Combined toxicity of chlorpyrifos, abamectin, imidacloprid, and acetamiprid on earthworms (*Eisenia fetida*)

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**Abstract**

Mixed pesticides have been broadly used in agriculture. However, assessing the combined effects of pesticides in the environment is essential for potential risk assessment, though the task is far from complete. Median lethal concentrations of pesticides as well as acetylcholinesterase (AChE) levels and cellulose activities were measured in earthworms (*Eisenia fetida*) individually and jointly exposed to pesticides imidacloprid (IMI), acetamiprid (ACE), chlorpyrifos (CRF), and abamectin (ABM)). A 3:1 mixture of CRF and IMI had additive effects, while a 3:1 mixture of CRF and ACE had synergic effects. The joint effects of ABM with IMI or with ACE were synergistic. As CRF concentration increased, AChE activities were significantly decreased. For high concentrations of IMI, AChE activities under combined CRF and IMI applications were significantly inhibited following increased exposure time. Moreover, the cellulase activities under combined applications of CRF with IMI or with ACE had similar effects. This study provides basic data for scientifically evaluating the environmental risk and safety of combined uses of pesticides.

**Keywords** Neonicotinoid · Chlorpyrifos · Abamectin · Combined toxicity · Earthworm

**Introduction**

The use of pesticides in the agricultural industry has continuously increased. In 2017, 2018, and 2019, the total amount of pesticides employed in agriculture use was more than 4.1 million tons (FAO). The wide use of pesticides and their potential effects upon entering into environments and subsequent hazards on the balance of ecosystems are drawing more attention (Bishop et al. 2020; Fang et al. 2019; Utami et al. 2020). Thorough knowledge of biochemical responses and combined effects of pesticides in the environment is essential for proper risk assessment (Sakthiselvi et al. 2020; Zhou et al. 2020).

Neonicotinoid pesticides are one of the most commonly used classes of insecticides and affect nicotinic acetylcholine receptors to influence insects’ nervous systems, ultimately resulting in their paralysis and death. Because of their efficient insecticidal activity and low toxicity to vertebrates and most invertebrates, neonicotinoids are widely used in crop applications, seed treatment, soil, and control of pests on household pets, underlying the world-leading sales of this class of pesticides (Cimino et al. 2017; Douglas and Tooker 2015). In China, 8000 tons of acetamiprid (ACE) and 14,000 tons of imidacloprid (IMI) are used every year (Xusheng et al. 2013). Owing to their high solubility and relatively low degradation in soil, water, and other chemical reagents, the environmental and health effects of neonicotinoids have gained research attention. In recent years, the negative effects of neonicotinoids in organisms have been studied, including their associated underlying risk to human health (Hallmann et al. 2014). It has been reported that neonicotinoids reduce the reproduction of bees and increase their mortality by inhibiting their homing ability (Rundlof et al. 2015; Stanley et al. 2015; Woodcock et al. 2017). The metabolism and dissipation fate of IMI and ACE on are relatively safe. The application of IMI in different soils at 0.5~1.0 kg Al/ha, and its metabolites were first detected at 30th day. The 50% degradation time (DT50) values of IMI in soil ranged from 28.7 to 47.8 days (Sarkar et al. 2001;...
Tomalski et al. 2010). Half-life of ACE in soil varied from 10.0 to 22.8 days in the laboratory (Pitam et al. 2013). However, differences in environmental conditions, microbial activity, and soil properties influence IMI and ACE dissipation in soil (Castillo Diaz et al. 2016; Xu et al. 2020). Additionally, neonicotinoid insecticides are assimilated by plants and transported to organs, including roots, thus contaminating soil and underground organisms. It is crucial to understand the impact of IMI and ACE on soil organisms to precisely assess risk.

Chlorpyrifos (CRF) is a typical organophosphorus pesticide and neurotoxic insecticide, an acetylcholinesterase inhibitor that overstimulates the nervous system. It is extensively applied to seeds, lawns, and crops. The total production of CRF exceeds 200,000 tons, with rates of use increasing by 10% annually in China (John and Shaike 2015). Previous studies have reported that use of CRF increased the population of fungi and the number of bacteria, while decreased nitrogen fixation and microorganism levels in soil (John and Shaike 2015; Pandey and Singh 2004; Singh et al. 2015). In addition, in 2019, CRF was noted to have potential developmental toxicity, neurotoxicity, and genotoxicity effects on mice and rat, which increased attention on its human health impacts by the European Food Safety Authority (EFSA) ((FSN) 2019).

ABM is generated by actinomycete fungi (Streptomyces avermitilis), belonging to the family of avermectins, and it is used to eliminate nasal bots, gastrointestinal nematodes, and lung worms in sheep and cattle (Campbell 2012). ABM is a mixture containing less than 20% avermectin B1b and more than 80% avermectin B1a. It interferes with neurophysiological activities and stimulates the release of γ-amino butyric acid (GABA), which has an inhibitory effect on nerve conduction in arthropods, resulting in their paralysis, and death. ABM is slightly soluble in water and has a lipophilic character that makes it difficult for mammals to metabolize, 80–98% of the chemical dose is eliminated in stools and transferred to soil (Sun et al. 2005). With the wide application of ABM in agriculture, it is essential to evaluate the toxicities of ABM on soil organisms.

The global population of earthworms has decreased gradually due to increasing environmental pollutants, habitat loss, and reduced microbial communities (Kwak and An 2021; Ren et al. 2018). Earthworms are the most populous terrestrial soil animal species (Bart et al. 2019). They have a critical role in breaking down organic matter, and thus contribute to soil fertility and soil formation (Mattsson et al. 2017; Van Groenigen et al. 2019). Earthworms inhabit moist soil, where they come in contact with pesticides via inhalation, swallowing soil, gut absorption, and skin contact (Pelosi et al. 2014). In recent decades, earthworms have been used as a typical model of invertebrate species in soil toxicology studies (Blouin et al. 2013). Earthworms (e.g., Eisenia fetida) has been selected for research because they are easy to culture in laboratory settings, have a high reproduction rate, short-life cycle, and small body size, and can be used as bioindicators of soil pollutants (Blouin et al. 2013). Despite the abundance of earthworms, their population size and diversity have both declined (Chan 2001). The wide use of pesticides could be a serious threat to earthworm survival in agricultural settings (Chagnon et al. 2015; Pelosi et al. 2014). Therefore, earthworms are suitable for the toxicological assessment of environmental pollutants.

IMI, ACE, CRF, and ABM can enter the environment and induce severe damage to soils (Hasenbein et al. 2015; Wang et al. 2015). Pesticides may disturb soil ecosystems and impact soil invertebrate structure (Yang et al. 2015). As a result, the wide use of insecticides is being questioned on environmental grounds in a number of countries, leading to usage restrictions. However, in spite of growing research efforts to understand insecticide use and its potential effects on a variety of organisms, we still lack assessments of the combined effects of insecticide pollutants in the environment, especially in soil, in order to evaluate their potential risks to underground organisms. To fill such a knowledge gap, we measured the individual and joint toxic effects of IMI, ACE, CRF, and ABM on earthworms, including effects on the activities of acetylcholinesterase (AChE) and cellulase. AChE is the target enzyme for pest control by insecticides, such as neonicotinoids and organophosphate pesticides. In earthworms, cellulase activity indicates relatively high activities of endoglucanase more broadly (Ikarashi et al. 2016).

In order to assess the toxic effects of pesticides to earthworms, artificial soil test protocols have been used (OECD 1984, 2004). Many studies do employ standardized artificial soil test to assess toxicity of contaminants on earthworms.

### Methods and materials

#### Chemicals and reagents

The following chemicals were obtained for the present study: ABM (95.75% purity; from Shanxi Xi’an Meibang Pesticide Co., Ltd., Xi’an, China), IMI [(2E)-1-((6-chloro-3-pyridinyl) methyl)-N-nitro-2-imidazolidinimine] (97.6% active ingredients, from Zhejiang Haizheng Pesticide Company, Taizhou, China), CRF (97% purity, from Sumitomo Chemical Corp., Tokyo, Japan), ACE (96% active ingredients, from Beijing Huarong Pesticide Company, Beijing, China). Four pesticides were dissolved separately in acetone and stored at 4 °C. All other reagents used were of analytical grade.
Earthworm acute toxicity test

Earthworms (Eisenia fetida) were obtained from the College of Resources and Environmental Sciences, China Agricultural University, Beijing, China. The artificial soil comprised of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat moss, and calcium carbonate to regulate the pH to 6.5 ± 0.5 (OECD 1984). After acclimation for 1 week in an environmental chamber maintained at 20 ± 0.5 °C, 80 ± 2% humidity, and 400–800 lux illumination. Two-month-old earthworms with well-developed clitella (0.30–0.45 g) were selected and deprived of food for 4 h before the experiment was conducted according to a previously described study (Wang et al. 2015). The soil was prepared by adding different concentrations (mg/kg, dry soil) of pesticides (90, 108, 129.60, 155.52, 186.62 mg/kg CRF, 4.44, 6.67, 10, 15, 22.5 mg/kg ABM, 1.50, 2.10, 2.94, 4.12, 5.76 mg/kg IMI, 1.40, 2.10, 3.15, 4.73, 7.09 mg/kg ACE, and their mixtures). The amounts of pesticide were carefully mixed into soil as an aqueous solution to yield the working concentrations of pesticides, and mixed soils were transferred into glass jars. Ten adult earthworms were placed into 1-L glass containers filled with 500 g soil, and test containers were enclosed with a polythene sheet with integrated gauze (+1 mm) to prevent the worms from escaping and to ensure optimal ventilation. After 1, 7, and 14 days of incubation, living worms were sorted by hand, and the test endpoint was mortality. Three replicates were conducted for acute toxicity tests. In parallel, solvent and control treatments were also conducted with three replicates.

Earthworm sample collection and AChE assay

Based on the lethal concentration results, different concentrations of pesticides were designated for testing the biochemical response of insecticides on AChE activity. AChE enzyme activity was assayed in triplicates (10 worms/replicate) following exposure to pesticides for 1, 3, 7, 14, and 21 days. During the exposed period, there is no dead earthworm. Live earthworms were depurated for 4 h on moist filter paper to empty their gut contents, rinsed, and then stored at −80°C for further study. AChE activity was determined according to a previously described method (Worek et al. 2012). Total protein contents were measured according to the manufacturer’s protocol (Bradford method, Beyotime Biotechnology, Shanghai, China). Earthworm samples were homogenized in phosphate buffer (pH=7.4; 1/4, w/v) and centrifuged at 8000×g, 4 °C for 15 min. Then, 50 μL of supernatant was combined with 150 μL of mixture containing 1.5 mL of 10 mM acetylthiocholine iodide and 1.2 mL of 6.2 mM 5,5′-dithiobis-2-nitrobenzoic acid in 30 mL of 100 mM phosphate buffer. Absorbance was read at 405 nm after 15 min.

Cellulase activity assay

Earthworms were collected following exposure to pesticides for 1, 7, and 14 days. After a 4-h depuration period, two earthworms from each treatment were randomly selected for homogenization with ice-cold phosphate buffer (Wang et al. 2015). Cellulase activity was measured using the carboxymethyl cellulose (CMC) method as described by Ghose (Ghose 1987).

Statistical analysis

Mortality and biochemical results were determined by one-way analysis of variance (ANOVA) using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Significant differences were analyzed by using Dunnett’s test, and a P < 0.05 threshold was applied to identify significant results versus control group (‘P < 0.05, ** P < 0.01).

The median lethal concentrations of chemicals (LC50) and associated 95% confidence limits were calculated by probit analysis.

The co-toxicity coefficient (CTC) of pesticides was calculated using the Sun method with the following equations (Sun and Johnson 1960):

\[
single\text{ toxic index (TI)} = \frac{\text{standard reagent LC}_{50} \times 100}{\text{test reagent LC}_{50}};
\]

\[
mixture\text{ actual toxic index (ATI)} = \frac{\text{standard reagent LC}_{50} \times 100}{\text{mixture reagents LC}_{50}};
\]

\[
theoretical\text{ toxic index (TTI)} = \frac{\text{ATI} \times A\% + B\% + C\% + \ldots}{\text{TTI}};
\]

\[
A\% = \frac{A}{A + B + C + \ldots}, \quad B\% = \frac{B}{A + B + C + \ldots}, \quad C\% = \frac{C}{A + B + C + \ldots}, \quad \text{co – toxic coefficients (CTC)} = \frac{\text{ATI} \times 100}{\text{TTI}}
\]
Results

Lethal effect of pesticides

The LC$_{50}$ results for earthworms after 14-day pesticide exposures are shown in Table 1. Different pesticides have different lethal effects on earthworms. The pesticides can be arranged in descending order of lethal sensitivity to earthworms as follows: ACE (2.69 mg/kg), IMI (2.96 mg/kg), ABM (9.68 mg/kg), CRF (1.31×10$^2$ mg/kg). The lethal effect of CRF was lower than that of other pesticides.

We then measured the acute toxicity of different ratios of pesticides between neonicotinoids and each pesticide, as well as their LC$_{50}$ values for earthworms. The mixture toxicities for 3:1, 6:1, and 9:1 ratios of mixes of CRF and IMI were 10.7, 17.6, and 26.4 mg/kg, respectively. The response to a mixture of CRF and IMI was additive. Additionally, 3:1, 6:1, and 9:1 ratios of mixes of CRF and ACE were 3.79, 10.2, and 10.1 mg/kg, respectively. CTC values ranged from 164 to 268 mg/kg. For the other two mixtures, the 14-day LC$_{50}$ of 1:3 mixes of ABM with IMI and ACE were 2.565 and 1.632 mg/kg, respectively, indicating synergistic responses.

IMI and ACE are neonicotinoids with similar chemical structures, though they differ in their acute toxicity after being combined with CRF. The joint toxicity of CRF and IMI is additive, while CRF and ACE have a synergistic response. Owing to the complexity of the joint toxicity of insecticides to earthworms and their similar action of mode, CRF and these two neonicotinoids were selected for subsequent biochemical analyses.

Effects on AChE activity

The activities of AChE under IMI exposure are presented in Fig. 1A. After a 1-day exposure, IMI treatments inhibited AChE activity, with higher concentrations of IMI causing significant decreases in AChE enzymatic activities compared to the control group. AChE activity under 0.0500 mg/kg IMI treatment led to significant increases following exposure for 3 days, while 0.100 mg/kg IMI treatment significantly decreased AChE activity. At 7 days, enzyme activity was significant inhibited in the 0.4000 mg/kg group. However, after a 21-day exposure, for the lower concentration groups (0.0125 and 0.0500 mg/kg), IMI caused significant increases in AChE activity. Similarly, 1.0000 mg/kg IMI significantly increased AChE activity in earthworms.

The changes in AChE activities under ACE exposure are summarized in Fig. 1B. The highest concentration of ACE induced a significant increase in AChE activity after 1-day exposure. After a 3-day exposure, 0.0125 mg/kg ACE significantly increased AChE activity, but significantly increased its activity under the 0.8000 mg/kg treatment. Unexpectedly, there was no significant change in AChE activity following exposure ACE for 7 day. AChE activity was lowered to the control level in earthworms exposed to 0.4000 mg/kg ACE for 14 days. A low pesticide dose, 0.0500 mg/kg CAE caused a significant increase in AChE activity following a 21-day exposure, while AChE activities were significantly inhibited in earthworms exposed to 0.4000 and 0.8000 mg/kg ACE.

The effects on earthworm AChE activity induced by CRF are summarized in Fig. 1C. After exposures of 1, 3, 7, 14, and 21 days, 5 mg/kg CRF induced significant increases in AChE activity.
activity. However, AChE activities were significantly reduced under the 40 mg/kg CRF treatment following exposures of 1, 3, 7, and 14 days, but not 21 days. After a 7-day exposure, 10 and 30 mg/kg CRF treatments significantly increased AChE activity. AChE activity under the 30 mg/kg CRF treatment for 14 days was inhibited. The 10 mg/kg CRF concentration caused marked increases under a 21-day exposure. AChE activity under CRF and IMI combined at a 3:1 ratio is summarized in Fig. 2A. AChE activity under combined pollutant after a 1-day exposure was significantly increased in the 0.800 mg/kg treatment, but inhibited in 1.000 mg/kg group. There were significant increases in AChE activities of earthworms following exposure to the combined application of CRF and IMI after 3 days. AChE activity was significant increased after a 7-day exposure to the combinations in the 0.800 mg/kg treatment groups. Unexpectedly, the combined pollutants did not result in
significant changes in AChE activities at any exposure concentration for 14 and 21 days. The joint toxicity on AChE activity of CRF and ACE combined at a ratio of 3:1 is summarized in Fig. 2B. AChE activity of the combined application was markedly increased under the 0.050 mg/kg exposure at 3 days. The 21-day combined exposure was the most sensitive stage among all treatment periods. In the 0.200 mg/kg co-exposure group, AChE activity was significantly increased, while the combined applications at 0.800 and 1.000 mg/kg significantly inhibited the activity of AChE.

**Effects on cellulase activity**

Pesticides were observed to affect cellulase activity in earthworms (Figs. 3 and 4). After 1- and 7-day IMI exposures, cellulase activities were significantly increased except in the 0.4000 mg/kg treatment group (Fig. 3A). After a 1-day exposure, ACE induced a higher cellulase activity level than the control group. In contrast, ACE induced lower cellulase activity. The activity of cellulase significantly increased following exposure to 0.4000 mg/kg ACE for 14 days (Fig. 3B). Similarly, after exposure to CRF for 1 day, cellulase activities significantly increased, except for the 30 mg/kg treatment (Fig. 3C). However, 10, 20, and 30 mg/kg CRF treatments induced lower cellulase activities than the control at 7 days. We determined cellulase activities of a 3:1 mixture of CRF and IMI (Fig. 4A). The combined application of CRF and IMI significantly increased cellulase activity in earthworms following 0.40 and 0.80 mg/kg treatments for 1 day, but significantly decreased cellulase activity after 0.05 and 0.10 mg/kg treatments for 7 days. The cellulase activities in earthworms were significantly increased after 1- and 14-day exposures to the 3:1 mixture of CRF and ACE (Fig. 4B) compared to the control and were inhibited by 0.05, 0.40, and 0.80 mg/kg treatments at 7 days.

**Discussion**

Applications of mixtures have become a major trend in pesticide use, as combinations of pesticides can expand the scope of use, improve efficiency, reduce the number of resistant
pests, and also help to control and prevent insects more generally. The combined application of pesticides is extensive, and while many studies have been conducted on the joint action mechanism of target organisms, few have examined the effects of combined pesticides in non-target organisms.

Our CRF, IMI, ACE, and ABM toxicity results from the present study match the range of LC50 values described by previous studies using *Eisenia fetida* (Dong-Mei et al. 2015; Karanjkar and Naik 2009; Tenorio Nunes and Gaeta Espindola 2012; Wang et al. 2015). However, the LC50 value for IMI in *Eisenia andrei* was 25.53 mg/kg, which is a discrepancy that can be interpreted as two different species having different responses to this chemical (Alves et al. 2013).

**Fig. 3** Cellulase activity in earthworms exposed to different concentrations of imidacloprid (IMI) (A), acetamiprid (ACE) (B), and chlorpyrifos (CRF) (C). Significant differences were assessed using a one-way ANOVA followed by Dunnett’s test (*P < 0.05, **P < 0.01) Results are presented as mean ± standard deviation (S.D.) values.
Two-pesticide combinations (i.e., CRF and ACE, ABM and IMI, and ABM and ACE) exhibited synergistic effects in the present soil toxicity test. It is known that IMI and ACE belong to neonicotinoids and have the same molecular mode of action (acetylcholine receptor, AChR) and CRF is an AChE agonist (Kousba et al. 2004; Tomizawa and Casida 2005). However, ABM has a different molecular mode of action (Liang et al. 2019) acting on similar targets through different biological processes and with synergistic interactions (Cedergreen 2014). Similar synergism was observed in Daphnia magna and Caenorhabditis elegans (Gomez-Eyles et al. 2009; Loureiro et al. 2010). Synergistic responses may impair soil organisms (such as earthworms), hindering survival in natural environments (Uwizeyimana et al. 2017). If one pollutant in the combined application triggers changes in the toxicity kinetics of an organism, then synergistic interactions may be observed in combinations including that pollutant (Rizzati et al. 2016). Synergistic responses can be affected by the co-occurrence of avermectin and neonicotinoid pesticides. However, direct evidence of target enzyme alterations needs to be investigated in the future studies.

The main function of AChE is to stop pulse transmission in the cholinergic synapse by rapidly hydrolyzing the neurotransmitter acetylcholine and cleaving ACh into acetate and choline (Soreq and Seidman 2001). Many studies have shown that the target of CRF and neonicotinoid pesticides is related to acetylcholinesterase (Rao et al. 2003; Reinecke and Reinecke 2007; Shao et al. 2013). CRF, as a cholinesterase inhibitor mainly inhibits AChE, causing neurological dysfunction and thus affecting neurodevelopment (John and Shaike 2015). In the current study, AChE activities were gradually inhibited in earthworms as CRF concentrations increased. The targets of neonicotinoid pesticides are the nicotinic acetylcholine receptors (nAChRs) of the postsynaptic membrane, a member of the family of ligand-gated ion channels in insects (Shao et al. 2013). The hydrolysis...
of acetylcholine is catalyzed by AChE, which is a crucial enzyme in the development and functions of nervous systems (Colovic et al. 2013). Additionally, the nervous system plays an important role in neurotransmission (Kaizer et al. 2017). In high concentration of IMI treatments, AChE activities of combined CRF and IMI application decreased significantly as exposure time increased. A decrease in AChE increases levels of the neurotransmitter acetylcholine, which then induces the over-stimulation of muscles overall and finally causes insects paralysis and death. Thus, changes in AChE activity could have toxic effects in earthworms.

However, the joint AChE activities under CRF and ACE are complex. For example, as ACE concentration increased, AChE activity gradually increased after a 1- and 7-day exposure, but its activity decreased significantly as concentrations increased. There was a difference between the two combined applications likely owing to the structure of ACE and IMI. CRF binds with monoxygenase to activate the molecules to varying degrees, resulting in alteration of AChE activity (Belden and Brain 2017).

Cellulase is a cold-tolerant digestive enzyme that is involved in cellulose digestion (Ikarashi et al. 2016). Notably, cellulase plays a major role in gut epithelial cells. In an artificial soil test, the gut absorbs environmental pollutants under controlled conditions (Nozaki et al. 2009). Increases in cellulase activity were induced by IMI and ACE exposure of 1 day and recovered to normal levels at 14 days. The recovery period for cellulase in these assays was longer than that for the CRF exposure. However, cellulase activities in the combined applications of CRF with IMI and ACE was similar. On the first day, cellulase was activated by the two combined applications, while the enzyme returned to normal levels after a 7-day exposure. Insecticides are assimilated by earthworms at their initial exposure and stimulated increased levels after a 7-day exposure. Insecticides are assimilated by earthworms at their initial exposure and stimulated increased levels after a 7-day exposure.

**Conclusion**

The development and application of mixtures of pesticides in China and elsewhere are extensive, and there are many studies on the combined action mechanism on target organisms. The screening of compound formulas and the determination of co-toxicity coefficients are necessary for assessing the effects of compound preparations. This study has focused on whether the combined effects of the compound preparations have effects on a critical soil organism, earthworms, and provides a basis for environmental risk assessment of these compound pesticides.

**Author contribution** Miaomiao Teng: investigation; formal analysis; validation; writing original draft. Xiaoli Zhao: conceptualization; investigation; supervision; resources; writing, review and editing. Chen Wang: conceptualization; writing, review and editing. Lingfeng Zhou: investigation; formal analysis. Xiaowei Wu: review and editing. Fengchang Wu: resources; writing—review and editing, project administration.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Competing interests** The authors declare no competing interest.

**References**

Alves PRL, Cardoso EJBNN, Martin AM, Sousa JP, Pasini A (2013) Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. Chemosphere 90:2674–2682

Bart S, Pelosi C, Barraud A, Pery ARR, Cheviron N, Grondin V, Mougin C, Crouzet O (2019) Earthworms mitigate pesticide effects on soil microbial activities. Front Microbiol 10:11

Belden JB, Brain RA (2017) Incorporating the joint toxicity of co-applied pesticides into the ecological risk assessment process. Integr Environ Assess Manag 14(1):79–91

Bishop CA, Woundneh MB, Maisonneuve F, Common J, Elliott JE, Moran AJ (2020) Determination of neonicotinoids and butenolide residues in avian and insect pollinators and their ambient environment in Western Canada (2017,2018). Sci Total Environ 737:139386

Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, Dai J, Dendooven L, Peres G, Tondoh JE, Cluzeau D, Brun JJ (2013) A review of earthworm impact on soil function and ecosystem services. Eur J Soil Sci 64:161–182

Campbell WC (2012): Ivermectin and abamectin. Springer Science & Business Media

Castillo Diaz JM, Martin-Laurent F, Beguet J, Nogales R, Romero E (2016) Fate and effect of imidacloprid on vermicompost-amended soils under dissimilar conditions: risk for soil functions, structure, and bacterial abundance. ence of the Total. Environment 579:1111–1119

Cedergreen N (2014) Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9:e96580

Chagnon M, Kreutzweiser D, Mitchell EA, Mitchell EA, Morrissey CA, Noome DA, Noome DA, Van der Sluijs JP, Van der Sluijs JP (2015) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res 22:119–134

Chan KY (2001) An overview of some tillage impacts on earthworm population abundance and diversity - implications for functioning in soils. Soil Tillage Res 57:179–191

Cimino AM, Boyles AL, Thayer KA, Perry MJ (2017) Effects of neonicotinoid pesticide exposure on human health: a systematic review. Environ Health Perspect 125:155–162
Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM (2013) Acetylcholinesterase Inhibitors: pharmacology and toxicology. Curr Neuropharmacol 11(3):315–335

Dong-Mei Xu, Yan-Hua W, Nan W, Gui-Wei R (2015) Effects of Single and Co-Exposure of Cu and Chlorpyrifos on the Toxicity of Earthworm. Environ Sci 36:280–285

Douglas MR, Tooker JF (2015) Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. Environ Sci Technol 49:5088–5097

Fang W, Peng Y, Muir D, Lin J, Zhang X (2019) A critical review of synthetic chemicals in surface waters of the US, the EU and China. Environ Int 131:104994

FAO (2021) Food and agriculture organization of the United Nations. http://www.fao.org/faostat/en/#data/RP

EFSA (2019): European votes against renewal chlorpyrifos approval. https://www.foodsafetynews.com/2019/12/eu-votes-against-renewing-chlorpyrifos-approval/

Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem 59(2):257–268

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. Ecotoxicol Environ Saf 72:71–79

Hallmann CA, Foppen RPB, Turnhout CAMV, Kroon HD, Jongejans E (2014) Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511:341–343

Hasenbein S, Lawler SP, Geist J, Connon RE (2015) The use of growth and behavioral endpoints to assess the effects of pesticide mixtures upon aquatic organisms. Ecotoxicology 24:746–759

Ikarashi Y, Yarimizu J, Yokoyama K, Kobayashi T, Nakazawa H (2016) Measurement of cellulase activities. Pure Appl Biochem 41:762–769

Ivanov DJ (2009) Measuring and modelling mixture toxicity of pesticides and earthworms. A review. Agron Sustain Dev 34:199–228

John EM, Shaike JM (2015) Chlorpyrifos: pollution and remediation. Chem Biol Interact 254:231–246

Karanjkar AS, Naik RL (2009) Acute toxicity: novel mode of pesticides and pest control. Recent Adv Pest Sci 80(1):55–66

Koutsba AA, Sultatos LG, Poet TS, Timchalk C (2013) Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. Toxicol Sci 80:239–248

Kwak JI, An Y-J (2021) Microplastic digestion generates fragmented nanoplastics in soils and damages earthworm sperrmatogenesis and coelomocyte viability. J Hazard Mater 402:124034

Liang Y, Dong B, Pang N, Hu J (2019) ROS generation and DNA damage contribute to abamectin-induced cytotoxicity in mouse macrophage cells. Chemosphere 234:328–337

Loureiro S, Svendsen C, Ferreira ALG, Pinheiro C, Ribeiro F, Soares AVM (2010) Toxicity of three binary mixtures to Daphnia magna: comparing chemical modes of action and deviations from conceptual models. Environ Toxicol Chem 29:1716–1726

Mattsson K, Johnson EV, Malmedal A, Linse S, Hansson LA, Cedervall T (2017) Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci Rep 7:11452

Nozaki M, Miura C, Tozawa Y, Miura T (2009) The contribution of endogenous cellulase to the cellulose digestion in the gut of earthworm (Pheretima hilgendorfi: Megascolecidae). Soil Biol Biochem 41:762–769

OECD (1984): Earthworm acute toxicity. OECD Guideline for Testig of Chemicals No. 207.

OECD (2004): Earthworm reproduction test (Eisenia fetida/Eisenia andrei). OECD Guideline for Testig of Chemicals No. 222.

Pandey S, Singh DK (2004) Total bacterial and fungal population after chlorpyrifos and quinalphos treatments in groundnut (Arachis hypogaea L.) soil. Chemosphere 55:197–205

Pelosi C, Barot S, Capowiez Y, Hedde M, Vandenbulcke F (2014) Pesticides and earthworms. A review. Agron Sustain Dev 34:199–228

Pitam S, Mukherjee I, Kumar A (2013) Evaluation of environmental fate of acetamiprid in the laboratory. Environ Monit Assess 185:2807–2816

Rao JV, Pavan YS, Madhavendra SS (2003) Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, Eisenia fetida. Ecotoxicol Environ Saf 54:296–301

Reinecke SA, Reinecke AJ (2007) The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. Ecoxicol Environ Saf 66:244–251

Ren X, Zeng G, Tang L, Wang J, Wan J, Feng H, Song B, Huang C, Tang X (2018) Effect of exogenous carbonaceous materials on the bioavailability of organic pollutants and their ecological risks. Soil Biol Biochem 116:70–81

Rizzato V, Briand O, Guillou H, Gamet-Payrastre L (2016) Effects of pesticide mixtures in human and animal models: an update of the recent literature. Chem Biol Interact 254:231–246

Rundlof M, Andersson GKS, Bommarco R, Fries L, Hederstrom V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521:77–U162

Sakthiselvi T, Paramasivam M, Vasanthi D, Bhuvaneswari K (2020) Persistence, dietary and ecological risk assessment of indoxacarb residue in/on tomato and soil using GC-MS. Food Chem 328:127134

Sarkar M, Roy S, Kole R, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. Pest Manag Sci 57:598–602

Shao X, Xia S, Durkin KA, Casida JE (2013) Insect nicotinic receptor interactions in vivo with neonicotinoid, organophosphorus, and methylcarbamate insecticides and a synergist. Proc Natl Acad Sci U S A 110(43):17273–17277

Singh S, Gupta R, Sharma S (2015) Effects of chemical and biological pesticides on plant growth parameters and rhizospheric bacterial community structure in Vigna radiata. J Hazard Mater 291:102–110

Sorop H, Seidman S (2001) Acetylcholinesterase - new roles for an old actor. Nat Rev Neurosci 2:294–302

Stanley DA, Garratt MPD, Wickens JB, Wickens VJ, Potts SG, Raine NE (2015) Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. Nature 528:548–U17

Sun Y, Johnson ER (1960) Analysis of joint action of insecticides against house flies. J Econ Entomol 53:887–892

Sun Y, Diao X, Zhang Q, Shen J (2005) Bioaccumulation and elimination of avermectin B1a in the earthworms (Eisenia fetida). Chemoecology 60:699–704

Tenorio Nunes ME, Gaeta Espindola EL (2012) Sensitivity of Eisenia andrei (Annelida, Oligochaeta) to a commercial formulation of abamectin in avoidance tests with artificial substrate and natural soil under tropical conditions. Ecotoxicology 21:1063–1071

Tomalski M, Leimkuehler W, Schal C, Vargo EL (2010) Metabolism of Imidacloprid in Workers of Reticulitermes flavipes (Isoptera: Rhinotermitidae). Ann Entomol Soc Am 103:84–95

Tomizawa M, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol 45:247

Utami RR, Geerling GW, Salami IRS, Notodarmojo S, Ragas AMJ (2020) Environmental prioritization of pesticide in the Upper Citarum River Basin, Indonesia, using predicted and measured concentrations. Sci Total Environ 738:140130–140130
Uwizeyimana H, Wang M, Chen W, Khan K (2017) The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil. Environ Toxicol Pharmacol 55:20–29
Van Groenigen JW, Van Groenigen KJ, Koopmans GF, Stokkermans L, Vos HMJ, Lubbers IM (2019) How fertile are earthworm casts? A meta-analysis. Geoderma 338:525–535
Wang K, Pang S, Mu X, Qi S, Li D, Cui F, Wang C (2015) Biological response of earthworm, Eisenia fetida, to five neonicotinoid insecticides. Chemosphere 132:120–126
Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Ridding L, Dean H, Sleep D, Henrys P, Peyton J, Hulmes S, Hulmes L, Sarospataki M, Saure C, Edwards M, Genersch E, Knaebe S, Pywell RF (2017) Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356:1393
Worek F, Eyer P, Thiermann H (2012) Determination of acetylcholinesterase activity by the Ellman assay: a versatile tool for in vitro research on medical countermeasures against organophosphate poisoning. Drug Test Anal 4:282–291
Xu B, Xue R, Zhou J, Wen X, Shi Z, Chen M, Xin F, Zhang W, Dong W, Jiang M (2020) Characterization of acetamiprid biodegradation by the microbial consortium ACE-3 enriched from contaminated soil. Front Microbiol 11:1429
Xusheng S, Zewen L, Xiaoyong X, Zhong L, Xuhong Q (2013) Overall status of neonicotinoid insecticides in China: production, application and innovation. J Pestic Sci 38:1–9
Yang G, Chen Y, Yijun Z, Huiyu W, Wen Y (2018) Combined effects of four pesticides and heavy metal chromium (VI) on the earthworm using avoidance behavior as an endpoint. Ecotoxicol Environ Saf 157:191–200
Zhou Y, Guo J, Wang Z, Zhang B, Sun Z, Yun X, Zhang J (2020) Levels and inhalation health risk of neonicotinoid insecticides in fine particulate matter (PM2.5) in urban and rural areas of China. Environ Int 142:105822

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