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Plant protection products in agricultural fields – residues in earthworms and assessment of potentially toxic effects to the environment

Sredstva za zaštitu bilja na poljoprivrednim površinama – rezidue u gujavicama i procjena potencijalno toksičnih učinaka na okoliš

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ABSTRACT

The environmental risk assessment of plant protection products for soil organisms is mainly based on the results of laboratory and extended laboratory studies while the link from the laboratory to realistic field conditions over several seasons is not well established. The current environmental risk assessment is applied to a single active ingredient and does not consider that soil organisms are exposed to varying degrees to a mixture of active ingredients from different pesticides. In this study, earthworm samples were collected from eight fields in Croatia during two growing seasons and analyzed for 300 active ingredients. The concentrations of 26 analyzed active ingredients ranged between 0.000 and 0.247 mg/kg earthworm fresh weight with a mean of 0.005 mg/kg earthworm fresh weight. The percentage of samples with values below the limit of detection (LOD = ½ LOQ), values below the limit of quantification (LOQ = 0.001 mg/kg) and values above LOQ were 33, 44 and 23 %, respectively. Based on publicly available draft assessment reports from European Commission and European Food Safety Authority, degradation parameters (DT_{50}, DT_{90}) were used to calculate degradation curves and the current concentration in soil at the date of earthworm sampling. Subsequently, compound-specific bioconcentration factors in soil were determined by dividing the analyzed pesticide residues in earthworms by the calculated concentrations in soil. The results of the study showed that most active ingredients do not pose a risk to earthworms and have no secondary poisoning potential to birds and mammals that feed on them. The retrospective analysis method of analytically measured neonicotinoid residues in earthworm samples can be reliably used to calculate degradation and concentration curves in soil at the time of sampling.

Keywords: bioaccumulation, bioconcentration factors, earthworms, environmental risk assessment, pesticide residues, secondary poisoning, toxicity-exposure ratio

SAŽETAK

Procjena ekološkog rizika sredstava za zaštitu bilja za organizme u tlu uglavnom se temelji na rezultatima laboratorijskih i proširenih laboratorijskih studija dok veza između laboratorija i realnih poljskih uvjeta tijekom nekoliko sezona nije dobro utvrđena. Trenutna procjena rizika za okoliš primjenjuje se na pojedinačne aktivne tvari i ne uzima u obzir da su organizmi u tlu izloženi mješavini mješavini aktivnih tvari različitih pesticida. U istraživanju su prikupljeni uzorci gujavica s osam polja u Hrvatskoj tijekom dvije vegetacijske sezone. Analizirani su na 300 aktivnih tvari. Koncentracije 26 analiziranih aktivnih tvari kretale su se od 0,000 do 0,247 mg/kg svježe mase gujavica sa srednjom vrijednosti od

0,005 mg/kg svježe mase gujavica. Postotak uzoraka s vrijednostima ispod granice detekcije (LOD = ½ LOQ), vrijednosti ispod granice kvantifikacije (LOQ = 0,001 mg/kg) i vrijednosti iznad LOQ iznosio je 33, 44 and 23%. Na temelju javno dostupnih nacrta izvješća o procjeni Europske komisije i Europske agencije za sigurnost hrane, parametri degradacije (DT_{50}, DT_{90}) korišteni su za izračunavanje krivulja razgradnje i koncentracije u tlu u vrijeme uzorkovanja gujavica. Potom su određeni faktori biokoncentracije specifičnih za spoj u tlu dijeljenjem analiziranih ostataka pesticida u gujavicama s izračunatim koncentracijama u tlu. Rezultati istraživanja pokazali su da većina aktivnih tvari ne predstavlja rizik za gujavice i nema sekundarni potencijal trovanja za ptice i sisavce koji se njima hrane. Metoda retrospektivne analize analitički izmjerenih rezidua neonikotinoida u uzorcima gujavica može se pouzdano koristiti za izračunavanje krivulja razgradnje i koncentracije u tlu u vrijeme uzorkovanja.

Ključne riječi: bioakumulacija, faktori biokoncentracije, gujavice, procjena rizika za okoliš, ostaci pesticida, sekundarno trovanje, omjer toksičnosti i izloženosti

INTRODUCTION

The environmental risk assessment of plant protection products on invertebrate soil organisms is based on the European Commission (EC) Guidance Document on Terrestrial Ecotoxicology (EC, 2002) and EC Regulation No 1107 (EC, 2009) with additional recommendations given by the European Food Safety Authority (EFSA) Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (Ockleford et al., 2017). In principle, acute and chronic effects of the active ingredient of a plant protection product or the plant protection product itself are tested by exposing a few soil species to treated artificial soil. If the toxicity-exposure ratio (TER) does not exceed the defined trigger value, the active ingredient in question is not considered to pose an unacceptable risk to soil organisms. Otherwise, the exposure scenario must be refined, or higher tier tests must be performed (e.g., terrestrial mesocosm or earthworm field studies) to study the potential impact of an active ingredient under more natural conditions. Only with the evidence of no effects at one level of the tiered testing approach, the active ingredient is allowed to be placed on market and used in the field following the recommended use pattern dependent on the crop species (Ockleford et al., 2017).

Whereas monitoring of a medicine after its approval (pharmacovigilance) is a requirement of the European Medicine Agency (Küster and Adler, 2014), post-registration monitoring of plant protection products (PPP's) is still not strictly required (Vijver et al., 2017). According to Hernandez-Jeret et al. (2021) if refined approaches have been used in the risk assessment of metal-containing PPP's, post-registration monitoring and controlled long-term studies should be conducted and assessed. For PPP's, residue data from monitoring studies in soil are rare in comparison to aquatic systems (Hommen et al., 2004, Rosenbom et al., 2016). In the case of heavy metals and a few persistent organic chemicals, historical data from permanent study fields are available (German Environmental Specimen Bank, 2018) and document the time series of concentrations in different matrices such as soil and earthworms. However, samples from different matrices are often not taken from the same site at the same time and cannot be compared directly, e.g., for using soil concentrations and earthworm concentrations to calculate bioaccumulation factors. Some monitoring projects measured soil biodiversity in relation to general land use pattern and not specifically dependent on soil concentrations of PPP's (Rutgers et al., 2009).

In this study, monitoring data on residues in earthworms are available from a two-year investigation in agricultural fields in Croatia, although most active ingredients were not analytically determined in the corresponding soils. Since the data on application time and amount of applied PPP's were delivered by the farmers, a retrospective analysis of analytically measured residues in earthworms and re-calculated soil concentrations was performed with the aim to answer the following questions:

a) can the concentrations of active ingredients in soil be reliably calculated based on information from farmers and soil dissipation studies from publicly available assessment reports;

b) are the "hybrid" bioaccumulation factors, calculated by using analytically measured residues in earthworms and recalculated soil concentrations, comparable to literature data;

c) are the "hybrid" bioaccumulation factors suitable for the assessment of the potential for secondary poisoning;

d) d) are the recalculated soil concentrations of active ingredients suitable for the assessment of their potential risk to the earthworms.

MATERIALS AND METHODS

Field site and cultivation

Four fields in each of two investigated regions (Tovarnik, Lukač) in Croatia were cultivated with alternative crops according to good agricultural practices in 2015 and 2016. The predominant crops were wheat, maize, or sugar beet. In the two seasons, 2 – 16 different pesticides were applied per field, namely 2 – 10 herbicides, 2 – 9 fungicides, and 0 – 6 insecticide active ingredients. Farmers provided information on the name of the pesticide used, application rate in the case of spray application or seed density in the case of sowing treated seed, as well as time of application.

Earthworm sampling and residue analysis

Earthworms were sampled three times during the two seasons (autumn 2015, spring 2016., and autumn 2016) following the sampling method of ISO 23611-1 (2006). The fresh weight of the earthworm samples was 5-17 g/sample. The earthworm samples were deep frozen until analysis. Analysis was done by liquid chromatography-tandem mass spectrometry (LC-MS/MS), with so called "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged and Safe) pre-treatment sample purification method (Anastassiades et al., 2003). Limit of quantification (LOQ) was 0.001 mg/kg in case of earthworm fresh weight and limit of detection (LOD) = ½ LOQ. LC-MS/MS is one of the most widely used techniques for pesticide multiresidue analysis in food due to their high sensitivity and selectivity and their ability to screen many pesticides from different chemical classes in a very complex matrix in a single run. LC-MS/MS is suitable for both more polar pesticides and pesticide metabolites, which are often more polar and less volatile than the pesticide itself (Stachniuk and Fornal, 2016).

Recalculation of soil concentrations

Substance specific dissipation curves in soil were calculated by using soil concentrations at DT_0, DT_{50} and DT_{90} (DT = dissipation time when 0, 50 and 90% of the substance has dissipated from the soil). The soil concentration at DT_0 was derived from the application rate on a study field by converting the application rate (g a.i./ha) to soil concentration (mg a.i./kg dry soil), considering the soil density of 1.5 g/cm^3 and a soil depth of 30 cm. The values for DT_{50} and DT_{90} were taken from data of soil dissipation field studies, publicly available in EC review reports for active substances (1998 – 2016) and EFSA scientific reports on conclusion on the peer review of active substances (2005 – 2016). Based on the soil concentration at DT_0, the soil concentrations at DT_{50} (i.e., 50% of soil concentration at DT_0) and DT_{90} (i.e., 10% of soil concentration) were derived, and the three soil concentrations at three different times were used for construction of a logarithmic dissipation curve following the formula

$$y = a * e^{(-b * x)}$$

y = concentration in soil at day x; a = soil concentration at day 0; b = substance – specific slope; x = time after application.

Calculation of bioaccumulation/bioconcentration factors

Bioaccumulation is the general uptake and storage of substances, while uptake from the surrounding medium as part of bioaccumulation is defined as bioconcentration (Franke et al., 1994, Fent, 2013).

Bioconcentration is a measure of the amount of pesticide residues in an organism's tissues relative to the concentration in the organism's environment (Zartarian and Schultz, 2009). This includes the uptake of pesticides through respiration and contact, but not through food sources. Bioconcentration factors (BCF) are calculated by considering pesticide tissue concentrations relative to pesticide concentrations in the environment.

BCF Values > 1 indicate that the concentration in the organism is higher than that of the medium (e.g., soil or water) from which the pesticide was taken (USEPA, 2021). In this study, bioconcentration cannot be separated from bioaccumulation, so the two terms are used interchangeably. The ratio of concentration in earthworms and concentration in soil was defined as bioconcentration factor. For nine active ingredients, data from investigated fields allowed the calculation of bioconcentration factor, using analyzed residues in earthworms and recalculated soil concentrations.

Assessment of potential for secondary poisoning

Secondary poisoning is defined by the transfer of the active ingredient within the food chain from earthworms to earthworm-eating birds and mammals. The assessment of the potential for secondary poisoning followed EFSA (2009) procedure. In a five-step calculation scheme, the predicted environmental concentration in soil (PEC_{soil}) was determined. In EFSA (2009), the theoretical bioconcentration factor for earthworms ($BCF_{earthworm}$) is calculated using the substance-specific partition coefficient in octanol/water (as a measure of lipophilicity) and the substance-specific partition coefficient in soil organic carbon/water (as a measure of adsorption). In this study the bioconcentration factor can be derived from the ratio of measured residues in earthworms and the calculated soil concentration at the time of sampling. The residues in earthworms as predicted environmental concentrations (PECearthworm) were estimated by multiplying PEC_{soil} and $BCF_{earthworm}$. The estimated residues in earthworms were converted to daily consumption doses for birds (factor 1.05) and mammals (factor 1.28) and finally toxicity-exposure ratios ($TER_{seconday\ poisoning}$) were calculated by using No-Oberserved-Adverse-Effect-Levels (NOAEL) from chronic dietary studies with birds and mammals, taken from the above-mentioned EC review reports (1998 – 2016) and EFSA scientific reports (2005 – 2016). The calculated daily consumption doses for birds and mammals. $TER_{seconday\ poisoning}$ values < 5 indicate a potential risk for secondary poisoning and would require further refinement.

Assessment of potentially toxic effects to earthworms

Data on laboratory reproduction tests with the compost earthworm *Eisenia fetida* Savigny were available from above mentioned EC review reports (1998 – 2016) and EFSA scientific reports (2005 – 2016). The toxicity endpoint was the No-Observed-Effect-Concentration (NOEC) where the number of juvenile worms did not significantly differ from the control. A toxicity-exposure ratio (TER_{worm}) was calculated by using the NOEC from the worm reproduction test and the soil concentration at the time of application. TER_{worm} values < 5 indicate a potential risk to earthworms and would require further refinement.

RESULTS AND DISCUSSION

Residues found in earthworms

The sampling resulted in 58 individual earthworm samples over the two-year investigation. The fresh weight of the samples was 5 -17 g/sample. Screening for 300 active ingredients was performed for each sample, of which 26 active ingredients were detected (9%). From 1566 analytical measurements, 33.2 % were < LOD, 43.5 % between LOD and LOQ and 23.3 % ≥ LOQ (Table 1). Three active ingredients, boscalid, fipronil, and difenoconazole, were detected, although farmers reported that they had not been applied during the two study years and were residues from previous year's applications.

Seven active ingredients were detected in 100% of earthworm samples (i.e. the insecticides imidacloprid, clothianidin and thiamethoxam, the fungicides azoxystrobin and cyproconazole, the herbicides tembotrione and ethofumesate) (Figure 1).

Table 1. Residues of active ingredients in earthworm samples 2015 and 2016

Number of analysed earthworm samples, Location: municipality Lukač, Croatia (45.8739° N, 17.4191° E)	34
Number of analysed earthworm samples, Location: municipality Tovarnik, Croatia (45.1649° N, 19.1522° E)	24
Earthworm number per sample	2-62 (mean ± SD: 18.4 ± 11.4)
Earthworm fresh weight per sample	5.0 – 27.0 g (mean ± SD: 10.1 ± 4.6 g)
Limit of quantification (LOQ)	0.001 mg/kg earthworm fresh weight
Limit of detection (LOD = ½ LOQ)	0.0005 mg/kg earthworm fresh weight
No. active ingredients (a.i.s) analysed	300
No. active ingredients detected	26
No. analytical measurements	1566
• Percentage < LOD	33.2
• Percentage between LOD and LOQ	43.5
• Percentage ≥ LOQ	23.3
No. a.i.s detected in one earthworm sample	12 - 20
No. a.i.s quantified in one earthworm sample	3 - 12

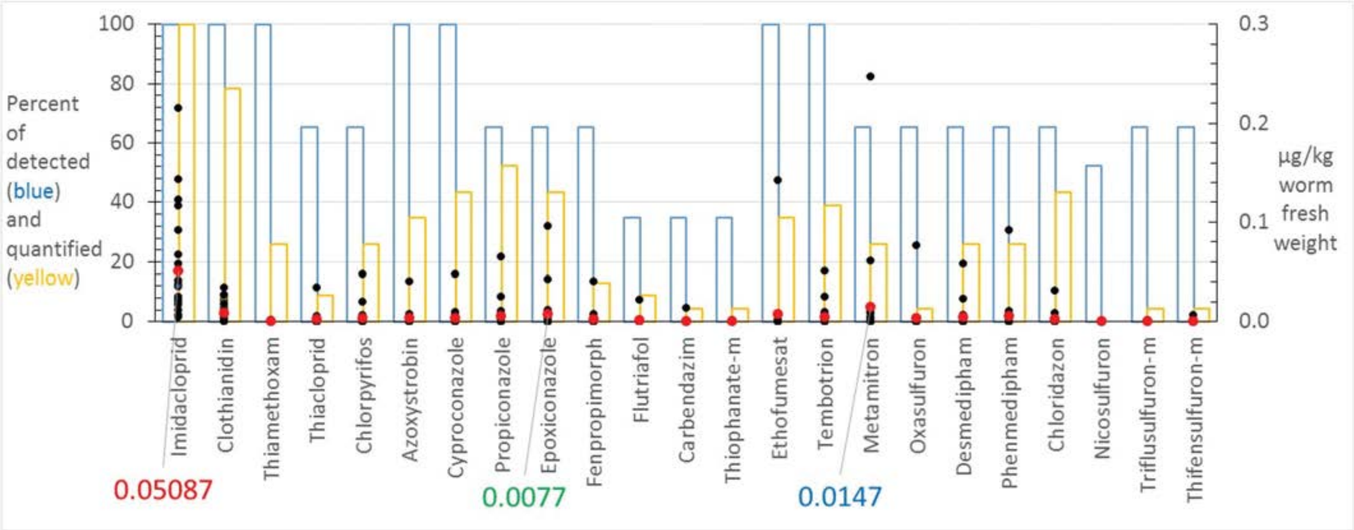

Figure 1. Frequency of detection and concentration of 23 active ingredients analyzed in earthworm samples from four plots of two fields (Lukač, Tovarnik) during three sampling dates (blue and yellow bars represent the percent of detection and quantification. Black dots denote individual concentrations per plot (only one subplot sampled on one sampling date) or mean concentrations per plot (2-4 subplots sampled on one sampling date. Red dots denote the overall mean of the analyzed concentrations)

Imidacloprid was the only active ingredient which was quantified in all earthworm samples. The highest mean concentrations of an insecticide, fungicide, and herbicide in one plot were: 0.05087 mg imidacloprid/kg earthworm fresh weight, 0.0147 mg metamitron/kg earthworm fresh weight and 0.0077 mg epoxiconazole/kg earthworm fresh weight, respectively.

Recalculation of soil concentrations

The dissipation behaviour of active ingredients in soil was calculated by using following data points: the soil concentration at the time of application as soil concentration at DT_0 and the soil concentrations at DT_{50} and DT_{90}, taken from EC reports (1998 – 2016) and EFSA reports (2005 – 2016). Examples are presented as Figure 2 for the herbicide ethofumesate, fungicide azoxystrobin and the insecticide imidacloprid. The coefficient of determination R^2 was > 0.95 for the majority of the active ingredients indicating that the used dissipation formula was reliable for estimating the soil concentration of an active ingredient at any time after application.

Calculation of bioaccumulation factors

Dividing the analysed residues of an active ingredient in earthworm samples by its corresponding calculated soil concentration at the time of earthworm sampling results in a ratio, the bioconcentration factor (BCF) (Figure 3). BCF values > 1.0 indicate an accumulation within the earthworms. For nine active ingredients, a variation of plot-spectific BCF values below and above the trigger value of 1.0 is observed. Therefore, the potential for bioconcentration cannot be considered as straight-forward but seems to depend on plots characteristics and the time between application and sampling. For imidaclopid, thiamethoxam, metamitron and phenmediphamthe the mean BCF value is > 1.0.

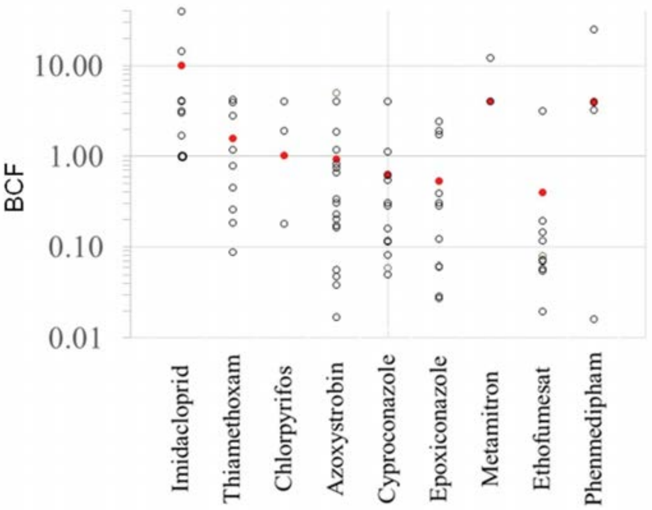

Figure 3. Bioconcentration factors (BCF) of nine active ingredients in earthworm samples derived from calculated soil concentrations at the time of sampling (white dots indicate BCF values from individual field plots; red dots indicate the resulting mean value)

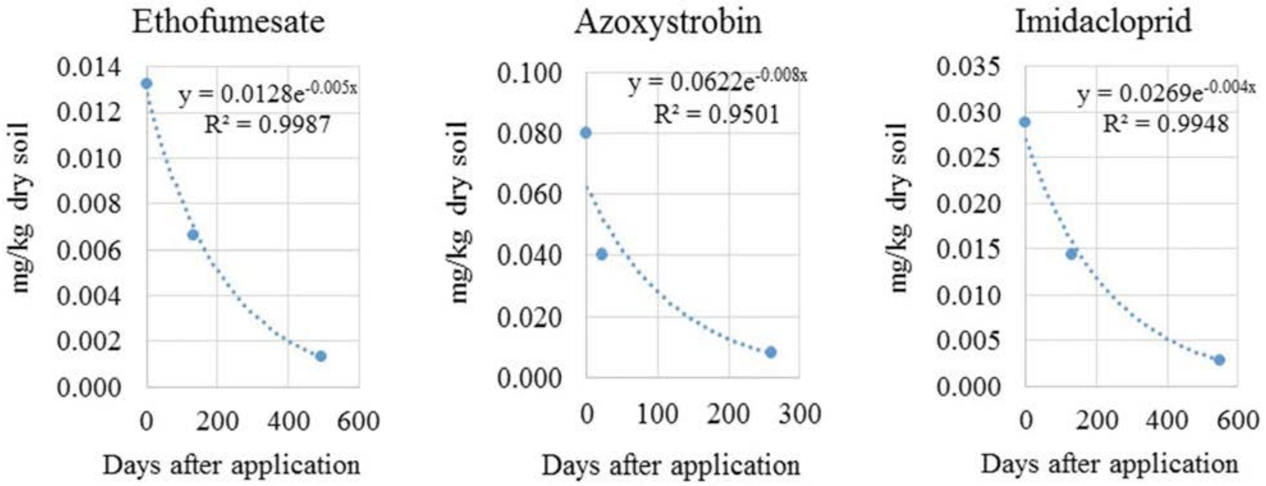

Figure 2. Three examples of soil dissipation curves for the active ingredients ethofumesate (left figure), azoxystrobin (middle figure) and imidacloprid (right figure)

The calculated BCF values of this study are comparable to values from the literature, as shown for imidacloprid (BCF = 15, Chevillot et al. 2017), thiamethoxam (BCF = 1-2, Douglas et al. 2015), azoxystrobin (BCF = low risk, EFSA 2009) and ethofumesate (BCF = 2.2, Xu et al. 2014).

Therefore, the information of farmers regarding the actual application rate and application time of a product is highly valuable for the calculation of the soil concentration at a specific time after the application and can be used for the calculation of bioconcentration factors.

Assessment of potential for secondary poisoning

Earthworms are considered as potential prey for mammals and birds. According to EFSA (2009), the predicted environmental concentration in earthworms (PEC_{worm}) is calculated based on a theoretical bioconcentration factor BCF(calc.) from substance-specific physicochemical data i.e., logarithm of the octanol-water partition (LogPow) and logarithm of the octanol-water partition (Koc) (Table 2.). For eight out of nine active ingredients, all $TER_{secondy\ poisoning}$ values were > 5 indicateing no potential for secondary poisoning to earthworm-feeding mammals and birds. In the case of chlorpyriphos, the high lipophilicity (LogPow = 7.0) triggers a high PEC_{worm} and accordingly a $TER_{secondy\ poisoning}$ value of < 5 meaning a high risk for secondary poisoning to mammals and birds.

As the previous section shows, the "hybrid" bioaccumulation factors derived from analytically measured earthworm concentrations and recalculated soil concentrations are reliable and can be used for further refinement. When replacing BCF (calc.) with the measured BCF, the $TER_{secondy\ poisoning}$ values for chlorpyriphos are > 5 and no longer pose a risk to birds and mammals. For the remaining eight active ingredients, $TER_{secondy\ poisoning}$ values generally decrease but still do not exceed the trigger of TER < 5.

Table 2. Potential of secondary poisoning using calculated BCF values

Active ingredient	Physchem data		Application		PEC_{soil} mg/kg dry soil at sampling	BCF (calc.)	PEC_{worm} = PEC_{soil} x BCF (calc.) mg/kg	Residue (mg/kg)		long-term NOAEL (mg/kg/day)		TER	
	Log Pow	Koc	g/ha	PEC_{soil} mg/kg dry soil				mamal	bird	mamal	bird	mamal	bird
imidacloprid	0.57	225	130	0.0289	0.0037	0.1966	0.0007	0.0009	0.0008	5.7	9.3	6123	12178
thiamethoxam	-0.13	56.2	36	0.0080	0.0001	0.7552	0.0001	0.0001	0.0001	2.6	29.4	26895	370740
chlorpyrifos	7.0	8151	850	0.1889	0.012	736.1	8.8	11.3	9.3	1.0	25.0	0.088	2.695
azoxystrobin	2.5	482	165	0.0367	0.0082	0.4808	0.004	0.0050	0.0041	20	1200	3963	289888
cyproconazole	3.09	711	64	0.0142	0.0118	1.0973	0.013	0.0166	0.0136	1.84	1.4	111	103
epoxiconazole	3.3	2647	112	0.0249	0.0049	0.4681	0.002	0.0029	0.0024	2.30	10.0	783	4152
metamitron	0.85	122	700	0.1556	0.0004	0.3791	0.0002	0.0002	0.0002	4.9	81.5	25246	511892
ethofumesat	2.7	147	60	0.0133	0.045	2.3314	0.1049	0.1343	0.1102	7.0	406	52	3686
phenmedipham	4.0	888	78	0.0173	0.0004	6.8041	0.0027	0.0035	0.0029	6.8	82	1952	28554

Log Pow: Lipophilicity (Log of partition between octanol and water);
Koc: Potential for adsorption (Distribution between organic carbon and water);
PEC_{soil}: Predicted Environmental Concentration in soil (30 cm soil depth, density 1.5 kg/L), BCF (calc.) Bioconcentration Factor (calculated: $BCF_{earthworm}$ = (0.84 + 0.012 * Pow) / (foc x Koc));
PEC_{worm}: Predicted Environmental Concentration in worms (PEC_{worm} = PEC_{soil} x BCF(calc.));
Residue in mammals: PEC_{worm} x 1.28;
Residue in birds: PEC_{worm} x 1.05;
NOAEL: No-observed-adverse-effect level from chronic studies with mammals and birds;
TER: Toxicity-Exposure-Ratio from NOAEL/Residue (risk is TER < 5)

Table 3. Potential of secondary poisoning using measured BCF values

Active ingredient	Application		PEC_{soil} mg/kg dry soil at sampling	Residues in worms (mg/kg)	Measured BCF (max)	PEC_{worm} = PEC_{soil} x BCF	Residue (mg/kg)		long-term NOAEL (mg/kg/day)		TER	
	g/ha	PEC_{soil} mg/kg dry soil					mamal	bird	mamal	bird	mamal	bird
imidacloprid	130	0.0289	0.0037	0.1427	38.6	0.1427	0.1827	0.1498	5.7	9.3	31	62
thiamethoxam	36	0.0080	0.0001	0.0005	5.0	0.0005	0.0006	0.0005	2.6	29.4	4063	56000
chlorpyrifos	850	0.1889	0.012	0.0475	4.0	0.0475	0.0608	0.0499	1.0	25.0	16	501
azoxystrobin	165	0.0367	0.0082	0.0408	5.0	0.0408	0.0522	0.0428	20	1200	383	28011
cyproconazole	64	0.0142	0.0118	0.0475	4.0	0.0475	0.0608	0.0499	1.84	1.4	30	28
epoxiconazole	112	0.0249	0.0049	0.0177	3.6	0.0177	0.0227	0.0186	2.30	10.0	102	538
metamitron	700	0.1556	0.0004	0.0043	10.8	0.0043	0.0055	0.0045	4.9	81.5	890	18051
ethofumesat	60	0.0133	0.045	0.142	3.2	0.1420	0.1818	0.1491	7.0	406	39	2723
phenmedipham	78	0.0173	0.0004	0.0105	26.3	0.0105	0.0134	0.0110	6.8	82	506	7401

PEC_{soil}: Predicted Environmental Concentration in soil (30 cm soil depth, density 1.5 kg/L);
BCFmax: Bioconcentration factor (maximum calculated value from measured earthworm residues and calculated soil concentration at the time of sampling);
PEC_{worm}: Predicted Environmental Concentration in worms (PEC_{worm} = PEC_{soil} x BCFmax);
Residue in mammals: PEC_{worm} x 1.28;
Residue in birds: PEC_{worm} x 1.05;
NOAEL: No-observed-adverse-effect level from chronic studies with mammals and birds;
TER: Toxicity-Exposure-Ratio from NOAEL/Residue (risk if TER < 5)

Nevertheless, the comparison of Table 2 and Table 3 shows that environmentally relevant values can be derived from compound-specific characteristics but should be taken with caution and verified by measured values as far as possible.

Assessment of potential toxic effects to earthworms

Recalculated soil concentrations, based on application information provided by farmers, are converted from application rates (g a.i./ha) to soil concentrations (mg a.i./ha). These expected soil concentrations directly after application are used for the assessment of the potential risk of plant protection products on earthworms in the field. The toxicity-exposure ratio (TER_{worm}) for earthworms was derived from the values of no-observed-effect-concentrations (NOEC) from earthworm laboratory reproduction studies and the expected soil concentrations directly after application (OECD, 1984; 2016).

For fungicides (Table 4), NOEC values from earthworm reproduction studies were available for all 12 fungicide active ingredients used and resulted in TER values of 1.5 - 241. The two fungicides epoxiconazole and thiophanate-methyl resulted in TER values of 1.5 and 4, respectively, and would need to be further evaluated for their potential risk to earthworms in the environment. Some fungicides are characterised by the same mode of action and may cause mixed toxicity to earthworms when applied in the same season. This needs to be further evaluated.

When replacing the expected soil concentration directly after application by the maximum calculated soil concentration at the time of earthworm sampling, the TER_{worm}-values increased as expected since the soil concentrations decreased continuously after application. This decrease was rather slow for epoxiconazole resulting into a still critical TER_{worm}-value.

Table 4. Fungicide active ingredients risk potential on earthworms in the field

Active ingredient	Number of fields	Number of applications	Application		Soil conc. at time of sampling	Toxicity to *Eisenia*			Mode of action
			g/ha	soil conc		repro NOEC	TER at DAT 0	TER at time of sampling	
				mg/kg dry soil					
azoxystrobin	8	8	165	0.083	0.0282	20	241	709	Respiration
carbendazim	1	1	250	0.125	0.0481	1	8.0	21	Mitosis and cell division
thiophanate-methyl	7	7	465	0.233	0.0001	0.85	3.6	8500	
chlorothalonil	2	2	500	0.25	0.0482	50	200	1037	Multi-site activity
copper oxychloride	2	3	750	0.375	0.0824	15	40	182	
cyproconazole	6	6	64	0.032	0.0118	0.75	23	64	Sterol biosynthesis
epoxiconazole	6	7	281	0.056	0.0274	0.084	1.5	3.1	
fenpropimorph	1	1	250	0.125	0.0544	4.7	38	86	
flutriafol	1	2	75	0.038	0.0164	6.1	161	372	
propiconazole	1	1	130	0.065	0.0285	0.833	13	29	
prothioconazole	2	2	100	0.05	0.0001	1.33	27	13300	
tebuconacole	2	2	100	0.05	0.0063	10	200	1587	

NOEC: Experimentally determined no-observed effect concentration from earthworm reproduction tests according to OECD 222;
TER: Toxicity-Exposure Ratio from soil concentration/repro-NOEC

Therefore, the environmental risk assessment on earthworms should consider that a slow degradation rate of an active ingrdient might impact earthworms over a longer time period.

CONCLUSIONS

Field dissipation curves (based on EU, EC and EFSA) reasonably predict the soil residue concentration of active ingredients at any time after application. Therefore, the analytically determined residues in earthworms from the two regions, Lukač and Tovarnik, can be reliably used for the calculation of bioconcentration factors. A comparison with literature data shows that these “hybrid” bioconcentration factors are reasonable and can be used for a basic assessment of the potential for bioaccumulation. Most active ingredients do not pose a risk to the earthworms and have no potential of secondary poisoning for earthworm-eating birds and mammals. The most important mitigation measure is to reduce the number of applications and/or the amount of application rates used.

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