Science of the Total Environment 644 (2018) 1452–1459

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Alcohol ethoxylates significantly synergize pesticides than alkylphenol ethoxylates considering bioactivity against three pests and joint toxicity to *Daphnia magna*

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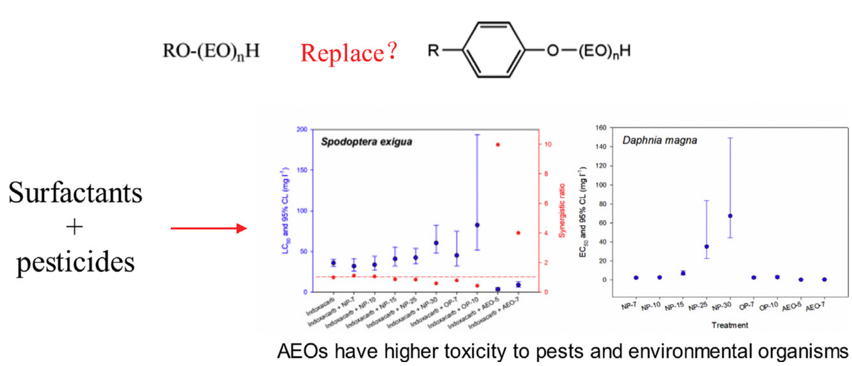
HIGHLIGHTS

- AEOs had higher synergistic effects on pesticides than APEOs.
- Additive index, toxicity unit, V value and isobologram methods were integrated.
- Synergistic ratios and toxicities of AEOs and APEOs decreased with the EO numbers.
- AEOs may be potential alternatives for APEOs in agricultural production system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 April 2018
Received in revised form 29 June 2018
Accepted 1 July 2018
Available online 23 July 2018

Editor: Henner Hollert

Keywords:
Alcohol ethoxylates
Alkylphenol ethoxylates
Indoxacarb
Acetamiprid
Synergistic effect
Joint toxicity

ABSTRACT

Seeking alternatives for alkylphenol ethoxylates (APEOs) have been a heavily researched topic in the surfactant industry and agricultural systems. In this study, the combined effects of different ethoxylates and pesticides on the bioactivity against three pests and toxicological risks to *Daphnia magna* were investigated. Results showed that alcohol ethoxylates (AEOs) had higher synergistic effects on the bioactivity of pesticides against *Spodoptera exigua*, *Agrotis ipsilon* and *Aphis citricola* than did APEOs. In terms of the joint toxicity of the ethoxylates and pesticides to *D. magna*, additive index method, toxicity unit method, V value method and isobologram method were used in the tests. All of these methods indicated that the joint effects of APEOs + acetamiprid and APEOs + indoxacarb upon *D. magna* turned from synergism to antagonism with the increasing EO (ethylene oxide) numbers. Those of AEOs exhibited similar trends. Overall, AEOs may be potential alternatives for APEOs in agriculture as they synergize pesticides against three pests significantly more than do APEOs. However, further research should investigate the compounds' environmental risks to aquatic organisms because the AEOs were highly toxic to *D. magna*.

1. Introduction

Alkylphenol ethoxylates (APEOs) are highly consumed surfactants in the textile, cosmetic, agrochemicals and other domains owing to

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https://doi.org/10.1016/j.scitotenv.2018.07.012
0048-9697/

their satisfactory wettability, emulsibility, and oxidative resistance (Andreu et al., 2007; Felício et al., 2016; Hannas et al., 2011). APEOs are always used as wetting agents for agrochemicals because they can dramatically reduce the surface tension of pesticide formulations (Björklund et al., 2009; Datta et al., 2002). They are also able to assist pesticides in penetrating the epidermis of target pests or epicuticular wax of crops and thus enhance the efficacies of pesticides. However, APEOs are among the hormonal compounds that threaten nervous system and immune system (Kinch et al., 2016). Moreover, APEOs are hard to biodegrade and the degradation product nonylphenol is also a typical endocrine-disrupting compound (Ward et al., 2013; Welshons et al., 2003; Ying, 2006). Therefore, we intended to seek potential alternatives for APEOs, especially in agrochemicals.

Alcohol ethoxylates (AEOs) are another series of highly consumed nonionic surfactants, with an annual output of approximately 1.1 million metric tons (Belanger et al., 2006). AEOs have certain advantages over APEOs for environmental risks because AEOs are less likely to cause endocrine-modulating activity (Motteran et al., 2014; Newsome et al., 1995). In addition, they are relatively easy to biodegrade compared with APEOs (Irena et al., 2016; Jardak et al., 2016; Motteran et al., 2014; Zembrzuska et al., 2016). Schönherr (1993) reported that low-molecular-weight monodisperse AEOs tend to be the most effective surfactants with favorable wettability and permeability. However, few reports have described whether AEOs have similar or better surfactant activity than APEOs in synergizing pesticides. In this study, we intended to compare the combined effects of APEOs + pesticides and AEOs + pesticides on their bioactivity against agricultural pests. Their toxicological risks to a model aquatic organism, *Daphnia magna*, were also evaluated for comprehensive consideration.

Indoxacarb is a widely used oxadiazine pesticide that has high bioactivity against various Lepidoptera and Coleoptera pests (Bird, 2017; Zlotkin, 1999). Acetamiprid is a highly-efficient neonicotinoid insecticide for the management of Hemiptera pests, such as aphids, leafhoppers and whiteflies (Zhang et al., 2017). In this study, these aforementioned pesticides were used as models to assess whether AEOs were able to replace APEOs in agrichemicals as synergists. Two Lepidoptera pests, *Spodoptera exigua* (Hübner) and *Agrotis ipsilon* (Rottemberg), and one Hemiptera pest *Aphis citricola* (von der Goot), were used as model pests in the bioassay (the immersion test was employed to evaluate the synergistic effects of surfactants on pesticides).

After the application of surfactants and pesticides in agricultural systems, chemicals may be partially washed out or drift into the water and thus threaten the health and survival of environmental organisms (Fenoll et al., 2014; Harris et al., 2009). *Daphnia magna* is recommended as a typical aquatic organism in OECD (Organization for Economic Cooperation and Development) guidelines for the environmental toxicity assessment of chemicals. Therefore, *D. magna* was used as a model to evaluate the single toxicity of APEOs and AEOs. These compounds' joint toxicity with pesticides to *D. magna* were also assessed in accordance with the protocols described in OECD Guidelines (OECD, 2004). The additive index method (Marking, 1977), the toxicity unit method (Brown, 1968; Sprague, 1970; Nirmalakhandan et al., 1997), the V value method (He and Xiong, 1994; Clausing and Bieleke, 1980), and the isobologram method (Altenburger et al., 1990; Li et al., 2018) were typical methods to evaluate the combined effects of chemical mixtures. Thus, these methods were integrated into the current study to provide comprehensive evaluations of the joint toxicity of mixtures.

2. Materials and methods

2.1. Test chemicals

All of the nonylphenol ethoxylates (NPEOs, including NP-7, NP-10, NP-15, NP-25 and NP-30), octylphenol ethoxylates (OP-7 and OP-10) and alcohol ethoxylates (AEO-5 and AEO-7) with different EO (ethylene oxide) numbers were purchased from Xingtai Lanxing Auxiliary Factory (Hebei, China). Acetamiprid (technical grade; purity of 99%) was purchased from Shandong Hailir Chemicals Co. Ltd. (Shandong, China) and dissolved in water to yield a 0.4% stock solution for further tests considering convenience. Indoxacarb (technical grade; purity of 98%) was generously supplied by Rainbow Chemical Co., Ltd. (Shandong, China) and fabricated into a 15% stock suspension.

2.2. Bioactivity of ethoxylates + indoxacarb against S. exigua and A. ipsilon

Long-term raised laboratory populations of two Lepidoptera pests (i.e. *S. exigua* and *A. ipsilon*) were used as models in the applied bioassays. Bioactivity of single indoxacarb and indoxacarb + ethoxylates against *S. exigua* and *A. ipsilon* were evaluated using standardized 3rd instar larvae in accordance with the protocols of the immersion test (Busvine, 1980). Preliminary tests were conducted as range-finding experiments in order to determine five concentrations of chemicals finally used in the definitive test (Table S1, Supporting Information). To evaluate the bioactivity of ethoxylates + indoxacarb, the concentration of ethoxylates was maintained at 1000 mg/L, slightly higher than the typical surfactant concentration adopted in agricultural system (Liu et al., 2011). Whether single ethoxylates had bioactivity upon *S. exigua* and *A. ipsilon* were also investigated. Water treatment served as a negative control. Briefly, the larvae were dipped into tested solutions for 5 s, dried with filter paper and transferred to 24-cell culture plates. Fresh cabbage leaves without any contaminants were used to feed the larvae. The mortality of the larvae was calculated at 48 h and the death of a larva was confirmed according to previous reports (Zhang et al., 2016). All experiments were repeated in triplicate and a total of 72 larvae were used for each concentration. The synergistic ratios were calculated as the 48 h-LC_{50} value of indoxacarb to those of ethoxylates + indoxacarb (Liu et al., 2011).

2.3. Bioactivity of ethoxylates + acetamiprid against A. citricola

Bioactivity of single acetamiprid and ethoxylates + acetamiprid against *A. citricola* were determined using previously reported methods (Li et al., 2017). To evaluate the bioactivity of ethoxylates + acetamiprid against *A. citricola*, the concentration of ethoxylates was also maintained at 1000 mg/L. Five concentrations of acetamiprid used in official tests were determined according to range-finding experiments and are displayed in Table S1 (Supporting Information). Water treatment was used as a negative control. The midget crabapple leaves with appropriate amount of wingless *A. citricola* were immersed in the solutions for 3 s. Then the mortality of *A. citricola* left on the leaves after immersion was calculated at 48 h and each treatment was repeated four times to enhance the experimental precision. The synergistic ratios were calculated in accordance with the aforementioned method.

In addition, the surface tension of the 1000 mg/L surfactant solutions were also measured as auxiliary information. The measurements were conducted using a BZY-1 automatic surface tension meter (Shanghai Hengping Instrument and Meter Factory, Shanghai, China) and repeated three times.

2.4. Acute single toxicity to D. magna

The *D. magna* used in the toxicity test were pure strain introduced from the National Institute of Environmental Health, Chinese Center for Disease Control and Prevention (Beijing, China). These crustaceans were bred and cultivated in accordance with the protocols described in OECD Guidelines (OECD, 2004). Briefly, the crustaceans were held in groundwater and fed daily with 1 mL of algae at a concentration of approximately 3×10^5 cells/ml (Fan et al., 2017). The groundwater were obtained from Tai'an, China (Site: 36°15′17″N, 117°06′15″E) with a pH of 6–8, a total hardness of 140–250 mg/L and the dissolved oxygen concentration of approximately 4.0 mg/L for seven days before

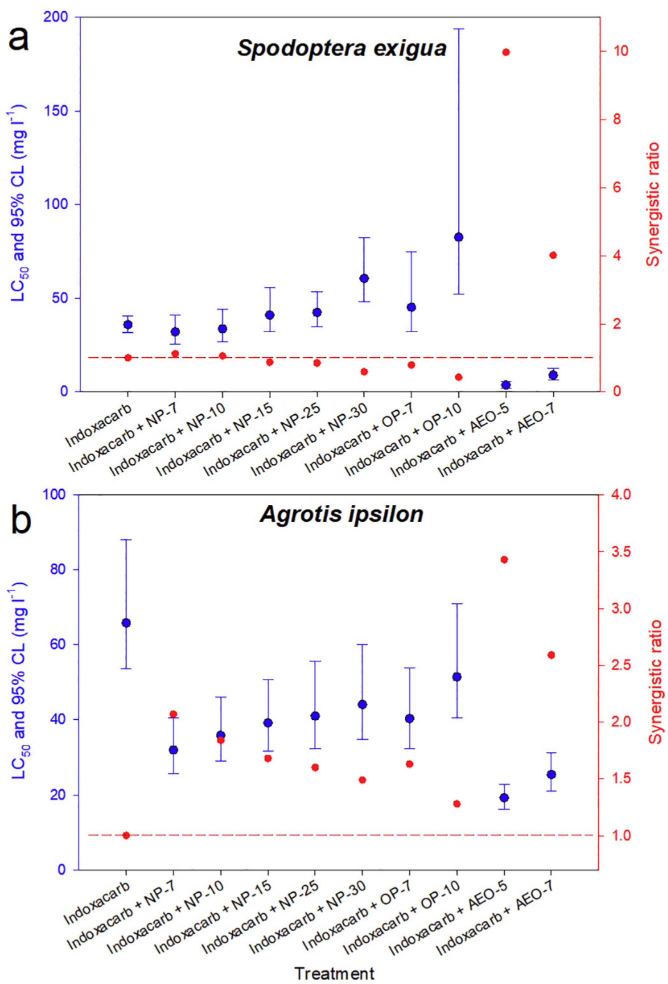

Fig. 1. LC_{50} values (blue dots), 95% confidence intervals, and synergistic ratios (red dots) of ethoxylates + indoxacarb against (a) *Spodoptera exigua* and (b) *Agrotis ipsilon*. The dotted lines indicate the synergistic ratio of 1.00. Synergistic ratios lower than 0.8, between 0.8 and 1.2, larger than 1.2 indicated antagonism, simple addition, and synergism, respectively. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

testing, which was valid according to the OECD Guideline. The standard protocols were described as follows. Tested chemicals (ethoxylates, indoxacarb and acetamiprid) were first diluted with groundwater to yield five or more concentrations (Table S2, Supporting Information). Then ten healthy and uniform *D. magna* (aged <24 h old) were exposed

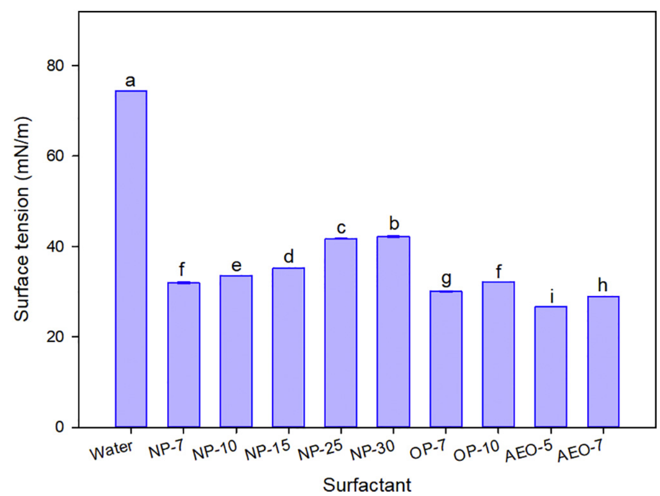

Fig. 2. Surface tension of surfactant solutions. Data are displayed as the mean ± SD and those with different lower case letters are significantly different at the $p < 0.05$ level by Tukey's test. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

to solutions contained different concentrations of toxicants. Pure groundwater served as the negative control. The test vessels were maintained at 20 ± 1 °C with a photoperiod of 16:8 h (L:D) until the end of experiment. The test was repeated in triplicate with 30 *D. magna* for each concentration. The examined endpoint was immobilization—*D. magna* were unable to swim after 15 s of gentle agitation. Immobilization was recorded at 48 h to calculate the EC_{50} value.

2.5. Acute joint toxicity to D. magna

In the joint toxicity test, the concentrations of each toxicant were chosen based on their single toxicity to *D. magna*. Four typical methods (a detailed description can be found in Table S3, Supporting Information), the additive index method (Marking, 1977), the toxicity unit method (Brown, 1968; Sprague, 1970; Nirmalakhandan et al., 1997), the V value method (He and Xiong, 1994; Clausing and Bieleke, 1980) and the isobologram method (Altenburger et al., 1990; Li et al., 2018), were used to evaluate the combined effects of ethoxylates and pesticides on *D. magna*. Joint concentrations were determined based on one toxic unit, which was defined as the ratio 1:1 of EC_{50}s of indoxacarb or acetamiprid (A) and ethoxylates (B). As shown in Table S4 (Supporting Information), the toxicant concentrations of 0.125 × ($EC_{50\text{-}A} + EC_{50\text{-}B}$), 0.25 × ($EC_{50\text{-}A} + EC_{50\text{-}B}$), 0.50 × ($EC_{50\text{-}A} + EC_{50\text{-}B}$), 0.75 × ($EC_{50\text{-}A} + EC_{50\text{-}B}$) and 1.00 × ($EC_{50\text{-}A} + EC_{50\text{-}B}$) were used in the test to calculate the joint toxicity of the mixtures (Li et al., 2018).

Table 1
Bioactivity of ethoxylates + acetamiprid against *Aphis citricola*. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers. Synergistic ratios lower than 0.8, between 0.8 and 1.2, larger than 1.2 indicated antagonism, simple addition, and synergism, respectively.

Chemicals	LC_{50} and 95% confidence interval (μg/L)	Regression	R^2	Synergistic ratio
Acetamiprid	105.1 (48.00–230.1)	Y = 5.294 + 1.301X	0.967	1.00
Acetamiprid + NP-7	2.342 (0.7383–7.427)	Y = 0.3606X + 5.9486	0.969	44.9
Acetamiprid + NP-10	2.676 (0.4586–15.62)	Y = 0.3285X + 5.8449	0.930	39.3
Acetamiprid + NP-15	2.895 (0.6750–12.42)	Y = 0.3152X + 5.800	0.950	36.3
Acetamiprid + NP-25	7.044 (2.367–20.97)	Y = 0.3162X + 5.6905	0.959	14.9
Acetamiprid + NP-30	22.48 (7.850–64.40)	Y = 0.2963X + 5.4884	0.946	4.70
Acetamiprid + OP-7	2.235 (0.2760–18.08)	Y = 0.3498X + 5.9272	0.912	47.0
Acetamiprid + OP-10	3.921 (1.418–10.84)	Y = 0.3201X + 5.7704	0.971	26.8
Acetamiprid + AEO-5	0.6076 (0.0685–5.391)	Y = 0.4053X + 6.3035	0.937	173
Acetamiprid + AEO-7	1.297 (0.1867–9.009)	Y = 0.4189X + 6.2095	0.936	81.0

Table 2
Single toxicity of ethoxylates, indoxacarb or acetamiprid to *Daphnia magna*. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

Chemicals	EC_{50} and 95% confidence interval (mg/L)	Regression	R^2
Indoxacarb	2.372×10^{-2} (1.253×10^{-2}–3.868×10^{-2})	Y = 1.105× + 1.791	0.941
Acetamiprid	27.54 (20.19–39.81)	Y = 1.637X − 2.357	0.957
NP-7	2.318 (1.728–3.006)	Y = 2.245X − 0.820	0.981
NP-10	2.650 (2.028–3.593)	Y = 1.966X − 0.832	0.936
NP-15	6.894 (5.320–9.306)	Y = 2.048X − 1.717	0.862
NP-25	35.13 (22.54–83.74)	Y = 1.384X − 2.139	0.916
NP-30	67.51 (44.37–149.3)	Y = 1.454X − 2.659	0.864
OP-7	2.518 (1.661–3.484)	Y = 1.556X − 0.624	0.988
OP-10	3.016 (1.834–4.497)	Y = 1.237X − 0.593	0.973
AEO-5	0.287 (0.192–0.377)	Y = 2.246X + 1.216	0.997
AEO-7	0.438 (0.301–0.585)	Y = 1.826X + 0.654	0.980

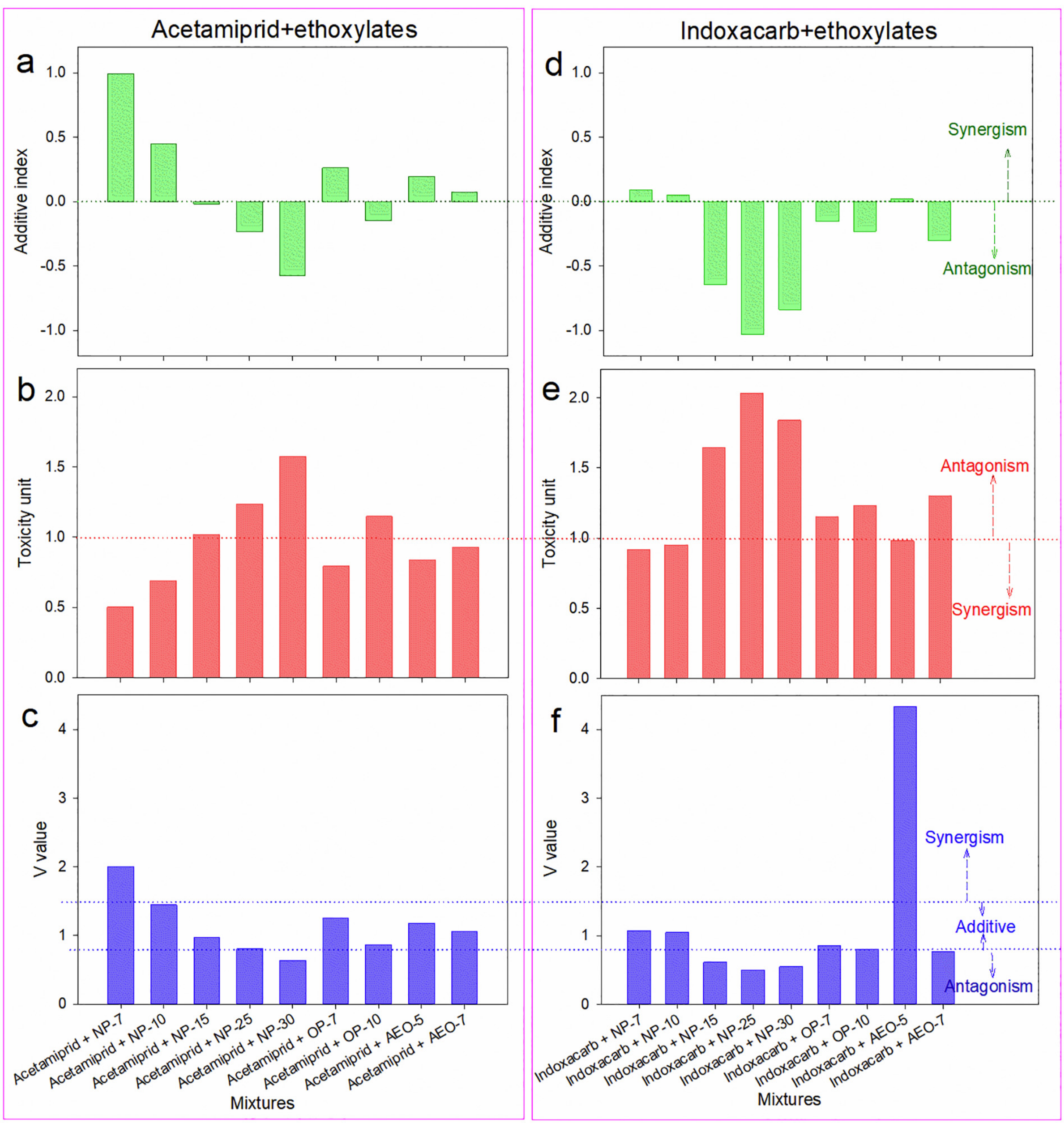

Fig. 3. (a, b and c) Joint toxicity of acetamiprid and ethoxylates to *Daphnia magna*; and (d, e and f) joint toxicity of indoxacarb and ethoxylates to *D. magna* evaluated by using the additive index, the toxicity unit and the V value models. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

2.6. Data analysis

The LC_{50} or EC_{50} values were calculated by the probit regression of the mortality of the pests or *D. magna* immobilization rates against the log10 values of toxicant concentrations. The synergistic ratios of the surfactants on pesticides were calculated as the 48 h-LC_{50} value of pesticides to those of ethoxylates + pesticides. Surface tension of the surfactant solutions are submitted for analysis of variance at the $p < 0.05$ level by Tukey's test and displayed as the mean ± standard deviation. All of the statistical analyses were carried out using the SPSS statistical package (ver.17.0, USA).

3. Results and analysis

3.1. Bioactivity of ethoxylates + pesticides in the control of S. exigua, A. ipsilon and Aphis citricola

In the first step, we have investigated the bioactivity of single ethoxylates against *S. exigua* and *A. ipsilon*. The results showed that all of the surfactant solutions had no lethal bioactivity at the concentration of 1000 mg/L. Subsequently, the bioactivity of ethoxylates + indoxacarb against *S. exigua* and *A. ipsilon* were determined to confirm whether ethoxylates had synergistic effects on indoxacarb. As shown in Fig. 1a, all of the APEOs (NP-7, NP-10, NP-15, NP-25, NP-30, OP-7 and OP-10) exhibited additive or antagonistic effects for indoxacarb in controlling *S. exigua* (synergistic ratio of 1.12, 1.06, 0.87, 0.85, 0.59, 0.79 and 0.43), whereas the AEOs (synergistic ratio of 9.97 and 4.01 for AEO-5 and AEO-7, respectively) always showed synergistic effects. In addition, synergistic ratios of all series of ethoxylates decreased with the EO number. However, the binary bioassay of ethoxylates + indoxacarb upon *A. ipsilon* showed different trends. The synergistic ratios of NP-7, NP-10, NP-15, NP-25, NP-30, OP-7, OP-10, AEO-5 and AEO-7 were 2.07, 1.84, 1.68, 1.60, 1.49, 1.63, 1.28, 3.43 and 2.59. All of the ethoxylates showed certain synergistic effects for indoxacarb against *A. ipsilon*, although the synergistic ratios gradually decreased with the EO number (Fig. 1b).

To verify whether the ethoxylates had asimilar influences on other pesticides, a Hemiptera pest *A. citricola* and a neonicotinoid insecticide acetamiprid were examined as experimental models. Much different from those on *S. exigua* and *A. ipsilon*, all ethoxylates showed highly synergistic effects for acetamiprid against *A. citricola*, although synergistic ratios greatly decreased with the EO number (Table 1). Moreover, AEOs had considerably higher synergistic effects for acetamiprid in the control of *A. citricola* than APEOs. Synergistic ratios of APEOs ranged from 44.9 to 4.70, whereas those of AEO-5 and AEO-7 were 173 and 81.0.

The surface tension of surfactant solutions also differed greatly. As depicted in Fig. 2, the surface tension of NP-7, NP-10, NP-15, NP-25, NP-30, OP-7, OP-10, AEO-5 and AEO-7 were 32.00, 33.50, 35.20, 41.75, 42.20, 30.04, 32.10, 26.60 and 28.80 mN/m, respectively. All of the tested APEOs and AEOs significantly reduced the surface tension of water (74.40 mN/m), especially AEOs. Besides, the surface tension of

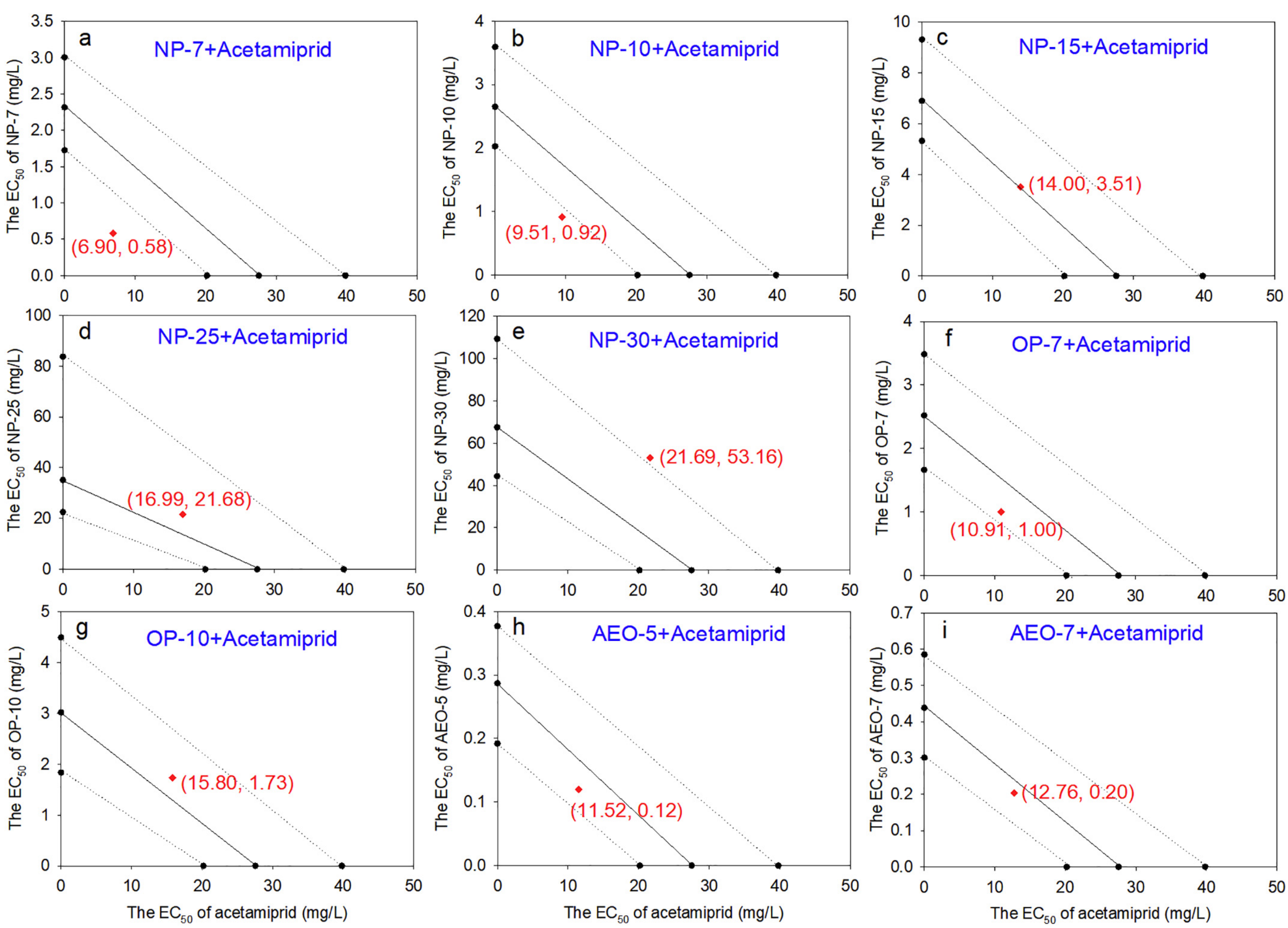

Fig. 4. Joint toxicity (at EC_{50} level) displayed by the isobologram model for the binary mixture of acetamiprid and ethoxylates. The upper and lower dotted lines represent the 95% confidence intervals, and that the labelled red dots represent where the joint toxicity of the binary mixture fall. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

all series of APEOs and AEOs greatly increased with the increasing EO number.

3.2. Single toxicity of ethoxylates, indoxacarb or acetamiprid to D. magna

As shown in Table 2, nonylphenol ethoxylates and octylphenol ethoxylates with the same EO numbers had similar acute toxicity to *D. magna*, whereas alcohol ethoxylates exhibited considerably higher toxicity. In addition, single toxicity of all series of ethoxylates to *D. magna* largely decreased with the EO number.

3.3. Joint toxicity of ethoxylates + pesticides to D. magna

Joint toxicity of ethoxylates + pesticides to *D. magna* were fully evaluated by the additive index, toxicity unit, V value and isobologram models. The results showed that the combined effects of NPEOs and acetamiprid turned from synergism to antagonism with the increasing EO number, regardless of which models were used (Fig. 3a, b and c; Fig. 4). Synergistic effects of octylphenol ethoxylates and AEOs on acetamiprid also decreased with the EO number, although results calculated using additive index and toxicity unit models were different from those calculated by the V value and isobologram models (Fig. 4a, b and c; Fig. 4). In terms of NPEOs and indoxacarb, the combined effects turned from synergism to antagonism with the EO number when using additive index and toxicity unit models (Fig. 3d and e). But V value and isobologram models were more conservative in the judgement of synergism. The combined effects of NPEOs and indoxacarb turned from additive effects to antagonism with the EO number (Fig. 3f; Fig. 5). Combined effects of octylphenol ethoxylates and indoxacarb were slightly different. A degree of antagonism was observed for OP-7 and OP-10 when using additive index and toxicity unit models (Fig. 3d and e), while those using V value and isobologram models were always additive effects (Fig. 3f; Fig. 5). As for AEOs and indoxacarb, the combined effects changed from synergism to antagonism with the EO number when using additive index, toxicity unit and V value models (Fig. 3d, e and f). In addition, additive effects were observed for the isobologram model (Fig. 5).

4. Discussion

In the current study, AEOs exhibited considerably higher synergistic effects than APEOs for indoxacarb in controlling *S. exigua* and *A. ipsilon*, although the synergistic ratios of all series of ethoxylates gradually decreased with the EO number. All of the ethoxylates displayed apparently synergistic effects for acetamiprid against *A. citricola*, with the synergistic ratios of APEOs ranging from 44.9 to 4.70, while those of AEO-5 and AEO-7 were 173 and 81.0, respectively. The significantly higher ability of AEOs over APEOs in reducing the surface tension of surfactant solutions may be an important factor, as depicted in Fig. 2. In fact, synergistic agents with favorable wettability are always required in the

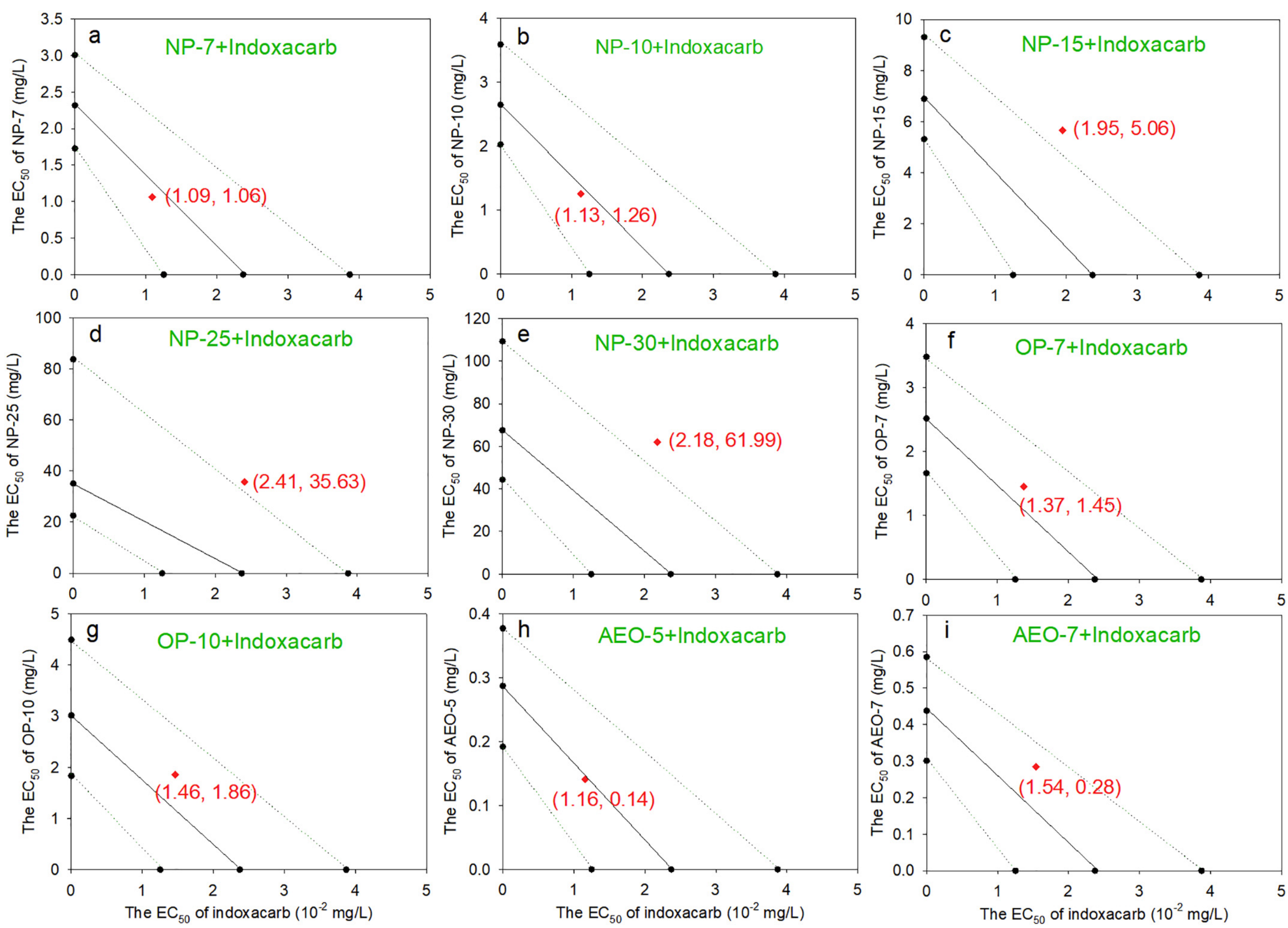

Fig. 5. Joint toxicity (at EC_{50} level) displayed by the isobologram model for the binary mixture of indoxacarb and ethoxylates. The upper and lower dotted lines represent the 95% confidence intervals, and that the labelled red dots represent where the joint toxicity of the binary mixture fall. NP-7, NP-10, NP-15, NP-25 and NP-30 (nonylphenol ethoxylates), OP-7 and OP-10 (octylphenol ethoxylates), AEO-5 and AEO-7 (alcohol ethoxylates) were ethoxylates with different ethylene oxide numbers.

management of agricultural pests to assist pesticides in adhering to the target surfaces and thus increase effective retention of active ingredients (Baur et al., 1999; Burton and Bhushan, 2006; Njobuenwu, 2007).

In this study, we have comprehensively assessed the potential of the addition of AEOs and APEOs as tank-mix surfactants in controlling various agricultural pests. The addition of agricultural surfactants into pesticide formulations or tank-mix would dramatically enhance the validity and efficacy of pesticides in pest control by promoting their penetrability through the compact wax layers of target surfaces (Antonious and Saito, 1981; Querejeta et al., 2012). Our results showed that AEOs and APEOs also have considerable application potential as tank-mix surfactants. In addition, we found that surfactants with lower surface tension tend to have higher synergistic effects on pesticides, which was consistent with the commonly used organosilicone. Therefore, we can also develop and select surfactants with lower surface tension to achieve higher bioactivity in pest management.

Remarkably, environmental risks of these surfactants showed similar laws to their surfactant activity in synergizing pesticides. The toxicity of APEOs and AEOs to *D. magna* varied from low toxic to highly toxic; and AEOs had much higher ecotoxicological effects on *D. magna*. In addition, both of APEOs and AEOs tend to show considerably lower toxicity to *D. magna* with the increasing EO number. The combined effects of most of the binary mixtures also turned from synergism to antagonism with the EO number. APEOs also had huge ecotoxicological effects on other aquatic species. Zoller (2006) reported that egg production of *Brachydanio rerio* was reduced up to 76.9% after exposure to 10 μg/L APEOs for 28 days. However, the NOECs (no observed effect concentrations) of AEOs on fish were reported to be much higher. The NOECs for the reproduction and larval survival of *Pimephales promelas* was approximately 0.73 mg/L AEOs, while the 30-day growth and survival with NOECs for *Lepomis macrochirus* were 5.7 mg/L (Dorn et al., 1997). Although they had certain environmental risks to *D. magna*, we still considered that AEOs might be potential alternatives for APEOs in agriculture as they significantly synergized pesticides against various Lepidoptera and Hemiptera pests.

In the current study, we have used four statistical methods to assess the combined effects of chemical mixtures to provide comprehensive evaluations. In general, these methods showed similar performance in assessing the joint toxicity of chemical mixtures except that V value and isobologram models were more conservative in the judgement of synergism or antagonism, compared with additive index and toxicity unit models. Many studies had compared the predictive accuracy of different models, but quantitative measures and extensively applicative models were rarely reported (Lydy and Austin, 2004; Belden et al., 2007). As clearly described in Table S3 (Supporting Information), V value and isobologram models had large buffers that were judged as additive effects. We suggested that using these two models in assessing the joint toxicity of chemical mixtures was more likely to avoid large deviation or bias.

5. Conclusion

In the current study, the synergistic effects of NPEOs, octylphenol ethoxylates and AEOs on the bioactivity of indoxacarb and acetamiprid were compared. The results showed that AEOs have considerably higher synergistic effects than APEOs for indoxacarb in controlling *S. exigua* and *A. ipsilon*, and for acetamiprid in controlling *A. citricola* although the synergistic ratios of all series of ethoxylates decreased with the EO number. Joint toxicity of ethoxylates + pesticides to *D. magna* were fully evaluated by the additive index, toxicity unit, V value and isobologram models. The results showed that the combined effects of most of the binary mixtures turned from synergism to antagonism with the increasing EO number. Overall, AEOs may be potential alternatives for APEOs considering their synergistic effects against three pests and toxicological risks to *D. magna*.

Acknowledgments

This work was supported by grants from National Natural Science Foundation of China (31772203) and National Key R&D Program of China (2017YFD0200307).

Declaration of interest statement

The authors declare no competing financial interest.

Appendix A. Supplementary data

Table S1 describes the concentrations of pesticides used in official tests for the bioassay of ethoxylates + indoxacarb against *S. exigua* and *A. ipsilon*, and those of ethoxylates + acetamiprid against *A. citricola*. Table S2 displays the concentrations of chemicals used for the determination of single toxicity to *D. magna*. Table S3 provides all-around description about the additive index (AI) method, toxicity unit method, V value method and isobologram method. Table S4 shows the concentrations of chemicals used for the determination of joint toxicities of indoxacarb + ethoxylates and acetamiprid + ethoxylates to *D. magna*.

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