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Binary mixtures of alcohol ethoxylates, nonylphenol ethoxylates and pesticides exhibit comparative bioactivity against three pests and toxicological risks to aquatic organisms

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HIGHLIGHTS

- Nonylphenol and alcohol ethoxylates had similar synergistic effects upon pests.
- Nonylphenol and alcohol ethoxylates had similar toxicities to *D. magna* or *B. rerio.*
- Synergistic ratios and toxicities of ethoxylates decreased with the EO numbers.
- Alcohol ethoxylates may be potential alternatives for nonylphenol ethoxylates.

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ABSTRACT

Nonylphenol ethoxylates are widely used surfactants in the industry and agriculture. However, seeking for alternatives has been imperative considering their effects of the hormonal and other toxicological risks. In the current study, the synergistic effects of nonylphenol ethoxylates or alcohol ethoxylates on the bioactivity of indoxacarb and acetamiprid were compared. Results showed that synergistic ratios of nonylphenol ethoxylates (TX-7~TX-30) and alcohol ethoxylates (MOA-5~MOA-20) against *Spodoptera exigua*, *Agrotis ipsilon* and *Aphis citricola* decreased with the EO (ethylene oxide) numbers, although different magnitudes of decreases were observed. Single toxicities of all ethoxylates to *Daphnia magna* and *Brachydanio rerio* also dramatically decreased with the EO numbers. In terms of joint toxicity, the combined effects of all ethoxylates and pesticides upon *D. magna* turned from synergism to antagonism with the increasing EO numbers; the combined effects of nonylphenol ethoxylates and pesticides turned from synergism to antagonism with the increasing EO numbers of ethoxylates, whereas alcohol ethoxylates and pesticides always showed antagonistic effects whatever EO numbers. Overall, alcohol ethoxylates may be potential alternatives for nonylphenol ethoxylates as they exhibited nearly comparative bioactivity against tested pests and toxicities to *D. magna* and *B. rerio*.

1. Introduction

Nonylphenol ethoxylates are widely used surfactants in textile, dyeing, paper production, cosmetic and agricultural chemicals owing to their favorable wettability, emulsibility, and oxidative resistance (Hannas et al., 2011; Felício et al., 2016). In agricultural chemical systems, nonylphenol ethoxylates are always used as wetting agents for pesticide emulsifiable concentrate, aqueous solution or suspension concentrate because their solutions have low surface tension (Datta et al., 2002; Siemering et al., 2008; Björklund et al., 2009). Besides, they can assist pesticides in permeating the wax layer of target pests or crops and thus provide satisfactory efficacy. However, nonylphenol surfactants are toxic to

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various *Daphnia* and fishes (Zhang et al., 2003). More seriously, biodegradation of nonylphenol ethoxylates is slow (Ying, 2006) and one of the stable degradation products, nonylphenol, is a typical endocrine-disrupting compound which can induce disordered cell proliferation, feminization and bad influences on nervous system and immune system (Kinch et al., 2016; Capaldo et al., 2012; Rubin, 2011; Welshons et al., 2003; Ward et al., 2013). Therefore, we wonder if there are potential alternatives for nonylphenol ethoxylates in agricultural systems. Alcohol ethoxylates are another series of high consumption and promising nonionic surfactants owing to their relatively easy biodegradation (Budnik et al., 2016; Jardak et al., 2016). Compared with nonylphenol surfactants, alcohol ethoxylates have less environmental risks. However, whether or not these alcohol ethoxylates have similar efficacies are rarely reported. In the current study, we intended to compare the synergistic effects of nonylphenol ethoxylates and alcohol ethoxylates on the bioactivity of pesticides. For comprehensive consideration, their toxicological risks to aquatic organisms, including single toxicity and joint toxicity combined with pesticides, were also evaluated.

Two frequently used representative pesticides were selected in this study as models to verify the synergistic effects and toxicological risks of nonylphenol ethoxylates and alcohol ethoxylates. Indoxacarb, an oxadiazine pesticide developed by DuPont Company, has favorable efficacy in controlling various Lepidoptera, Homoptera and Coleoptera pests as it works as an efficient sodium channel blocker (Bird, 2017; Narahashi, 2001; Zlotkin, 1999). Acetamiprid is a widely used and highly-efficient neonicotinoid insecticide for the management of Hemiptera pests such as aphids, leafhoppers, whiteflies (Sun et al., 2017; Zhang et al., 2017). However, various pests had developed different levels of resistance to indoxacarb and acetamiprid because of frequent or improper use (Romero and Anderson, 2016; Ghodki et al., 2009; Roditakis et al., 2017). The addition of synergistic agents may be an effective approach in the management of resistant populations of pests, although it is not a sustainable solution. Therefore, the above-mentioned two pesticides were used to compare the synergistic effects of nonylphenol ethoxylates and alcohol ethoxylates on pesticides, with two Lepidoptera pests *Spodoptera exigua* (Hübner) and *Agrotis ipsilon* (Rottemberg) and one Hemiptera pest *Aphis citricola* as models.

As large amount of surfactants and pesticides are used in agricultural production, part of the chemicals would be washed out and drift into the water, and then persist for a long period in groundwater (Brantley and Holmes, 2017; Fenoll et al., 2014; Harris et al., 2009). Their influences of the environmental organisms would also not be neglected. *Daphnia magna* and *Brachydanio rerio* are two typical aquatic model organisms recommended in the environmental toxicity assessments of chemicals in OECD guidelines. Thus, in the current study, they were used to evaluate the single toxicity of two series of surfactants and their joint toxicity combined with pesticides.

2. Materials and methods

2.1. Test chemicals

A series of nonylphenol ethoxylates (TX-7, TX-10, TX-15, TX-21 and TX-30) and a series of alcohol ethoxylates (MOA-5, MOA-7, MOA-9 and MOA-20) with different EO (ethylene oxide) numbers were purchased from Jiangsu Haian Petroleum Chemical Factory (Jiangsu, China). These ethoxylates were synthesized by the additive reactions between nonylphenol and EO or aliphatic alcohols and EO. Therefore, ethoxylates with different polymerization degrees (EO numbers) may have distinct surfactant properties (Dillan, 1985). Technical material of acetamiprid (purity of 99%) was purchased from Shandong Hailir Chemicals Co. Ltd. (Shandong, China). As acetamiprid is slightly soluble in water, it was dissolved in water to yield a 4000 mg L^{-1} stock solution for the sake of convenience. Technical material of indoxacarb with a high purity of 98% was supplied by Rainbow Chemical Co., Ltd (Shandong, China), and then it was fabricated to a 15% stock suspension.

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

The tested pests, *S. exigua* and *A. ipsilon*, were long-term raised populations in the Key Laboratory of Pesticide Toxicology and Application Techniques in Shandong Agricultural University, Shandong Province, China. Bioassays of indoxacarb and the mixtures with ethoxylates were carried out using newly emerged, uniform and standardized 3rd instar larvae of *S. exigua* and *A. ipsilon* according to the protocols of the immersion test for insecticide activity (Busvine, 1980). General concentrations of the chemicals used for official tests were determined based on preliminary range-finding tests. In terms of the evaluation of ethoxylates + indoxacarb, the concentrations of ethoxylates were kept at 1000 mg L^{-1}, slightly higher than typical surfactant concentration used in agricultural production (Li et al., 2018). The sole effects of the surfactants were also measured by adding ethoxylates at the same concentration. Simultaneously, distilled water without any chemicals was regarded as a control treatment. After the larvae were dipped into tested solutions for 5 s, they were allowed to dry using two pieces of filter paper and then transferred to 24-cell culture plates. Fresh cabbage leaves without any chemicals were used to feed the larvae. Forty-eight hours later, the mortality of the larvae was calculated and a larva was considered dead if it cannot move when prodded with a camel hair brush (Zhang et al., 2016). The experiment was repeated three times and a total of 72 larvae were used for each concentration. The synergistic ratios of ethoxylates to indoxacarb were defined as the LC_{50} values (48 h) of indoxacarb to those of ethoxylates + indoxacarb (Liu et al., 2011). When the synergistic ratios were larger than 1.2, between 0.8 and 1.2, and lower than 0.8, the combined effects were defined as synergistic effects, additive effects and antagonistic effects, respectively.

2.3. Bioactivity of ethoxylates + acetamiprid against A. citricola

Bioassays of acetamiprid and the mixtures with ethoxylates against *A. citricola* were conducted according to previously reported methods (Li et al., 2017). The midget crabapple leaves with appropriate amount of *A. citricola* were collected from experimental stations of Shandong Agricultural University for the bioassay of acetamiprid and its mixtures. Briefly, acetamiprid was diluted into five series of concentrations based on preliminary tests. Then midget crabapple leaves with wingless *A. citricola* were immersed in the solutions for 3 s. Forty-eight hours after treatment, the mortality of *A. citricola* was calculated. Each concentration treatment was repeated in quadruplicate. The bioactivity of ethoxylates + acetamiprid against *A. citricola* was determined by using 1000 mg L^{-1} surfactant solutions to dilute acetamiprid into five series of concentrations. The synergistic ratios of ethoxylates to acetamiprid were calculated as the LC_{50} values (48 h) of acetamiprid to those of ethoxylates + acetamiprid (Liu et al., 2011).

2.4. Measurements of the surface tension and spreadability of the surfactants

Prior to the measurements of the physical properties of the surfactant solutions, deionized water was used to prepare 1000 mg L^{-1} surfactant solutions. Then a BZY-1 automatic surface tension meter (Shanghai Hengping Instrument and Meter Factory, Shanghai, China) was used to determine the surface tension of the solutions. The measurements were repeated in triplicate. The spreadability of the surfactant solutions were measured on the surface of hydrophilic substrates according to the protocols reported by Liu et al. (2014) and the measurements were repeated four times.

2.5. Acute single toxicity to Brachydanio rerio and Daphnia magna

The *B. rerio* were cultivated according to OECD Guideline—Test No. 203: Fish, Acute Toxicity Test and held in groundwater 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^{-1} and the dissolved oxygen concentration of approximately 4.0 mg L^{-1} for seven days before testing. The standard protocol is described as follows. Firstly, the chemicals (ethoxylates, indoxacarb and acetamiprid) were diluted with groundwater to yield five or more concentrations in the toxicity test. Then ten healthy and uniform *B. rerio* with a body length of 2.3 ± 0.3 cm were exposed to each concentration of the tested chemicals. A control treatment contains only groundwater. The exposure duration was 96 h and the mortality of *B. rerio* was recorded at 24, 48, 72 and 96 h. Subsequently, the chemical concentrations that killed fifty percent of the fish (LC_{50}, 96 h) were calculated by the probit regression of the *B. rerio* mortality (96 h) against the log10 values of chemical concentrations using SPSS. All experiments were repeated three times.

The *D. magna* was the pure strain introduced from the National Institute of Environmental Health, Chinese Center for Disease Control and Prevention (Beijing, China). They were bred and cultivated according to OECD Guideline—Test No. 202: *Daphnia* sp. Acute Immobilisation Test and fed daily with 1 mL of algae *Scenedesmus obliquus* at a concentration of 2.0×10^5–3×10^5 cells mL^{-1} (Wu et al., 2017). Similar to toxicity test on *B. rerio*, tested chemicals were first diluted with abovementioned groundwater to yield five or more concentrations and groundwater with no chemicals were regarded as control. Then ten neonatal *D. magna* (aged less than 24 h old) were exposed to solutions contained different concentrations of toxicants. The test vessels were maintained at 20 ± 1 °C with a photoperiod of 16:8 h (L:D) until the end of experiment. The examined endpoint was immobilisation, which was defined as the inability to swim after 15 s of gentle agitation. The measurements were repeated three times with 30 *D. magna for* each concentration. Immobilisation was recorded at 48 h to calculate the EC_{50} value.

2.6. Acute joint toxicity on to B. rerio and D. magna

In joint toxicity test, the concentrations of each chemical were designed according to their independent toxicities to *B. rerio* and *D. magna*. An additive index (Marking, 1977) concept was adopted in the joint toxicity test of ethoxylates and pesticides. Joint concentrations were designed 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). Concentrations of $0.125 \times (EC_{50\text{-}A} + EC_{50\text{-}B})$, $0.25 \times (EC_{50\text{-}A} + EC_{50\text{-}B})$, $0.50 \times (EC_{50\text{-}A} + EC_{50\text{-}B})$, $0.75 \times (EC_{50\text{-}A} + EC_{50\text{-}B})$ and $1.00 \times (EC_{50\text{-}A} + EC_{50\text{-}B})$ were used in the test to calculate the joint toxicity of the mixtures (Li et al., 2018).

2.7. Data analysis

The EC_{50} or LC_{50} values were calculated by the probit regression of the *D. magna* immobilisation rates or *B. rerio* mortality against the log10 values of chemical concentrations. All of the statistical analyses were performed using the SPSS statistical package (ver.17.0, USA).

3. Results and analysis

3.1. Bioactivity of ethoxylates + pesticides in controlling S. exigua, A. ipsilon and A. citricola

Two kinds of Lepidoptera pests, *S. exigua* and *A. ipsilon*, were first used to confirm whether ethoxylates had synergistic effect on the bioactivity of indoxacarb. Firstly, we have investigated the bioactivity of ethoxylates against *S. exigua* and *A. ipsilon*. All of the surfactant solutions had no lethal bioactivity at the concentration of 1000 mg L^{-1}. Then, the bioactivity of ethoxylates + pesticides against pests were determined. As shown in Table 1, both nonylphenol ethoxylates and alcohol ethoxylates with small EO numbers (TX-7~TX-15; MOA-5~MOA-7) had certain synergistic effects for indoxacarb in controlling *S. exigua*. However, antagonistic effects were observed when EO numbers exceeded approximately 20 for nonylphenol ethoxylates, whereas indoxacarb + MOA-20 showed additive effect against *S. exigua*. The binary bioassay of ethoxylates + indoxacarb against *A. ipsilon* showed similar trends (Table 2). Synergistic ratios of nonylphenol ethoxylates and alcohol ethoxylates decreased with the EO number, although all of the binary combinations displayed synergistic effects.

To verify whether the EO numbers of ethoxylates had similar influences on the bioactivity of other pesticides, a hemipteran pest *A. ipsilon* and a neonicotinoid insecticide *A. citricola* were regarded as experimental models. Much different to those on *S. exigua* and *A. ipsilon*, all of the ethoxylates showed extremely synergistic effects for acetamiprid against *A. citricola* (Table 3). Synergistic ratios of TX-7~TX-30 ranged from 70.0 to 4.60, whereas those of MOA-5~MOA-20 varied from 97.1 to 22.3. Synergistic ratios of nonylphenol ethoxylates and alcohol ethoxylates dramatically decreased with the EO number, although all of the binary combinations displayed significantly synergistic effects. We deduced that this may be related to the differences in the physicochemical parameters of surfactant solutions. As depicted in Fig. 1, the surface tension of water dramatically decreased after the addition of the ethoxylates surfactants. Surface tension of the surfactant solutions increased significantly with the increasing EO number of nonylphenol ethoxylates and alcohol ethoxylates. Variations in the spreadability of the surfactant solutions provided supplementary evidence. Ethoxylates with lower EO number had more favorable spreadability on the surface of hydrophilic substrate. In addition, spreadability of the surfactant solutions dropped with EO number and those of TX-15, TX-21, TX-30 and MOA-20 solutions were not significantly different from water.

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

As shown in Table 4, *D. magna* was much more sensitive to indoxacarb (highly toxic, with EC_{50} of 2.372×10^{-2} mg/L) than acetamiprid (low toxic, with EC_{50} of 27.54 mg L^{-1}). Single toxicities of nonylphenol ethoxylates and alcohol ethoxylates to *D. magna* dramatically decreased with the EO number. The single toxicities of ethoxylates, indoxacarb or acetamiprid to *B. rerio* showed similar trends. As described in Table 5, single toxicities of the two series of ethoxylates to *B. rerio* also dramatically decreased with the EO number, except that the decreased magnitude was more significant.

Table 1
Bioactivity of ethoxylates + indoxacarb against *Spodoptera exigua*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

Chemicals	48 h-LC_{50} and 95% confidence interval (mg L^{-1})	Regression	R^2	Synergistic ratio
Indoxacarb	35.85 (31.53–40.37)	Y = 3.057X-4.752	0.963	1.00
Indoxacarb + TX-7	18.23 (13.63–23.92)	Y = 1.164X-1.468	0.991	1.97
Indoxacarb + TX-10	22.33 (16.84–29.86)	Y = 1.135X-1.531	0.988	1.61
Indoxacarb + TX-15	25.44 (19.10–34.90)	Y = 1.095X-1.539	0.974	1.41
Indoxacarb + TX-21	40.78 (31.67–56.28)	Y = 1.324X-2.132	0.985	0.88
Indoxacarb + TX-30	79.58 (57.24–131.9)	Y = 1.303X-2.476	0.961	0.45
Indoxacarb + MOA-5	10.09 (6.781–15.16)	Y = 0.808X-0.811	0.965	3.55
Indoxacarb + MOA-7	26.35 (17.80–45.84)	Y = 0.865X-1.228	0.977	1.36
Indoxacarb + MOA-9	30.15 (19.54–57.89)	Y = 0.801X-1.185	0.991	1.19
Indoxacarb + MOA-20	42.72 (29.65–73.48)	Y = 1.120X-1.827	0.978	0.84

Table 2
Bioactivity of ethoxylates + indoxacarb against *Agrotis ipsilon*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

Chemicals	48 h-LC_{50} and 95% confidence interval (mg L^{-1})	Regression	R^2	Synergistic ratio
Indoxacarb	65.83 (53.61–88.00)	Y = 1.755X-3.192	0.984	1.00
Indoxacarb + TX-7	29.12 (23.74–36.70)	Y = 1.599X-2.342	0.978	2.26
Indoxacarb + TX-10	32.85 (26.61–42.15)	Y = 1.568X-2.379	0.981	2.00
Indoxacarb + TX-15	39.10 (24.32–92.45)	Y = 0.649X-1.034	0.980	1.68
Indoxacarb + TX-21	39.51 (28.17–64.91)	Y = 0.954X-1.523	0.984	1.67
Indoxacarb + TX-30	42.57 (35.42–53.16)	Y = 1.989X-3.240	0.983	1.55
Indoxacarb + MOA-5	27.31 (22.47–33.88)	Y = 1.682X-2.416	0.979	2.41
Indoxacarb + MOA-7	33.61 (27.02–43.68)	Y = 1.514X-2.311	0.980	1.96
Indoxacarb + MOA-9	35.58 (29.26–44.85)	Y = 1.748X-2.711	0.979	1.85
Indoxacarb + MOA-20	38.75 (32.83–46.90)	Y = 2.188X-3.475	0.973	1.70

Table 3
Bioactivity of ethoxylates + indoxacarb against *Aphis citricola*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

Chemicals	48 h-LC_{50} and 95% confidence interval (mg L^{-1})	Regression	R^2	Synergistic ratio
Acetamiprid	1.051×10^{-1} (4.800×10^{-2}~2.301×10^{-1})	Y = 5.294 + 1.301X	0.967	1.00
Acetamiprid + TX-7	1.502×10^{-3} (3.624×10^{-4}~6.223×10^{-3})	Y = 0.3784X+6.068	0.962	70.0
Acetamiprid + TX-10	1.995×10^{-3} (4.685×10^{-4}~8.496×10^{-3})	Y = 0.3477X+5.939	0.956	52.7
Acetamiprid + TX-15	2.435×10^{-3} (3.330×10^{-4}~1.782×10^{-2})	Y = 0.3520X+5.920	0.916	43.2
Acetamiprid + TX-21	5.345×10^{-3} (1.665×10^{-3}~1.715×10^{-2})	Y = 0.3636X+5.826	0.958	19.7
Acetamiprid + TX-30	2.287×10^{-2} (6.706×10^{-3}~7.800×10^{-2})	Y = 0.4047X+5.664	0.928	4.60
Acetamiprid + MOA-5	1.082×10^{-3} (1.046×10^{-4}~8.014×10^{-3})	Y = 0.4259X+6.263	0.936	97.1
Acetamiprid + MOA-7	2.034×10^{-3} (4.776×10^{-4}~8.663×10^{-3})	Y = 0.4428X+6.192	0.956	51.7
Acetamiprid + MOA-9	3.963×10^{-3} (1.023×10^{-3}~1.536×10^{-2})	Y = 0.5000X+6.201	0.951	26.5
Acetamiprid + MOA-20	4.716×10^{-3} (1.222×10^{-3}~1.820×10^{-2})	Y = 0.3925X+5.913	0.948	22.3

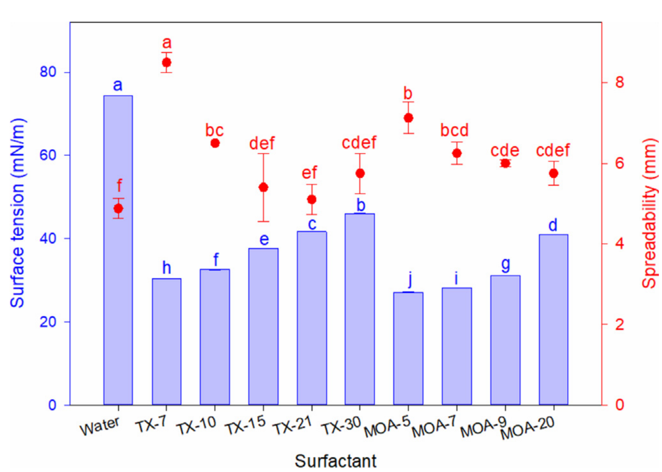

Fig. 1. Physicochemical parameters of surfactant solutions. (a) Surface tension; and (b) spreadability of adjuvant solutions. Data are displayed as the mean ± SD, and that with the same lower case letters are not significantly different at the $p < 0.05$ level by Tukey's test.

3.3. Joint toxicity of ethoxylates + pesticides to *D. magna* and *B. rerio*

Joint toxicities of ethoxylates + pesticides to *D. magna* and *B. rerio* were assessed by the additive index model. The results indicated that the combined effects of ethoxylates + indoxacarb and ethoxylates + acetamiprid upon *D. magna* turned from synergism to antagonism with the increasing EO number of ethoxylates (Fig. 2 **a and b**). In terms of the combined effect of ethoxylates + pesticides upon *B. rerio*, there were slight differences. The combined effects of nonylphenol ethoxylates (TX-7~TX-30) and pesticides turned from synergism to antagonism with the increasing EO number of ethoxylates, whereas alcohol ethoxylates (MOA-5~MOA-20) and pesticides always showed antagonistic effects whatever EO numbers.

4. Discussion

In pesticide application systems, droplets without surfactants can hardly infiltrate and adhere to the surfaces of pests or crops

Table 4
Single toxicity of ethoxylates, indoxacarb or acetamiprid to *Daphnia magna*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

Chemicals	48 h-EC_{50} and 95% confidence interval (mg L^{-1})	Regression	R^2	Toxicity
Indoxacarb	2.372×10^{-2} (1.253×10^{-2}~3.868×10^{-2})	Y = 1.105X+1.791	0.941	Highly toxic
Acetamiprid	27.54 (20.19–39.81)	Y = 1.637X-2.357	0.957	Low toxic
TX-7	1.822 (1.205–3.210)	Y = 2.021X-0.527	0.947	Moderate toxic
TX-10	2.011 (1.553–2.623)	Y = 2.111X-0.641	0.883	Moderate toxic
TX-15	5.043 (3.767–7.147)	Y = 1.672X-1.175	0.871	Moderate toxic
TX-21	21.98 (14.28–46.75)	Y = 1.180X-1.584	0.918	Low toxic
TX-30	25.98 (18.67–39.28)	Y = 1.428X-2.020	0.870	Low toxic
MOA-5	0.446 (0.333–0.561)	Y = 2.601X+0.913	0.983	Highly toxic
MOA-7	0.687 (0.509–0.892)	Y = 2.031X+0.332	0.998	Highly toxic
MOA-9	0.770 (0.545–0.965)	Y = 3.091X+0.350	0.970	Highly toxic
MOA-20	4.891 (3.408–6.544)	Y = 1.781X-1.228	0.982	Moderate toxic

Table 5
Single toxicity of ethoxylates, indoxacarb or acetamiprid to *Brachydanio rerio*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

Chemicals	96 h-LC_{50} and 95% confidence interval (mg L^{-1})	Regression	R^2	Toxicity
Indoxacarb	0.452 (0.382–0.496)	Y = 6.164X+2.128	0.954	Highly toxic
Acetamiprid	59.87 (50.97–67.58)	Y = 4.327X-7.690	0.987	Low toxic
TX-7	1.343 (1.223–1.428)	Y = 9.287X-1.189	0.937	Moderate toxic
TX-10	2.899 (2.342–3.485)	Y = 2.971X-1.373	0.981	Moderate toxic
TX-15	13.11 (11.76–14.30)	Y = 5.807X-6.490	0.963	Low toxic
TX-21	33.29 (26.84–40.84)	Y = 2.617X-3.984	0.966	Low toxic
TX-30	85.58 (77.80–93.72)	Y = 5.903X-11.41	0.966	Low toxic
MOA-5	1.139 (1.096–0.177)	Y = 15.97X-0.904	0.975	Moderate toxic
MOA-7	1.353 (1.238–1.437)	Y = 9.470X-1.244	0.969	Moderate toxic
MOA-9	5.976 (4.910–6.868)	Y = 3.648X-2.832	0.959	Moderate toxic
MOA-20	12.82 (11.73–13.79)	Y = 7.295X+8.083	0.966	Low toxic

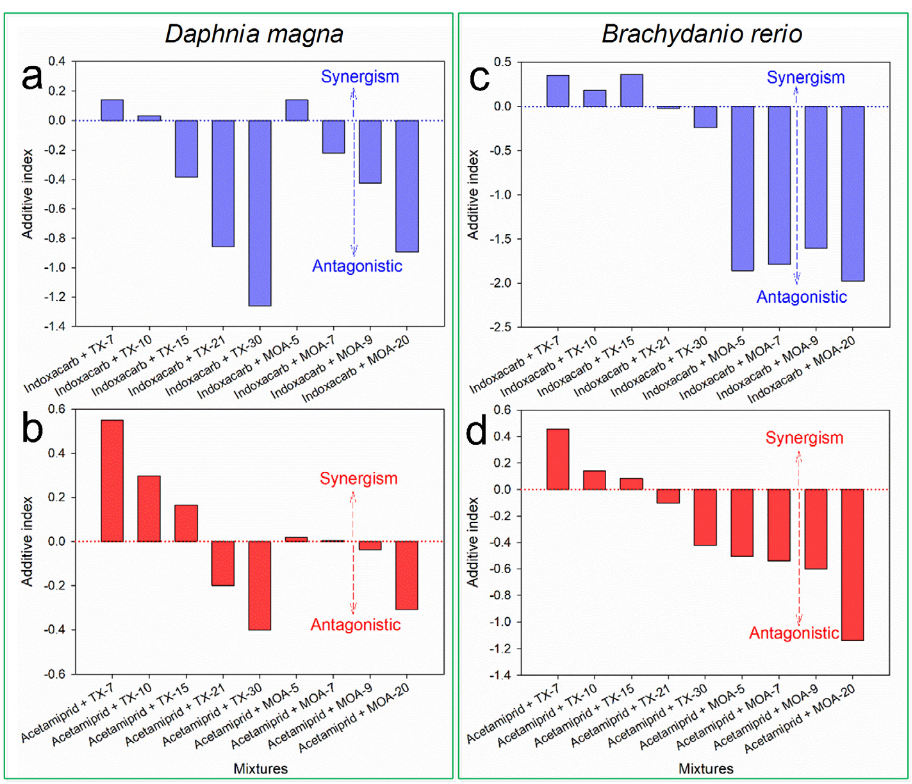

Fig. 2. Joint toxicity of (a) ethoxylates + indoxacarb to *Daphnia magna*; (b) ethoxylates + acetamiprid to *Daphnia magna*; (c) ethoxylates + indoxacarb to *Brachydanio rerio*; (d) ethoxylates + acetamiprid to *Brachydanio rerio*. TX-7~TX-30 were a series of nonylphenol ethoxylates with different ethylene oxide numbers, while MOA-5~MOA-20 were a series of alcohol ethoxylates.

because of the compact wax layers that coating them (Burton and Bhushan, 2006; Njobuenwu, 2007; Baur et al., 1999). And thus, decreased efficacies were always observed owing to the runoff of the active ingredients (Querejeta et al., 2012). The addition of wetting agents in the pesticide formulations or tank-mix has been an indispensable approach as they can significantly enhance the efficacy by increasing utilization efficiency of pesticides (Antonious and Saito, 2008).

In the current study, strong synergistic effects of surfactants were only observed for *A. citricola* and not for *S. exigua* and *A. ipsilon*. We deduced that the penetrability and retention capabilities of different ethoxylates may account for these differences. According to PubChem Compound Database, acetamprid has a water solubility of 4250 mg L^{-1} and a log Kow value of 0.8 at 25 °C, while indoxacarb has a water solubility of 0.2 mg L^{-1} and a log Kow value of 4.65. As acetamprid has lower log Kow value, it possesses inferior affinity with hydrophobic wax layers of the pests and can hardly penetrate these compact wax layers. With the use of surfactants, the uptake or adherence of acetamprid on the wax layer are much likely to be enhanced and thus lead to higher synergistic effects. But in terms of indoxacarb, which is expected to have a high affinity for wax in the first place, adding a surfactant may have much less effect as its uptake is already high. As a consequence, the interactions of indoxacarb and ethoxylates for *S. exigua* and *A. ipsilon* were much weaker. To the best of our knowledge, the interactions is general rather than only relevant for the specific experimental conditions, although different mixtures of surfactants and pesticides have different synergistic effects on various pests.

In the current study, both of nonylphenol ethoxylates and alcohol ethoxylates can significantly reduce the surface tension of water and showed certain synergistic effects on the bioactivity of indoxacarb and acetamiprid at low polymerization degrees (small EO numbers). However, their synergistic effects on the bioactivity of pesticides also dramatically decreased with the EO number. Simultaneously, their single toxicities and joint toxicities to *D. magna* and *B. rerio* also dropped with EO number. Furthermore, nonylphenol ethoxylates and alcohol ethoxylates showed nearly considerable bioactivity against tested pests and toxicities to *D. magna* and *B. rerio*. Therefore, we consider that alcohol ethoxylates may be potential alternatives for nonylphenol ethoxylates.

The bioactivity and toxicity mediated by the EO number of ethoxylates were another interesting phenomena. Previously articles reported that the hydrophilic-lipophilic balance (HLB value) and hydrophilicity of ethoxylates increased with the EO number, whereas the surface tension of their aqueous solutions decreased with the EO number (Qin et al., 2014). Wettability and permeability of alcohol ethoxylates were related with their length of the carbon chains and the EO numbers (Schönherr, 1993; Moore et al., 2006). When the EO numbers of alcohol ethoxylates were kept at 7–10, they were always soluble in water and this range was appropriate for ensuring their favorable wettability and permeability. This was consistent with our findings in this manuscript. However, the surfactants were harder to degrade with increasing EO numbers or phenolic groups (Steber and Wierich, 1983; Federle and Itrich, 2006; Krogh et al., 2003), let alone the antagonistic effects against *S. exigua* observed above. We deduced that MOA-9 may be the best choice considering the balanced bioactivity and toxicological risks.

5. Conclusion

In the current study, the synergistic effects of nonylphenol ethoxylates or alcohol ethoxylates on the bioactivity of indoxacarb and acetamiprid were compared. Results showed that synergistic ratios of nonylphenol ethoxylates and alcohol ethoxylates against *S. exigua*, *A. ipsilon* and *A. citricola* decreased with the EO numbers, although different magnitudes of decreases were observed. Single and joint toxicities of ethoxylates to *D. magna* and *B. rerio* also dramatically decreased with the EO numbers. Overall, alcohol ethoxylates may be potential alternatives for nonylphenol ethoxylates. In addition, *D. magna* and *B. rerio* may be potential models organisms for the screening of synergists as the toxicities of adjuvant + pesticides to them were consistent with their bioactivities.

Declaration of interest statement

The authors declare no competing financial interest.

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