquatic effect data and the two indices are strongly auto-correlated. For the other ranking criteria, in particular the soil endpoints, the information on the ranks from the different calculations showed different aspects of the toxic pressures caused by the spray series in apple, winter wheat, potato and winter oilseed rape. However, no systematic deviation of correlated indicators can be concluded (Fig. 17), which would mean that one indicator would always rank lower in cases where the other ranks higher, and vice versa. It is concluded that the two priority criteria “toxic unit soil” and “toxic unit aquatic” allow a valid description of toxic pressures.

Figure 17: Correlation between ranks of main ranking criteria and validation criteria for data of four focal crop types: apple, potato, winter oilseed rape winter wheat

Ranks from toxic units based on regulatory acceptable concentrations $AQRAC_{rank}$; ranks from toxic units based on effects on reproduction of earthworms $SOIL_{rank}$; ranks from treatment index TI_{rank}.





3.4 Focal crops and characteristic of selected spray series

With the focal crops, and with the typical- and worst-case spray series readily defined, a closer look to the patterns of pesticide use was possible. Subsequently, the patterns were translated into descriptions of environmental risks; the results are described in the following chapter 5.

3.4.1 Patterns in apple

3.4.1.1 General patterns and generic treatment regime

The permanent crop apple is characterized by intense use of plant protection products. The pesticide class of fungicides dominate the use patterns. Plant protection measures mainly aim to prevent a reduction of the qualities due to apple scab and other fungal pathogens (Table 10). The application of fungicides is often preventive and based on early warnings of the public services. Consequently, the actual application patterns in apple varied significantly between years (Fig. 18). In the dataset at hand, a total of 50 spray series in apple were available. All data for Germany came from one federal state (Sachsen-Anhalt), complemented by data from Austria, which is considered climatically comparable to German conditions. During the growth period from April to September or October, the mean break between two spray events was not longer than 10 days but was often much shorter.

Table 10: General characteristics of apple crops (Germany and Austria)

Spray series in apple start in spring of the growth period (March/April) and end in autumn or early winter (October to December, see also Fig. 18). Comparisons with the other cultures can be found in chapter 3.2.

Characteristic feature	Indication
Active substances applied in total	57
Spray series	50
Farms	23
Growth periods covered	2007 – 2009, 2012 – 2014
Substances per tank mixture	up to 5 (59% tank mixtures)
Spray events per spray series	up to 39 (17 applications most frequent)
Fungicides dominant (almost 80%), followed by insecticides (20%) and herbicides (3%)	
Average time span between spray events	5 days (2008) – 10 days (2014)

The generic treatment regime for apple includes fungicide treatments from the first day of budding to the date of harvest. Herbicides and insecticides are expected to be applied during spring season only (Fig. 19). This pattern was confirmed by own data (Fig. 14).

Figure 18: Total number of PPP spray events per year and month for 50 spray series in apple

Bars show the sum of all spray events during the course of a growth period over all spray series in apple. The data came from five years between 2007 and 2014, which are grouped in the figure. Winter periods are indicated as well.

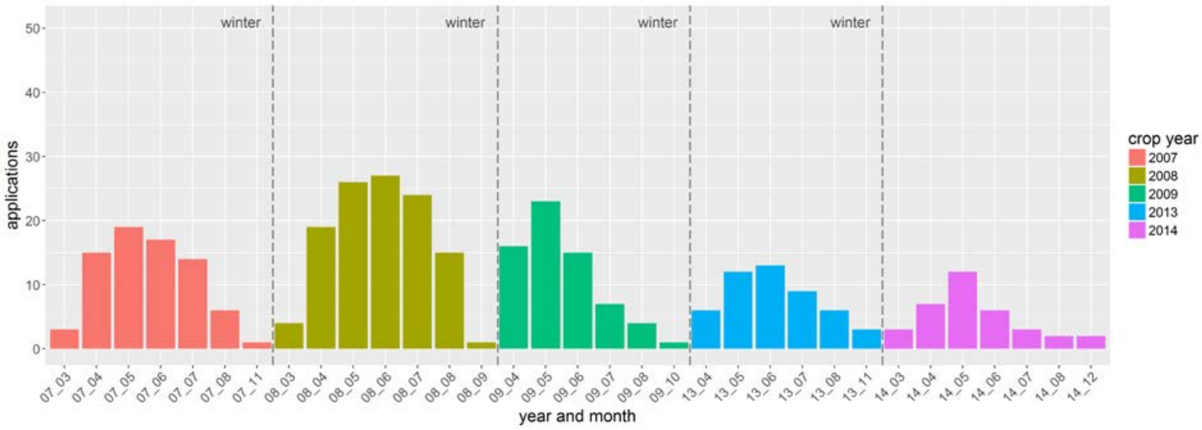
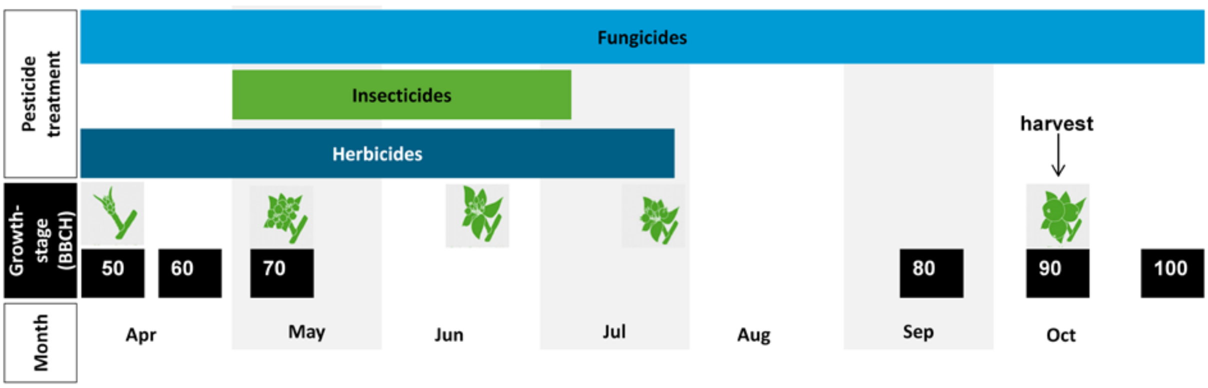


Figure 19*:* Generic treatment regime for apple*

A generic treatment regime was compiled from agricultural companions. It shows the time course of plant protection measures during the growing season. Given are the month (Apr = April, May = May, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October) and the corresponding standardized growth stage (BBCH-stage) as a pictorial representation of the crop and a number between 50 (first flowers) and 100 (harvest of fruits). The pesticide treatment can be one of fungicides (light blue bar), insecticides (green bar) and herbicides (dark blue bar).



* adapted from Weber and Kruse (2015)

3.4.1.2 Worst-case spray series in apple

The worst-case spray series in apple was chosen as a good compromise between aquatic and terrestrial indicators of toxic pressures, as well as it fit fairly well to the additional indices used for validation (especially the treatment index). It was ranked first for soil and for aquatic indicators (chapter 3.3.4) and was intensively revised during discussions as an example for possible caveats of risk characterization. Most striking was the multiple application of the fungicidal a.s. captan, which was applied 14-times between beginning of May and end of August via two products, Malvin WG and Merpan 80 WDG (Fig. 20). The latter was allowed to be sprayed 5-times during a growing season with a minimum break of 7-10 days; the former can be applied 13-times (without time gap). Hence, this treatment was well within the amounts assessed and registered and not an example of excess spraying (Table 11, Fig. 21). Fig. 21 gives indication whether there were exceedances of the maximum allowed application rates by more than factor 2. The application of Envidor in the worst-case apple spray series was the only exception of the rule that the plant protection practice was well-advised and well-executed.

Figure 20: Profile of the worst-case spray series in apple: pattern over time

The figure shows the application rates of all products and active substances on the abscissa applied during the growing period with date of application on the ordinate axis. Orange colors depict fungicide, red colors insecticides. Symbols stand for the various products; the names of active substances are given with the symbols. Symbols that are vertically aligned, represent one spray event since all products have been applied at one date.

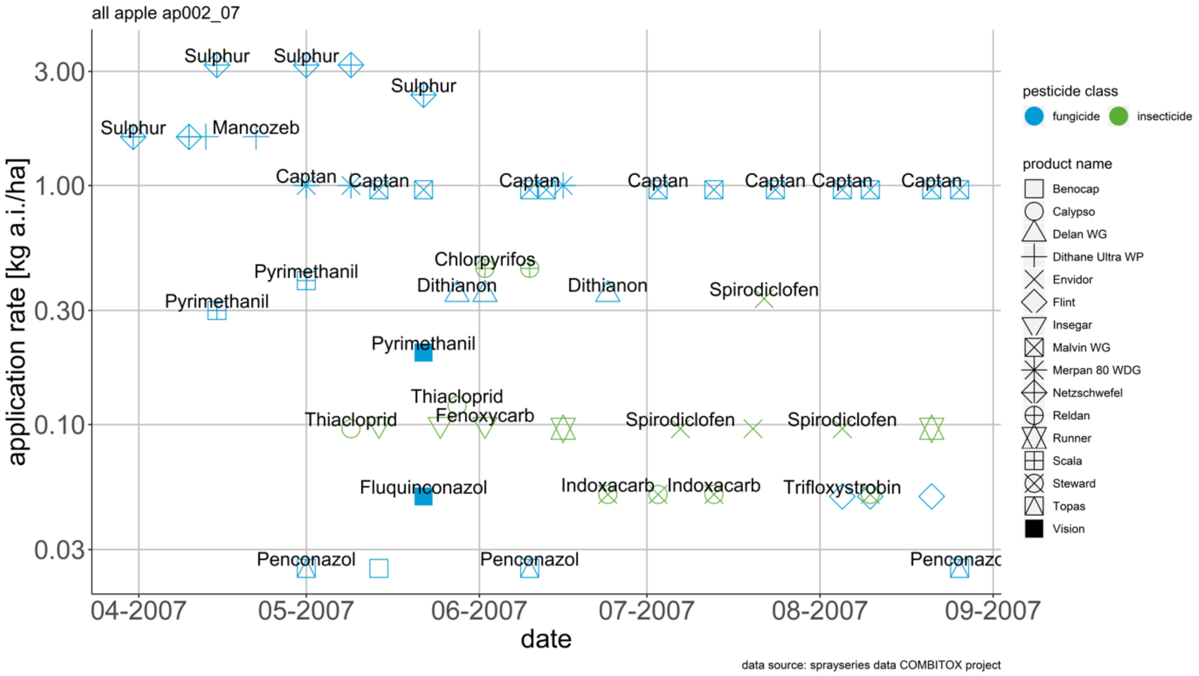


Table 11: Profile of the worst-case spray series in apple: characteristic features

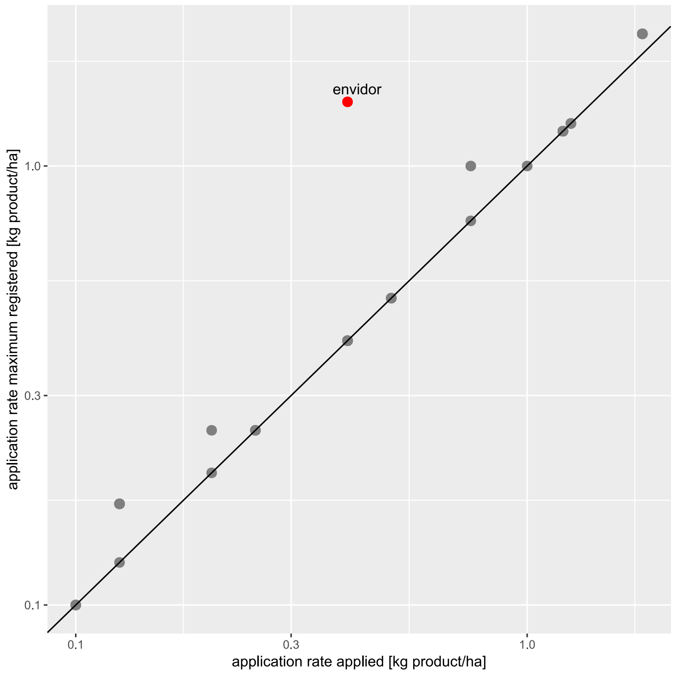
Historical data was analyzed, therefore the actual registration status of the products applied in the worst-case spray series in apple is listed. The products and until when they were registered: Benocap 2007-12-30, Calypso 2018-04-29, Delan WG2017-03-30, Dithane Ultra WP 2008-12-30, Envidor 2023-12-30, Flint 2017-01-30, INSEGAR 2013-12-30, Malvin WG 2017-12-30, Merpan 80 WDG 2016-12-30, Runner 2015-12-30, Scala 2019-12-30, STEWARD 2018-10-30, Topas 2021-12-30, Vision 2011-12-30.

Characteristic feature	Indication
crop	apple
crop protection period	April 2007 – September 2007
days between first & last application	148
spray events	26
average days break between two spray events [winter break omitted]	5.9 [-/-]
active substances	15
products	16

Characteristic feature	Indication
combination products	1
spray events with combination products	1
spray events with tank mixtures	18
spray events with fungicide, insecticide, herbicide	22, 16, 0
treatment index JKI	34.1
ranking TU_{RAC}; TU_{soil}	2; 1 [out of 50]

Figure 21: Actual versus maximum registered application rates in worst-case spray series in apple

All points on the diagonal match the allowed application rates perfectly. Red-labelled points indicate deviations of actually applied application rates from allowed application rates by more than factor 2 (exceedance, above the diagonal) or of less than factor 0.5 (no full exploitation, below the diagonal). Deviation of plotted number of products compared to total number applied in the spray series shown here is due to non-matches in the registration database. Rates are given as kg product / hectare. Representation of axes is on log10-scale.



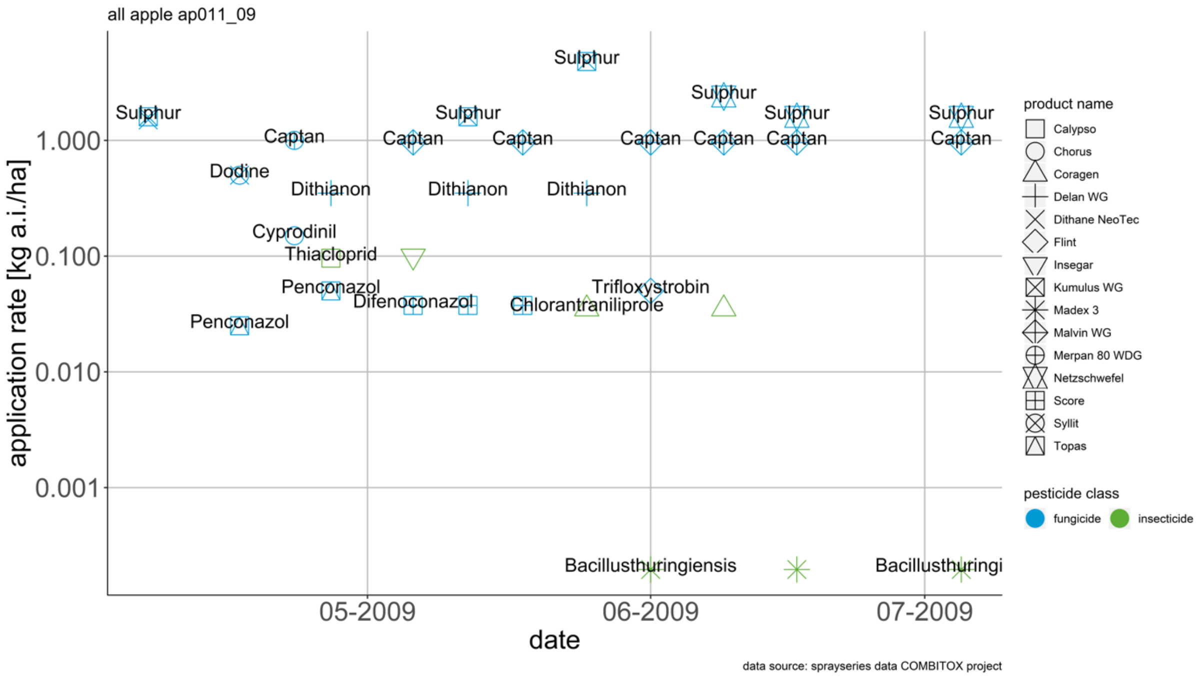
3.4.1.3 Typical-case spray series in apple

The typical-case spray series in apple lasted four months only, which was much shorter in time compared to the worst-case (Fig. 22, Table 12). In addition, spray events and with it the application of single products were much less frequent than in the worst-case spray series. For example, the substance captan was applied seven-times versus fourteen-times in worst-case spray series, Fig. 22). The farmer applied a product containing the bacterium *Bacillus thuriengiensis* that operates under the label "biological pest control". It was already expectable from the use pattern in Fig. 22 that risk indices would be much lower for this spray series in contrast to the worst-case (refer to the following chapter 5). It may certainly be questioned to what extent the possible range of agricultural practices in apple was represented by the COMBITOX dataset at hand. Well-advised farmers as in this study have better options and opportunities to take decisions towards the requirements of an integrated pest management, which is strictly stipulated by the regulatory authorities to be the normal agricultural practice. The average treatment index in apple as reported by the German Julius-Kühn-Institut (Roßberg and Harzer 2015) was between 28 and 33 for the years 2001, 2004, 2007, 2011-2013, with later years being more intensively treated. This fact leads to the assumption that even the worst-case spray series was not at the upper edge of usual plant protection practice in apple rather than representing mean intensities of plant protection.

No herbicides were applied in worst- and typical-case apple spray series. This contrasts findings from literature that considered herbicide treatments in apple orchards as normal agricultural practice (see generic treatment regime 3.4.1.1, Fig. 19). It was concluded that the stands reported in our dataset were older than 3-4 years and thus were better accessible to mechanical weed removal and less sensitive on the development of roots.

Figure 22: Profile of the typical-case spray series in apple: pattern over time

The figure shows the application rates of all products and active substances on the abscissa applied during the growing period with date of application on the ordinate axis. Orange colors depict fungicide, red colors insecticides. Symbols stand for the various products; the names of active substances are given with the symbols. Symbols that are vertically aligned, represent one spray event since all products have been applied at one date.



For the typical-case spray series, deviations of actual from maximum allowed application rates involved two products, Madex 3 was applied in much lower rates than allowed and Coragen exceeded maximum allowed rates by a factor of two (Fig. 23).

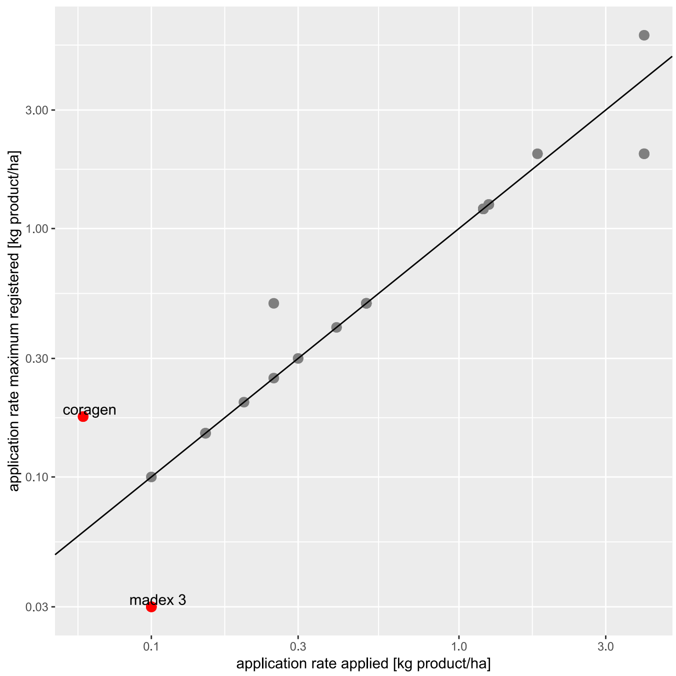
Table 12: Profile of the typical-case spray series in apple: characteristic features

Historical data was analyzed, therefore the actual registration status of the products applied in the typical-case spray series in apple is listed. The products and until when they were registered: Calypso 2018-04-29, Chorus 2009-10-29, Chorus 2019-12-30, Coragen 2025-12-30, Delan WG 2017-03-30, Dithane NeoTec 2017-05-30, Flint 2017-01-30, Insegar 2013-12-30, Kumulus WG 2020-12-30, Madex 3 2016-12-30, Malvin WG 2017-12-30, Merpan 80 WDG 2016-12-30, Score 2020-12-30, Syllit 2017-12-30, Topas 2021-12-30.

Characteristic feature	Indication
crop	apple
crop protection period	April 2009 – July 2009
days between first & last application	89
spray events	12
average days break between two spray events [winter break omitted]	8.1
active substances	13
products	15
combination products	0
spray events with combination products	0
spray events with tank mixtures	12
spray events with fungicide, insecticide, herbicide	12, 7, 0
treatment index JKI	28.2
ranking TU_{RAC}; TU_{soil}	29, 25 [out of 50]

Figure 23: Actual versus maximum registered application rates in typical-case spray series in apple

All points on the diagonal match the allowed application rates perfectly. Red-labelled points indicate deviations of actually applied application rates from allowed application rates by more than factor 2 (exceedance, above the diagonal) or of less than factor 0.5 (no full exploitation, below the diagonal). Deviation of plotted number of products compared to total number applied in the spray series shown here is due to non-matches in the registration database. Rates are given as kg product / hectare. Representation of axes is on log10-scale.



3.4.2 Patterns in winter oilseed rape

3.4.2.1 General patterns and generic treatment regime

The general treatment pattern in winter oilseed rape was clearly different from apple under various aspects. In principle, the treatment season of winter vegetable crops is interrupted by a winter break approximately from October to April. Moreover, the crop was marked by a nearly equally frequent use of herbicides fungicides and insecticides, which was considerably different compared to apple (Table 13). The fact that during the winter break of crop growth, also recovery and degradation processes will come to halt is also described in chapter 5 in connection with the interpretation of risk indices.

Table 13: General characteristics of winter oilseed crops

Spray series usually start in spring of the growth period (March/April) and ends in autumn or early winter (October to December, see also Fig. 24)

Characteristic feature	Indication
Active substances applied	52
Spray series	80
Farms	14
Growth periods covered	2011 – 2014

Characteristic feature	Indication
Substances per tank mixture	up to 8 (60 % tank mixtures)
Spray events per spray series	up to 11 (8 applications most frequent)
Fungicides and herbicides dominant (> 30% each), followed by insecticides (> 20%)	
Average time span between spray events	15 days (2013) – 19 days (2012)

In winter oilseed rape, a remarkably high number of application events occurred over the years, with considerable variability (Fig. 24).

Figure 24: Total number of PPP spray events per year and month for 80 spray series in winter oilseed rape

Bars show the sum of all spray events during the course of a growth period over all spray series in apple. The data came from five years between 2007 and 2014, which are grouped in the figure with winter periods indicated.

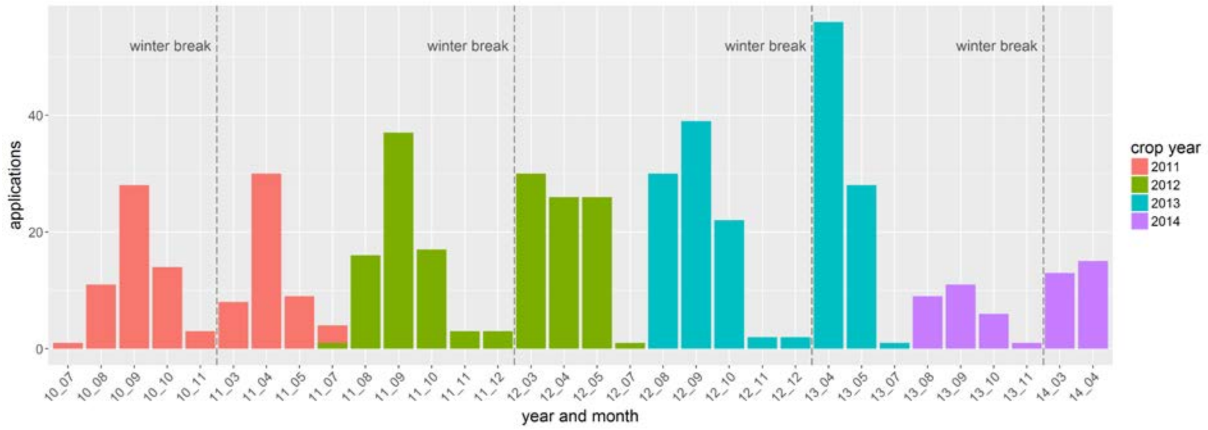
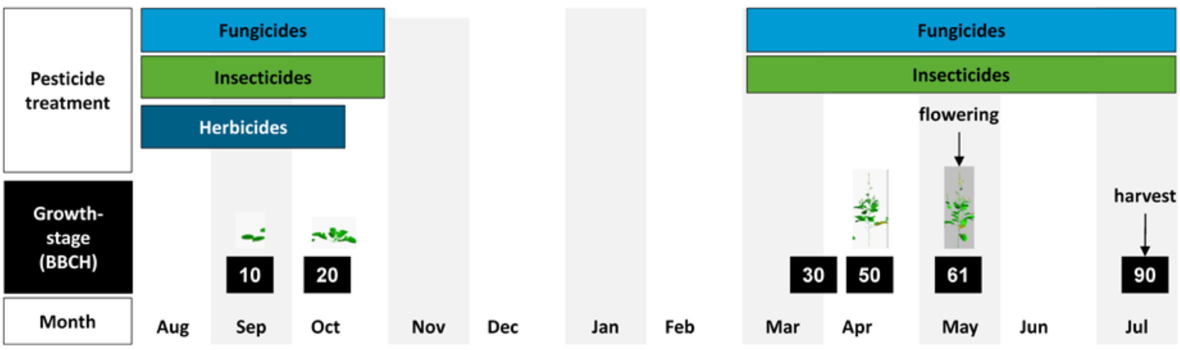


Figure 25*: Generic treatment regime for winter oilseed rape**

A generic treatment regime was compiled from agricultural companions. It shows the time course of plant protection measures during the growing season. Given are the month (Aug = August, Sep = September, Oct = October, Nov= November, Dec = December, Jan = January, Feb = February, Mar = March, Apr = April, May = May, Jun = June, Jul = July) and the corresponding standardized growth stage (BBCH-stage) as a pictorial representation of the crop and a number between 50 (first flowers) and 100 (harvest of fruits). The pesticide treatment can be one of fungicides (light blue bar), insecticides (green bar) and herbicides (dark blue bar).



* adapted from Landwirtschaftskammer Nordrhein-Westfalen (2015)

3.4.2.2 Worst-case spray series in winter oilseed rape

The worst-case in winter oilseed rape showed a higher proportion of combination products compared to the worst-case spray series in apple (Table 14). The reason for the high rank of this spray series was the insecticide treatment shortly before harvest (Fig. 26). However, the underlying reason of this insecticide application was not given in the provided data sets.

Figure 26: Profile of the worst-case spray series in winter oilseed rape: pattern over time

The figure shows the application rates of all products and active substances on the abscissa applied during the growing period with date of application on the ordinate axis. Orange colors depict fungicide, red colors insecticides, blue are molluscicides, green herbicides, and grey other preparations. Symbols stand for the various products; the names of active substances are given with the symbols. Symbols that are vertically aligned, represent one spray event since all products have been applied at one date.

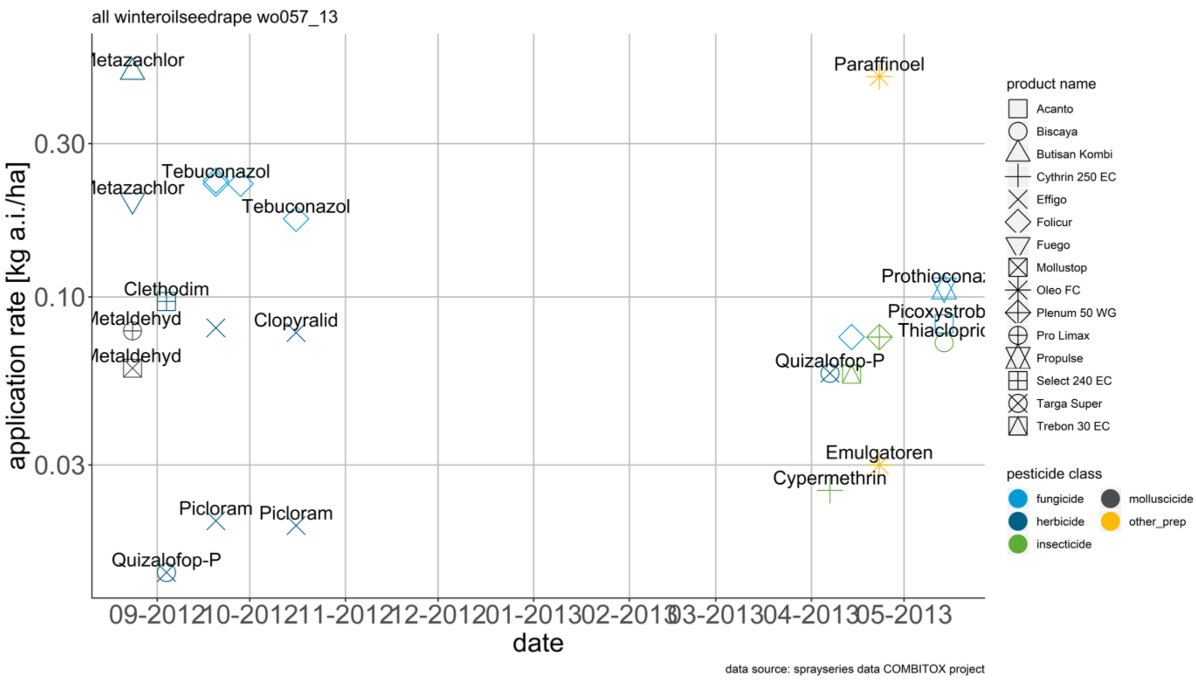


Table 14: Profile of the worst-case spray series in winter oilseed rape: characteristic features

Historical data was analyzed, therefore the actual registration status of the products applied in the worst-case spray series in apple is listed. The products and until when they were registered: Acanto 2018-10-30, Biscaya 2016-12-30, Butisan Kombi 2019-12-30, Cythrin 250 EC 2019-03-31, EFFIGO 2019-04-29, Folicur2020-12-30, Fuego 2018-12-30, Mollustop 2017-05-30, Plenum 50 WG 2017-02-27, Pro Limax 2015-12-30, Propulse 2017-02-27, Select 240 EC 2024-12-30, Targa Super 2020-11-29, Trebon 30 EC2018-12-30

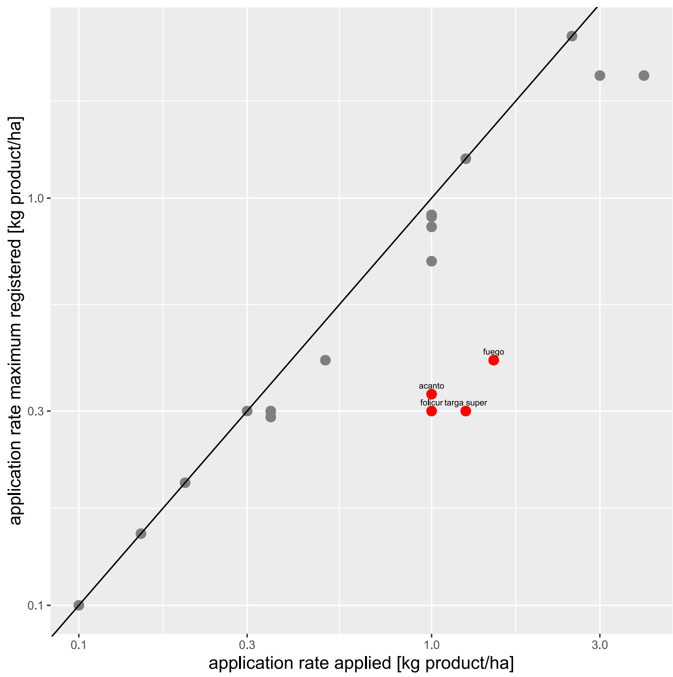
Characteristic feature	Indication
crop	winter oilseed rape
crop protection period	August 2012- May 2013
days between first & last application	263 [2012: 53, 2013: 37]
spray events	9 [2012:5, 2013: 4]
average days break between two spray events [winter break omitted]	32.9 [9.9]

Characteristic feature	Indication
active substances	16
Products	15
combination products	4
spray events with combination products	9
spray events with tank mixtures	8
spray events with fungicide, insecticide, herbicide	5, 4, 5
treatment index JKI	14.1
ranking TU_{RAC}; TU_{soil}	6; 2 [out of 80]

For the worst-case spray-series in winter oilseed rape, no overdose of PPP was seen (Fig. 27). The low amounts of for example the product Fuego were seen for reasons of not exceeding the total maximum allowed amounts of the a.s. metazachlor, which is also one of two active substances of the product Butisan Kombi. Both products were only allowed to be applied once during one whole season.

Figure 27: Actual versus maximum registered application rates in worst-case spray series in winter oilseed rape

All points on the diagonal match the allowed application rates perfectly. Red-labelled points indicate deviations of actually applied application rates from allowed application rates by more than factor 2 (exceedance, above the diagonal) or of less than factor 0.5 (no full exploitation, below the diagonal). Deviation of plotted number of products compared to total number applied in the spray series shown here is due to non-matches in the registration database. Rates are given as kg product / hectare. Representation of axes is on log10-scale.



3.4.2.3 Typical-case spray series in winter oilseed rape

The typical-case spray series in winter oilseed rape differed from the worst-case spray series in terms of the frequency of spray events with tank mixtures in relation to the total of spray events (Table 15). Only two spray events out of seven in the typical-case contained tank mixtures, compared to eight out of nine in the winter oilseed rape worst-case spray series. Risk indices were expected to be low, because the allowed application rates were not exhausted in most cases (Fig. 29). The typical pattern showed that the farmer protected the crop within a short period immediately pre- and post-emergence by measures applying molluscicides, herbicides and fungicide within a short period of time in autumn (Fig. 28).

Figure 28: Profile of the typical-case spray series in winter oilseed rape: pattern over time

The figure shows the application rates of all products and active substances on the abscissa applied during the growing period with date of application on the ordinate axis. Orange colors depict fungicide, red colors insecticides, blue are molluscicides, green herbicides, and grey other preparations. Symbols stand for the various products; the names of active substances are given with the symbols. Symbols that are vertically aligned, represent one spray event since all products have been applied at one date.

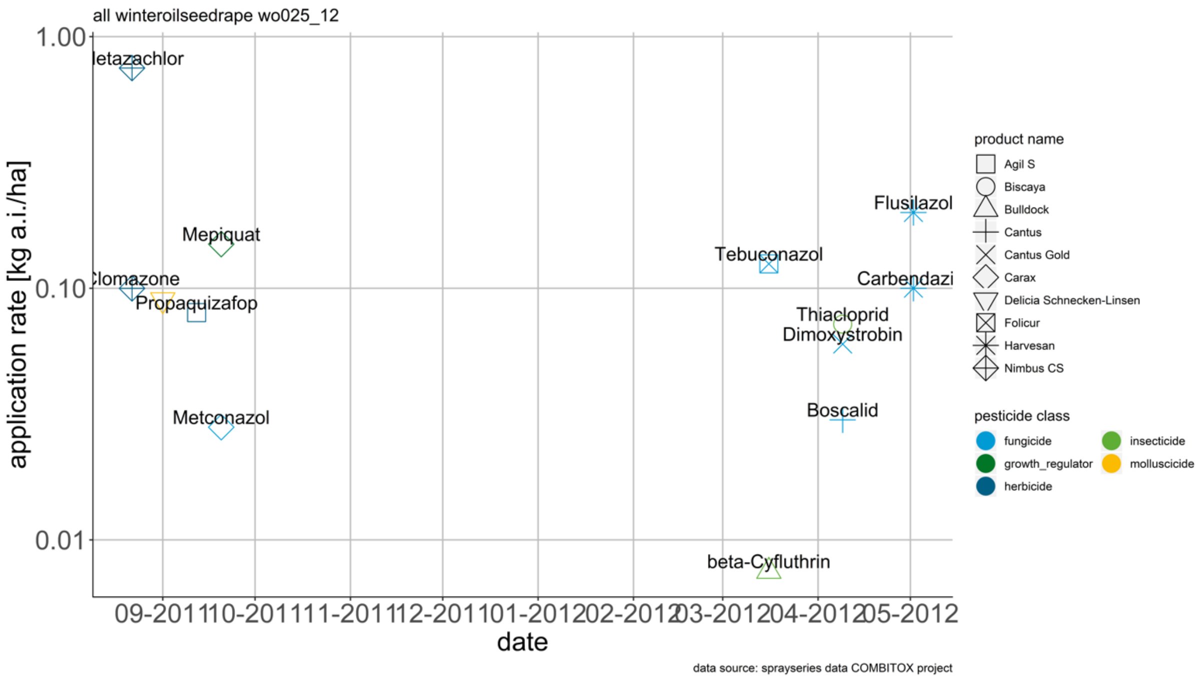


Table 15: Profile of the typical-case spray series in winter oilseed rape: characteristic features

Historical data was analyzed, therefore the actual registration status of the products applied in the typical-case spray series in apple is listed. The products and until when they were registered: Acanto 2018-10-30, Biscaya 2016-12-30, Butisan Kombi 2019-12-30, Cythrin 250 EC 2019-03-31, EFFIGO 2019-04-29, Folicur 2020-12-30, Fuego 2018-12-30, Mollustop 2017-05-30, Plenum 50 WG 2017-02-27, Pro Limax 2015-12-30, Propulse 2017-02-27, Select 240 EC 2024-12-30, Targa Super 2020-11-29, Trebon 30 EC 2018-12-30

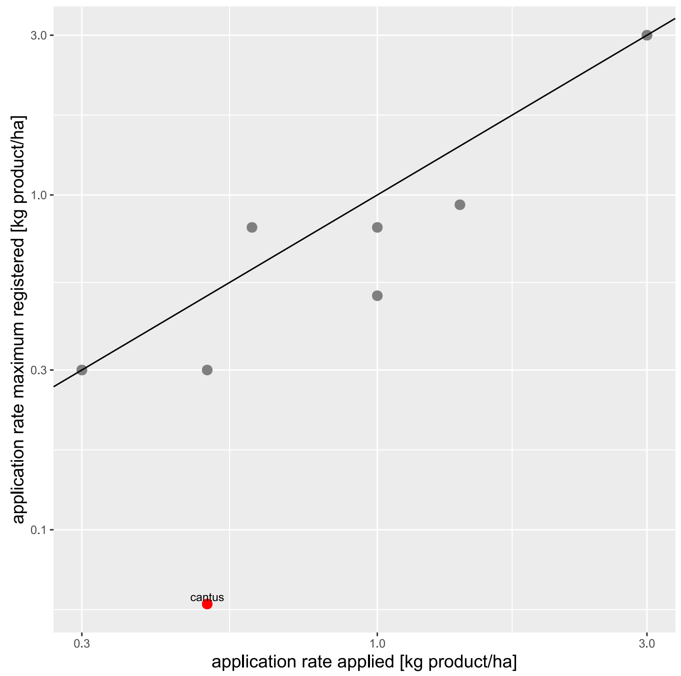
Characteristic feature	Indication
crop	winter oilseed rape
crop protection period	August 2011 – May 2012
days between first & last application	254 [2011: 29, 2012: 47]

Characteristic feature	Indication
spray events	7 [2011:4, 2012: 3]
average days break between two spray events [winter break omitted]	42 [11.5]
active substances	13
products	14
combination products	4
spray events with combination products	7
spray events with tank mixtures	2
spray events with fungicide, insecticide, herbicide	4, 2, 2
treatment index JKI	8.0
ranking TURAC; TUsoil	39; 38 [out of 80]

As shown in Fig. 29, no clear exceedance of the allowed application rates was registered for the typical spray series in winter oilseed rape. The product Cantus (a.s. boscalid) was applied in very low amounts, which was probably because the combination product Cantus Gold (boscalid and dimoxystrobin) was applied at the same spray event.

Figure 29: Actual versus maximum registered application rates in typical-case spray series in winter oilseed rape

All points on the diagonal match the allowed application rates perfectly. Red-labelled points indicate deviations of actually applied application rates from allowed application rates by more than factor 2 (exceedance, above the diagonal) or of less than factor 0.5 (no full exploitation, below the diagonal). Deviation of plotted number of products compared to total number applied in the spray series shown here is due to non-matches in the registration database. Rates are given as kg product / hectare. Representation of axes is on log10-scale.



Main findings chapter 3

Representative, standardized & unique application data were gathered for twelve main crops in Germany and Austria. A total number of 873 spray series was collected.

With focus on the analysis of the 873 spray series, the best-represented crop was winter wheat, comprising 133 spray series, followed by sugar beet (149) and potatoes (59). The least represented crops were spring wheat and triticale (six and 21, respectively). Two thirds of the spray series originated from a three years period between 2012 and 2014. The total number of active substances within permanent crops, cereals and other arable crops were comparable, whereas maize applications are characterized by few substances. In contrast, apple and winter wheat are treated by several PPP classes. Apple, vine and potato were dominated by fungicide use, cereals received a wide variety of PPP classes. Cereals receive constant numbers of spray events between two to six (50 % probability) per spray series. With five to nine and four to ten spray events per year, the application frequency for the crops winter oilseed rape and potatoes was slightly higher than for cereals. The most frequently treated crop is the vertical, permanent culture apple. The other permanent crop, vine, ranks with potato and rape at medium levels of treatment frequencies.

63% of spray events, across all spray series and crop types, were realized through tank mixtures of several PPPs. In apple particularly, critical fungicide-insecticide mixtures were applied at 14% of all spray events. However, most tank mixtures contained PPP-classes of the same PPP class (i.e. e.g. herbicide-herbicide-combinations).

Major differences in overall treatment patterns in terms of the total number of spray events and the pesticide classes applied were identified between crop types. Maize showed a very low intensity of mainly herbicide use, whereas apple and vine were marked by the frequent and intense use of fungicides.

Surrogate spray series were derived out of four focal crops (apple, winter wheat, winter oilseed rape and potato) for typical and worst-case situations to evaluate the risk assessment and additional risk of mixture toxicity in spray series.

In apple, the treatment indices of both the worst-case spray series (TI=34) and the typical-case spray series (TI=28) were close to the range of the treatment index reported by the JKI for the period between the years 2011 and 2013 (TI = 28-33). This finding suggests that the data at hand reflects more typical situation for apple and does not include realistic extremes. For the crop winter oilseed rape, worst- and typical case spray series were in closer agreement with the corresponding treatment indices.

4 Comparison of existing approaches and models for the environmental risk assessment of pesticide mixtures and sequential applications

In the first project phase, current assessment models for mixture toxicity were evaluated and compared based on two questions: (i) prediction from empirical data, and (ii) are mechanistic (process-based) approaches available to predict time-dependent toxicity of pesticide mixtures? In this report we refer to corresponding mathematical approximations as "models". Models implemented as software packages, including additional possibilities to consider application practices (like crop types, details of spray series etc.) and environmental boundary conditions (like exposure and fate parameters) are referred to as "tools". This subproject was carried out in the context of a master thesis (Alexandra Sybertz).

4.1 Evaluation of pesticide treatment regimes

A literature search was conducted on the effects of pesticide treatment regimes on populations of aquatic and terrestrial non-target organisms and biocenoses. Search items in "Web of Science" were for example: "study mixture effect spray series", "pesticide mixture effect spray series" and "effect pesticide treatment non-target organisms ". Numerous references reported the effects on pest organisms instead of non-target organisms, but some examples regarding non-target organisms could be identified.

Verbruggen and van den Brink (2010) concluded in their review on aquatic mixture toxicity that effects of realistic treatment regimes can be sufficiently described by the most toxic compound in the mixture. However, the authors based this conclusion only on a few studies, such as by Van Wijngaarden (2004), Arts et al. (2006) and Wendt-Rasch et al. (2004). These studies investigated the effects of a realistic pesticide application scenario including insecticides, fungicides and herbicides. In detail, Wijngaarden et al. (2004) assessed in indoor microcosms the risk of PPPs applied in bulb crops. Wijngaarden et al. (2004) calculated the sum of toxic units (TU_{sum}) based on the effective concentration (EC_{50}) of four different active substances for daphnids, algae and macrophytes in order to analyze the effects for different biological groups. According to this analysis, lambda-cyhalothrin presented by far the highest toxicity (up to 0.8 $TU_{Daphnia}$) for macroinvertebrates and zooplankton and, thus, mainly explained the decreasing effect of these taxa. Wijngaarden et al. (2004) also summarized that the concept of TU_{sum} can be used to describe direct effects of treatment regimes. Wendt-Rasch et al. (2004) investigated the same mixture of active substances as Wijngaarden et al. (2004) to assess the effects of tulip treatments on algae communities and the growth of *Myriophyllum spicatum* in outdoor microcosms. However, the authors mainly described indirect effects of the insecticide exposure on the algae communities and *M. spicatum* under mesotrophic and eutrophic conditions. Hence, the outcomes are difficult to evaluate the prediction of mixture toxicity using simulated treatment regimes.

The study by Arts et al. (2006) investigated the effects of a simulated treatment regime for potato in mesocosms comprising structural (phytoplankton, zooplankton, macroinvertebrates, macrophytes) and functional endpoints (leaf litter breakdown, chlorophyll-a). The study included a total of 15 treatments with herbicides, insecticides and fungicides applied as spray drift. As in the study of Wijngaarden et al. (2004), single TUs and TU_{sum} (based on CA) were calculated using daphnids and algae as reference organisms. Arts et al. (2006) concluded that the identified thresholds are similar to semi-natural experiments with individual compounds despite repeated exposure. The authors explain this finding with fast dissipation rates and only few simultaneous exposures. However, the comparison of thresholds between community experiments with or one or several compounds several is based on very few studies.

In addition to the studies included in the review by Verbruggen and van den Brink (2010), Cross and Berrie (1996) investigated the effects of treatment regimes in apple orchards (West Malling, Kent) on populations of the predatory mite *Typhlodromus pyri* (Cross and Berrie, 1996). Foliar spray applications were performed with various pesticides as single applications, as repeated application of the same pesticide and as application of two pesticides. Chlorpyrifos applied together with mancozeb or with thiophanate-methyl resulted in a greater reduction of the *T. pyri* population than any of the chemicals alone (Cross and Berrie 1996). The study also revealed that multiple applications of mancozeb caused adverse effects on *T. pyri*.

Finally, an aquatic pond community exposed to multiple low pesticide pulses was investigated by Talk et al. (2016). A real pesticide application protocol for apples in the Bodensee region, Germany, was used including eleven different pesticides applied within 60 days. The authors assessed the fungi colonization of the litter layer (*Alnus glutinosa*) using a molecular fingerprinting approach. No difference between the treatment and control was observed in terms of fungal pattern and litter degradation probably because of the low pesticide concentration that was used according to good agricultural practice (Talk et al. 2016).

Generally, there are some issues that question the generalization of outcomes from the mentioned studies with simulated treatment regimes:

- Wijngaarden et al. (2004) and Arts et al. (2006) only considered the pathway drift in one possible treatment scenario. PPP runoff is another important exposure pathway that may also lead to a change in exposure scenarios.
- Reported endpoints are not always sensitive enough to enable the prediction of direct mixture effects (i.e. Wendt-Rasch et al. 2004, Talk et al. 2016).
- We detected a variety of treatment scenarios in terms of applied PPPs (type and dosage) and the number of applications. This variety may lead to different risks of treatment regimes (*e.g. worst-case versus typical-case spray series,* see also chapter 3 and 5).
- Regarding the concept of TU_{sum}, the selection of reference organisms must be adapted according to the applied PPPs and the community under consideration. For example, neonicotinoid insecticides are far less toxic to daphnids than to insects (PPDB, Lewis et al. 2016).
- Some studies observed direct and indirect effects (e.g. Wijngaarden et al. 2004, Wendt-Rasch et al. 2004, Arts et al. 2006). However, indirect effects are more complex to predict and findings could be mainly linked to direct effects.

Another approach that goes beyond the evaluation of spray sequences is the “Chemical footprint”. It is an approach to consider the overall risk of chemical pollution (Posthuma et al. 2014). In general, such a “Chemical footprint” includes the following points (Zijp et al. 2014):

- Exposure assessment
- Impact assessment
- Boundary conditions

- Dilution volume needed to reach the set boundary condition

A case study was conducted at the Rhine/Meuse/Scheldt River Catchments (Zijp et al. 2014). The observed and predicted pesticide concentrations in this study were of the same order of magnitude. The chemical footprint is a hypothetical value, which should primarily consider the development of the chemical load over time. The aspects of the chemical footprint are important elements of environmental risk assessment.

4.2 Approaches for the prediction of mixture toxicity

We conducted a comprehensive literature search in "Web of Science" and "Google Scholar", searching for documents containing the keywords "mixture toxicity", "toxicity prediction model" or "toxicity models". In addition, we used specific keywords such as “concentration addition” or “independent action” to refine the search. We then sorted the results by relevance and restricted to articles in English language.

4.2.1 Mathematical approaches

The concentration addition model by Loewe (CA) (1926) and the independent action model by Bliss (IA, 1939) are fundamental models for the environmental risk assessment of mixture toxicity. The first is the concentration addition model, suitable for mixtures that contain substances with similar modes of action. The other, the independent action model, can be applied to mixtures of substances with dissimilar modes of action. Assuming concentration addition is appropriate if mainly additive effects are to be expected. Subsequently, by merging the concepts of concentration addition and independent action, the heuristic model (HM) was developed, in which the components with similar modes of action are assigned into one “cassette”. Within these cassettes, the toxicity is calculated based on the concept of concentration addition. Toxicity between the cassettes is calculated assuming the concept of independent action. When using such models, only the toxicity of mixtures containing non-interacting substances is considered (Olmstead and LeBlanc, 2005). Consequently, the heuristic model was extended by including so called k-functions, which consider possible interactions (synergism and antagonism) between components of a mixture. The k-function describes the degree to which the concentration of one compound changes the effective concentration of a second compound in the mixture. However, it is necessary to calculate these k-functions from experimental data of the individual compounds and of the mixture (Rider and LeBlanc, 2005). This approach is currently not able to estimate synergisms, as both values for each individual substance and the mixture must be known.

A further model relevant for mixtures of non-interacting substances is the effect summation model (ES). This model evaluates the effects of mixtures as arithmetic sum of the effects of all mixture components (Ge,Liu,Su, et al. 2013). Yet another approach is the integrated fuzzy concentration addition-Independent action model (IFCAM), using QSARs (Quantitative Structure-Activity Relationships) and fuzzy set membership functions for the prediction of the toxicity of mixtures with non-interacting substances (Mwense et al. 2004). The IFCAM approach is suitable for mixtures of similar and dissimilar components. The fuzzy set theory is used to characterize the degree of similarity and dissimilarity of the mixture components. In addition, the model integrates the concepts of concentration addition and independent action.

Several indices have been defined to calculate the ecological risk, like Hazard Index or Margin of Exposure (Wilkinson et al. 2000). These approaches, however, neglect different modes of action of mixture components. The Hazard Index, defined as sum of quotients consisting of exposure and maximum acceptable concentration level (Table 29, Appendix B), for instance represents only a general risk indicator of mixtures. The Maximum Toxic Unit concept (Nowell et al. 2014) is based on the simplified assumption, that the most toxic component of a mixture is a proxy for the toxicity of the mixture.

Our literature search did not reveal any further mathematical approaches to assess mixture toxicity not already reported in Coors et al. (2014) and Kortenkamp et al. (2009). It can be assumed that the most frequently applied models are the concentration addition and independent action model. There are very few models, such as the heuristic model with k-functions, which attempts to predict the mixture toxicity of interacting mixtures. An overview of models identified so far and a small selection of existing risk indicators can be found in Appendix B (Table 29).

4.2.2 Mechanistic approaches

Our evaluation of mechanistic model approaches includes the following questions. (1) Which tools are available and which models they are based on? (2) In which context are these tools used and what are the calculated results? (3) Have the tools already been validated in a representative study? (4) To what extent is mixture toxicity considered in these tools?

Three tools are described below regarding whether they consider pesticide treatment intensity, type of application, tank mixtures and combination products. These tools are PRIME beta („Pesticide Risk Mitigation Engine“) (ipmPRIME), SYNOPS-WEB („Synoptische Bewertung des Risikopotentials chemischer Pflanzenschutzmittel“, v.1.0) (JKI) and HAIR 2014 (“Harmonised environmental Indicators for pesticide Risk”) (HAIR), respectively.

PRIME-beta

PRIME-beta (Guzy et al. 2014) was developed in 2008 and is an online tool, designed as a relative risk ranking method (http://IpmPrime.org). The PRIME project team consisted of Oregon State University, the IPM Institute of North America, the University of Arizona and the UN FAO/GEF (UN Food and Agriculture Organization/ Global Environment Facility) (Pesticide Research Institute, accessed 15.02.2018). The Integrated Plant Protection Center of Oregon State University (IPPC) is responsible for maintenance of the tool. Due to the breadth of possible applications, the partners focus on specific objectives: IPM Institute of North America focuses on applications for food industry supply chains and other commercial sectors with the Pesticide Risk Tool (ipmPRiME.com). Oregon State University's Integrated Crop Center focuses on the public sector (ipmPRiME.org), i.e., governments, agencies and farmers (Pesticide Research Institute, accessed 15.02.2018).

The risk indicators of the Prime Model can be specified in great detail and even information on aquatic and terrestrial endpoints can be considered. However, the tool lacks several parameters needed to predict pesticide exposure, for instance important soil parameters or interception (ipmPRIME, accessed 24.07.2016). With regard to mixture toxicity, each individual application of a pesticide is considered as independent event. No accumulation of substances in the soil nor additive effects are considered (ipmPRIME, accessed 24.07.2016). As an advantage of PRIME some risk indexes are based on SSD-data (Species Sensitivity Distribution), although the SSD-data source is not reported. The tool illustrates the risk of each substance applied and the cumulative risk for each endpoint. Cumulative risk represents the probability that at least one of the applications has an adverse effect, and is based on the assumption of Independent Action (PRIME, 2009; ipmPRIME, accessed 24.07.2016). The aim of the PRIME-project is to demonstrate the benefits of Integrated Pest Management (IPM) (IPM PRiME Mission Statement). The online tool was designed as a decision-making tool for people who use pesticides, such as farmers for planning the selection of pesticide products and pesticide mitigation measures

(Pesticide Risk Mitigation Engine at http://IpmPrime.org). The substance database includes nearly all registered pesticides in the US (ipmPRIME, accessed 24.07.2016). The last update of the pesticide database was on 29. September 2014.

SYNOPS-WEB

SYNOPS-WEB has been developed by the Julius Kühn-Institute (JKI) to estimate the environmental risk of pesticide applications (JKI, accessed 27.07.2017). The tool considers three compartments soil, surface water and field margins (Strassemeyer et al. 2017). In terms of surface water, the SYNOPS-WEB tool has already been validated. SYNOPS-WEB was evaluated on the Lamme River (Germany) over a period of four years (1995-1999) (Strassemeyer et al. 2017). An automatic sampler was used to take surface samples at different intervals. Eight different active substances were analyzed in 143 samples. Three substances showed a good to moderate agreement with the predicted concentration (Lin's concordance coefficient (CCC) > 0.74), while four substances showed very poor or no agreement with the predicted concentration (CCC < 0.35) (Strassemeyer et al. 2017). In this case, the results could not validate the model. So far, the tools for soil and field margins have not been validated. SYNOPS-WEB can be used to predict the environmental risk of an application series of pesticides and to decide on which pesticides should be applied. The main objective of SYNOPS-WEB is to assist the farmers in including further mitigation factors such as the width of the field margins and drift reducing application techniques. Properties of the active substances are derived from the PPDB (Pesticides Properties DataBase)(Lewis et al. 2016). To calculate the risk for soil organisms over one year the tool predicts time-dependent exposure curves and also includes temperature-dependent degradation of the pesticides. The parameter describing the overall mixture risk is the ETR (Exposure Toxicity Ratio), i.e. the ratio of the exposure concentration (PEC) and the concentration of a species-specific ecotoxicological endpoint (e.g.LC 50). The acute risk of an application series is defined as the risk of the most toxic active substance of the whole application series. Whereas the chronic risk is estimated by concentration addition by using the time-weighted (seven days) average concentrations of each substance. The time-weighted concentrations are added on a daily basis and the highest mixture risk on a certain time point is defined as the overall chronic risk. The relevance of short and high concentrations is therefore not taken into account. However, the effects on soil organisms will only be really predictable when the data for earthworms in the data source are complete. SYNOPS-WEB generates tables, no diagrams, including the calculated risk values for the different endpoints. The tool defines risk thresholds ranking the resulting acute and chronic risk values from low to high risk (Strassemeyer et al. 2017).

HAIR2014

Alterra Wageningen has developed a tool called HAIR2014 (Harmonised environmental Indicators for pesticide Risk) which can be applied to assess the effectiveness of EU sustainable agriculture policy (Kruijne et al. 2014). The calculation of risk indicators is based on the HAIR consortium within the framework of the 6th Environmental Action Programme (Contract No. SSP-CT-2003-501997). Aim of the HAIR consortium was to generate an European scientific expertise on the consequences of agricultural use for the environment and human health (Kruijne et al. 2011).

HAIR2014 is no online tool and must be downloaded on the project's homepage (http://www.pesticidemodels.eu/hair/home). HAIR2014 uses four different databases. The first one, the Hair Database, includes for example information about crops or model parameters. The database is maintained by Alterra and is part of the software package (Kruijne et al. 2014). The second database (Geographical database) contains GIS data from Europe, while the third database, the compound database, contains information on various pesticide active substances and their physicochemical and ecotoxicological properties. The fourth database, the usage database, contains the scenario of a simulated pesticide application. The evaluation in HAIR is based on a regional scale depending on the used geographical database. ETRs as regional indicators are calculated for every grid cell (Kruijne et al. 2011; Kruijne et al. 2014).

The toxicity for different aquatic and terrestrial organisms is based on a detailed and comprehensible calculation in HAIR2014 (PesticideModels.eu, accessed 24.08.2016). Like in SYNOPS-WEB, the risk in HAIR2014 is defined as quotient of the exposure and a species-specific ecotoxicological endpoint. Usually the maximum exposure is taken to predict the risk. Standard test organisms are taken into account for the risk to aquatic and terrestrial organisms. The tool includes also some effect endpoints for human toxicity. The use of the transparent program and the input of variables and parameters is user-friendly. As disadvantage HAIR2014 only considers several applications of the same pesticide, i.e., it cannot be used for the risk assessment of pesticide mixtures. Exposure of soils by multiple use is defined as the exposure after the last application, taking into account residues from previous applications (Kruijne et al. 2011). Table 16 shows that HAIR2014 and SYNOPS-WEB share similar input-information in comparison to PRIME.

During the project, a new tool, called MITAS (Mixture Toxicity of Application Series) was developed by RWTH Aachen University to consider the different aspects of pesticide spray series in soil. The tool is able to estimate the risk of pesticide mixtures, so far only for earthworms. Aim of MITAS is to predict the time-dependent risk of an entire pesticide application series taking also in account the degradation of the pesticides. MITAS provides several advantages compared to the tools described above. PEC-calculation and risk calculation of mixtures are comprehensive and transparent. The PEC-calculation is based on FOCUS soil persistence models (Boesten et al. 1997). The tool uses three databases to receive information about the applied pesticide application series called *compoundtab, croptab* and *maintab*. *Compoundtab* and *croptab* incorporate data of compound and crop properties, like degradation kinetics or toxicity endpoints. Additionally, the *compoundtab* discriminates different modes of action of pesticides, which are derived from IRAC (Insecticide Resistance Action Committee) and the PPDB-Database (Pesticide Properties DataBase) (Lewis et al. 2016), the latter also used in PRIZM (Pesticide Root Zone Model), which is used inside SYNOPS to assess environmental risk (Lewis et al. 2016). In MITAS it is possible to integrate further information in the databases received from different sources. Databases can be extended individually in structure, for example with additional risk indices like indices for human toxicology or community characteristics. The *maintab* includes important application information like the application rate, the application date or the applied compounds. Moreover, this database incorporates environmental information, which can also be adjusted individually.

Table 16: Comparison of the three tools PRIME-beta, HAIR2014 and SYNOPS-WEB relating to the input-data

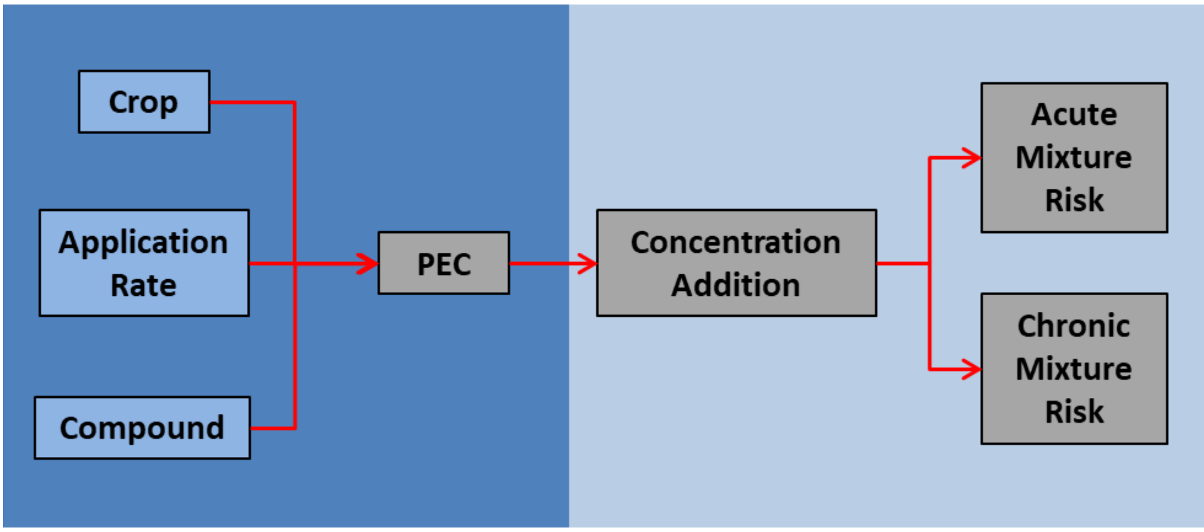
The table displays the names of the various tools in the heading. The first column lists the different input-parameters. The word "yes" indicates that the user is able to enter the value. The table is separated into the two subgroups treated area and application.

	PRIME-beta	HAIR2014	SYNOPS-WEB (v 1.0)
		Treated area	
Exact location	no GIS-input possible as yet	user chooses a NUTS1 region	possible to mark a chosen area (some data are automatically added)
Type of application area	yes	/	/
Area size	yes	yes	fixed (10x10 km^2 or 1x1 km^2)
Average slope	/	automated entry: slope map (Europe)	yes
Width of field margin	/	yes	yes
Buffer zone (min. distance to surface	/	yes	yes
Width of surface water	/	standard cross-sectional dimensions are assumed	yes
Depth of surface water	/	standard cross-sectional dimensions are assumed	yes
Scenario for crop development (FOCUS)	/	partially based on FOCUS	FOCUS
Flowing surface waters	/	automatically calculated	yes
Existence of hedge between site and	/	/	yes
Organic carbon content of top soil (0-0.3 m)	/	automated entry: soil organic carbon map	automated entry possible
Organic carbon of soil profile (0-1 m)	/	automatically calculated	automated entry possible
Dry bulk density of top soil (0-0.3 m)	/	automatically calculated	automated entry possible
Water content at field capacity of top soil	/	automated entry: depends on texture	automated entry possible
pH of top soil	/	automated entry: soil pH map (Europe)	automated entry possible

	PRIME-beta	HAIR2014	SYNOPS-WEB (v 1.0)
Texture class	/	automated entry: texture class map (Europe)	automated entry possible
Hydrologic soil class	/	automated entry: hydrological soil class map	SCS classification (Stolbovoy et al. (2007))
Depth of groundwater level	/	/	user specific
Drainage scenario	/	automated entry: FOCUS	FOCUS
Precipitation annual	/	taken from background information	taken from background information
Annual temp (mean of month)	/	taken from background information	taken from background information
Crop	yes	user specific	user specific
		Application	
Application Date	yes	yes	yes
Plant protection product/Active	yes	yes	yes
Active substance code (CAS-number)	/	yes	yes
Application rate	yes	yes	yes
Application method	yes	yes	yes
Drift reduction of sprayer	/	yes	yes
Buffer zone	/	yes	yes
Driving speed	/	/	yes
Flowering weeds in crop	/	*yes*	/
Price	/	/	*yes*

MITAS allows transforming special application scenarios into continuous time series of PEC-values, considering first order degradation kinetics of the different compounds. MITAS simulations lead to the following results on a time-dependent (daily) scale: (1) the acute and chronic risk of the whole mixture assuming concentration addition (Loewe and Muischnek 1926) (Fig. 30) (2) the acute and chronic risk and PECs of each individual component of a mixture. Calculations for more than one year are possible. The tool is in the prototype stage and was programmed in R (The R-Project).

Figure 30: Flowchart of MITAS (Mixture Toxicity of Application Series)



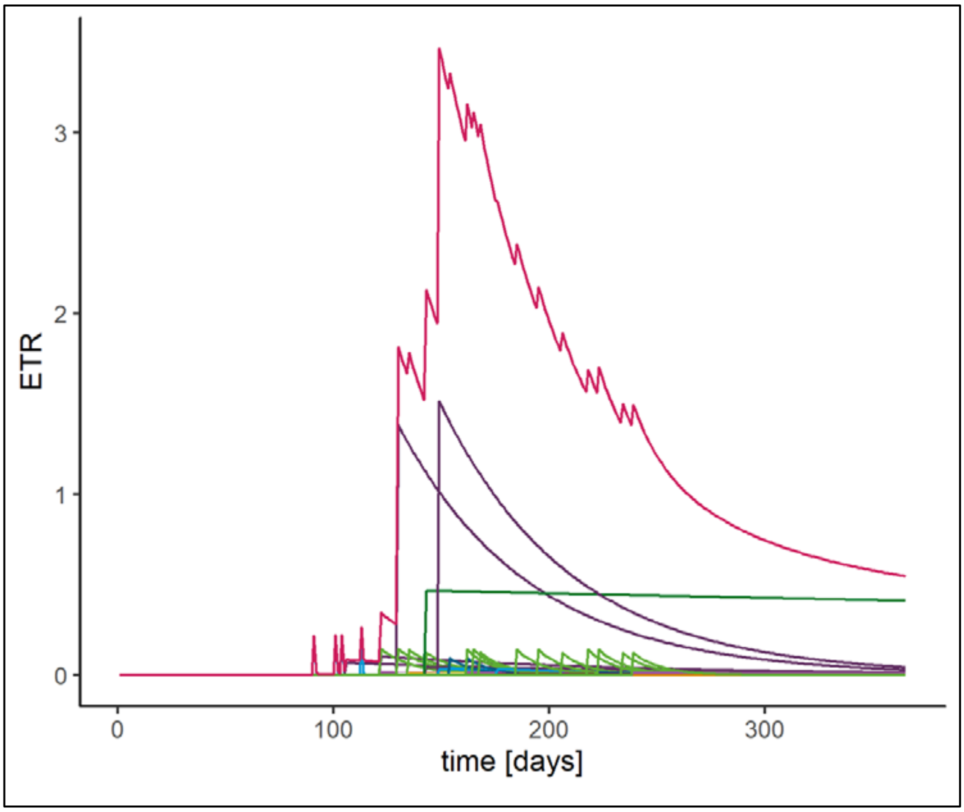
Like in SYNOPS-WEB and HAIR, the risk is calculated as quotient of PEC and an ecotoxicological endpoint (=ETR). The reciprocal toxicological value is the TER (Toxicity Exposure Ratio), which is also determined in MITAS. Regarding acute toxicity, median-lethal Concentration (LC_{50}) is used, the chronic endpoint is the No Observed Effects Concentration (NOEC). In MITAS, the concentration of each substance in soil at a specific day is simulated. Of course, remaining residues of previously applied substances are considered. ETR of each compound of a mixture are summed up to obtain the mixture risk (Berenbaum 1985). Results of MITAS are the acute ETR_{mix} (Exposure Toxicity Ratio Mixture) based on acute ETR-values and the chronic ETR_{mix} based on chronic ETR-values.

We used the MITAS model to simulate a worst case application series in apple crops selected from all available spray series (chapter 3). Fifteen different active substances were applied in this spray series at 26 different dates in one growing season. A variable mixing depth in soil was selected for the calculation depending on the Kfoc-values (Freundlich sorption constant normalized for organic carbon content) of the individual pesticides. Based on a common assumption of the national authorization procedure, initial PEC-values for substances with Kfoc above 500 or below 500 L/kg were calculated for soil depth of 1 cm or 2.5 cm, respectively. High Kfoc-values here defined above 500 L/kg, indicate high adsorption of the substance to the solid matrix and, thus a low leaching potential. A mixing depth of 1 cm leads to 2.5 higher initial exposure concentrations compared to a mixing depth of 2.5 cm. Data for the substance-specific ecotoxicological endpoints and degradation half-lives of the substances were provided for this spray series by the German Environment Agency (UBA). Fig.31 and Fig. 32 are generated with MITAS and demonstrate the time-dependent chronic mixture risk of the apple worst-case spray series.

The maximum mixture risk (red graph) is higher (3.46) than the maximum risk of the individual pesticide thiacloprid (1.51, violet graph) (Fig. 31), since other compounds like the slowly degrading fluquinconazole (green graph) influence the overall mixture risk of this application series.

Figure 31: Time-dependent chronic mixture risk of the spray series apple (worst-case spray series) for earthworms

The x-axis represents the single days of a year, whereas the y-axis represents the ETR-values. The single graphs show the chronic ETR-development of the applied substances in consideration of the degradation. The different colors highlight the various applied substances. The red graph represents the chronic mixture risk calculated with concentration addition.



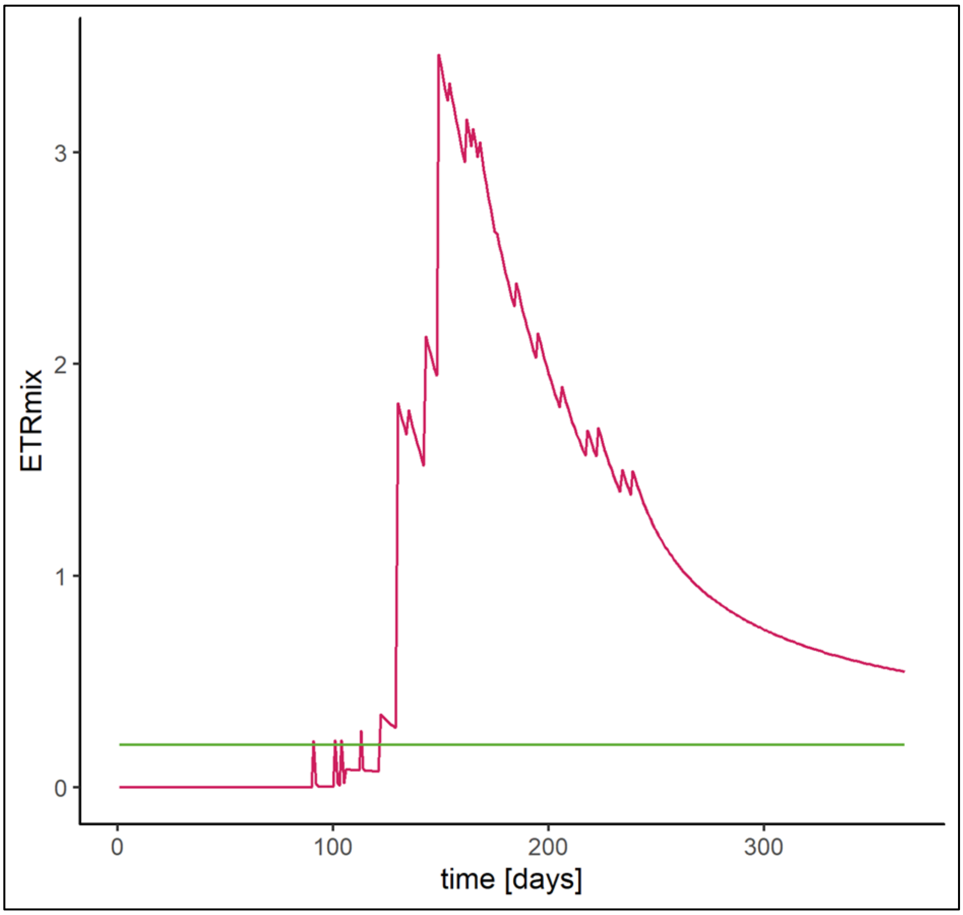
The calculation of the ETR_{mix} value, enables the mixture risk to be assessed with the thresholds of the European Commission (European Commission 2002, EFSA 2009a). The chronic mixture risk of the worst case spray series in apple for earthworms exceeds already the chronic threshold of 0.2 (TER = 5) after less than 150 days of the simulation period (Fig. 32) and remains below the threshold value for more than half a year. Apple is a permanent crop, so a similar spraying sequence is possible next year. This sequence would already start above the threshold value.

In MITAS, the exposure from the spray series is represented dynamically over time and is related to an effect endpoint (mainly NOEC 56 d values are available). Nevertheless, a effect endpoint would be required that takes into account previous exposure of organisms, different exposure times and application scenarios. As it is very unlikely that such NOEC-values will be available ever, an approximation of time-dependent effects must be used.

MITAS allows transforming discrete application scenarios into continuous time series. There is still a need for research on the validation of the calculated exposure and effects. Monthly climate data can be entered manually or taken from a database of the German Weather Service (average air temperature Germany) (DWD 2018).

Figure 32: Chronic mixture risk of the spray series apple (worst-case spray series) for earthworms

The x-axis represents the single days of a year, whereas the y-axis represents the ETR-values of the mixture. The red graph shows the chronic mixture risk predicted with concentration addition. The threshold value for chronic risk is visualized with the green horizontal line.



The calculation in MITAS is transparent and comprises PEC calculation, includes substance properties, climate and soil data. The tool allows a fast comparison of different time-dependent spray series in consideration of the mixture risk in soils. Table 17 and Table 18 provide a comparison of MITAS with the tools PRIME, HAIR2014 and SYNOPS-WEB.

Table 17: Comparison of the tools PRIME-beta, HAIR2014, SYNOPS-WEB and MITAS with respect to the risk indicators considered

The table displays the names of the various tools in the heading. The first column lists the different risk indicators of the tools. The tick indicates the risk indicators considered in the tool.

	PRIME-beta		HAIR2014		SYNOPS-WEB		MITAS	
				Risk indicators				
Aquatic	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**
Algae	✓		✓	✓	✓*	✓*		
Daphnia			✓	✓	✓	✓		
Fish		✓	✓	✓	✓	✓		

	PRIME-beta		HAIR2014		SYNOPS-WEB		MITAS	
Lemna					✓	✓		
Chironomus					✓	✓		
Aquatic invertebrates	✓							
Soil	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**
Birds	✓	✓	✓	✓				
Mammals	✓		✓	✓				
Earthworm	✓		✓	✓		✓	✓	✓
NTA (Non-Target	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**
Bee			✓		✓			
Groundwater								
Leaching			✓		✓			
Workers and	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**
Operators				✓				
Reentry workers			✓	✓				
Greenhouse workers			✓	✓				
Bystander	✓		✓					
Child bystander			✓					

*Algae acute and chronic vary in their endpoint: algae acute (EC_{50}) and algae chronic (NOEC)

SYNOPS-WEB and HAIR2014 consider many aquatic endpoints such as acute and chronic effect data for daphnids (Table 17). The chronic risk for earthworms and the acute risk for bees are the only terrestrial endpoints that are used by SYNOPS-WEB. HAIR2014 is able to predict the risk for birds and mammals as well. PRIME considers different aquatic and terrestrial endpoints and one human risk endpoint (for bystander). HAIR2014 includes various human risk indicators (e.g. operators, bystander). Since MITAS is in the prototype stage, so far risk assessment can be predicted only for earthworms, but other terrestrial endpoints will be considered in the future.

All tools except HAIR2014 include mixture risk calculation and risk thresholds to classify the results (Table 18). PRIME-beta takes only partial or no account of substance degradation and multiple applications. HAIR2014 and SYNOPS-WEB calculate risk indicators for three different compartments (soil, surface waters and field margins). Currently MITAS calculates risk indicators for soil organisms only. It is possible to simulate the risk for more than one year in MITAS and HAIR2014, whereas SYNOPS-WEB has a fixed simulation time of one year. All models provide ETRs as output except PRIME-beta. With MITAS also TER-value can be calculated. Only the new MITAS tool is able to calculate and visualize the time-dependent overall risk of pesticide mixtures and illustrates the period during which a risk threshold is fallen below.

None of the previously existing tools so far is capable to consider an application series in its complexity (multiple applications, mixture toxicity, sequences and substance degradation). MITAS, however, incorporates such important aspects and thereby assess the exposure (PEC) and the impact (ETR/TER) representing points 1 and 2 in the concept of "Chemical footprint".

Table 18: General comparison of the tools PRIME-beta, HAIR2014, SYNOPS-WEB and MITAS

The table displays the names of the various tools in the heading. The first column lists the different aspects of the tools. Among others, the symbols ✓ (true), ~ (partly true) and ✗ (does not apply) are used to compare the tools.

	PRIME-beta	HAIR	SYNOPS-WEB (v.1.0)	MITAS
Substance degradation	~	✓	✓	✓
Multiple application	✗	✓	✓	✓
Risk classes / risk thresholds	✓	✗	✓	✓
Calculation of mixture risk	✓	✗	✓	✓
Time-dependent mixture risk (ETR mix)	✗	✗	✗	✓
Period exceeding a risk threshold (ETR mix)	✗	✗	✗	✓
Simulation time		more than 1 year	1 year	more than 1 year
Risk indicator		ETR	ETR	ETR / TER
Compartments	Soil, surface waters	Soil, surface waters, field margin biotopes	Soil, surface waters, field margin biotopes	Soil

Main findings chapter 4

In general, we identified only few studies with regard to the effects of treatment regimes on non-target organisms at the populations or community level. Two studies applied and suggested the use of toxic units (TU, single TUs and TU_{sum}) to describe direct effects of applied pesticides (Wijngaarden et al. 2004, Arts et al. 2006). The other studies did not include direct pesticide effects on the investigated endpoints or did not use an approach to estimate the overall effect of the treatment regime.

Most of the models investigated are not able to predict the mixture toxicity from individual pesticides within a spray series and do not consider interacting substances such as synergisms or indirect effects. The heuristic model with k-functions is one of the few models predicting mixture toxicity for interacting substances (e.g. synergism). However, this requires a lot of experimental information.

Out of the mechanistic tools examined here (HAIR, SYNOPS-WEB, PRIME-beta, MITAS), only two (SYNOPS-WEB and MITAS) have included the complexity of a pesticide spray series with multiple applications and time-dependent degradation. The results of the investigated tools were presented as risk endpoint. The investigated tools in this chapter that predict mixture effects use either the approach of Concentration Addition or Independent Action.

For an apple spray series, the MITAS tool was used to simulate the course of the overall risk of a spray sequence. The threshold value of the chronic risk was not met over a longer period (TER_{mix}), which can be partly explained by the varying degradation rates of the applied substances. MITAS shows that residues from earlier applications may remain in the soil and consequently could influence the mixture risk of subsequent applications.

None of the three models (HAIR, SYNOPS-WEB, PRIME-beta) examined so far is able to show the complexity of a spray series (multiple applications, mixture toxicity, sequences, degradation). MITAS does integrate these aspects and the model is still expandable.

With regard to the simulation of effects on populations or communities over time, no suitable models could be found. This means that indirect effects are not considered in any of the reviewed approaches.

However, none of the models (including MITAS) is able to integrate effects over time on communities, containing organisms interacting with each other and with the environment.

5 Risk characterization of selected spray series

We described in chapter 3 the selection of four spray series from the large data set on real treatment regimes. The four spray series represent typical- and worst-case scenarios for treatment regimes in apple and winter oilseed rape. For these spray series, we determined 28 commonly used risk indicators for the risk assessment of plant protection products (PPPs). Risk indicators are the combination of an entry pathway (e.g. direct overspray in case of soil organisms or run-off into surface water for aquatic organisms), the organism tested (e.g. birds, terrestrial non-target arthropods) and the endpoint (e.g. acute, chronic). The aim was to check the protectivity of the current environmental risk assessment (ERA) performed for individual PPP applications considering real treatment regimes. Risk indices were based on predicted environmental concentrations (PECs) and regulatory relevant endpoint measures for active substances (a.s.) and PPPs, respectively. It was crucial for the comprehensibility and reproducibility of the analyses that a transparent conceptual approach and workflow of data processing and computation was developed and documented. Further, it was considered important that the approach could be easily adapted to new spray series as soon as high-quality data on measures of ecotoxicity become available.

The analyses of risk indicators were used to answer a number of three key questions:

- To what extent is the risk assessment of PPPs protective for terrestrial and aquatic ecosystems based on Tier 1- and higher Tier-risk indicators?
- What kind of mixture risks do we expect from the exposure to tank mixtures and PPP sequences based on existing lower- and higher Tier studies and the predicted exposure patterns?
- Which crops and treatment regimes cause the highest predicted mixture effects and risks for the environment in general or for specific ecosystems? The application of which substances mainly indicate non-acceptable risk?

In this chapter specific terms are defined and used

TER_{single} – Toxicity to exposure ratio for each PPP application in one spray series expressed in equivalents of active substance. TER_{single} were determined as the ratio of regulatory relevant effective concentrations (endpoint measures) for each acceptability criterion and the predicted environmental concentrations (PEC). TER_{single} were determined for 28 risk indicators including terrestrial organisms (e.g. earthworms, birds, non-target arthropods) and aquatic organisms (e.g. daphnids, algae, macrophytes, fish). Accepted registration data and regulatory exposure models provided by the German Environment Agency by served to determine relevant PECs.

TER_{min} per tank mixture or spray series – TER_{min} represented the lowest TER_{single} and, hence, the TER_{single} with the highest risk per tank mixture (spray event) or spray series.

Threshold of acceptable risk – The threshold of acceptable risk was specified for each of the 28 risk indicators according to current ERA schemes and guidance documents (Table 30). We considered TER indicators to pose a risk if they do not achieve the threshold of acceptable risk.

TER_{mix} per tank mixture and spray series – TER_{mix} represents the toxicity exposure ratio of one tank mixture, i.e. all applications on one day, or all applications in one spray series. We calculated TER_{mix} for all applications in terms of a.s. per time point (tank mixture) and for all applications of a.s. per spray series applying the TER_{mix} approach. TER_{mix} assumes additive effects (CA, Equation 5) as the most basic approach and without considering synergistic or antagonistic mixture effects.

5.1 Data processing before the risk assessment of the selected spray series

The following describes the use of spray series data, which was necessary to perform a risk assessment of the four selected spray series. We executed several consecutive steps of data pre-processing, TER-calculation and TER-processing steps within a generic workflow (Figure 33) This approach allows for comprehending the computational steps and for the transfer to other situations and new treatment scenarios.

Spray series data held information on the amounts applied of the product and the active substances. This resulted in a matrix of 43 columns with the variable names and one row that represented the case "application of an active substance within a product at one specific date, crop". For example, the worst-case spray series in apple consisted of 55 rows.

5.1.1 Calculation of risk indicators

We calculated Tier 1- and higher Tier-risk indicators (TER_{single}-values) using the software tool "risk profiler", originally developed by the German Environment Agency within the frame of a comparative product assessment. Technically speaking, we calculated the risk indicator TER_{single} for each application of a.s. and/or product (i.e. depending on data availability) and for each of the endpoints separately. The availability of toxicity data for both the a.s. and the products partly varied between the risk assessment areas. Data for a.s. and products were often available for aquatic endpoints, while for NTA-studies mostly only product data was present. The tool needed input effect data for the a.s. and mono- or combination products (Table 19). The German Environment Agency provided the necessary data and included the effect values relevant for the risk assessment as used for the national registration of PPP in Germany. All effect value units were converted prior the calculations to contents of the a.s..

Further input data were needed on intrinsic and experimentally deduced substance properties (e.g. degradation rates in water and soil) to calculate predicted environmental concentrations (PECs). Ecotoxicological effect data (endpoint measures) were aligned to EU-agreed effect measures.

Figure 33: Generic workflow of spray series data processing and mixture risk indices calculations

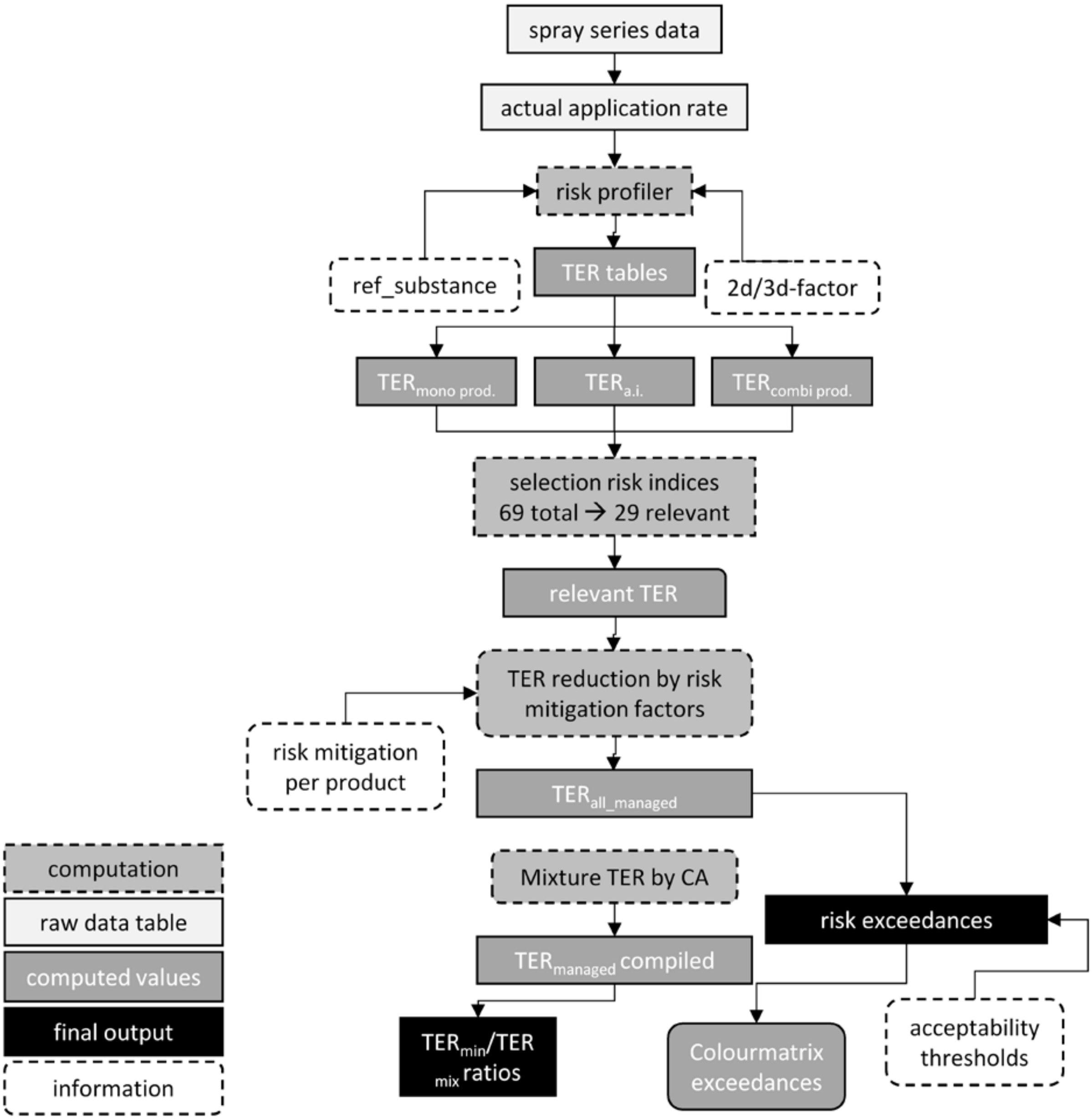


Table 19: Input data for calculations of risk indicators by means of the tool “risk profiler”

The software tool “risk profiler” requires substance and product intrinsic properties (density, water solubility, vapor pressure) and fate data (degradation half-times). Effect data are not intrinsic but come from standard and non-standard laboratory, semi-field and field studies from different assessment areas. (lt. = long-term, ac. = acute endpoints, BCF = bioconcentration factor, DT_{50} = degradation time 50%, $disT_{50}$ = disappearance time, LD = lethal dose, LR = lethal rate, ER = effect rate, EC = effect concentration, NOAEL = No Observed Adverse Effect Level, NOEC = No Observed Effect Concentration). Units are given in round brackets. Mean and worst-case refers to all available field experimental data on degradation in soil. “Corrected value” refers to soil endpoint measures divided by two for substances with high log K_{OW}.

Category input data	Input data measure
substance/product	substance name
	substance type
	product density (kg/L)
physico-chemical properties	molar mass (g/mol)
	log P_{ow}
	vapour pressure (Pa)
	Henry's law constant (Pa × m³ × mol^{-1})
	K(f)oc (L/kg)
	Freundlich coefficient (1/n)
environmental fate	soil DT_{50}, mean (d)
	soil DT_{50}, worst case (d)
	water $DisT_{50}$ (d)
	BCF (fish)
vertebrates, effects	LD_{50} birds
	NOAEL birds
	LD_{50} mammals
	NOAEL mammals
terrestrial arthropods, effects	LD_{50} honeybees, oral
	LD_{50} honeybees, contact
	LR_{50} NTA, laboratory
	ER_{50} NTA, ext. laboratory
aquatic organism, effects	LC_{50} fish (ac.)
	NOEC fish (lt.)
	EC_{50} daphnids (ac.)
	NOEC daphnids (lt.)
	EC_{50} other inverteb. (ac.)

Category input data	Input data measure
	NOEC other inverteb. (lt.)
	ErC_{50} algae
	$E(r)C_{50}$ aq. macrophytes
	overall RAC
soil organism, effects	NOEC earthworms (corrected value)
	NOEC soil arthropods (corrected value)
terrestrial plants effects	ER_{50} seedling emergence
	ER_{50} vegetative vigour

Information on the relevant crop scenario was provided by the German Environment Agency for exposure calculation. This included the interception of a crop during the growing season (relevant for soil) or specific drift percentiles (relevant for aquatic assessments). We simulated only one application in a row, i.e. no multiple application factors were taken into consideration. We chose the generic standard scenarios for winter oilseed rape (field crops) and for apple.

For spray drift to adjacent surface waters and terrestrial non-target areas, the 90st percentile of the distribution of deposited a.i. via drift was assumed (standard assumption, implemented in EVA). This amounts for “arable crops” scenarios 2.77%, for “orchards early” 29.20% and for “orchards late” 15.73% of the original application rates. Run-off and erosion depend highly on the K(f)oc-value of the active substance. This value was therefore considered for PEC-calculations for surface water bodies.

The computation of risk indicators by the risk profiler for all relevant assessment areas required further input on the application patterns. The aim of the studies was to describe the Tier 1- and higher Tier-risks at the level of each single application. Therefore, the number of applications was set to 1. No multiple application factor (MAF) was used. The sequential use of PPP has been addressed in a separate approach that is described in chapter 5.3. The application rate was used as reported for the actual treatment regimes. The start (and the end) of the application window was chosen according the growth stage of the crop at the time of application. Values from 0-10 were possible, corresponding to the BBCH- or EC-stages between 0 (sowing) and 100 (harvest). We assumed that the treatment was directed towards the crop.

In a final step, we expressed risk indices as TER (toxicity exposure ratios), which were calculated as the quotient of the given effect values (EC_X, NOEC, etc.) and the modelled PEC-values (Table 30, Appendix C). For bees, the regulatory used “hazard quotient - HQ” was determined, which was for reasons of better compatibility with other endpoints recalculated as the reciprocal of the HQ. In total, TER-single values for 28 risk indicators were exported for subsequent analyses (Table 20).

Table 20: Assessment factors of the 28 risk indicators

Risk indicators combine the entry pathways (e.g. direct overspray in case of soil organisms or run-off into surface water for aquatic organisms), the organism tested (e.g. birds, terrestrial non-target arthropods), and the endpoint (e.g. acute, chronic). An assessment factor represents the threshold of acceptable risk and is therefore the risk indicator. Risk indicators relate to an assessment area regarding a specific organism group and exposure scenario. Reference numbers to guidance for the determination of PEC and TER values are given for each assessment factor in square brackets. Code of reference numbers: [1] EFSA/2009/1438, [2] EFSA (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J 11(7):3290, 268 implemented as software: EVA3 and Exposit, [3] EU Commission SANCO/10329/200, [4] EU Commission SANCO/10329/2002. Rationales of risk management (column RM = risk management applied, x= "yes") implementation are described in section 5.1.2.

Risk indicator	Assessment factor [reference]	RM
Birds dietary acute	10 [1]	
Birds dietary long-term	5 [1]	
Mammals dietary acute	10 [1]	
Mammals dietary long-term	5 [1]	
Fish acute / drift / runoff	100 [2]	x
Fish long-term / drift / runoff	10 [2]	x
Daphnia acute / drift / runoff	100 [2]	x
Daphnia long-term / drift / runoff	10 [2]	x
Aquatic invertebrate acute / drift / runoff	100 [2]	x
Aquatic invertebrate long-term / drift / runoff	10 [2]	x
Algae / drift / runoff	10 [2]	x
Aquatic macrophytes / drift / runoff	10 [2]	x
RAC / drift / runoff	1 [2]	x
Honey bees oral	50 [3]	
Honey bees contact	50 [3]	
Terrestrial non-target arthropods – standard lab / in-field	2 [4]	
Terrestrial non-target arthropods – standard lab / off-field (DE approach)	2 [4]	x
Terrestrial non-target arthropods – extended lab / in field	1 [3]	
Terrestrial non-target arthropods – extended lab / off-field (DE approach)	5 [4]	x
Earthworm long-term	5 [3]	
Soil arthropods long-term	5 [4]	
Non-target terrestrial plants – seedling emergence	10 [3]	x
Non-target terrestrial plants – vegetative vigour	10 [3]	x

The TER_{single}-output data of the risk profiler were processed and made-ready for risk analysis. The following steps were performed for this purpose:

- TER-values were compiled to three preliminary tables for the a.s., the mono-formulations and the combination-products.
- The tables were used in the above order reflecting the order of prioritization and relevance to compile a final TER-table with all available information. For all empty cells (in cases where no effect measure was available) in the table of TER-values for a.s., the table for mono-products was looked-up. If there were additional TER-values found, these were added to the compiled table. The last step of compilation was to look for TER-values calculated for combination-formulations. These did not fit the design requirements of the compiled table because only one entry for the two or more a.s. was provided. For this reason, TER-values for combination-products were split into its parts according to their proportion of the amounts of active substances in the formulated products. E.g. the product Vision contained 0.2 kg pyrimethanil / L product and 0.05 kg fluquinconazol / L product. This signifies a proportion of 80:20 between the two components. A TER-value of 10 would be split to 8 for pyrimethanil and 2 for fluquinconazol. During later computational steps, while calculating the mixture risk for the product Vision, the original TER-value of 10 would be re-established (refer to Equation 5).

5.1.2 Risk management implementation

Depending on the assessment endpoints and exposure pathways, predicted environmental concentrations (PEC) can be mitigated. Techniques for drift reduction and distance requirements are meant to reduce risks from PPP. Combinations of drift reducing nozzles and distance requirements reduce PEC-values for drift pathways (Table 31, Appendix C). In comparison, distance requirements concerning riparian buffer strips for run-off can reduce corresponding PEC-values by a factor of up to 5. The specific requirements are laid down by the Federal Office of Consumer Protection and Food Safety of Germany (BVL) and depend on the PPP and active substance. Regarding the application of tank-mixtures, the product with the strictest requirements for risk management was decisive for the application of management measures.

In order to characterize the risk of applied active substances realistically, risk management was integrated for the determination of TER_{single} for the risk indicators indicated in Table 21 above. The PPP specific requirements for drift and runoff as well as expected reduction factors were provided by the German Environment Agency for all PPP of the focal spray series. In case of multiple possible combinations of drift reducing nozzles and distance requirements, the most effective combination in terms of drift reduction was considered as the best case. Furthermore, reduction factors according to field crops and permanent cultures (apple) were differentiated. Apple again was assigned to early and late PPP applications, what influenced the respective reduction factor. The overview on the applied reduction factor per PPP and active substance is given in the Appendix C (Table 31). Regarding aquatic invertebrates, the most sensitive TER-value out of daphnids and other aquatic invertebrates was selected. In order to determine managed TER_{single} –values, we calculated the managed TER_{single} (risk management factor was taken from Appendix C, Table 31).

Equation 4: Calculation of TER_{single} managed

With f_m as the most effective risk management factor per tank mixture m and TERsingle_unmanaged as a single TER-value within m.

$$TER_{single} = TER_{single\_unmanaged} * 1/ f_m$$

Subsequent analyses and chapters are always based on TER-values considering the risk management of drift and runoff. Following the implementation of reduction factors, the endpoint daphnia and the endpoint aquatic invertebrates was merged to general "aquatic invertebrates". Only the most sensitive TER-value from both endpoints regarding acute/ chronic endpoints and drift or runoff pathways was considered.

5.1.3 Compliance of TER_{single} with thresholds of acceptable risk

We analyzed four focal spray series and acceptability criteria (risk indicators) over time regarding the presence of risks. By doing so, we documented TER_{single} that did not meet the risk thresholds across all Tier 1-risk indicators. For this aim, we standardized TER_{single} – values by dividing each TER_{single} with the corresponding threshold of acceptable risk. We then selected the lowest standardized TER_{single} per application and active substance (Fig. 34).

Fig. 34 highlights that most PPP applications (presented as active substance) indicated a non-compliance with Tier-I thresholds up to several orders of magnitude in at least one area of risk assessment. More interestingly, the presence of risks was independent of the treatment scenario (worst-case, typical-case, see Fig. 34) and crop type (data on winter oilseed rape not shown). In addition to Tier 1, we also identified PPPs in terms of active substances (a.s.) that presented a risk for Tier 2 risk indicators (RACs). Remarkably, thiacloprid presented a considerable risk for apple and winter oilseed rape for both treatment scenarios and exposure pathways (drift and runoff). Further substances that caused unacceptable risks comprised for example chlorpyrifos (apple, drift and runoff), mancozeb (apple drift), captan (apple drift) or cypermethrin (winter oilseed rape, drift). TER_{single} for RACs (exposure pathway runoff) are also shown in Figure 35 and 36.

In addition, we determined the number of risk indicators (Tier 1 and higher-tier) showing TER-values that did not meet the threshold of acceptable risk for each single application (Table 21). Despite the application of risk management factors for drift and runoff pathways, we detected risks for the application of 17 and 14 different a.s. in the worst-case or typical-case spray series of apple, respectively (Table 21). We observed the highest number of risk indicators beyond the threshold of acceptable risk for applications of the fungicide mancozeb and the insecticides thiacloprid or chlorpyrifos (Table 21). Regarding winter oilseed rape, we detected a non-compliance of risk indicators for the application of twelve different a.s. for at least one risk indicator in both spray series (Table 21). Application of insecticides (e.g. beta-cyfluthrin, cypermethrin, etofenprox, thiacloprid), fungicides (picoxystrobin, dimoxystrobin, carbendazim) and the herbicide metazachlor presented TER-values causing a risk for the highest number of investigated risk indicators.

Table 21: Number of risk indicators with unacceptable risks

Given are the numbers of risk indicators for all four spray series. We considered the presence of a risk per risk indicator if the TERsingle-value was below the corresponding threshold of acceptable risk for at least one PPP application. The maximum number of risk indicators with expected risks beyond the risk thresholds

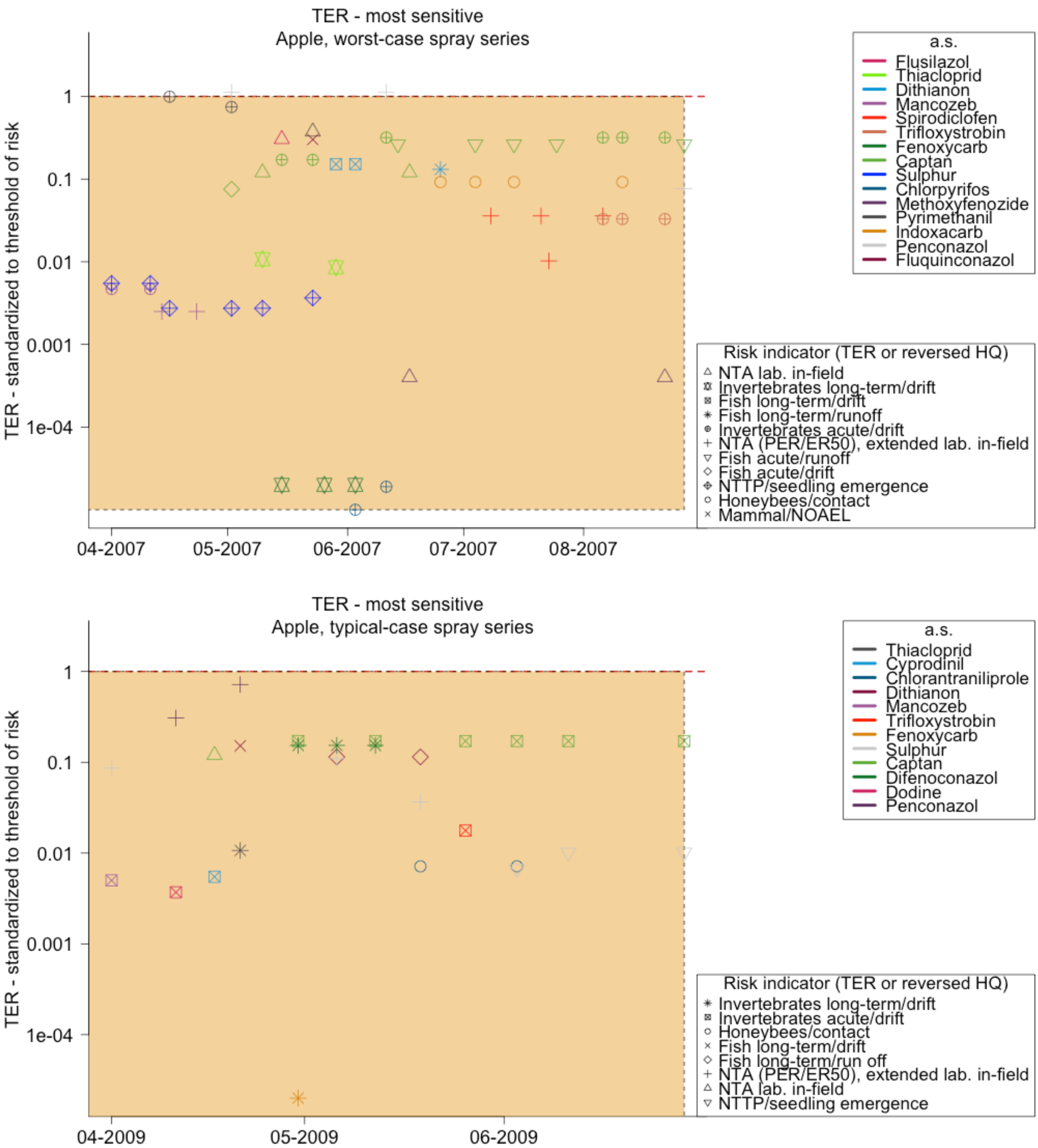
Product	Active substance	Apple, worst-case spray series	Apple, typical-case spray series	Oilseed rape, worst-case spray series	Oilseed rape, typical-case spray series
Acanto	picoxystrobin	NA	NA	7	NA
Benocap	flusilazole	2	NA	NA	NA
Biscaya	thiacloprid	NA	NA	6	6
Bulldock	beta-cyfluthrin	NA	NA	NA	10
Butisan Kombi	dimethenamid-P	NA	NA	1	NA
Butisan Kombi	metazachlor	NA	NA	3	NA
Calypso	thiacloprid	12	10	NA	NA
Cantus Gold	boscalid	NA	NA	NA	2
Cantus Gold	dimoxystrobin	NA	NA	NA	6
Carax	mepiquat	NA	NA	NA	2
Carax	metconazole	NA	NA	NA	1
Chorus	cyprodinil	NA	8	NA	NA
Coragen	chlorantraniliprole	NA	5	NA	NA
Cythrin 250 EC	cypermethrin	NA	NA	9	NA
Delan WG	dithianon	5	5	NA	NA
Dithane NeoTec	mancozeb	NA	14	NA	NA
Dithane Ultra WP	mancozeb	14	NA	NA	NA
Effigo	clopyralid	NA	NA	1	NA
Effigo	picloram	NA	NA	1	NA
Envidor	spirodiclofen	6	NA	NA	NA
Flint	trifloxystrobin	4	5	NA	NA
Folicur	tebuconazol	NA	NA	5	3
Fuego	metazachlor	NA	NA	1	NA
Harvesan	carbendazim	NA	NA	NA	6
Harvesan	flusilazole	NA	NA	NA	6

Product	Active substance	Apple, worst-case spray series	Apple, typical-case spray series	Oilseed rape, worst-case spray series	Oilseed rape, typical-case spray series
Insegar	fenoxycarb	5	5	NA	NA
Kumulus WG	sulphur	NA	3	NA	NA
Malvin WG	captan	4	5	NA	NA
Merpan 80 WDG	captan	6	5	NA	NA
Netzschwefel	sulphur	5	4	NA	NA
Nimbus CS	clomazone	NA	NA	NA	2
Nimbus CS	metazachlor	NA	NA	NA	5
Plenum 50 WG	pymetrozin	NA	NA	1	NA
Propulse	fluopyram	NA	NA	1	NA
Propulse	prothioconazole	NA	NA	1	NA
Reldan	chlorpyrifos	14	NA	NA	NA
Runner	methoxyfenozide	5	NA	NA	NA
Scala	pyrimethanil	2	NA	NA	NA
Score	difenoconazole	NA	4	NA	NA
Select 240 EC	clethodim	NA	NA	2	NA
Steward	indoxacarb	6	NA	NA	NA
Syllit	dodine	NA	6	NA	NA
Targa Super	quizalofop-P	NA	NA	2	NA
Topas	penconazole	1	1		NA
Trebon 30 EC	etofenprox	NA	NA	9	NA
Vision	pyrimethanil	1	NA	NA	NA
Vision	fluquinconazol	4	NA	NA	NA

As a conclusion, we observed that almost every PPP application in the selected spray series was predicted to cause an environmental risk for one or several of the 28 risk indicators. We mainly focused on the Tier 1-risk assessment including usual risk mitigation measures for the exposure by drift and run-off. Therefore, we did not consider subsequently refined or meanwhile outdated risk assessment (for further details, refer to 5.1.3.1). However, we also detected risks at higher-tiers (RACs) especially for the active substance thiacloprid. Thiacloprid belongs to the group of neonicotinoids that have been previously reported to cause strong negative impact on bees (Rundlöf et al. 2015) and aquatic ecosystems (Münze et al. 2017, Knillmann et al. 2018, Shahid et al. 2018).

Figure 34: Most sensitive TER-value for each PPP application across all assessment areas for both apple spray series (upper graph: worst-case spray series, lower graph: typical-case spray series)

TER_{single}- values were standardized to the corresponding threshold of acceptable risk to select the most sensitive TER_{single}. The dashed line displays the standardized TER-threshold of acceptable risk indicating a potential risk with values < 1. The area below this threshold is colored orange to highlight TER that present a risk.



5.1.3.1 Authorization despite indication of risk? – Possible explanations

In some cases, TERs calculated for the application of single PPPs in a spray series do not meet the standardized acceptability criteria (Table 21, Fig. 34, critical TER/ETR-trigger values according to COMMISSION REGULATION (EU) No 546/2011 as regards uniform principles for evaluation and authorization of plant protection products), although the actual application rates were often below the authorized maximum application rates. This conflicts with their state of authorization which requires compliance with the standardized acceptability criteria in each risk assessment area (at the Tier 1-level). A cross-check between the Tier 1- TERs calculated here with the results reported in the official authorization report explain this putative contradiction. The following general facts might clarify this discrepancy:

Outdated risk assessment

The standard authorization period for PPPs in Germany is 10 years. Authorizations remain even in case of new regulatory requirements or advances in science and technology - unless there is indication for specific and concrete unacceptable impacts on the environment. As a consequence of the general continuation permit, former risk assessments might have become outdated for different reasons. For example, the requirements for conducting a comprehensive risk assessment in the terrestrial compartments changed during the years 1990 – 2000, as the former directive 91/414/EEC including the uniform principles has not come into force until 1998. In the meantime, the Commission regulation R (EU) No 546/2011 has replaced the directive 91/414/EEC. Further, the principles of risk assessment changed profoundly in some areas due to the introduction of new technical guidance documents, e.g. three SANCO Guidance documents in the early 2000 years (SANCO/414/2000-final – birds and mammals, SANCO/3268/2001 rev. 4 (final) – Aquatic Ecotoxicology and SANCO/10329/2002 rev. 2 (final) –Terrestrial Ecotoxicology). The renewals of technical guidance documents are constantly ongoing in multi-annual cycles (e.g. EFSA-PPR-Panel 2013c). With the updated guidance documents, especially the options for risk refinement changed. Finally, risk assessments are outdated if new studies / relevant endpoints have become available in the meantime. A situation which quite frequently occurs (e.g. submission of an independent data package for active substances / a new PPP by another applicant).

Refinement of Tier 1-risk

In this study (apart from the RAC) only the standardized risk indicators of Tier 1-level are considered. However, typically, if a Tier 1-risk is indicated, the risk assessment is refined already by the applicant seeking the PPP authorization. Respective so-called "higher-tier" refinement options comprise both the exposure and effect assessment (e.g. more comprehensive modelling of exposure, semi-field or field studies exploring the effects of the PPP under more realistic conditions of use). Almost each refined risk assessment does disburden the Tier 1-risk such that an authorization is possible.

Emergency authorization

A temporary emergency authorization by BVL in 2007 was the reason for the legal use of one not generally authorized PPP failing the acceptability criteria in several risk assessment areas. Thus, this temporary emergency authorization was despite the indication of an unacceptable environmental risk. (Note: UBA has not the right to veto in the procedure for emergency authorization).

Risk-benefit-analysis

The authorization decision for two PPPs based on a risk-benefit analysis. An environmental risk by the use of these PPPs was thus indicated, but accepted due the high benefit for agriculture. Partly, this decision included additional risk management measures to further reduce the environmental risk.

5.2 Toxicity and maximum cumulative ratio (MCR) of tank mixtures

To assess the additional toxicity due to tank mixtures we calculated the mixture toxicity as TER_{mix} based on all available TER_{single} per spray event (i.e. missing TER_{single} for one or several a.s. per spray event were not considered) using the concept of concentration addition (CA). TER_{mix} was determined for the four selected spray series and each of the 28 risk indicators. In detail, the formula for the calculation of TER_{mix} was applied as described in the following equation 5.

Equation 5: Calculation of TER_{mix}

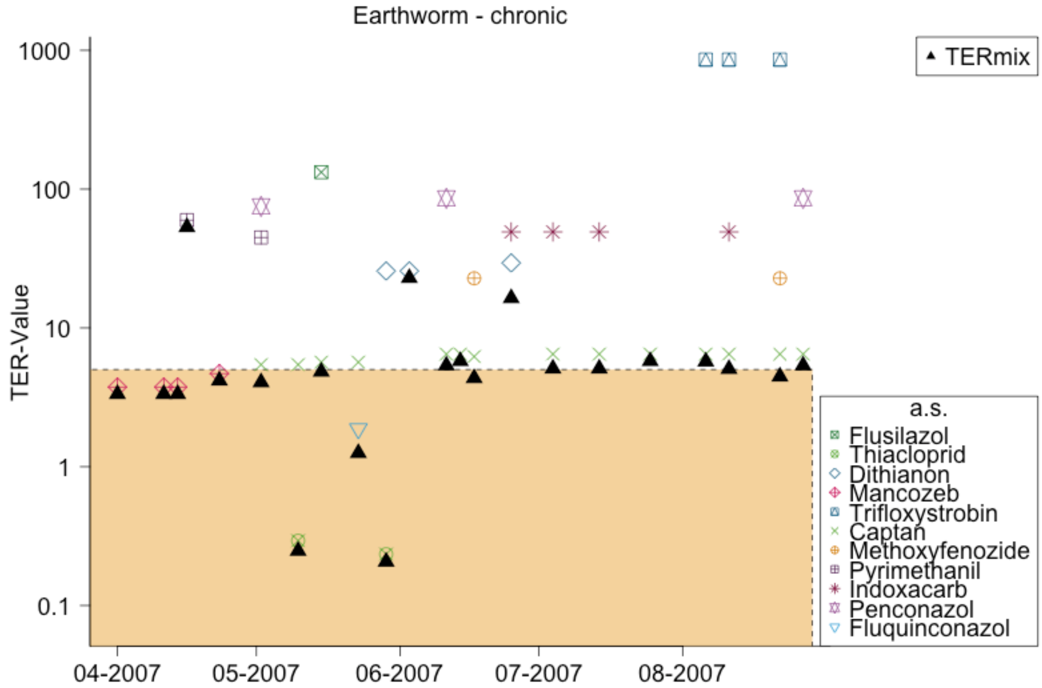
$$TER_{mix}^{CA} = \frac{ECx_{mix}^{CA}}{PEC_{mix}} = \frac{\frac{1}{\sum_{i=1}^{n} \frac{p_i}{ECx_i}}}{\sum_{i=1}^{n} PEC_i} = \frac{1}{\sum_{i=1}^{n} \frac{1}{TER_i}} \quad if: p_i = \frac{PEC_i}{PEC_{mix}} = \frac{PEC_i}{\sum_{i=1}^{n} PEC_i}$$

with n as the number of mixture components, i as the index from 1 to n mixture components, ECx_i as the concentration of component i causing x% effect, TER_i as the TER_{single} for each applied component i and PEC_i as the predicted environmental concentration of component i.

In Fig. 35 and Fig. 36, TER_{single} and TER_{mix} based on the available a.s. data are shown over time of the worst-case spray series in oilseed rape and apple for one aquatic and one terrestrial endpoint. The a.s. applications per time point included one to four a.s. for the selected treatment scenarios. The graph displays the distribution of risk indices within each applied tank mixture for the exemplary risk indicators, which is in most cases relatively close to the TER_{min} of the corresponding mixture (Fig. 35 and Fig. 36). The figures also highlight that TER_{mix} fall below the given risk threshold, if TER_{min} of the given tank mixture are already close to this threshold (e.g. 1 or 5) (Fig. 35, Earthworm chronic). We further determined the maximum cumulative ratio (MCR, according to Han and Price 2011) as TER_{min}/TER_{mix} per tank mixture for all 28 risk indicators. Application events with one single compound were removed from this analysis. The results indicate that the MCR was rarely higher than 2 for all spray series and acceptability criteria (Fig. 37 and 38). Fig. 37 and 38 further show slight differences between the single risk indicators. However, the differences of the MCRs between the acceptability criteria are not consistent for the treatment scenarios or crop type. In a final step, we checked the influence of the number of compounds within one tank mixture on the resulting MCR. For this, we determined the median MCR out of all acceptability criteria for each application event with more than one a.s.. As described above, the exemplary spray series included tank mixtures with two to four compounds. As a result, the compound number proved a significant influence across all four spray series on the median MCR per application event (ANOVA, P = 0.001). The median MCR increased from 1.05 (two a.s.) to 1.32 (four a.s.). The median MCR slightly differed between apple (1.28 and 1.19, Fig. 37) and oilseed rape (1.43 and 1.20, Fig. 38).

Figure 35: TER_{single} for all PPP applications and TER_{mix} per spray event for one terrestrial (earthworm, upper graph) and one aquatic risk indicator (regulatory acceptable concentration - RAC, runoff, lower graph) in the apple worst-case spray series

Each point displays the application of an active substance (a.s.) with available TER_{single} values. No TER_{single} values were available for the applied a.s. sulphur, fenoxycarb, spirodiclofen (RAC runoff and earthworm chronic), indoxacarb (only RAC runoff) and chlorpyrifos (only earthworm chronic). TER_{mix} was determined based on all available TER_{single} values per application date. A break in the TER_{mix} line indicates applications of a single a.s. without available TER_{single}-value (fenoxycarb, spirodiclofen). The dashed line at TER = 5 (upper graph) or TER = 1 (lower graph) represents the threshold of acceptable risk for the risk indicator TER earthworm chronic and TER RAC – pathway run off, respectively. The area below this threshold is colored orange to highlight TER-values that present a risk.



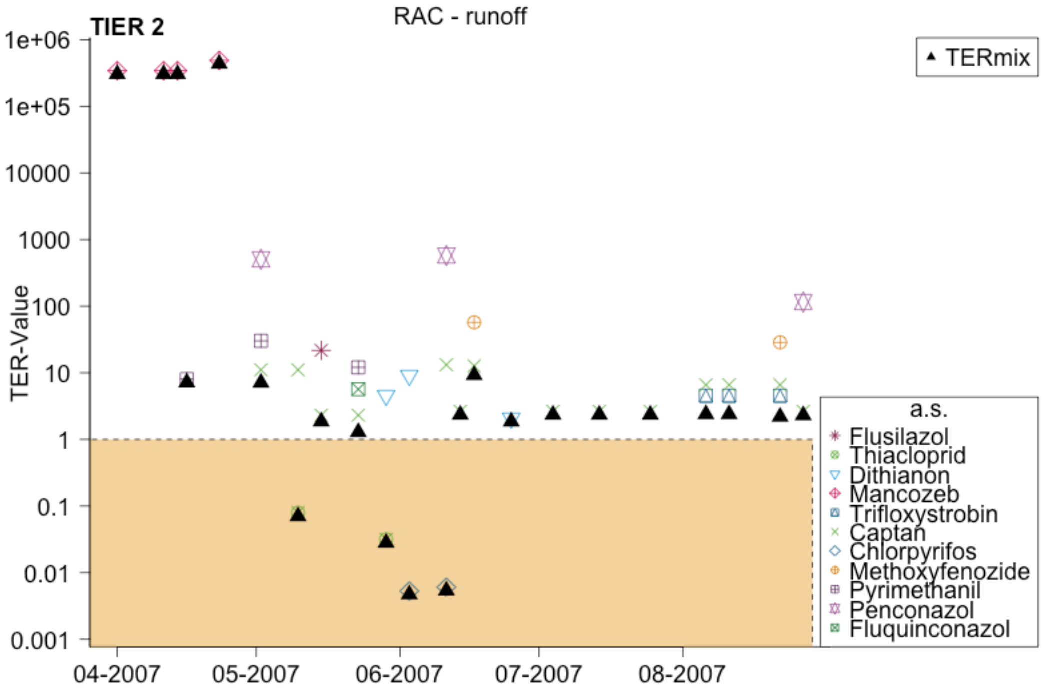


Figure 36: TER_{single} for all PPP applications and TER_{mix} per spray event for one terrestrial (earthworm, upper graph) and one aquatic risk indicator (regulatory acceptable concentration - RAC, runoff, lower graph) in the winter oilseed rape worst-case spray series

Each colored point displays the application of an active substance (a.s.) with available TER_{single} values. No TER_{single} values were available for the applied a.s. cypermethrin, metazachlor (earthworm chronic) and clopyralid, dimethenamid-P (RAC runoff). TER_{mix} was determined based on all available TER_{single} values per application date. The dashed line at TER = 5 (upper graph) or TER = 1 (lower graph) represents the risk threshold for the criteria earthworm chronic and RAC – pathway run off. The area below this threshold is colored orange to highlight TER that present a risk.

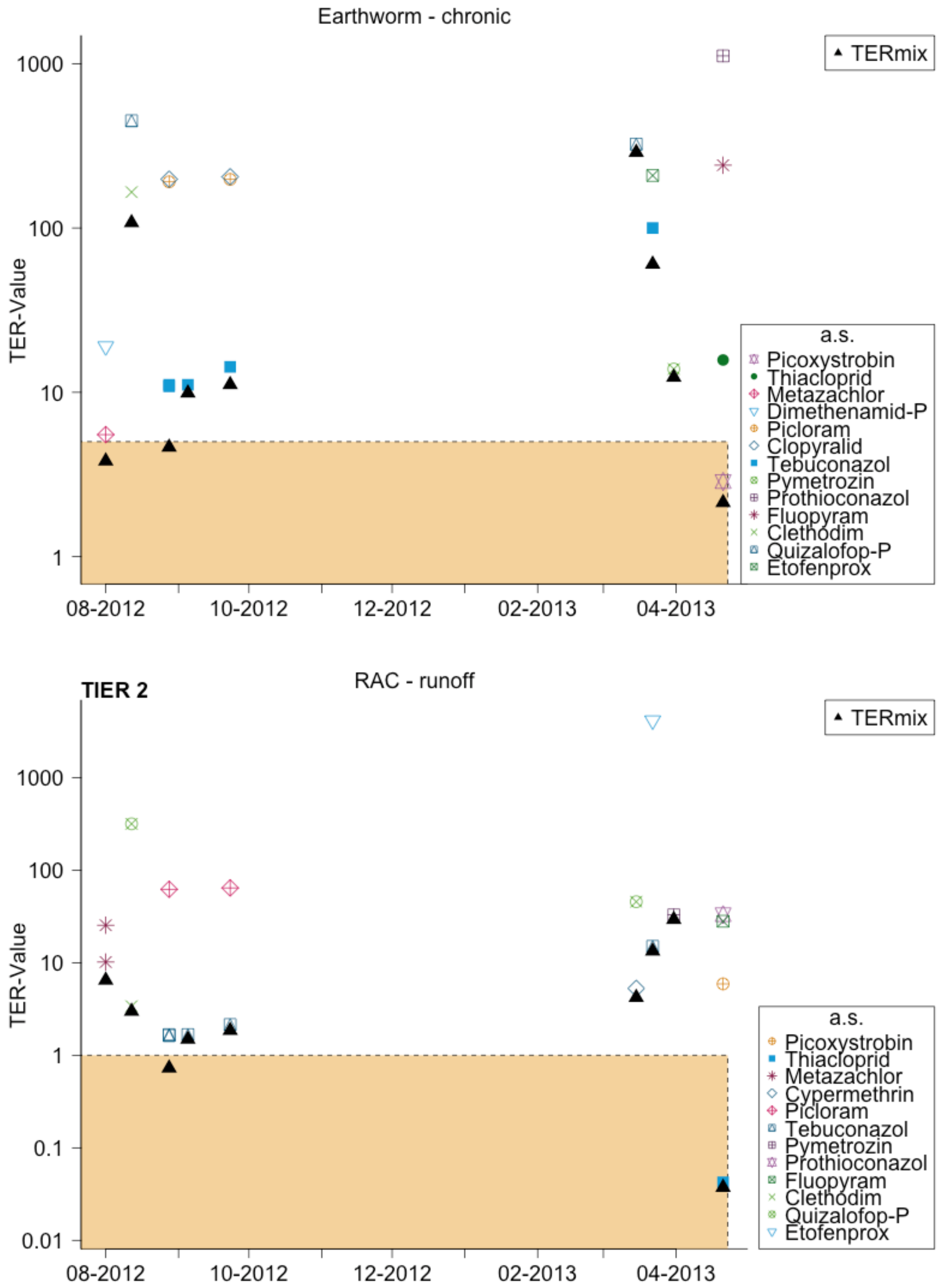
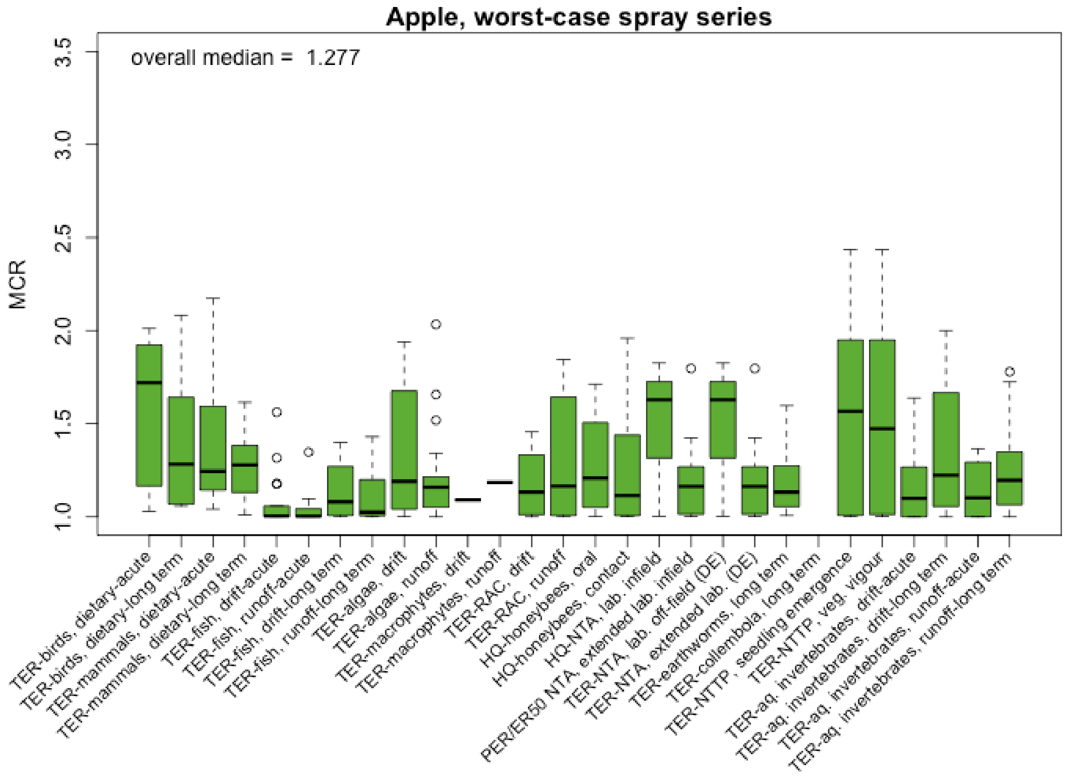


Figure 37: Maximum cumulative ratio (MCR) between TER_{min} and TER_{mix} for all tank mixtures and all 28 risk indicators of the spray series apple: worst-case (upper graph) and typical-case (lower graph)

TER_{min} and TER_{mix} were determined based on all available TER_{single} per tank mixture and risk indicator. We excluded MCR with only one available TER_{single} per application time point.



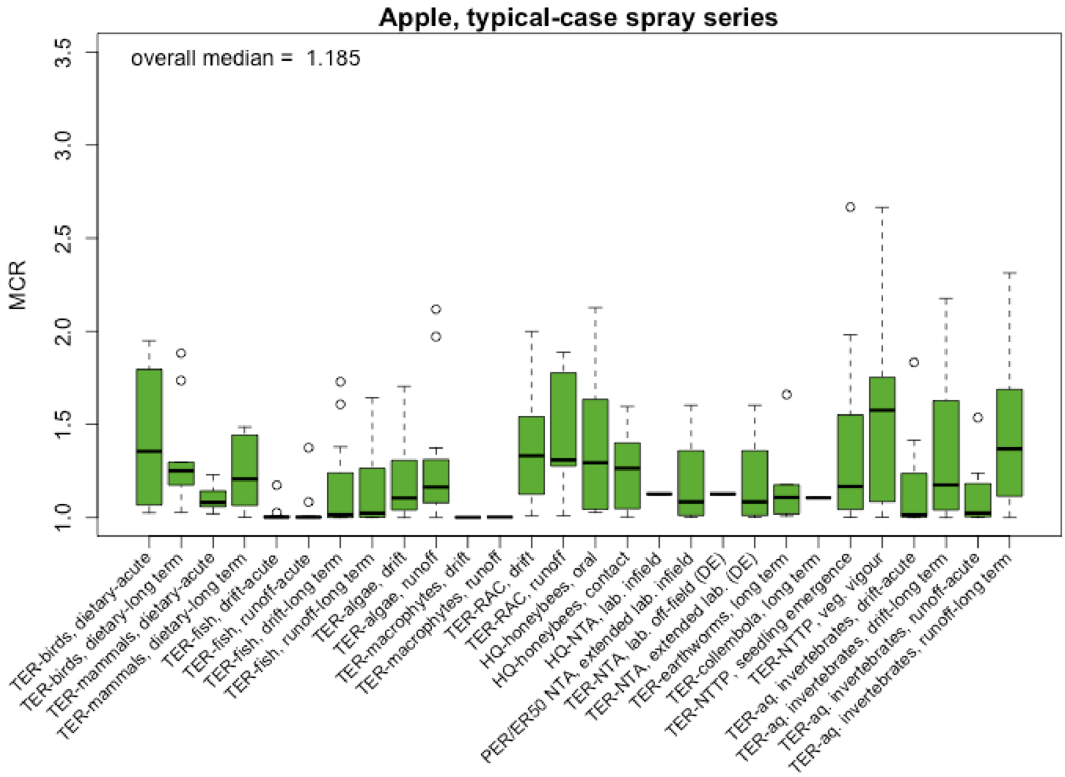
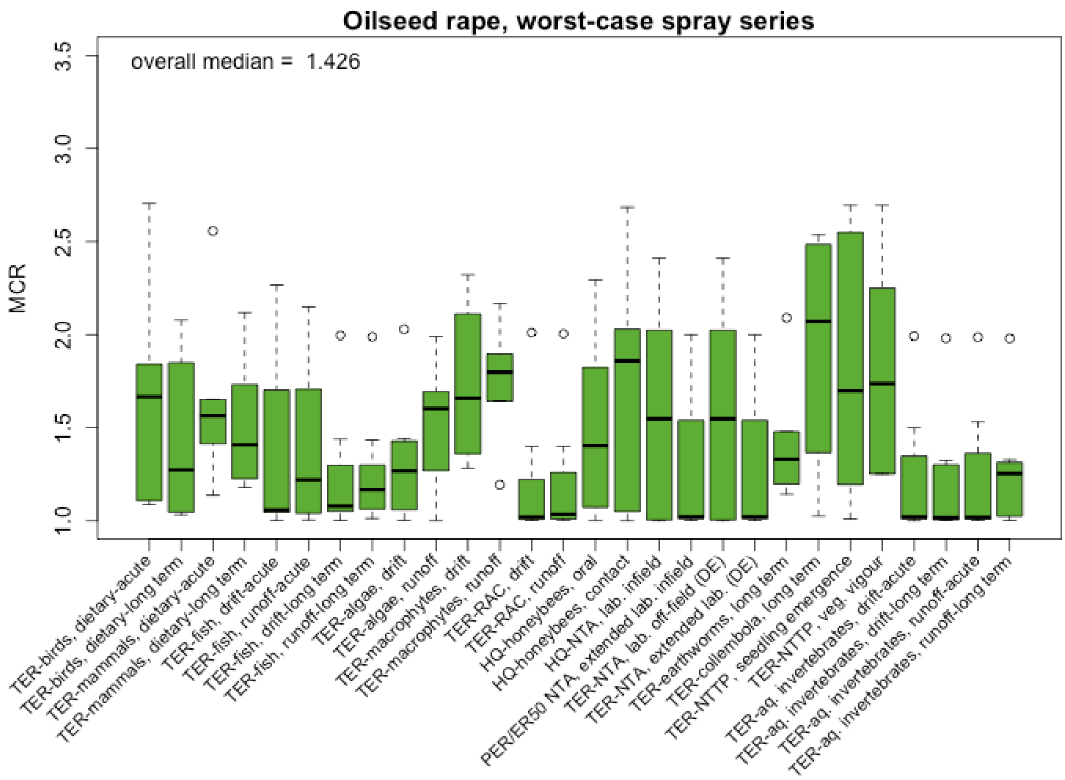
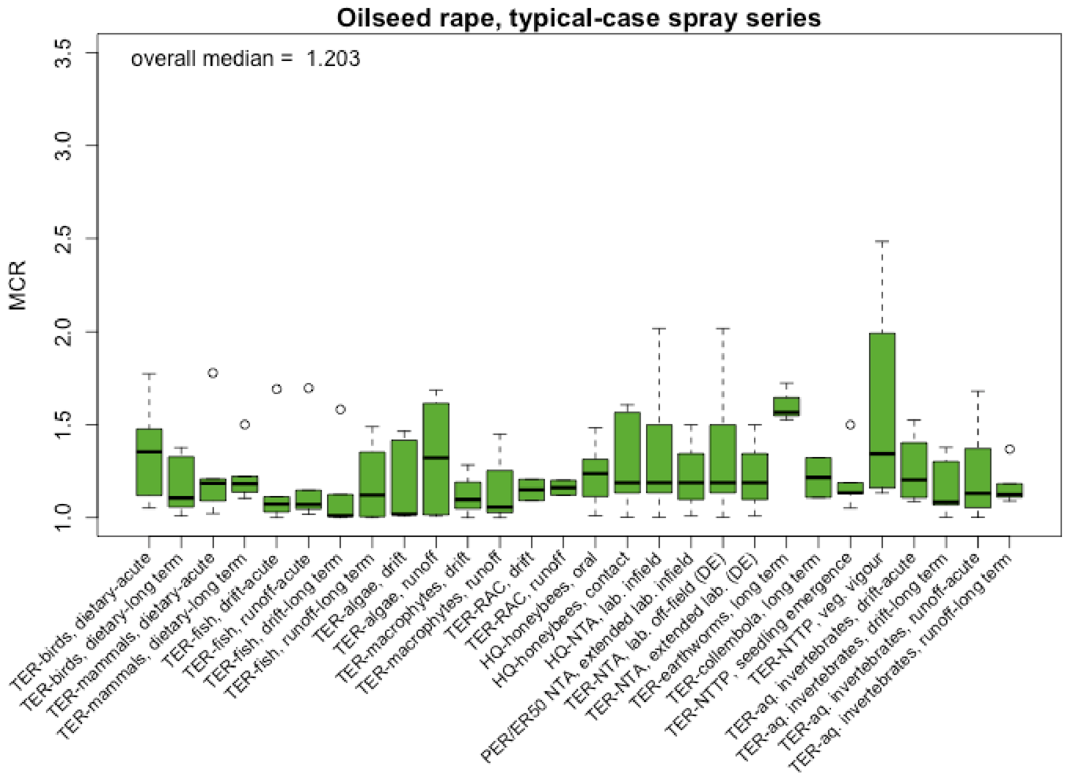


Figure 38: Maximum cumulative ratio (MCR) between TER_{min} and TER_{mix} for all tank mixtures and all 28 risk indicators of the spray series winter oilseed rape: worst-case (upper graph) and typical-case (lower graph)

TER_{min} and TER_{mix} were determined based on all available TER_{single} per tank mixture and risk indicator. We excluded MCR with only one available TER_{single} per application time point.





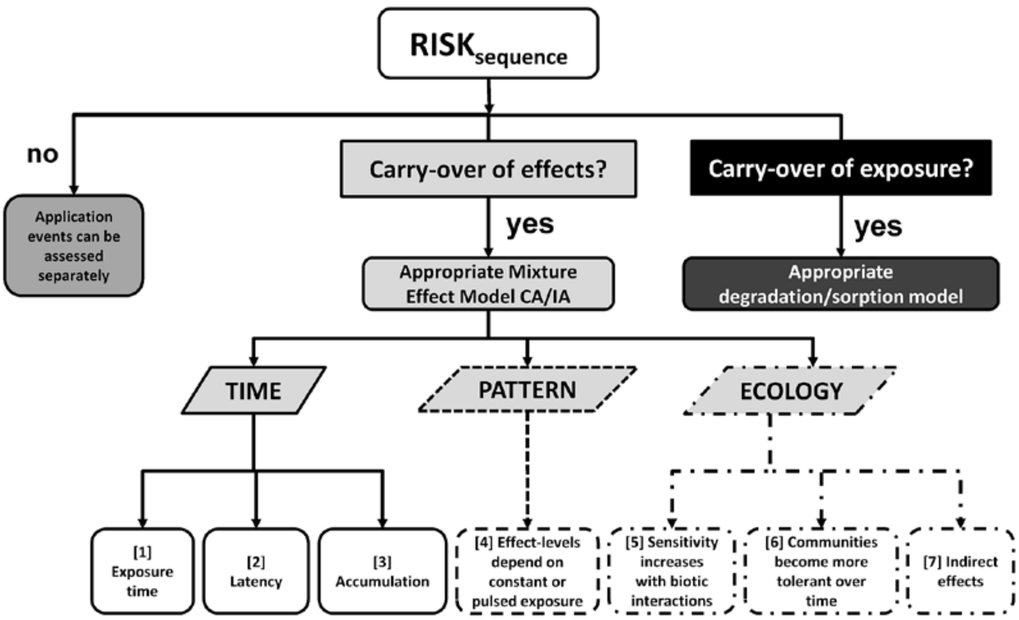
The median MCR between 1.19 and 1.43 seems at first glance not relevant for the overall risk of one applied PPP substance. However, the true TER_{mix} and, hence, MCR is underestimated due to missing ecotoxicological data for selected a.s. and endpoints (Fig. 35 and 36). Moreover, the ERA of single PPP applications is often just above the acceptable TER-threshold of risk as shown in for the selected spray series. We observed this phenomenon for Tier 1- and especially also for Tier 2 (RACs, reference to Fig. 35 and 36). As a consequence, the additional toxicity due to spray series and tank mixtures can cause risks beyond the threshold compared to the evaluation of single PPPs. Finally, the additional toxicity will further increase with the number of compounds in a tank mixture. This outcome is important for the quantification of additional toxicities due to tank mixtures in a prospective RA.

5.3 From tank mixtures to spray series – Sequential exposure and effects

To characterize the risk of spray series it is necessary to go beyond tank mixtures and address the potential impact of sequential exposure. The investigated spray series show that each spray event is usually followed by the next event in short sequences (in apple e.g. 26 spray events within 148 days of a growth period or treatment period). For the worst-case spray series in apple, this means that an application of one to six different active substances took place each week. We assume that neither the effect nor the exposure will disappear from the scene in such short periods of time. Therefore, we expected carry-over effects. In the following chapters, we first give a summary on existing knowledge regarding sequential exposure and effects. The scheme in Fig. 39 allows for the identification of relevant aspects and approaches, which are presented in the following. Subsequently, we describe and apply selected approaches to quantify the additional toxicity of sequential PPP exposure and suggest generic uncertainty factors for the RA of PPPs. In a final step, we discuss the uncertainties of the applied approaches

Figure 39: Important aspects for the risk assessment of sequential exposure of PPPs and effects aiming at the protection of non-target organisms

The numbers in the boxes refer to the following references [1] Schulz and Liess (2000); [2] Jager (2011); Drost (2011); [3] Tennekes and Sánchez-Bayo (2011); [4] Independent action model: EFSA (2015); [5] Reynaldi and Liess (2005); [6] Liess et al. (2013); [7] Becker and Liess et al. (2015).



5.3.1 State of the art

Sequential exposure in terrestrial and aquatic ecosystems

A sequence of PPP applications can lead to different patterns of carry-over exposure or accumulation in the environmental media, such as for example in aquatic sediments or soil. In soils PPPs accumulate and may persist for long periods of time (Chiaia-Hernandez et al. 2017). Therefore, persistent exposure due to predominantly long degradation half-rates of more than the average time-gap between two application events will cause higher cumulative exposure concentrations at later application events. In comparison, PPPs also accumulate in aquatic sediments over time to high concentrations (McKnight et al. 2015). However, several studies show (Liess and von der Ohe 2005, Liess and Schulz 1999, Schäfer et al. 2008) that the most ecological effective exposure takes place via short-lived concentration peaks in the water phase following for example surface runoff. In contrast, there are to our knowledge no terrestrial studies that analyzed the ecological impact of accumulated PPPs in soils compared to high concentrations peaks of PPPs. Hence, additional research is necessary to further understand the (dis)similarities between relevant aquatic and terrestrial exposure scenarios for the prediction of effects due to PPP treatment regimes.

Effects due to sequential exposure

The search for literature did not yield relevant reviews or reports on sequential exposure and effects. Therefore, we gathered information from single experimental studies to identify important principles that should be considered when assessing repeated toxicant exposure. As outlined by a study of Mohr et al. (2012) the effect due to repeated pulses of PPPs depends on a range of variables including toxicity and duration of the exposure, number of pulses, time between pulses and taxon specific traits for the organisms under consideration. Also, it should be considered that the application of a PPP does not take effect immediately after application, in particular when considering chronic endpoints. For example, insects are found to strongly respond several months after a short PPP exposure in terms of emergence (Liess and Schulz 1996, Liess 2002, Beketov and Liess 2005). This latency, i.e. time from exposure to onset of effects, might play a crucial role for the choice of risk indicators to assess effects from sequential exposure (Tennekes and Sanchez-Bayo 2011).

More specifically, a study by Liess et al. (2013) further observed that repeated exposure to thiacloprid increased the effects on mosquito larvae if they were also experiencing interspecific competition with less sensitive daphnids. In a similar study with a two-species-systems, Dolciotti et al. (2014) identified that repeated exposure of pirimicarb with several generations between the pulses and competition with less sensitive mosquito larvae impeded the recovery of daphnids on the long-term.

In contrast to an increase of effects or sensitivities, repeated pesticide exposure can also lead to a tolerance development as it has been observed for macroinvertebrate populations that were sampled in several PPP-contaminated streams in Central Germany (Becker and Liess 2017). The authors tested the acute sensitivity towards clothianidin under laboratory conditions and detected an up to eight-fold increase in tolerance compared to reference sites without pesticide exposure. However, the tolerance development was stronger for sites with low biodiversity and, hence, high interspecific competition. Another study by Shahid et al. (2018) observed an adaptation of crustaceans in agricultural streams with low or no possible recolonization from unpolluted stream sections. Similarly, Russo et al. (2018) detected an increase in sensitivity of vulnerable population under repeated pesticide exposure, which recolonized polluted stream sections from unpolluted stream sections. Therefore, the context of biotic interactions plays a crucial role for the response of non-target organisms to sequential exposures.

Due to this very limited genetic adaptation at the population level under natural conditions of biotic and abiotic stress, repeated exposure to toxicants leads to changes in the community structure as observed for aquatic communities (Liess and von der Ohe 2005, Molander et al. 1990, Knillmann et al. 2018). Approaches including the pollution-induced community tolerance (PICT, Blanck et al. 1988) or the SPEARpesticides indicator (Liess and von der Ohe 2005) use this change in aquatic communities to indicate toxicant exposure.

As a conclusion we summarize three scenarios how sequential exposure can influence effects on or sensitivity of organisms to toxicants (i.e. including lethal and also sublethal impairments of, population growth, competitive strength, weakening and multigenerational genetic adaptation):

- Each toxic stress event causes a similar effect on the population and therefore culminates to population extinction already at such low concentrations that in a one-time exposure would only cause negligible effects. This scenario only occurs under high interspecific competition when recovery is reduced. Under low interspecific competition only concentrations that cause high acute mortality result in an increasing effect on populations (Liess et al. 2013).
- Long-term occurrence of sequential exposure leads to genetic adaptation. The degree of adaptation is low when interspecific competition or environmental stressors effect populations as identified in laboratory experiments (Becker and Liess 2015) and in the field (Becker and Liess 2017).
- Under natural conditions interspecific competition or environmental stressors are relevant in many non-target communities. Accordingly, the degree of genetic adaptation is low (Becker and Liess 2017, Shahid et al. 2018). Consequently, the long-term exposure towards toxicants with a varying degree of repeated exposure lead to a change in the community structure towards insensitive species as identified by the PICT (Blanck et al. 1988) and the SPEAR approach (Liess and Ohe 2015).

5.3.2 Approaches to assess the additional risk of sequential exposure to PPPs

5.3.2.1 TER_{min} of all applications in one spray series

Assuming that the toxicity in one spray series is often driven by the dominating toxicity of one or few compounds (i.e. refer to Figure 35 or 36), TER_{min} is a parsimonious approach to describe the toxicity of a whole spray series with this highest toxicity. However, as a prerequisite for this approach, the TER_{min} must be known. Significant relations between the most toxic compound of one season and ecological effects on aquatic invertebrate communities have been described for the SPEAR-indicator by Liess and von der Ohe (2005) or Schäfer et al. (2012). Nevertheless, TER_{min} can only be considered as a relative descriptor of pesticide risk and does not reflect the absolute toxicity pressure or the additional risk due to a sequence of PPP applications. Hence, we mention TER_{min} for matters of completeness, but we will not explicitly link TER_{min} to the modelled spray series data.

5.3.2.2 The maximum cumulative ratio (MCR) for a spray series assuming simultaneous exposure to all PPP applications over time

As the most basic approach to evaluate the additional toxicity of all PPP applications in one spray series, we applied the Maximum Cumulative Ratio (MCR, proportion between TER_{min} and TER_{mix} according to Price and Han 2011). We determined the TER_{mix} and MCR for spray series the same way as for tank mixtures assuming additive effects (CA). TER_{mix} for all single applications of PPP substances within one spray series represents a conservative approach (except the presence of synergistic mixtures), because we supposed that all single applications of PPP as a.s. act simultaneously on the given risk indicator without recovery and degradation.

With respect to the analyzed crop types, winter oilseed rape and other winter sown cultures follow a different spraying pattern over the year than for example spring sown crops or permanent crops. Regarding winter sown cultures, the treatment of the crop with PPP starts in late summer or autumn. Subsequently, farmers pause treatments during the winter months and restart in spring until the harvest in summer. For the following analyses, we assumed that processes of degradation and recovery are low during the winter break due to low temperature and applied the same concepts (MCR for TER_{min}, TER_{mix}) for apple and winter oilseed rape.

The MCR per area of risk assessment and spray series is displayed in Table 22. In general, the treatment intensive culture apple shows higher MCRs than oilseed rape, what is mainly due to the higher number of a.s. applications in apple (55 or 29 for apple vs. 22 or 9 applications for oilseed rape, worst-case and typical-case, respectively). When comparing the single spray series with the MCR for all risk indicators, the MCR of apple worst-case spray series was significantly higher than all other spray series (ANOVA followed by a pairwise t-test, $P < 0.05$). In comparison, winter oilseed rape had the lowest number of a.s.-applications and the MCR was significantly lower than for the apple spray series (ANOVA followed by a pairwise t-test, $P < 0.05$). Consequently, as shown for the tank mixtures the number of PPPs affects the additional toxicity of a spray series compared to single PPP applications.

Regarding differences between acceptability criteria, we observed the highest MCR (MCR = 13.61) for the risk assessment for mammals/LD_{50} in the apple worst-case spray series. Comparing all four spray series, we detected the highest ratios for the TER-endpoints birds, mammals and partly fish or algae. In addition, also the risk indicators macrophytes, invertebrates, NTTP or NTA (extended lab. test) showed relatively high MCRs in apple worst case (MCR > 4). Apart from these risk indicators in the apple worst-case spray series, a high proportion of indicators in apple typical-case and oilseed rape present an MCR of less than 3 (apple typical-case spray series: 19 risk indicators, oilseed rape worst-case spray series: 23 risk indicators, oilseed rape typical-case spray series: 26 risk indicators).

Finally, we analyzed the distribution of MCR for all four spray series and for all risk indicators. By doing so, we identified an overall median MCR of 2.18 (90^{th} percentile = 5.26). As discussed for the tank mixtures (refer to chapter Toxicity and maximum cumulative ratio (MCR) of tank mixtures) this MCR is relatively low compared to other additional stressors, such as for example environmental stressors (factor of sensitivity increase: 10-100, Liess et al. 2016) or effects due to repeated exposure (factor of toxicity increase 10, Liess et al. 2013). The increase in risk may cause a noncompliance of risk thresholds that are not detectable when only single PPP applications are considered. This outcome holds especially true for the MCR based on RACs as the higher-tier risk indicator for aquatic ecosystems. Moreover, the MCR approach based additive effects contains uncertainties that might underestimate the additional risk of spray series. Uncertainties include for example the influence of synergistic mixtures or unknown exposure to seed coating in rapeseed oil (see also 5.3.3.1).

Table 22: MCR between TER_{min} and TER_{mix} across all PPP applications within one spray series

Given is the MCR between TER_{min} and TER_{mix} per spray series for all 28 risk indicators and the four spray series from apple and oilseed rape. Risk indicators marked with * are usually determined as the hazard quotient. These indicators were recalculated as the reciprocal of the HQ to treat them the same as the TER- risk indicators.

Risk indicator	Apple worst-case	Apple typical-case	Oilseed rape worst-case	Oilseed rape typical-case
Bird/LD_{50}/dietary	9.98	6.37	3.39	2.01
Bird/NOAEL/dietary	8.83	3.71	4.97	2.19
Mammal/LD_{50}	13.61	7.82	2.60	2.69
Mammal/NOAEL	8.07	3.31	7.35	3.66
Fish acute/drift	5.64	4.13	2.05	1.50
Fish acute/runoff	9.40	7.32	2.18	1.09
Fish long term/drift	3.31	1.71	1.48	1.80
Fish long term/runoff	2.21	2.71	4.35	1.42
Algae/drift	3.31	2.21	2.16	1.06
Algae/runoff	3.10	7.06	2.24	1.05
Macrophytes/drift	2.21	1.86	1.70	1.01
Macrophytes/runoff	5.29	1.96	1.78	1.00
RAC aquatic/drift	1.82	1.89	1.39	1.12
RAC aquatic/runoff	2.14	1.18	1.14	1.14
Honeybees/oral*	2.32	2.22	1.31	1.28
Honeybees/contact*	3.48	2.03	1.33	1.03
NTA lab., in-field*	2.45	3.21	1.03	1.01
NTA (PER/ER50) extended lab., in-field*	5.04	2.05	2.17	1.17
NTA lab., off-field (DE-approach)	2.26	1.51	1.91	1.09
NTA extended lab., off-field (DE approach)	4.47	1.91	2.17	1.17
Earthworms long-term/annual	2.78	2.35	3.18	3.08
Collembola long-term/annual	2.44	2.20	2.09	2.37
NTTP/seedling emergence	4.80	2.40	2.13	1.59
NTTP/vegetative vigour	4.81	2.41	2.50	1.50
Invertebrates acute/drift	1.54	3.12	1.01	1.00

Risk indicator	Apple worst-case	Apple typical-case	Oilseed rape worst-case	Oilseed rape typical-case
Invertebrates long-term/drift	4.51	1.01	2.16	1.01
Invertebrates acute/runoff	1.88	2.26	2.01	1.04
Invertebrates long-term/runoff	3.80	1.01	2.83	2.85
Median across all risk indicators	**3.40**	**2.24**	**2.15**	**1.17**

Alternatively, the effects of a sequence can be also modelled by the independent action (IA) model as suggested for example for the RA of PPPs on terrestrial non-target arthropods (EFSA 2015) and assuming many different modes of action of the compounds within a spray series. The IA approach (also known as effect addition) has been originally described by Bliss (1939) and is based on the addition of effects following the equation 6. Bliss assumes that sensitivity distribution does not change over the single exposure events (i.e. no selection of sensitive organisms from one exposure event to another).

Equation 6: Independent action

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i))$$

where $E(c_{mix})$ is the total effect of the all single effects E(ci) and n the number of exposure events.

To cumulate effects of different a.s., known dose or concentration-response curves are required, which are not present for all substances and endpoints in the selected spray series. The use of modelled curves presents one possibility to overcome this lack of data, but causes further uncertainties for the assessment of sequential exposure. Therefore, we mainly focused on the CA approach and did not apply IA to evaluate the additional impact of the selected spray series. Nevertheless, we present a hypothetical example to outline the use of the concept. For the hypothetical example we assume a simplified model spray series with repeated exposure (i.e. n =10) to the same concentration/dosage of a selected acceptability criterion over time. The expected exposure per time point of application alone only causes 10% effect and does not indicate a risk depending on the risk area under consideration (i.e. in crop risk indicators). However, if we now add the repeated effects together according to the Equation 7, we get the following result:

Equation 7: Independent action, hypothetical example

$$E(c_{mix}) = 1 - \prod_{1}^{10} (1 - 0.1) = 0.65$$

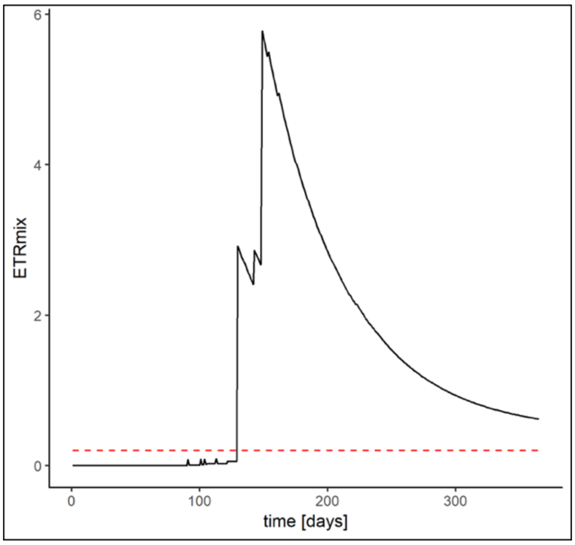
Hence, if we do not consider recovery or adaptation (i.e. proportion of sensitive individuals or species decline from one exposure event to the next) over time to the sequential exposure, with this approach, will cause a total effect of 65% on the considered risk indicator. A certain effect recovery or adaptation from one exposure event to the next is, however, likely and might reduce the total effect. Recovery and adaptation do not only apply to the example with IA or MCR based on CA. Nevertheless, the total effect from the sequential exposure can be far beyond acceptable risk thresholds for several risk indicators.

5.3.2.3 Dynamic ETR_{mix} including degradation of PPPs

We calculated the time-dependent chronic mixture risk for earthworms using CA (Fig. 40). The results from the model MITAS and the mixture risk calculation for the apple worst-case spray series is described in chapter 4. The aim was to predict the time-dependent mixture risk of an entire real spray series, also considering substance degradation based on first-order degradation kinetics. Data for the substance-specific physicochemical and ecotoxicological endpoints of the substances were provided for this apple spray series by the German Environment Agency (UBA) (see chapter 4 for further details). In the Tier 1 standardized risk assessment for pesticides in the EU approval procedure, the threshold for an environmentally acceptable ETR is 0.2 (EFSA 2009a; European Commission 2002).

Figure 40: Time-dependent chronic mixture risk of the apple worst-case spray series for earthworms

The x-axis represents the single days of a year, whereas the y-axis represents the ETR_{mix}-values. The black graph represents the chronic mixture risk calculated based on concentration addition. Visualization of the threshold value for chronic risk is done through the red bar in the plot.



Visualization of the time-dependent development of a real spray series illustrates the importance of the spray sequence. Although the last pesticide application is at day 239, MITAS calculates a chronic mixture risk exceeding the threshold till the end of the year. Therefore, adverse effects on earthworms for subsequent pesticide applications cannot be excluded.

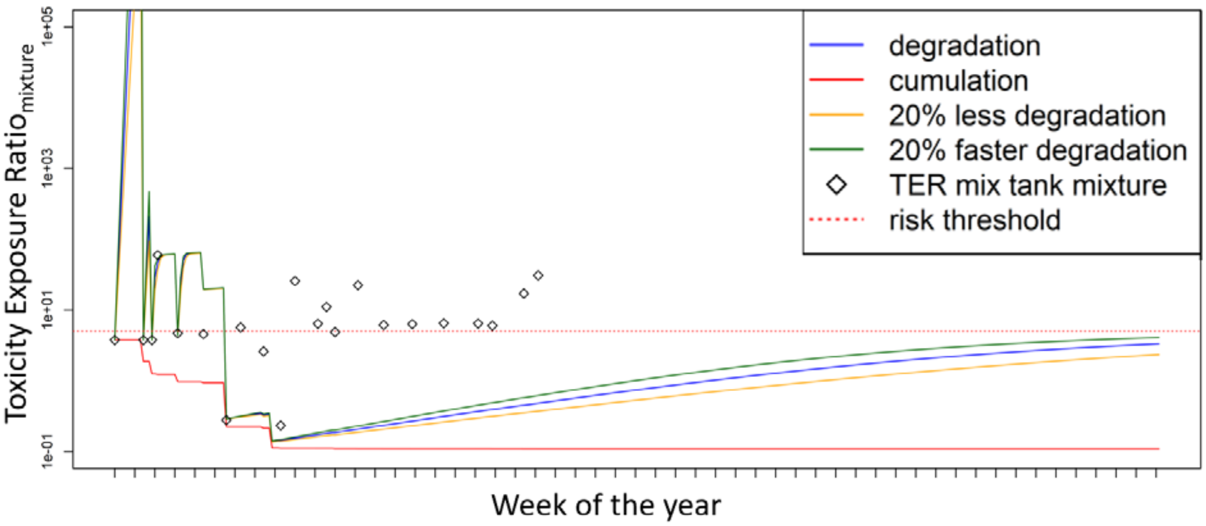
Modifications of the calculation above "fit for risk profiler"

The calculation above uses degradation and effect data from public databases. The risk indicators of the present report make use of the relevant endpoints for the regulatory risk assessment in Germany and the EU. This results in discrepancies that could overcome for the four surrogate example spray series in apple and winter oilseed rape that had been used for the calculation risk indices of chapter 5. For this, the calculation was modified.

Sensitivity of the model

The MITAS model (chapter 4) describes the progression of exposure over time by means of standard data for degradation and other parameters used for common FOCUS models. It computes risk indicators for single active substances, as well as mixture risks applying the concept of concentration addition. Fig. 41 shows an example how sensitive the model responds to simply changing a single parameter, here the degradation half-times of all substances in a spray-series in soil. Degradation rates were changed to values 20% higher- or lower than originally used.

Figure 41: Progression of mixture risks of the worst-case spray series in apple. The endpoint used for risk characterization was "earthworm reproduction after 56 days of exposure"



It can be seen from the Fig. 41 that the mixture risks fell below the threshold of acceptability soon after the first spray event. However, by means of rapid degradability of the a.i. used at early spray events, the mixture risk remained shortly below the threshold. This pattern changed after the sixth spray event in May: after this date, when the earthworm-toxic substance thiacloprid was applied, the mixture risk did not cross the threshold anymore. The risk remained unacceptable until the year was over and the next spraying season commenced.

5.3.3 Data uncertainties and link to aquatic monitoring data

5.3.3.1 Data uncertainties related to the risk characterization of selected spray series

Several aspects related to database and data analysis contain uncertainties that might influence the derivation of safety factors of tank mixtures and whole spray series. As described in chapter 5.3.1 sequential exposure can increase or decrease sensitivities to PPPs of non-target organisms. However, due to general data scarcity and the complexity of this aspect we assumed constant sensitivities of the evaluated test species. In the following we outline additional aspects and their potential impact on the outcome of the before presented results.

Selected treatment regimes

The present risk characterization is based on the four selected spray series of two crop types. We have run several analyses to selected spray series representing typical and worst-case scenarios (chapter 3). However, other spray series might give different results depending on different crop types or climatic and farm-specific conditions (see also chapter 3). Therefore, the analysis of further crop types and spray series is necessary to deduce an even more reliable MCR for spray series. For the present project, the risk characterization of additional spray series exceeded the frame and should be the focus of future projects.

Seed coating

The investigated spray series of oilseed rape did not include data on seed coatings with PPPs. Seeds are frequently treated with insecticides and fungicides (ISIP 2011). Usually, seeds are sold pre-treated and, hence, single famers do not report applications regarding seed coating. In addition, other data resources (e.g. BVL, JKI) do not allow a realistic estimation of seed coating and the corresponding environmental risk for spray series. As a result, the TER-indices presented here presumably underestimate the true overall risk of the single spray series of winter oilseed rape.

Synergistic mixtures

As described above, we assessed the additional impact of sequential exposure of the selected spray series assuming additive effects (concentration addition - CA). However, the CA approach does not consider potential synergistic mixtures, which might lead to an underestimation of mixture effects. Synergistic mixtures are defined to cause higher than predicted mixture effects with CA or IA and are well described in several studies (i.e. reviews by Cedergreen 2014 or Kortenkamp et al. 2009). Cedergreen (2014) also concluded from her review that synergistic mixtures are rare and often occur only at high concentrations. Despite this conclusion, a very recent study observed that food stress strongly decreases the threshold for synergistic effects between prochloraz and esfenvalerate to environmentally realistic concentrations in terms of survival of Daphnia magna (Shahid et al. in submitted).

Based on this knowledge, we aimed to assess the role of synergistic mixtures for the four crop types apple, winter oilseed rape, potato and winter wheat. We evaluated the frequency of synergistic tank mixtures as shown in Table 23. We screened all spray series for combined applications per time point (= tank mixtures) of exemplary synergistic mixtures such as azol fungicides and pyrethroids insecticides or neonicotinoid insecticides. More specifically, we considered imidazoles (prochloraz) and triazoles (propiconazol, epoxiconazol, penconazol) in mixture with pyrethroids (cypermethrin, alpha-cypermethrin, lambda-cyhalomethrin, esfenvalerate, beta-cyfluthrin, gamma-cyhalothrin, etofenprox, deltamethrin) or neonicotinoids (thiacloprid, acetamiprid) using the database on treatment regimes (chapter 3). We selected the substances according to existing studies (Cedergreen 2014, Norgaard and Cedergreen 2010, Iwasa et al. 2004). We identified, especially for winter wheat and apple, the frequent application of synergistic mixtures (33% and 18% or spray series, respectively, Table 23). Hence, this result implies that the consideration of such mixture effects should be addressed in future studies.

Table 23: Presence of potentially synergistic mixtures in the focal crops

Regarding potentially synergistic effects we considered the presence of tank-mixtures with at least one azol fungicide and at least one pyrethroide insecticide or neonicotinoid insecticide.

Crop type	Proportion of spray series with at least one potentially synergistic azol-pyrethroide-mix over all spray series per crop type	Proportion of spray series with at least one potentially synergistic azol-neonicotinoid-mix over all spray series per crop type
Apple	0%	18%
Winter oilseed rape	4%	0%
Potato	0%	0%
Winter wheat	33%	8%

Multiple application factor (MAF)

The MAF has been developed to take multiple applications of PPP as a.s. into account for the determination of predicted environmental concentrations (PECs) as for example outlined in the EFSA guidance document on birds and mammals (EFSA 2009a). MAFs are based on the number of applications of a given a.s., application interval and the dissipation rates of the a.s. (refer to exemplary MAFs in Table 24). In contrast, we included PECs for each PPP application individually without considering a MAF for repeated applications. As a result, our analyses may lead to an overestimation of the risk of sequential exposure when considering a simultaneous exposure to all PPP applications in a spray series without degradation of the substances (refer to chapter 5.3.2.2). Nevertheless, MAFs are based on the number of application events and dissipation rates, but do not consider recovery of the affected non-target organisms with generation times longer than DT_{50} of a.s..

Table 24: Exemplary MAFs for the acute dietary assessment of birds*

Application interval (d)	MAF90 for 90th percentile residue data for n applications							
	1	2	3	4	5	6	7	8	infinite
7	1.0	1.4	1.6	1.8	1.9	1.9	1.9	1.9	2.0
10	1.0	1.3	1.5	1.5	1.6	1.6	1.6	1.6	1.6
14	1.0	1.2	1.3	1.3	1.4	1.4	1.4	1.4	1.4

* EFSA guidance document (2009a)

Sequential exposure over several years

Terrestrial and aquatic ecosystems are exposed to a sequence of PPP applications during one season and to a sequence of spray series over many years, which are likely to cause long-term effects on non-target organisms. Hence, sequential exposure can be considered (i) during one growing season (= one spray series) and (ii) as a repeated exposure over several years. While, the exposure of environmental media during one season mainly depends on the treatment regime of the adjacent field, the multi-annual exposure is more difficult to assess. From aquatic field monitoring, we know that sequential exposures to PPPs continue over years in terms of pressure intensity. This was shown for example by Liess and von der Ohe (2005) or Gustavsson et al. (2017), who investigated pesticide exposure in streams over a period of 3 to 12 years. The results from both studies also show that the variation in PPP toxicity between sites of single years extends over several orders of magnitude. In contrast, the toxicity varies from one year to the other only within one order of magnitude indicating a relative constant exposure pattern over several years. Hence, not only sequences within one season need to be considered, but also across several seasons to account for the realistic assessment of treatment regimes and effects from agricultural spray series.

5.3.3.2 Linking modelled spray series to aquatic monitoring data

In addition to the analysis on PPP spray series, we evaluated aquatic monitoring data from freshwater streams in Central Germany with surrounding agricultural land use (database Knillmann et al. 2018). The monitoring data resulted from three field campaigns that were collected between 1998 and 2013 comprising a total of 41 stream sites (Liess and von der Ohe 2005, Münze et al. 2017 and Knillmann et al. 2018). The different studies describe the relations between pesticide mixtures and effects on aquatic macro-invertebrate communities at these sites and can be used for a comparative risk prediction. The monitoring of PPP substances was conducted using event sampling techniques and passive samplers. The investigated substances covered a broad range of insecticides, fungicides, and herbicides (Knillmann et al. 2018). The monitoring period per season took always place during the main period of PPP applications from spring to summer (Liess et al. 1999).

The aim of the following analysis was to assess the maximum cumulative ratio (MCR) for detected mixtures during one year or single exposure events at the investigated stream sites. Furthermore, we related the sum of all toxic units (TU_{sum}) and the maximum toxic unit (TU_{max}) to the trait-based indicator SPEAR (Liess and von der Ohe 2005). TU_{max} and TU_{sum} represent toxicity exposure ratios (TERs) with the difference that toxicity data is divided by the exposure concentration and PPP pressure is increasing with increasing TU. As a result, TU_{max} can be considered as $1/TER_{min}$ and TU_{sum} as $1/TER_{mix}$. We changed from TER to TU, because pesticide pressure is usually based on TU for the link to the ecological indicator SPEAR.

MCR for single exposure events and the whole season between May and July

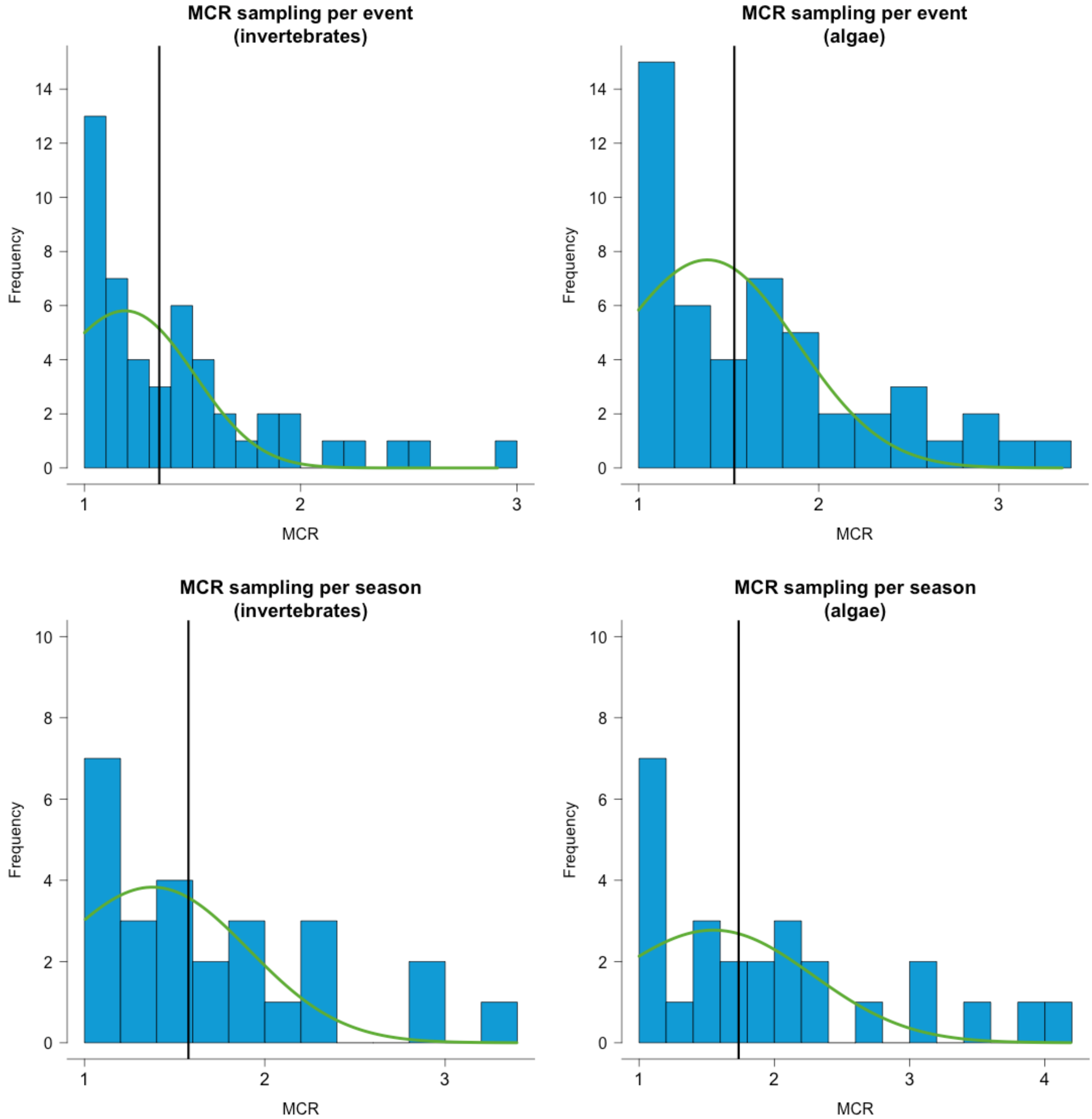
First, we calculated TU for all detected compounds for each field site and sampling season. Acute LC50-values for *D. magna, C. riparius* and algae served as reference values to calculate TU. Subsequently, we determined the MCR (TU_{sum}/TU_{max}) per sampling time point and the whole sampling season per site in assess the additional influence of mixture toxicity compared to the substance with the highest risk (TU_{max}). Since not in all three field campaigns the same substances were measured, we only selected the field campaigns from 2013 that measured the same 88 PPP substances using identical analytical methods (32 herbicides, four herbicide metabolites, 30 fungicides, one fungicide metabolite, 18 insecticides, two plant growth regulators, and one acaricide according to Münze et al. 2017, Knillmann et al. 2018). Fig. 42 shows that the median MCR was 1.35 for invertebrates (90^{th} percentile = 2.00) and 1.53 for algae (90^{th} percentile = 2.67) when considering all measured environmental concentration of one sampling time point (~ tank mixture). The MCR was slightly higher with 1.58 for invertebrates (90^{th} percentile = 2.54) and 1.74 for algae (90^{th} percentile = 3.29) when considering simultaneous exposure to all MECs in one sampling season (~ spray series).

We conclude that the ratio for the field monitoring of streams is in the range of MCRs identified for the TER-values based on modelled PECs for the selected spray series. As described before for spray series and tank mixtures, the factor of increase in risk will be particularly important when the detected TU_{max} (i.e. the most toxic single substance concentration) is just below the ecologically relevant TU-threshold in the field. Vallotton and Price (2016) observed similar results of MCR based on CA for a large set of aquatic monitoring data with 3099 samples across the United States. The authors identified most MCR between 1 and 3 using Tier 1- risk indicator. The authors also showed a large set of mixtures with risks driven by one PPP. Nevertheless, the analyses of Vallotton and Price (2016) as well as the above analyzed monitoring data only consider additive mixtures and might underestimate the influence of synergistic mixtures on non-target organisms.

We further compared the dependence of $SPEAR_{pesticides}$ from the substance with the highest risk TU_{max} and TU_{sum} of all detected PPP concentrations in the sampling season for the field campaigns in 1998-2000 and 2013. The relationship was significant for TU_{max} and TU_{sum}, and the same for TU_{max} ($r^2 = 0.57$, $P < 0.05$) and TU_{sum} ($r^2 = 0.57$, $P < = 0.05$) as shown in Fig. 43 (for further details, see also Knillmann et al. 2018). However, the TU-values for TU_{sum} are shifted to the right on the x-axis by half an order of magnitude. This shift indicates that similar effects are detected at higher concentrations than identified for TU_{max}. Hence, TU_{sum} and TU_{max} similarly describe the effect on aquatic invertebrates, but the absolute effect levels differ as shown above (i.e. MCR) by a factor of about 3. So far, the results on the observed differences between TU_{sum} and TU_{max} are only identified for aquatic invertebrate communities (see also chapter 2.2.1.1). Further analyses considering the effect of PPP mixtures on other communities of non-target organisms need to be the focus of future studies.

Figure 42: MCR between TU_{max} and TU_{sum} to account for mixture toxicity for sampling events (runoff events, upper graph) and for the whole sampling season from April until July per site (lower graph)

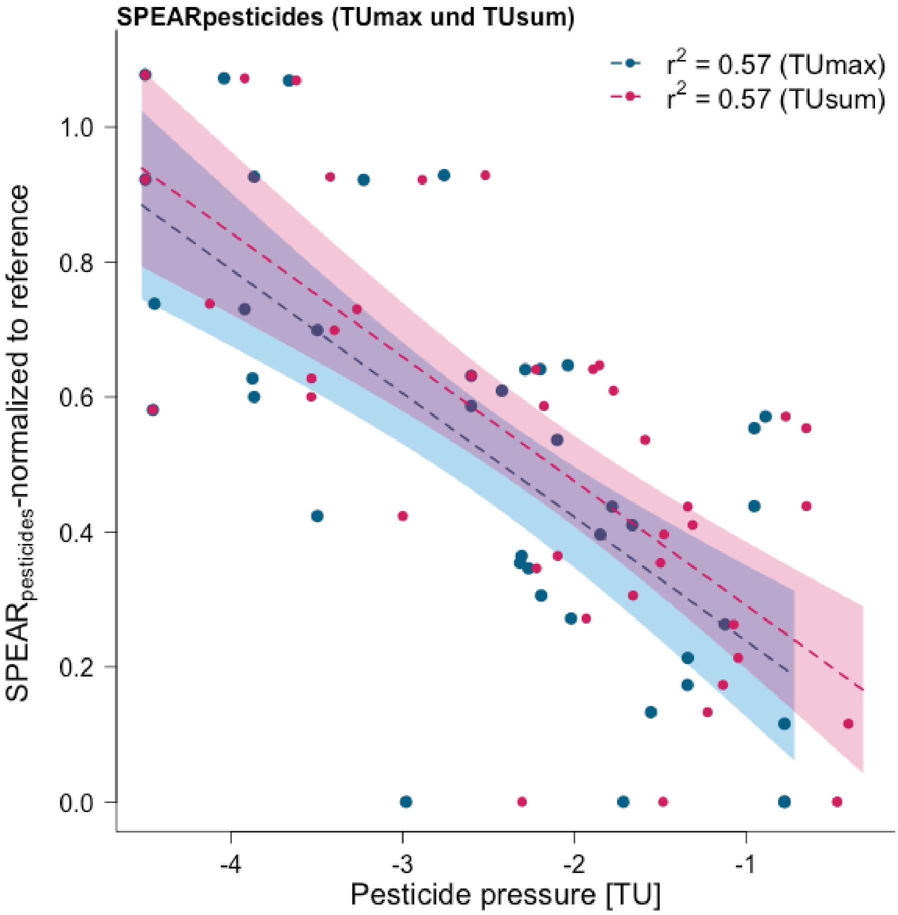
Given are the frequency distributions of MCRs per sampling event and sampling season. The distribution is displayed with bars and a distribution curve (green line). MCRs were determined using acute LC_{50}-values* for invertebrates (left side, see also chapter 3) or chronic EC_{50}-values for algae (right side). The vertical black line indicates the median MCR.



*Toxicity data were taken from the Pesticide Properties DataBase (Lewis et al. 2016) and Ecotox database (EPA 2017).

Figure 43: $SPEAR_{pesticides}$ in relation to TU_{sum} and TU_{max} shortly after the contamination period (June)

Each data point represents the measured $SPEAR_{pesticides}$ and pesticide pressure at one site and year. Red points display the relation between $SPEAR_{pesticides}$ and TU_{sum}, blue points display the relation between $SPEAR_{pesticides}$ and TU_{max}. Linear regressions are indicated with regression line, confidence interval (level = 0.95) and the regression coefficient r^2.



Main findings chapter 5

A risk characterization of four selected spray series from the crops apple and winter oilseed rape was performed. PECs and TER-values (or reversed HQ-values) were determined using German accepted guidelines for 28 endpoints and the corresponding exposure pathways.

Tier 1-threshold of acceptable risk is exceeded for most PPP applications for at least one risk assessment area in all four analyzed spray series of apple and winter oilseed rape. Regarding higher-tier risk indicators (i.e. regulatory acceptable concentration – RAC), we determined unacceptable risks for the application of thiacloprid in apple and winter oilseed rape. The unacceptable risks of PPP applications can be explained with (i) outdated risk assessment, (ii) not considered risk refinements for Tier 1-risk indicators, (iii) emergency authorizations or (iv) the results of a Risk-benefit-analysis.

We observed that tank mixtures of the investigated spray series include mixtures of two to four active substances and mixture toxicity is often dominated by the toxicity of TER_{min}. The median maximum cumulative ratio (MCR, ratio of TER_{min} and TER_{mix}) indicates additional risks between 1.19 and 1.43 for the single spray series. We identified the highest median MCR for winter oilseed rape, worst-case spray series. Nevertheless, the true increase in the risk of mixture compared to single a.s. applications might be slightly underestimated, because ecotoxicological data were partly not available for all a.s. and endpoints in the present study. In few tank mixtures, where similar toxic substances were applied, the additional risk of the mixture can be also higher than the median increase and contribute considerably to the total toxicity in addition to the substance with the highest toxicity. However, this only applies if the degradation of the substances is not considered. Toxic pesticides which are more persistent than the substance with the highest toxicity, may significantly contribute to the overall risk over time.

Similarly, we assessed the sequential exposure of spray series using MCR and CA as the most straightforward approach (assuming a simultaneous exposure to all active substances in a spray series without recovery or degradation as well as without increased or decreased sensitivity of non-target organisms). MCRs for the single risk indicators are detected to be highest for most of risk indicators in the spray series of apple, worst-case spray series. The apple worst-case spray series showed also the highest number of spray events (26) compared to other three spray series. The median MCR across all risk indicators ranged between 3.40 (apple, worst-case spray series) and 1.17 (winter oilseed rape, typical case spray series). The overall median MCR for the four spray series and risk indicators was 2.18 (90^{th} percentile = 5.26). As for tank mixtures, the true MCR might be slightly higher due to missing ecotoxicological values regarding some PPPs and endpoints. The MCR for tank mixtures and spray series is minor compared to the scale of detected TER_{single}-values. Nonetheless, the factor is already very crucial when risk assessment areas are regulated very close to the threshold of acceptable risk.

MITAS enables the mixture risk (ETR) of a spray sequence in apple cultivation to be observed over time. In this case, the threshold value for the chronic risk (earthworm, ETR = 0.2) for the spray sequence is exceeded from approximately half of the simulation time until the end. Adverse effects on the organism cannot be excluded for about half of the simulation period.

The comparison of the selected spray series and field monitoring data revealed similar MCR for exposure events (median MCR 1.35 and 1.53 for invertebrates and algae, respectively) compared to tank mixtures. We also identified similar MCR for all measured pesticide concentrations in one spraying season (median MCR 1.58 and 1.74 for invertebrates and algae, respectively) compared to the MCR of one spray series.

6 Overall conclusions and outlook

We conclude that tank-mixtures and sequential exposure in typical treatment regimes with PPPs cause additional risks for terrestrial and aquatic environments. A maximum cumulative ratio (MCR = TER_{min}/TER_{mix} per spray series) of 2.18 (median value) or 5.26 (90^{th} percentile) for spray series could be derived using selected spray series and a comparative analysis with aquatic monitoring data. The additional risk is specifically important, because spray series are common in agricultural practice, but PPPs are often regulated based on the assessment of single products and in a way that final TER-values are close to the threshold of acceptable risk. Hence, even comparatively low MCR may cause non-acceptable risks of the concerning assessment criteria.

The additional risk due to tank mixtures and sequential exposure alone cannot explain the shift between acute effective concentrations identified for standard test organisms in the laboratory and effective concentrations in the field. It has been shown that the proportion of sensitive aquatic invertebrates is already affected at concentrations with a factor of 100-1000 below acute LC_{50} values obtained in laboratory tests (Schäfer et al. 2012, Liess and von der Ohe 2005, Knillmann et al. 2018). In order to deduce protective thresholds of acceptable risk (i.e. regulatory acceptable concentrations (RAC) or environmental quality standards (UQN) for individual PPP compounds), further influential factors such as environmental stress (i.e. abiotic/biotic stress, other contaminants) need to be considered. In addition, further monitoring data and modelling approaches in terrestrial and aquatic environments are necessary to understand resulting field exposure of complex PPP application patterns and related effects on non-target organisms including indirect effects. This also holds true for repeated treatment sequences over several years that cause carry-over exposure and, thus, multi-annual carry-over effects in terrestrial and aquatic ecosystems.

In the current report, the additional risk of tank mixtures and spraying sequences was analyzed for a set of four spray series in apple and winter oilseed rape. Regarding crop types other than apple or winter oilseed rape and a potential variation between regions and years, it would be highly beneficial to also investigate additional real treatment regimes. For the present project we had the opportunity to obtain real data on treatment regimes from a private institute and a single farm. However, data on treatment regimes are documented yearly, as for example in different networks of reference farms for plant protection that are coordinated by the JKI. However, these data are currently only available in highly aggregated version and do not allow a proper environmental risk assessment of treatment regimes (i.e. Panel Pflanzenschutzmittel-Anwendungen -PAPA, data available at: https://papa.julius-kuehn.de/, accessed: 05.06.2019).

Analyses regarding mixture toxicity in the present project mainly focused on additive toxicity effects using the concept of concentration addition (CA). The concept of independent action (IA) represents an alternative to include effects for the assessment of tank-mixtures and spray series. However, this approach requires known dose-response-relationships, which are often not available and, thus, need to be modelled. In addition, there is an ongoing debate about the relevance of synergistic mixtures for non-target organisms. Synergistic effects of toxicant mixtures are assumed to occur mainly at high toxicant concentrations as for example reported in a review on mixture toxicity by Cedergreen (2014). In contrast, recent findings show that synergistic effects might be already present at environmentally realistic concentrations under conditions of additional stress (Shahid et al. under review). The investigated spray series in the present report show that potential synergistic mixtures were frequently applied in crops of winter wheat, apple and winter oilseed rape. Therefore, synergistic effects need to be better considered and quantified for the future risk assessment of culture related spray series.

Some modelling tools (SYNOPS-WEB, MITAS) calculate exposure for spray series. As outlined above relevant ecological processes that translate exposure to effect are not included in any of these tools. Their use in risk assessment requires the identification of such field relevant transfer mechanisms as well as a thorough validation of modelled results. The model MITAS, developed in the course of this project, addresses the complexity of pesticide spray series with time-dependent exposure and mixture risk. To be able to assess the validity of the predictions, the tool has to be verified by experimental studies. The model, developed so far only for earthworm toxicity, could also be extended to consider the effects on other organisms and field populations.

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A Literature research for mixture effects on aquatic and terrestrial communities

A.1 Literature research

A list of keywords was compiled in agreement with the contracting authority and converted into search phrases with truncations and Boolean operators. The "Web of Science Core Collection" was queried via http://apps.webofknowledge.com/WOS_AdvancedSearch_input.do?product=WOS&SID=S1GnNqgPbqGWm6lMVHF&search_mode=AdvancedSearch in the fields PY for the year of publication, TS for looking up the generic fields "title", "keywords" and "abstract" and SU for the research area.

Table 25: Search phrases and resulting query set that were used with the "Web of Science Core Collection"

No.	Search phrase
#9	TS = ("aquatic communit*" OR „freshwater communit*" OR "zooplankton communit*" OR "phytoplankton communit*")
#8	TS = ("daphni*" OR "fish" OR "invertebrate" OR "aquatic insect" OR "chironom*" OR "aquatic organism")
#7	TS = ("time-varying exposur*" OR "chronic*" OR "long term" OR transgenerational* OR "repeated exposure*")
#6	TS = ("multiple species*" OR "species assemblage*" OR "multiple species*" OR "species assemblage*" OR communit* OR "species sensitivity distribution*" OR SSD)
#5	SU=(Environmental Sciences OR Agriculture, Multidisciplinary OR Biodiversity Conservation OR Biology OR Ecology OR Entomology OR Environmental Sciences OR Environmental Studies OR Horticulture OR Medicine, Research & Experimental OR Microbiology OR Mycology OR Plant Sciences OR Soil Science OR Toxicology OR Zoology)
#4	TS = (earthworm* OR collembol* OR mite* OR "soil dwelling organism*" OR "soil invertebrate*" OR "terr* invertebrate" OR microarthropod* OR bird* OR mammal* OR "non-target*" OR springtail* OR mesofauna)
#3	TS = ("combined effect*" OR "joint action*" OR "mixture tox*" OR "concentration addition" OR "independent action" OR "tank mix*" OR "two chemicals" OR "binary mixture" OR "multiple chem*" OR "interactions between chemicals" OR "multiple exp*" OR "Loewe additivity" OR "dose addition" OR "The Additive Dose Model" OR "Bliss Independence" OR "Response Multiplication" OR "Response Addition" OR "Effect Addition")
#2	TS = (antifouling* OR "wood preservative*" OR contaminant* OR pesticid* OR pollutant* OR biocid* OR chemical* OR metal* OR toxicant*)
#1	PY=1987-2016

The single query sets could then be combined by the Boolean operator AND. The combination of set #1, #2, #3 and #4 and #5 represents the maximum number of hits for mixture toxicity studies (#2, #3) and for the terrestrial compartment (filtered out by query set #4) within the period between 1987-2016. It was aimed at further filtering for community or field studies by using the query sets #6 and for chronic studies query set #7 (Table 26).

Following the query, the literature had to be further screened manually for its usability in the context of the project at hand. The literature was complemented by various sources not resulting from systematic ISI-listed literature.

A.2 Identified studies from literature on aquatic and terrestrial communities and mixture toxicity

Table 26: Identified studies on aquatic communities

Given are the references and characteristics of identified studies that investigated the influence of toxicant mixtures on aquatic community endpoints for the single compounds and the mixture.

Year	Reference	Community	Endpoint	Applied concept for mixture toxicity	Investigated substances	Comment
1998	Carder, J. P., Hoagland, K. D. (1998): Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. *Environmental Toxicology and Chemistry*, *17*(7), 1415-1420.	benthic algal communities	Biovolume of viable algal cells, relative abundance of the six dominant algal species	not applied	alachlor, atrazine	part of Review by Verbruggen and van den Brink 2010
1994	Fairchild, J.F., LaPoint, T.W., Schwartz, T.R. (1994): Effects of an herbicide and insecticide mixture in aquatic mesocosms. Archives of Environmental Contamination and Toxicology 27, 527-533.	zooplankton, Algae, macrophytes, Fish (bluegill)	macrophyte biomass, photosynthesis, respiration, abundances	not applied	atrazin, esfenvalerate	part of Review by Verbruggen and van den Brink 2010
2003	Grünwald, H.M. (2003): Effects of a Pesticide Mixture on Plankton in Freshwater Mesocosms – From Single Substance Studies to Combination Impacts. Technischen Universität München, München.	zooplankton, Phytoplankton	abundances, functional parameters, recovery, species composition (PRC)	IA	alpha-cypermethrin, isoproturon	part of Review by Verbruggen and van den Brink 2010
1993	Hoagland, K.D., Drenner, R.W., Smith, J.D., Cross, D.R. (1993): Freshwater community responses to mixtures of agricultural pesticides: effects of atrazine and bifenthrin. Environmental Toxicology and Chemistry 12, 627-637.	plankton assemblages and bluegill	primary productivity, chlorophyll, green algal colonies, zooplankton taxa	not applied	atrazine, bifenthrin	part of Review by Verbruggen and van den Brink 2010
2008	Knauert, S., Escher, B., Singer, H., Hollender, J., Knauer, K. (2008): Mixture toxicity of three photosystem II inhibitors (atrazine, isoproturon,	freshwater phytoplankton	photosynthetic activity	CA, SSD	atrazin, asoproturon, diuron	part of Review by Verbruggen

Year	Reference	Community	Endpoint	Applied concept for mixture toxicity	Investigated substances	Comment
	and diuron) toward photosynthesis of freshwater phytoplankton studied in outdoor mesocosms. Environmental Science & Technology 42(17), 6424-6430.					and van den Brink 2010
2009	Relyea, R.A. (2009): A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159(2), 363-376.	zooplankton, phytoplankton, periphyton, and larval amphibians	zooplankton abundances, phytoplankton mass (chl a), periphyton biomass, time to metamorphosis, amphibian abundance	not applied	5 insecticides (malathion, carbaryl, chlorpyrifos, diazinon, and endosulfan) and 5 herbicides (glyphosate, atrazine, acetochlor, metolachlor, and 2,4-D)	part of Review by Verbruggen and van den Brink 2010
2003	Wendt-Rasch, L., Pirzadeh, P., Woin, P. (2003): Effects of metsulfuron methyl and cypermethrin exposure on freshwater model ecosystems. Aquatic Toxicology 63(3), 243-256.	macrophytes, periphyton	species composition (RDA)		aetsulfuron-methyl, cypermethrin	
2006	Arrhenius, Å., Backhaus, T., Grönvall, F., Junghans, M., Scholze, M., Blanck, H. (2006): Effects of Three Antifouling Agents on Algal Communities and Algal Reproduction: Mixture Toxicity Studies with TBT, Irgarol, and Sea-Nine. Archives of Environmental Contamination and Toxicology, 50, 335-345.	periphyton community	inhibition of photosynthesis and algal reproduction	CA, IA	3 antifoulants (Sea-Nine, Irgarol, and TBT)	
2004	Arrhenius, Å., Grönvall, F., Scholze, M., Backhaus, T., Blanck, H. (2004): Predictability of the mixture toxicity of 12 similarly acting congeneric inhibitors of photosystem II in marine periphyton and epipsammon communities. Aquatic Toxicology, 68, 351-367.	marine periphyton and epipsammon communities	Inhibition of photosynthesis in short-term tests	CA, IA	12 phenylurea herbicides, similar acting	

Year	Reference	Community	Endpoint	Applied concept for mixture toxicity	Investigated substances	Comment
2013	Choung, C.B., Hyne, R.V., Stevens, M.M. and Hose, G.C. (2013): The ecological effects of a herbicide–insecticide mixture on an experimental freshwater ecosystem. Environmental Pollution 172, 264-274.	aquatic algal and invertebrate assemblages in microcosms	Chironomid emergence, development time and adult size; phytoplankton abundance, community structure (PRC), abundances of single invertebrate taxa	not applied	herbicide (atrazine) + insecticide (terbufos)	
2011	Backhaus, T., Porsbring, T., Arrhenius, Å., Brosche, S., Johansson, P. and Blanck, H. (2011): Single-substance and mixture toxicity of five pharmaceuticals and personal care products to marine periphyton communities. Environmental Toxicology and Chemistry 30(9), 2030-2040.	periphyton communities	inhibition of final biomass	CA, IA	fluoxetine, propranolol, triclosan, zinc-pyrithione, and clotrimazole	
2009	Knauert, S., Dawo, U., Hollender, J., Hommen, U., Knauer, K. (2009): Effects of photosystem II inhibitors and their mixture on freshwater phytoplankton succession in outdoor mesocosms. Environmental Toxicology and Chemistry 28(4), 836-845.	freshwater phytoplankton	Total abundance, species composition, diversity and recovery of the community	CA	atrazine, isoproturon, and diuron	
2011	Bjergager, M. B. A., Hanson, M. L., Lissemore, L., Henriquez, N., Solomon, K. R., Cedergreen, N. (2011): Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate. Aquatic Toxicology, 101(2), 412-422.	zooplankton	Zooplankton diversity, Analysis of chlorophyll-a, Recovery, extinction rates, EC20	ratio of potentiation of esfenvalerate toxicity	prochloraz, esfenvalerate	
2010	Porsbring, T., Backhaus, T., Johansson, P., Kuylenstierna, M., Blanck, H. (2010): Mixture toxicity from photosystem II inhibitors on	microalgal community	photosystem II inhibiti, species species composition	CA	4 herbicides	

Year	Reference	Community	Endpoint	Applied concept for mixture toxicity	Investigated substances	Comment
	microalgal community succession is predictable by concentration addition. Environmental Toxicology and Chemistry 29(12), 2806-2813.					

Table 27: Terrestrial communities and mixture effects

Year	Reference	Assessment area	Article type	Endpoint	Mixture toxicity concept	Investigated substance	Conclusion	Comment
1991	Aggarwal M, Naraharisetti SB, Dandapat S, Degen GH, Malik JK (2008) Perturbations in immune responses induced by concurrent subchronic exposure to arsenic and endosulfan. Toxicology, 251, 51-60.	field, birds	Original paper	Nestling weight gain, Parenting behavior, Brain cholinesterase activities	toxicity-frequency value	Arsenic, endosulfan	Results suggest that increasing exposure to pesticides may reduce songbird reproduction productivity.	Monitoring of nests
2003	Bel'skii EA, Lyakhov AG (2003) Response of the avifauna to technogenic environmental pollution in the southern taiga zone of the Middle Urals. Russian Journal of Ecology, 34, 181-187.	field, birds	Review	combined effect of sulfur dioxide and heavy metals	none	sulfur dioxide, heavy metals	environmental pollution results in the reduction of species richness, biomass, and stability of the nesting bird fauna. In degraded areas, the structure of the bird community changes: (I) typical forest species are replaced by the species of open habitats, and (2) the proportion of species nesting in the upper tree layer decreases, whereas that of ground-nesting species increases.	
2000	Frampton GK, Wratten SD (2000) Effects of benzimidazole and triazole fungicide use on epigeic species of collembola in wheat. Ecotoxicology and Environmental Safety, 46, 64-72.	field, collembolans	Original paper	Population abundance	None	carbendazim, propiconazole, and triadimenol	"Fewer significant treatment effects were obtained in enclosed than in open plots and no consistent effects of carbendazim were detected."	discussion of considerations of fungicide tank-mixes and the risk for collembolans, no specific information on mixture toxicity

Year	Reference	Assessment area	Article type	Endpoint	Mixture toxicity concept	Investigated substance	Conclusion	Comment
2006	Maraldo K, Christensen B, Strandberg B, Holmstrup M (2006) Effects of copper on enchytraeids in the field under differing soil moisture regimes. Environmental Toxicology and Chemistry, 25, 604-612.	field and lab, enchytraeids	Original paper	combined effects of drought and copper in the field and in the lab	None	Heavy metals	field population density and species composition were highly affected by copper at concentrations in the range 300 to 500 mg Cu/kg dry soil and higher. In particular, a greatly impoverished species diversity was found in the copper-polluted areas Surprisingly, possible effects of summer drought in the field were not detected in the autumn sampling, perhaps because of rapid recovery of the enchytraeid populations in both unpolluted and copper-polluted areas	
2015	Sasaki K, Lesbarreres D, Watson G, Litzgus J (2015) Mining-caused changes to habitat structure affect amphibian and reptile population ecology more than metal pollution. Ecological Applications, 25, 2240-2254.	field, reptiles and amphibians	Original paper	role and relative effects of structural alterations of terrestrial habitat and metal pollution caused by century-long smelting operations on amphibian and reptile communities by collecting environmental and time- and area-standardized multivariate abundance data along three spatially replicated impact gradients.	none	Heavy metals	species richness, diversity, and abundance declined progressively with increasing levels of metals (As, Cu, and Ni) and soil temperature (T-s) and decreasing canopy cover, amount of coarse woody debris (CWD), and relative humidity (RH). The composite habitat variable (which included canopy cover, CWD, T-s, and RH) was more strongly associated with most response metrics than the composite metal variable (As, Cu, and Ni), and canopy cover alone explained 19-74% of the variance. Moreover, species that use terrestrial habitat for specific behaviors (e.g., hibernation, dispersal), especially forest-dependent species, were more severely affected than largely aquatic species results suggest that structural alterations of terrestrial habitat and concomitant changes in the resource availability and microclimate have stronger effects than metal pollution per se. Furthermore, much of the variation in response metrics was explained by the joint action of several environmental variables, implying synergistic effects (e.g., exacerbation of metal toxicity by elevated temperatures in sites with reduced canopy cover).	

Year	Reference	Assessment area	Article type	Endpoint	Mixture toxicity concept	Investigated substance	Conclusion	Comment
2014	Schmitz J, Hahn M, Brühl CA (2014) Agrochemicals in field margins - An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. Agriculture Ecosystems & Environment, 193, 60-69.	field, ntp	Original paper	To investigate individual and combined effects of fertilizer, herbicide, and insecticide inputs on the plant community of field margins, a 3-year field study with a randomized block design was performed. The applied fertilizer rates (25% of the field rate) and pesticide rates (30% of the field rate) were consistent with their average input rates (drift+overspray) in the first meter of a field margin directly adjacent to the field.	ANOVA for effects in block design with single and combined treatments	sulfonyl-urea, mesosulfuron-methyl, iodosulfuron-methyl-natrium, mefenpyr-diethyl, pyrethroid, lambda-cyhalothrin; fertilizer	significant herbicide-fertilizer interaction effects were also observed and could not be extrapolated from individual effects. The impacts of both agrochemicals became stronger over time, led to shifts in plant community compositions, and caused significantly lower species diversities than in the control plots. The insecticide application significantly affected the frequencies of two plant species. The results suggest that a continuous annual application of agrochemicals would cause further plant community shifts.	

Year	Reference	Assessment area	Article type	Endpoint	Mixture toxicity concept	Investigated substance	Conclusion	Comment
2015	Schnug L, Ergon T, Jakob L, Scott-Fordsmand JJ, Joner EJ, Leinaas HP (2015) Responses of earthworms to repeated exposure to three biocides applied singly and as a mixture in an agricultural field. Science of the Total Environment, 505, 223-235.	field, earthworms	Original paper	differently acting biocides; the insecticide esfenvalerate, the fungicide picoxystrobin and the bactericide triclosan, applied individually and as a mixture, on an earthworm community in the field. A concentration-response design was chosen and results were analyzed using univariate and multivariate approaches	GLMM for treatment effects with plot and replicate as random, year and season as fixed effects	Esfenvalerate, picoxystrobin, triclosan	Esfenvalerate and picoxystrobin appeared to be the main drivers for the mixture's toxicity. Species-specific toxicity patterns question the reliability of mixture toxicity predictions derived on E. fetida for field earthworms. Biocide concentrations equaling EC5Os (reproduction) for E. fetida provoked effects on the field earthworms mainly exceeding 50%, indicating effect intensification from the laboratory to field as well as the influence of indirect effects produced by species interactions.	
2011	Vaj C, Barmaz S, Sorensen PB, Spurgeon D, Vighi M (2011) Assessing, mapping and validating site-specific ecotoxicological risk for pesticide mixtures: A case study for small scale hot spots in aquatic and terrestrial environments. Ecotoxicology and	field, soil and pollinator communities (aquatic)	Original paper	general overview of an integrated methodological approach for assessing and mapping pesticide risk in different systems The work presented in the paper addresses the real world effects of pesticide mixtures on natural	CA	Mancozeb, Sulphur, Dimethomorph, Glyphosate, Oxadiazon, Folpet, Iprovalicarb, Cyprodinil, Fludioxonil, Thiamethoxam, Copper oxychloride, Chlorpyrifos,		in general not focused on terestrial env. Regarding conclusions... good reference for approaches on PEC-calculation in complex spray-series,

Year	Reference	Assessment area	Article type	Endpoint	Mixture toxicity concept	Investigated substance	Conclusion	Comment
	Environmental Safety, 74, 2156-2166.			communities; exposure models are georeferenced. Terrestrial Risk assessments are fully based on acute laboratory data for earthworms and bees		Copper sulphate, Mepanipyrim		

A.3 Literature research for chronic mixture effects

Table 28: Identified studies from literature on chronic endpoints and mixture toxicity (terrestrial)

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2008	Arnold KE, Boxall ABA, Brown AR et al. (2013) Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. Biology Letters, 9.	cmbtrr_0009	acute, earthworms	Original paper	lethality of toluene and methyl tert-butyl ether towards Asian earthworm Perionyx excavatus on filter paper and in natural soil	concentration addition	The combined effect of toluene and MTBE in filter paper contact test was not consistent with the results in soil toxicity tests. This phenomenon may be associated with the interaction of soil salts with pollutants. Treatment with toluene and MTBE also affected the behavior and morphology of P. excavatus. It indicates that the VOCs induce metabolic and functional damages in earthworms. Combined effects of gasoline components should be taken into account to soil risk assessment.	test duration not longer than 48h
2013	Ben Fredj F, Irie M, Han J et al. (2010) Stress Response Of Heavy Metal Mixture Present In Wastewater And Leachate On Heat-Shock Protein 47-Transfected Cells. Environmental Toxicology and Chemistry, 29, 1637-1647.	cmbtrr_0010	birds	Review	effects of pharmaceuticals on wildlife	none	The near extinction of Asian vultures following exposure to diclofenac is the key example where exposure to a pharmaceutical caused a population-level impact on non-target wildlife. However, more subtle changes to behaviour and physiology are rarely studied and poorly understood.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2008	Amorim MJB, Rombke J, Scheffczyk A, Soares A (2005) Effect of different soil types on the enchytraeids Enchytraeus albidus and Enchytraeus luxuriosus using the herbicide Phenmedipham. Chemosphere, 61, 1102-1114.	cmbtrr_0004	chronic, birds	Original paper	arsenic and endosulfan at environmentally relevant concentrations; weights of spleen, thymus, bursa, total weight, further immunological markers	none	metalloid and insecticide combination significantly depressed the ability of peripheral blood and splenic lymphocytes to proliferate in response to antigen RD-F and mitogen Con A. The delayed type hypersensitivity response to 2,4-dinitro-1-chlorobenzene or to PHA-P was also significantly decreased. Nitric oxide production by RD-F or lipopolysaccharide-stimulated peripheral blood and splenic mononuclear cells was significantly suppressed following concurrent exposure to arsenic and endosulfan. Furthermore, the combined exposure also decreased the antibody response to RDF.	
2009	Bednarska AJ, Portka I, Kramarz PE, Laskowski R (2009) COMBINED EFFECT OF ENVIRONMENTAL POLLUTANTS (NICKEL, CHLORPYRIFOS) AND TEMPERATURE ON THE GROUND BEETLE, PTEROSTICHUS OBLONGOPUNCTATUS (COLEOPTERA: CARABIDAE). Environmental Toxicology and Chemistry, 28, 864-872.	cmbtrr_0007	chronic, carabid beetles	Original paper	Ni, chlorpyrifos (CPF), and temperature in the ground beetle, Pterostichus oblongopunctatus; life-cycle parameters of the beetles (survival and reproduction)	GLM on effects of toxicants, temperature, biomass	Significant three-factor interactions were found for effects on beetle survival, indicating that the combined negative effect of Ni and CPF was temperature dependent. In addition, significant effects of body mass were found: The survival of beetles treated with CPF and the reproduction of beetles exposed to Ni were positively correlated with body mass. All studied endpoints were affected by temperature. The results indicate that understanding interactions between temperature and toxicants, as well as among chemicals themselves, is essential for proper environmental risk assessment.	same combination Nickel/Chlorpyrifos as in cmbtrr_0006, check reference list

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2012	Amorim MJB, Pereira C, Menezes-Oliveira VB, Campos B, Soares A, Loureiro S (2012) Assessing single and joint effects of chemicals on the survival and reproduction of Folsomia candida (Collembola) in soil. Environmental Pollution, 160, 145-152.	cmbtrr_0002	chronic, collembolans	Original paper	single and mixture toxicity of atrazine, dimethoate, lindane, zinc and cadmium were studied in Folsomia candida, assessing survival and reproduction.	CA, IA	Different response patterns were observed for the different endpoints and synergistic patterns were observed when pesticides were present. Compared with the previously tested Enchytraeus albidus and Porcellionides pruinosus, the mixture toxicity pattern for F. candida was species specific.	
2013	De Boer ME, Ellers J, Van Gestel CaM, Den Dunnen JT, Van Straalen NM, Roelofs D (2013) Transcriptional responses indicate attenuated oxidative stress in the springtail Folsomia candida exposed to mixtures of cadmium and phenanthrene. Ecotoxicology, 22, 619-631.	cmbtrr_0012	chronic, collembolans	Original paper	effect of binary mixtures of cadmium and phenanthrene on the reproduction of Folsomia candida and investigated the cellular mechanisms underlying this response	CA, IA by MixTox model developed by Jonker et al. (2005)	Mixture toxicity modeling showed an antagonistic deviation from concentration addition for reproduction effects of the mixtures Specifically associated with the mixture treatments were a biotransformation phase II gene, four mitochondrial related genes and a gene involved in the biosynthesis of antioxidant selenoproteins The antagonism found for inhibition of reproduction may partially originate from these differences. Mechanistic studies on mixture toxicity can ultimately aid risk assessment by defining relevant toxicity pathways in organisms exposed to real-world mixture exposures present in the field.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2012	Baylay AJ, Spurgeon DJ, Svendsen C, Griffin JL, Swain SC, Sturzenbaum SR, Jones OaH (2012) A metabolomics based test of independent action and concentration addition using the earthworm Lumbricus rubellus. Ecotoxicology, 21, 1436-1447.	cmbtrr_0006	chronic, earthworms	Original paper	effects on survival, weight change, cocoon production and metabolism caused by exposure to two similarly acting (imidacloprid/thiacloprid) and two dissimilarly acting (chlorpyrifos/Nickel) chemicals on the earthworm Lumbricus rubellus.	CA, IA, metabolomics based approach to elucidate mechanisms of effect	0.5 toxic unit equitoxic mixture demonstrated metabolic effects intermediate between those for each pesticide, indicating a non-interactive, independent joint effect. 1 and 1.5 toxic units), metabolite changes associated with thiacloprid exposure began to dominate metabolomic effects of the two dissimilarly acting chemicals were distinct, confirming separate modes of action and both proved more toxic than anticipated from previous studies	same combination Nickel/Chlorpyrifos as in cmbtrr_0006
2016	Fisker KV, Holmstrup M, Sorensen JG (2016) Freezing of body fluids induces metallothionein gene expression in earthworms (Dendrobaena octaedra). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, 179, 44-48.	cmbtrr_0016	chronic, earthworms	Original paper	freeze-tolerant earthworm Dendrobaena octaedra, combineds freezing and sublethal copper; transcription of genes coding for heat shock proteins (hsp10 and hsp70), metallothioneins (mt1 and mt2), and glutathione-S-transferase (gst), and that the combined effects of these two stressors would be additive.	mixed model with ‘copper’ and ‘freezing’ as fixed factors and ‘population’ as a random factor was used to test for the effect of copper and freezing, and their interaction, on relative normalized expression of the target genes using R (version 3.1.2)	there was a significant interaction causing more than additive transcription rates of mt1 in the copper/freezing treatment suggesting that freeze-induced cellular dehydration increases the concentration of free copper ions in the cytosol. This metallothionein response to freezing is likely adaptive and possibly provides protection against freeze-induced elevated metal concentrations in the cytosol and excess ROS levels due to hypoxia during freezing.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2016	Gao YH, Li HS, Li XM, Sun ZJ (2016) Combined subacute toxicity of copper and antiparasitic albendazole to the earthworm (Eisenia fetida). Environmental Science and Pollution Research, 23, 4387-4396.	cmbtrr_0021	chronic, earthworms	Original paper	combined subacute toxicity of Cu exposure (0, 80, 120, 160 mg kg(-1)) and ABZ exposure (0, 3, 9 mg kg(-1)) in earthworms (Eisenia fetida) were observed using three approaches, namely chronic growth and reproduction, antioxidant enzyme activity, and earthworm Cu residue.	ANOVA	sensitivity of the earthworms' reproduction to Cu increased with the presence of high concentrations of ABZ (9 mg kg(-1)), indicating a reduction beginning at a Cu concentration of 80 mg kg(-1), in the cocoon number, hatching success, and biomass. In addition, the three enzyme activities exhibited different responsive patterns, indicating inducement in the catalase and glutathione peroxidase, and inhibition in the superoxide dismutase, which were dependent on the exposure times and concentrations. In regard to the earthworm Cu residue, when increasing Cu exposure concentrations, the internal Cu concentrations tended to level off, exhibited a linear pattern at the Cu concentration range of 40 to 120 mg kg(-1), and showed a stable trend above 120 mg kg(-1)	
2005	An YJ, Lee WM (2008) Comparative and combined toxicities of toluene and methyl tert-butyl ether to an Asian earthworm Perionyx excavatus. Chemosphere, 71, 407-411.	cmbtrr_0005	chronic, enchytraeids	Original paper	reproduction of Enchytraeus albidus and Enchytraeus luxuriosus in 18 different soils	none	EC(50)s in enchytraeids changed by a factor of 9 for juveniles and nearly 30 for the adults; effect of soil properties very important (especially M (Organic Matter) and WHC (Water Holding Capacity) or pH, CEC (Cation Exchange Capacity), C/N (Carbon/Nitrogen ratio) and clay content)	important mix-partner substance Phenmedipham was tested in different Lufa soils

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2010	Broerse M, Van Gestel CaM (2010) Mixture effects of nickel and chlorpyrifos on Folsomia candida (Collembola) explained from development of toxicity in time. Chemosphere, 79, 953-957.	cmbtrr_0001	chronic, hamster	Original paper	three heavy metals nickel (Ni), cadmium (Cd), and lead (Pb)-and their combined effect on mammalian cells, using Chinese hamster ovary cells transfected with the heat-shock protein (HSP) 47 promoter.	statistical mixture model	single heavy metals induced the stress response on HSP() cells even at concentrations lower than the local and international guidelines mixture characterization discovered the key role played by the high levels of Ni or combination of Cd and Pb to induce the highest stress response following 3-h incubation. combination of a bioassay system with a statistical model proved extremely useful for better understanding the major contributors to the stress response of the mixture	consider the use of statistical mixed models (as in cmbtrr_0006) additionally to IA, CA
1997	Diawara MM, Kulkosky P, Williams DE, Mccrory S, Allison TG, Martinez LA (1997) Mammalian toxicity of 5-methoxypsoralen and 8-methoxypsoralen, two compounds used in skin photochemotherapy. Journal of Natural Toxins, 6, 183-192.	cmbtrr_0013	chronic, mammals	Original paper	ncreasing concentrations (0, 250, 1250, and 2500 ppm) of each of the two chemicals were mixed into the powdered diet of male and female outbred Wistar rats, Combining the two chemicals at 1,250 ppm; growth inhibition, birthrate	none	Combining the two chemicals at 1,250 ppm each resulted in a greater growth inhibition than when individual chemicals were added alone to the diet at 2,500 ppm. All psoralen-containing diets, except for the lowest dose (250 ppm), significantly reduced female birthrate.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2000	Diawara MM, Williams DE, Oganesian A, Spitsbergen J (2000) Dietary psoralens induce hepatotoxicity in C57 mice. Journal of Natural Toxins, 9, 179-195.	cmbtrr_0014	chronic, mammals	Original paper	subacute toxicity of bergapten and xanthotoxin in a mammalian model by mixing individual chemicals into mouse diet at 0, 250, and 1000 ppm, and in combination at 500 ppm each	none	only the combined diet induced a significant weight reduction in females compared with the control diet Neither bergapten nor xanthotoxin, however, induced a significant dose-dependent toxicity in either male or female mice, suggesting that mice may not represent a good laboratory animal model for evaluating the toxicolgical effects of psoralens.	
2009	Gomez-Eyles JL, Svendsen C, Lister L, Martin H, Hodson ME, Spurgeon DJ (2009) Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on Caenorhabditis elegans and Eisenia fetida. Ecotoxicology and Environmental Safety, 72, 71-79.	cmbtrr_0019	chronic, nematodes and earthworms		earthworm Eisenia fetida and the nematode Caenorhabditis elegans were exposed to a full range of mixtures of the similar acting neonicotinoid pesticides imidacloprid and thiacloprid.	CA with dose level-dependent deviation term	The effect of the mixtures on C. elegans was described significantly better ($p<0.01$) by a dose level-dependent deviation from the concentration addition model than by the reference model alone, while the reference model description of the effects on E. fetida could not be significantly improved. These results highlight that deviations from concentration addition are possible even with similar acting compounds, but that the nature of such deviations are species dependent.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2007	Cedergreen N, Kudsk P, Mathiassen SK, Streibig JC (2007) Combination effects of herbicides on plants and algae: do species and test systems matter? Pest Management Science, 63, 282-295.	cmbtrr_0011	chronic, ntp	Original paper	mixtures were tested on the terrestrial species Tripleurospermum inodorum (L.) Schultz-Bip. (Scentless Mayweed) and Stellaria media (L.) Vill. (Common Chickweed), and on the aquatic species Lemna minor L. (Lesser duckweed) and the alga Pseudokirchneriella subcapitata (Korschikov) Hindak.	CA, IA	mixed aquatic and terrestrial experiments, good example for a design: The ray design consists of dose–response curves of the two individual herbicides tested alone and a number of dose–response curves of the pesticides mixed at predefined mixture ratios. The mixture ratios were chosen with the aim of obtaining a contribution to the overall effect of the two pesticides of 100:0%, 75:25%, 50:50%, 25:75% and 0:100% for five- mixture ratio experiments, and of 100:0%, 83:17%, 67:33%, 50:50%, 33:67%, 83:17% and 0:100% for seven-mixture ratio experiments.	
2012	Van Gestel CaM (2012) Soil ecotoxicology: state of the art and future directions. Zookeys, 275-296.	cmbtrr_0027	general overview	Review	-/-	-/-		directions are given towards soil ecotoxicology is heading in the future

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2013	Gao HX, Dong YD, Meng CY, Guan WJ, Liu YL, Xing GZ (2013) Investigation of organic pollutants in wastewater-irrigated soil and its DNA damage and oxidative damage on mice. Environmental Monitoring and Assessment, 185, 2475-2482.	cmbtrr_0020	mammals	Original paper	Organic pollutants from Wastewater-irrigated soil samples in the vicinity of an industrial area in Tangshan, China were extracted from the soil using ultrasonic oscillation; DNA damage on mice was determined by the Comet assay after oral gavage with the extracts, and changes in total superoxide dismutase (T-SOD) activity, glutathione peroxidase glutathione, GSH peroxidase (GSH-P-X) activity and malondialdehyde content in serum of mice were investigated	-/-	The toxicity test of mice showed that compared with reagent control group, the activities of T-SOD and GSH-P-X decreased; the tailing rate of peripheral blood lymphocyte of mice increased and was more than that of the control group. This shows that mammalian toxicity end points can be used to determine the joint toxicity of organic pollutants in soil. When there is no means to identify each and every pollutant in soil, it is feasible to evaluate the combined effects of various pollutants to determine the extent to which the soil is polluted.	
2010	Gault N, Sandre C, Poncy JL, Moulin C, Lefaix JL, Bresson C (2010) Cobalt toxicity: Chemical and radiological combined effects on HaCaT keratinocyte cell line. Toxicology in Vitro, 24, 92-98.	cmbtrr_0022	mammals	Original paper	Combined chemical and radiochemical toxicity of cobalt toxicity in a model human keratinocyte cell line, HaCaT	-/-	Additive effects of cobalt and irradiation were demonstrated. The underlying mechanism of cobalt toxicity is not clearly established, but our results seem to indicate that the toxicity of Co(II) and of irradiation arises from production of reactive oxygen species.	

Year	Reference	Label intern	Assessment area	Article type	Endpoint	Mixture toxicity concept	Conclusion	Comment
2010	Druart C, Scheifler R, De Vaufleury A (2010) Towards the development of an embryotoxicity bioassay with terrestrial snails: Screening approach for cadmium and pesticides. Journal of Hazardous Materials, 184, 26-33.	cmbtrr_0015	snail eggs	Original paper	Reglone (R) (active ingredient (a.i.), diquat) and Roundup (R) or its a.i., glyphosate. of a surfactant (Agral (R) 90, a.i., nonylphenol polyethoxylates) and of cadmium (Cd)	CA	An antagonistic interaction between the two substances reglone and agral was found	
2015	Fountain MT, Medd N (2015) Integrating pesticides and predatory mites in soft fruit crops. Phytoparasitica, 43, 657-667.	cmbtrr_0017	various, predatory mites	Review	-/-	-/-	-/-	some information on tank mixtures, review does not focus our main research goals

B Overview on identified models

Table 29: Models identified for predicting mixture toxicity and mixture effects

The table displays the model types of the various mixture toxicity models and mixture risk models in the heading. The first column lists the different aspects of the model.

model type	**non-interaction model**			**interaction model**
model	**Concentration addition (CA)**	**Independent action (IA)**	**Heuristic model**	**Integrated addition and interaction model (IAI)**
synonyms	Loewe additivity	Bliss independence; Response addition; Effect multiplication	Integrated Addition Model; Two-stage prediction (TSP)	
source	Loewe & Muischnek, 1926; Berenbaum,1985	Bliss, 1939	Olmstead & LeBlanc, 2005; Rider & LeBlanc, 2005	Rider & LeBlanc, 2005
idea/ application	Substances with the same mode of action behave like dilutions of the same substance.	Substances with different modes of action can only act on that part of the test population which has not yet been affected by the other substances in the mixture. In contrast to a simple addition of effects, no effects >100% can result.	The chemicals were divided into "cassettes" based on their modes of action. The mixture toxicity within a "cassette" is calculated with CA and the mixture toxicity between the "cassettes" is calculated with IA.	
equation	$\sum_{i=1}^{N} \frac{c_i}{ECx_i} = 1$	$E_{mix} = 1 - \prod_{i=1}^{N} (1 - E_i)$	$R = 1 - \prod_{I=1}^{N} \left(1 - \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{C_i}{EC50_i}\right)^{p'}}}\right)$	$R = 1 - \prod_{I=1}^{N} \left\{1 - \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{k_{a,i}(CA) * C_i}{EC50_i}\right)^{p'}}}\right\}$
variables	**C_i** = concentration of chemical i in the mixture	**E** = effect [0<E<1]	**R** = response to the mixture	**R** = response to the mixture
	$Ec_{x,i}$ = concentration of component i that produces x% effect	**E_{mix}** = mixture effect	**C_i** = concentration of chemical i in the mixture	**C_i** = concentration of chemical i in the mixture
			$EC50_i$ = concentration of the chemical i, which produces 50% response	**$EC50_i$** = concentration of the chemical i, which produces 50% response
			p' = average power associated with the chemicals in the cassette	**p'** = average power associated with the chemicals in the cassette

model type	non-interaction model			interaction model
				R_i = response of chemicals in cassette i
				$k_{a,i}$ = represents a function describing the extent to which the chemical a (present in the mixture at a concentration of CA) alters the effective concentration of the chemical i

model type	non-interaction model			
model	**Effect Summation (ES)**	**ES with the exponent e (ESE)**	**ES with power of number of components n (ESN)**	**Integrated Fuzzy Concentration addition - Independent action Model (INFCIM oder IFCA-IA)**
source	Ge, Liu, Su, et al., 2013; Ge, Liu, Xie, et al., 2013	Ge, Liu, Su, et al., 2013; Ge, Liu, Xie, et al., 2013	Ge, Liu, Su, et al., 2013; Ge, Liu, Xie, et al., 2013	Mwense et al., 2004; Wang et al., 2009
idea/ application	It is assumed that the mixture effect is equal to the arithmetic sum of the effects of the individual mixture components.	Procedure to ensure that the predicted effect does not exceed 100%.	Procedure to ensure that the predicted effect does not exceed 100%.	QSAR approach for predicting mixture toxicity.
equation	$E^{ES}{}_{mix} = \sum_{i=1}^{n} E_i$	$E^{ESE}{}_{mix} = 1 - \exp\left(-\sum_{i=1}^{n} E_i\right)$	$E^{ESN}{}_{mix} = 1 - \left(1 - \sum_{i=1}^{n} E_i/n\right)^n$	$EC_{x,mix} = \omega_A * (CA) + \omega_B * (IA)$
variables	**n** = number of mixture components	**n** = number of mixture components	**n** = number of mixture components	**$EC_{x,mix}$** = mixture effect concentration
	E_i = effect of ith component in a mixture	**E_i** = effect of ith component in a mixture	**E_i** = effect of ith component in a mixture	**ω_A/ω_B** = weightings for the introduction of the concentration addition and independent effect (the weightings are calculated by molecular descriptors and fuzzy membership functions) [ωA=αsim=intrasimilarity / wB=αdis=intradissimilarity]
	E_{mix} = mixture effect	**E_{mix}** = mixture effect	**E_{mix}** = mixture effect	

model	Toxic Equivalents (TEQ)	Hazard Index (HI)	Margin of Exposure (MOE):
source	Safe, 1998	USEPA, 1986	EFSA, 2005
idea/ application	The individual substances act all in the same biological or toxic pathway.		Applied for risk assessment of genotoxic and carcinogenic substances in nutrition.
equation/ calculation	$TEQ = \sum(c_i \times TEF_i)$	$HI = E_1/AL_1 + E_2/AL_2 + \cdots + E_i/AL_i$	The MOE calculates the ratio between a predefined point on the dose-response curve for the adverse effect and human intake.
variables	**TEQ** = Toxic Equivalents	**HI** = Hazard Index	
	c_i = concentrations of the individual components	**E** = exposure level to the ith toxicant	
	TEF = Toxic Equivalency Factor	**AL** = maximum acceptable level for the ith toxicant	

C Determination of toxicity exposure ratios for the environmental risk assessment of selected spray series

Table 30: Risk Profiler for risk characterization of spray events/ series within the project - description of input data and calculations

Wider risk assessment areas: I: Birds and mammals, II: Aquatic, III: Non-target arthropods, IV: Soil organisms, V: Non-target plants.

Not included in description (as considered not relevant – in general or in a first approach – within the context of the project):

1. Secondary poisoning of birds and mammals (via consumption of earthworm/fish)
2. Aquatic risk assessment for exposure pathways total outflow (i.e. run-off + erosion) and drainage (PEC-sw, total outflow / PEC-sw, drainage)
3. Groundwater-relevant calculations (PEC-gw, run-off/drainage/ PEC-gw, leaching)
4. Multiple applications (multiple application factors, drift percentiles for multiple applications)
5. Accumulation over time in soil
6. Volatilization/deposition

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
I Birds dietary acute	• $DDD = SV_{90} \times AR$ • DDD = dietary daily dose (in mg a.s./kg bw/d) • SV_{90} = Shortcut value (90th percentile) of generic focal species relevant for the crop scenario/growth stage (cf. EFSA/2009/1438) • AR = application rate (in kg a.s./ha)	• LD_{50} (mg a.s./kg bw) • standard study (oral by gavage, 1 day), e.g. OECD 223 • lowest available LD_{50} (or geomean) if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	$TER_{acute} = LD_{50} / DDD$	10	EFSA/2009/1438: Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA", EFSA Journal 2009; 7(12):1438
I Birds dietary long-term	• $DDD = SV_{mean} \times TWA \times AR$ • DDD = dietary daily dose (in mg a.s./kg bw/d) • SV_{mean} = Shortcut value (mean) of generic focal species relevant for the crop scenario/growth stage (cf. EFSA/2009/1438)	• NO(A)EL (mg a.s./kg bw/d) • reproduction study (dietary exposure, several weeks), e.g. OECD 206 • lowest available ecologically relevant NO(A)EL	$TER_{long\ term}$ = NO(A)EL / DDD	5	EFSA/2009/1438: Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA", EFSA Journal 2009; 7(12):1438

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
	• TWA = factor for estimated time weighted average exposure (over 21 days, assuming a default DT_{50} of 10 days = 0.53) • AR = application rate (in kg a.s./ha)	• (typically no formulation data available)			
I Mammals dietary acute	• DDD = SV_{90} × AR • DDD = dietary daily dose (in mg a.s./kg bw/d) • SV_{90} = Shortcut value (90^{th} percentile) of generic focal species relevant for the crop scenario/growth stage (cf. EFSA/2009/1438) • AR = application rate (in kg a.s./ha)	• LD_{50} (mg a.s./kg bw) • standard study (oral by gavage/bolus, 1 day), e.g. OECD 401 • lowest available LD_{50} (or geomean) • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	TER_{acute} = LD_{50} / DDD	10	EFSA/2009/1438: Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA", EFSA Journal 2009; 7(12):1438
I Mammals dietary long-term	• DDD = SV_{mean} × TWA × AR • DDD = dietary daily dose (in mg a.s./kg bw/d) • SV_{mean} = Shortcut value (mean) of generic focal species relevant for the crop scenario/growth stage (cf. EFSA/2009/1438) • TWA = factor for estimated time weighted average exposure (over 21 days, assuming a default DT_{50} of 10 days = 0.53) • AR = application rate (in kg a.s./ha)	• NO(A)EL (mg a.s./kg bw/d) • Developmental study (oral by gavage/bolus, several weeks) or 1-/2-generation study (dietary exposure, several weeks), e.g. OECD 414/ OECD 416 • lowest available ecologically relevant NO(A)EL • (typically no formulation data available)	$TER_{long-term}$ = NO(A)EL / DDD	5	EFSA/2009/1438: Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA", EFSA Journal 2009; 7(12):1438
II Fish acute/ drift/runoff		• LC_{50} (µg a.s./L)	TER_{acute} = LC_{50} / PEC-sw_{drift}	100	EFSA (2013) Guidance on tiered risk assessment

Assessment area/organism group/ exposure scenario	**Exposure assessment – relevant input data and calculations**	**Effect assessment – relevant (eco)toxicity data**	**Risk assessment – calculation of risk quotient**	**Relevant decision criterion (assessment / uncertainty factor)**	**Relevant guidance document**
	• PEC-sw, drift = AR × Drift × Conversion Factor • PEC-sw, drift = predicted environmental concentration in edge-of-field surface waters resulting from spray drift at 1 m (arable crops) / 3 m (high crops) distance from edge of the field without risk mitigation measures, i.e. no buffer zone/ drift-reducing nozzles (in µg a.s./L) • AR = application rate (in kg a.s./ha; for formulation data with > 1 a.s. recalculate as sum of a.s.) • Drift = crop-specific drift values, 90%-ile for single application (in %) at 1 m (arable crops) / 3 m (high crops) distance from edge of the field • Conversion Factor = 333,33 (scenario: deposit on ditch surface 100 m × 1 m, 30 m^3 water volume stagnant in ditch) • PEC-sw, runoff = (Soil residues at rain event × Runoff)/Conversion factor • PEC-sw, runoff = predicted environmental concentration in edge-of-field surface waters resulting from runoff without risk mitigation measures, i.e. no vegetated buffer strips (in µg a.s./L)	• standard study (96 hours constant exposure), e.g. OECD 203 • several test species (e.g. Oncorhynchus mykiss) • lowest available LC_{50} • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	$TER_{acute} = LC_{50}$ / $PEC\text{-}sw_{runoff}$		for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J 11(7):3290, 268 Regulatory approach for drift exposure assessment for PPP authorization in Germany according to EVA3, available via: www.bvl.bund.de) based on drift values published by Rautmann et al. (2001). Regulatory approach for runoff exposure assessment for PPP authorization in Germany according to EXPOSIT 3.01, available via: www.bvl.de
II Fish long-term/drift/ runoff		• NOEC (µg a.s/L) • several study types with differing growth stages and exposure times, e.g. OECD 210 • several test species (e.g. Oncorhynchus mykiss) • lowest available (ecologically relevant) NOEC • (typically no formulation data available)	$TER_{long\text{-}term}$ = NOEC / $PEC\text{-}sw_{drift}$ $TER_{long\text{-}term}$ = NOEC / $PEC\text{-}sw_{runoff}$	10	
II Daphnia acute/ drift/runoff		• EC_{50} (µg a.s./L) • standard study (48 hours constant exposure), e.g. OECD 202 • standard test species: Daphnia magna • lowest available EC_{50} • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	$TER_{acute} = EC_{50}$ / $PEC\text{-}sw_{drift}$ $TER_{acute} = EC_{50}$ / $PEC\text{-}sw_{runoff}$	100	

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
II Daphnia long-term/drift/ runoff	• Soil residues at rain event (in g a.s./ha, 3 days following application) = AR × ((100-Interception/100) × EXP(-(LN(2)/$DT50_{soil}$) × 3) • Runoff = fraction of soil residues at rain event being washed off diluted in rainwater (in %), runoff potential determined by K_{OC} (L/kg) value (arithmetic mean of available values) of the a.s., assignment to respective K_{OC} class (12 classes defined) as tabulated in EXPOSIT 3.01 • AR = application rate (in kg a.s./ha; runoff calculation only for individual a.s. • Interception = fraction of application rate remaining on crop plants and thus not reaching the soil (in %), determined by crop type and growth stage (assignment according to FOCUS/ AppDate) • $DT50_{soil}$ = half-life for degradation of the a.s. in soil (SFO kinetics; in days), if field studies are available: worst case (non normalized) for representative sites;	• NOEC (µg a.s/L) • standard study (21 days constant exposure), e.g. OECD 211 • standard test species: Daphnia magna • lowest available (ecologically relevant) NOEC • (typically no formulation data available)	$TER_{long\text{-}term}$ = NOEC / PEC-sw_{drift} $TER_{long\text{-}term}$ = NOEC / PEC-sw_{runoff}	10	
II Aquatic invertebrate acute/drift/ runoff		• L/EC_{50} (µg a.s./L) • several study types differing exposure regimes/ times, e.g. US EPA Test Method 1007.0 • several test species, e.g. Americamysis bahia, Chironomus spec. • lowest available L/EC_{50} • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	TER_{acute} = L/EC_{50} / PEC-sw_{drift} TER_{acute} = L/EC_{50} / PEC-sw_{runoff}	100	
II Aquatic invertebrate long-term/drift/ runoff		• NOEC (µg a.s./L) • several study types with differing exposure regimes/ times, e.g. OECD 218 • several test species, e.g. Chironomus spec.	$TER_{long\text{-}term}$ = NOEC / PEC-sw_{drift} $TER_{long\text{-}term}$ = NOEC / PEC-sw_{runoff}	10	

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
	• if determined in standard lab testing (20°C, pF2), if ≥ 4 DT_{50}-values 90th percentile, if < 4 DT_{50}-values maximum; • Conversion factor = 260000 (scenario: rain event 3 days following application, 200 m^3 per ha within 24 hours, 50 % (100 m^3) runoff into adjacent ditch containing already 30 m^3, dilution by a factor of 2 due to slow flowing/ connected ditches)	• lowest available (ecologically relevant) NOEC • (typically no formulation data available)			
II Algae/drift/ runoff		• EC_{50} (µg a.s./L) • standard study (72-96 hours constant exposure), e.g. OECD 201 • several test species of green algae (e.g. Pseudokirchneriella subcapitata) as well as from other orders (e.g. diatoms) • lowest available EC_{50} (preferably calculated from growth rate, i.e. E_rC_{50}; otherwise E_bC_{50}) • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	$TER_{acute} = EC_{50} / PEC\text{-}SW_{drift}$ $TER_{acute} = EC_{50} / PEC\text{-}SW_{runoff}$	10	
II Aquatic macrophytes/ drift/runoff		• EC_{50} (µg a.s./L) • standard study (7 – 14 days constant exposure), e.g. OECD 221 • standard test species (Lemna spec.) or several other species (e.g. Myriophyllum spec.) • lowest available EC_{50} (preferably calculated from growth rate, i.e. E_rC_{50}; otherwise E_bC_{50})	$TER_{acute} = EC_{50} / PEC\text{-}SW_{drift}$ $TER_{acute} = EC_{50} / PEC\text{-}SW_{runoff}$	10	

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
		• if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)			
II RAC/drift/ runoff		• RAC = Regulatory acceptable concentration (in µg a.s./L), a.s. specific regulatory threshold covering both acute and chronic effects, derived by UBA reflecting the entire knowledge/ data set available for a.s. (i.e. including lower and higher-tier data), taking remaining uncertainties of the respective effect assessment into account by an appropriate assessment factor (i.e. RAC = decisive toxicity endpoint in µg a.s./L divided by assessment factor) • If decisive toxicity endpoint derived from formulation data, re-calculate to a.s.	TER = RAC / PEC-sw_{drift} TER = RAC / PEC-sw_{runoff}	1	
III Honey bees oral	Maximum single application rate (g a.s./ha)	• LD_{50} oral (µg a.s./bee) • standard study (48 hours, oral exposure), e.g. OECD 213 • standard test species: Apis mellifera • lowest available LD_{50}	HQ oral = Maximum single application rate / LD_{50} oral	50	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
		• if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)			on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC)
III Honey bees contact	Maximum single application rate (g a.s./ha)	• LD_{50} contact (µg a.s./bee) • standard study (48 hours, topical contact exposure), e.g. OECD 214 • standard test species: Apis mellifera • lowest available LD_{50} • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	HQ contact = Maximum single application rate / LD_{50} contact	50	
III Terrestrial non-target arthropods – standard lab/in-field	Application rate (g a.s./ha)	• LR_{50} (g a.s./ha) • standard study (exposure via dried residues on glass plates), e.g. according to Mead-Briggs et al./ IOBC Guideline for non-target arthropods, 2000 • standard test species: Aphidius rhopalosiphi (48 h exposure mortality + 10-12 days observation of reproduction of surviving females) • *Typhlodromus pyri* (7 days exposure mortality + 7 days observation of reproduction of surviving females)	HQ in-field = Application rate / LR_{50}	2	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC)

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
		• lowest available LR_{50} from dose-response-test if available, otherwise estimate LR_{50} from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)			
III Terrestrial non-target arthropods – standard lab/off-field	• PERoff-field = AR × (Drift/100) • PERoff-field = predicted environmental rate in edge-of-field terrestrial non-target habitats resulting from spray drift without risk mitigation measures at 1 m (arable crops) / 3 m (high crops) distance, i.e. no buffer zone/ drift-reducing nozzles (in g a.s./ha) • AR = application rate (in kg a.s./ha; for formulation data with > 1 a.s. recalculate as sum of a.s.) • Drift = crop-specific drift values, 90%-ile for single application (in %) at 1 m (arable crops) / 3 m (orchards) distance from edge of the field	• LR_{50} (g a.s./ha) • standard study (exposure via dried residues on glass plates), e.g. according to Mead-Briggs et al./ IOBC Guideline for non-target arthropods, 2000 • standard test species: *Aphidius rhopalosiphi* (48 h exposure mortality + 10-12 days observation of reproduction of surviving females) • *Typhlodromus pyri* (7 days exposure mortality + 7 days observation of reproduction of surviving females) • lowest available LR_{50} from dose-response-test if available, otherwise estimate LR_{50} from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	HQ off-field = PERoff-field / LR_{50}	2	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC)

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
III Terrestrial non-target arthropods – extended lab/in field	Application rate (g a.s./ha)	• $L(E)R_{50}$ (g a.s./ha) • extended lab study (e.g. exposure via dried residues on natural substrates, 2-dimensional detached plant leave design or 3-dimensional whole plant design) or (semi-)field study, e.g. according to IOBC Guideline for non-target arthropods, 2000 • conducted with standard (*Aphidius rhopalosiphi*, *Typhlodromus pyri*) and several other species (e.g. Chrysoperla carnea, Coccinella septempunctata, etc.) or natural populations (field studies) • exposure design /study duration differing, assessment of both mortality (LR_{50}) and effects on reproduction/ sublethal toxicity (ER_{50}) • lowest available $L(E)R_{50}$ from dose-response-test if available, otherwise estimate $L(E)R_{50}$ from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	Risk Quotient $PER/L(E)R_{50}$ in-field = Application rate / $L(E)R_{50}$	1	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC)

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
III Terrestrial non-target arthropods – extended lab/ off-field	• PERoff-field = (AR × (Drift/100))/(vegetation distribution factor) • PERoff-field = predicted environmental rate in edge-of-field terrestrial non-target habitats resulting from spray drift without risk mitigation measures at 1 m (arable crops) / 3 m (high crops) distance, i.e. no buffer zone/ drift-reducing nozzles (in g a.s./ha) • AR = application rate (in kg a.s./ha; for formulation data with > 1 a.s. recalculate as sum of a.s.) • Drift = crop-specific drift values, 90%-ile for single application (in %) at 1 m (arable crops) / 3 m (high crops) distance from edge of the field • Vegetation distribution factor = factor of 5, accounting for difference in exposure between toxicity studies with 2-dimensional design and more realistic 3-dimensional plant structures in off-field habitats (i.e. dilution of drift input); not applicable if toxicity study with 3-dimensional design	• $L(E)R_{50}$ (g a.s./ha) • extended lab study (e.g. exposure via dried residues on natural substrates, 2-dimensional detached plant leave design or 3-dimensional whole plant design) or (semi-)field study, e.g. according to IOBC Guideline for non-target arthropods, 2000 • conducted with standard (*Aphidius rhopalosiphi*, *Typhlodromus pyri*) and several other species (e.g. Chrysoperla carnea, Coccinella septempunctata, etc.) or natural populations (field studies) • exposure design /study duration differing, assessment of both mortality (LR_{50}) and effects on reproduction/ sublethal toxicity (ER_{50}) • lowest available $L(E)R_{50}$ from dose-response-test if available, otherwise estimate $L(E)R_{50}$ from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	TER = $L(E)R_{50}$ / PERoff-field	5	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC) Regulatory approach for NTA risk assessment for PPP authorization in Germany (TER-approach, vdf): Schulte et al. UWSF (5) 261-266 (1999), Bewertungskriterien des Umweltbundesamtes: Auswirkungen von Pflanzenschutzmitteln auf terrestrische Arthropoden.

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
IV Earthworm long-term	• $PEC\text{-}soil_{ini}$ = (AR × (100-Interception/100)) × (Conversion factor/Soil layer) • $PEC\text{-}soil_{ini}$= predicted environmental concentration in soils (in-field) immediately following application (i.e. realistic worst-case initial soil concentration), in mg a.s./kg dry weight • AR = application rate (in kg a.s./ha • Interception = fraction of application rate remaining on crop plants and thus not reaching the soil (in %), determined by crop type and growth stage (assignment according to FOCUS/ AppDate) • Conversion factor (normalized for 1 cm soil layer depth) = 0.0066667 (scenario: application area 1 ha, soil density 1.5 kg/L, equal distribution within soil layer) • Soil layer = Relevant soil layer for PEC-calculation, dependent on the soil-adsorption behavior of the a.s. (i.e. K_{FOC} value: arithmetic mean [German approach: default value of 2,5 cm; if K_{FOC} value > 500: 1 cm])	• NOEC (mg a.s./kg dry weight) • standard study (56 days), e.g. OECD 222 • standard test species: Eisenia fetida • lowest available (ecologically relevant) NOEC • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.) • if $\log K_{ow}$ of a.s. > 2 AND 10 % peat in OECD artificial soil: divide NOEC value by a factor of 2 (correction for unrealistically high organic carbon content, and thus reduced bioavailability compared to natural soils); if formulation data, apply correction, if “toxicity driving” a.s. has $\log K_{ow}$ > 2	TER = NOEC / $PEC\text{-}soil_{ini}$	5	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC) Regulatory approach for risk assessment for non-target soil organism (earthworm) for PPP authorization in Germany

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
IV Soil arthropods long-term	• $PEC\text{-}soil_{ini}$ = (AR × (100-Interception/100)) × (Conversion factor/Soil layer) • $PEC\text{-}soil_{ini}$= predicted environmental concentration in soils (in-field) immediately following application (i.e. realistic worst-case initial soil concentration), in mg a.s./kg dry weight • AR = application rate (in kg a.s./ha • Interception = fraction of application rate remaining on crop plants and thus not reaching the soil (in %), determined by crop type and growth stage (assignment according to FOCUS/ AppDate) • Conversion factor (normalized for 1 cm soil layer depth) = 0.0066667 (scenario: application area 1 ha, soil density 1.5 kg/L, equal distribution within soil layer depth) • Soil layer = Relevant soil layer for PEC-calculation, dependent on the soil-adsorption behavior of the a.s. (i.e. K_{FOC} value: arithmetic mean [German approach: default value of 2,5 cm; if K_{FOC} value > 500: 1 cm])	• NOEC (mg a.s./kg dry weight) • standard study (28 days/ 14 days), e.g. OECD 226/ OECD 232 • standard test species: Folsomia candida/ Hypoaspis aculeifer • lowest available (ecologically relevant) NOEC • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.) • if $logK_{ow}$ of a.s. > 2 AND 10 % peat in OECD artificial soil: divide NOEC value by a factor of 2 (correction for not realistic high organic carbon content, and thus reduced bioavailability compared to natural soils); if formulation data, apply correction, if “toxicity driving” a.s. has $logK_{ow}$ > 2	TER = NOEC / $PEC\text{-}soil_{ini}$	5	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC) Regulatory approach for risk assessment for non-target soil organism (arthropods) for PPP authorization in Germany

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
V Non-target terrestrial plants – seedling emergence	• PERoff-field = AR × (Drift/100) • PERoff-field = predicted environmental rate in edge-of-field terrestrial non-target habitats resulting from spray drift without risk mitigation measures at 1 m (arable crops) / 3 m (high crops) distance, i.e. no buffer zone/ drift-reducing nozzles (in g a.s./ha) • AR = application rate (in kg a.s./ha; for formulation data with > 1 a.s. recalculate as sum of a.s.) • Drift = crop-specific drift values, 90%-ile for single application (in %) at 1 m (arable crops) / 3 m (high crops) distance from edge of the field field	• ER_{50} (g a.s./ha) • standard study (14 – 21 days), e.g. OECD 208 • several standard (crop) test species (at least 6 species including mono- and dikotyledones) • lowest available ER_{50} from dose-response-test if available, otherwise estimate ER_{50} from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)	TER = ER_{50} / PERoff-field	10	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC) Regulatory approach for risk assessment for non-target terrestrial plants for PPP authorization in Germany: Füll et al. UWSF (11) 145-149 (1999), Prüfanforderungen des Umweltbundesamtes zur Bewertung der Auswirkungen von Pflanzenschutzmitteln auf terrestrische Pflanzen.
V Non-target terrestrial plants – vegetative vigour	• PERoff-field = AR × (Drift/100) • PERoff-field = predicted environmental rate in edge-of-field terrestrial non-target habitats resulting from spray drift without risk mitigation measures	• ER_{50} (g a.s./ha) • standard study (28 days), e.g. OECD 227	TER = ER_{50} / PERoff-field	10	EU Commission SANCO/10329/2002 rev 2 final (Guidance Document on Terrestrial Ecotoxicology

Assessment area/organism group/ exposure scenario	Exposure assessment – relevant input data and calculations	Effect assessment – relevant (eco)toxicity data	Risk assessment – calculation of risk quotient	Relevant decision criterion (assessment / uncertainty factor)	Relevant guidance document
	at 1 m (arable crops) / 3 m (high crops) distance, i.e. no buffer zone/ drift-reducing nozzles (in g a.s./ha) • AR = application rate (in kg a.s./ha; for formulation data with > 1 a.s. recalculate as sum of a.s.) • Drift = crop-specific drift values, 90%-ile for single application (in %) at 1 m (arable crops) / 3 m (high crops) distance from edge of the field field	• several standard (crop) test species (at least 6 species including mono- and dikotyledones) • lowest available ER_{50} from dose-response-test if available, otherwise estimate ER_{50} from limit-test • if formulation data, re-calculate to a.s. (in case > 1 a.s. calculate as sum of a.s.)			Under Council Directive 91/414/EEC) Regulatory approach for risk assessment for non-target terrestrial plants for PPP authorization in Germany: Füll et al. UWSF (11) 145-149 (1999), Prüfanforderungen des Umweltbundesamtes zur Bewertung der Auswirkungen von Pflanzenschutzmitteln auf terrestrische Pflanzen.

Table 31: Overview on the applied risk management factors for drift and runoff

Drift reduction was further differentiated for apple (early and late applications) and arable crops. The factor represents the most effective required risk reduction as the best case. The algorithm of calculating managed TER-values searches for the highest reduction factor within a tank mixture; T = terrestrial, A = aquatic, Oilseed rape = OR, apple = AP.

PPP	Indication	Active substance	Drift/ Apple/ early / T	Drift/ Apple/ late / T	Drift/ Arable/ T	Drift/ Apple/ early / A	Drift/ Apple/ late / A	Drift/ Arable/ A	Runoff/ A
Acanto	OR	Picoxystrobin	1.000	1.000	1.000	0.100	0.100	0.051	1.000
Agil-S	OR	Propaquizafop	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Benocap	AP	Flusilazol	1.000	1.000	1.000	0.095	0.069	0.054	1.000
Biscaya	OR	Thiacloprid	1.000	1.000	1.000	0.100	0.100	0.100	1.000
Bulldock	OR	beta-Cyfluthrin	0.100	0.100	0.100	0.068	0.053	0.021	1.000
Butisan Kombi	OR	Metazachlor, Dimethenamid-P	0.500	0.500	0.500	0.100	0.100	0.100	0.200
Calypso	AP	Thiacloprid	0.100	0.100	0.100	0.009	0.007	0.005	0.400
Cantus	OR	Boscalid	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cantus Gold	OR	Boscalid, Dimoxystrobin	1.000	1.000	1.000	0.100	0.100	0.100	1.000
Carax	OR	Mepiquat, Metconazol	1.000	1.000	1.000	0.681	0.535	0.206	1.000
Chorus	AP	Cyprodinil	1.000	1.000	1.000	0.019	0.012	0.007	0.200
Coragen	AP	Chlorantraniliprole	0.500	0.500	0.206	0.047	0.029	0.018	1.000
Cythrin 250 EC	OR	Cypermethrin	0.068	0.053	0.021	0.040	0.023	0.010	1.000
Delan WG	AP	Dithianon	0.053	1.000	1.000	0.009	0.007	0.005	1.000
Dithane NeoTec	AP	Mancozeb	1.000	1.000	1.000	0.095	0.069	0.054	1.000

PPP	Indication	Active substance	Drift/ Apple/ early / T	Drift/ Apple/ late / T	Drift/ Arable/ T	Drift/ Apple/ early / A	Drift/ Apple/ late / A	Drift/ Arable/ A	Runoff/ A
Dithane Ultra WP	AP	Mancozeb	1.000	1.000	1.000	0.095	0.069	0.054	1.000
Effigo	OR	Picloram, Clopyralid	0.500	0.500	0.500	1.000	1.000	1.000	1.000
Envidor	AP	Spirodiclofen	0.170	0.134	0.051	0.100	0.100	0.051	1.000
Flint	AP	Trifloxystrobin	0.500	0.500	0.500	0.095	0.057	0.026	0.400
Folicur	OR	Tebuconazol	0.500	0.500	0.500	0.068	0.053	0.021	0.400
Fuego	OR	Metazachlor	0.250	0.250	0.250	0.100	0.100	0.100	0.200
Harvesan	OR	Carbendazim, Flusilazole	1.000	1.000	1.000	0.681	0.535	0.206	1.000
Insegar	AP	Fenoxycarb	0.100	0.100	0.100	0.009	0.007	0.005	1.000
Kumulus WG	AP	Schwefel	0.068	0.053	0.021	0.048	0.029	0.018	1.000
Malvin WG	AP	Captan	1.000	1.000	1.000	0.019	0.012	0.007	1.000
Merpan 80 WDG	AP	Captan	1.000	1.000	1.000	0.068	0.053	0.021	0.200
Netzschwefel	AP	Schwefel	0.068	0.053	0.021	0.068	0.053	0.021	1.000
Nimbus CS	OR	Metazachlor, Clomazone	0.100	0.100	0.100	0.100	0.100	0.100	0.200
Plenum 50 WG	OR	Pymetrozin	0.500	0.500	0.500	1.000	1.000	1.000	1.000
Propulse	OR	Prothioconazol, Fluopyram	1.000	1.000	1.000	0.681	0.535	0.206	1.000
Reldan 22	OR, AP	Chlorpyrifos-methyl	0.068	0.053	0.021	0.009	0.007	0.005	0.200

PPP	Indication	Active substance	Drift/ Apple/ early / T	Drift/ Apple/ late / T	Drift/ Arable/ T	Drift/ Apple/ early / A	Drift/ Apple/ late / A	Drift/ Arable/ A	Runoff/ A
Runner	AP	Methoxyfenozide	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Scala	AP	Pyrimethanil	0.500	0.500	0.206	0.068	0.053	0.021	1.000
Score	AP	Difenoconazol	1.000	1.000	1.000	0.019	0.012	0.007	1.000
Select 240 EC	OR	Clethodim	0.250	0.250	0.250	0.068	0.053	0.021	0.200