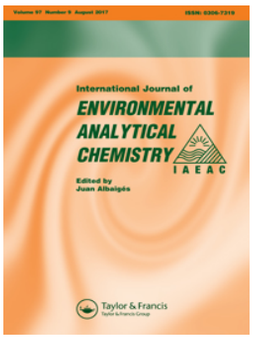


International Journal of Environmental Analytical Chemistry

ISSN: 0306-7319 (Print) 1029-0397 (Online) Journal homepage: www.tandfonline.com/journals/geac20

Effects of neonicotinoid insecticide formulations and their components on *Daphnia magna* – the role of active ingredients and co-formulants

Eszter Takács, Szandra Klátyik, Mária Mörtl, Gergely Rácz, Krisztina Kovács, Béla Darvas & András Székács

To cite this article: Eszter Takács, Szandra Klátyik, Mária Mörtl, Gergely Rácz, Krisztina Kovács, Béla Darvas & András Székács (2017) Effects of neonicotinoid insecticide formulations and their components on *Daphnia magna* – the role of active ingredients and co-formulants, International Journal of Environmental Analytical Chemistry, 97:9, 885-900, DOI: 10.1080/03067319.2017.1363196

To link to this article: https://doi.org/10.1080/03067319.2017.1363196

Published online: 22 Aug 2017.

Submit your article to this journal

Article views: 382

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=geac20

INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY, 2017
VOL. 97, NO. 9, 885–900
https://doi.org/10.1080/03067319.2017.1363196

Taylor & Francis
Taylor & Francis Group

ARTICLE

Check for updates

Effects of neonicotinoid insecticide formulations and their components on *Daphnia magna* – the role of active ingredients and co-formulants

Eszter Takács[a], Szandra Klátyik[a], Mária Mörtl[a], Gergely Rácz[b], Krisztina Kovács[b], Béla Darvas[a] and András Székács[a]

[a]Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, Budapest, Hungary; [b]Institute for Energy Security and Environmental Safety, Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary

ABSTRACT

Formulating agents used in pesticide products have been regarded as inactive/inert components; however, several studies confirmed additive, synergistic or antagonistic effects between the active ingredients (AIs) and additives, or the individual toxicity of the formulating agents. The worldwide used neonicotinoid insecticides and their components can reach surface waters, due to their physico-chemical properties (e.g. water solubility) or improper application in chemical plant protection technology, and can adversely affect non-target aquatic organisms. Formulated pesticides were analysed for alkane sulphonate surfactants by using liquid chromatography coupled with tandem mass spectrometry. In acute immobilisation tests on *Daphnia magna*, differences were found among the toxicity of the investigated neonicotinoid AIs and formulations. The toxicity of a formulated insecticide (Apache 50 WG®– AI: clothianidin) was found to be 46.5 times more toxic than explained by its AI, probably due to toxic effect of the formulating agents on *D. magna*. In contrast, two preparations (Calypso 480 SC® – AI: thiacloprid, Actara 240 SC®– AI: thiamethoxam) were 2–3 times less toxic than their AIs. Results indicate possible synergistic/antagonistic interaction with the AIs.

ARTICLE HISTORY
Received 14 January 2017
Accepted 26 July 2017

KEYWORDS
Neonicotinoid; adjuvant; surfactant; liquid chromatography–mass spectrometry; *Daphnia magna*; toxicity

1. Introduction

Neonicotinoids are currently the most frequently used insecticides worldwide, commercialised in more than 200 formulations, and widely used in aerosols and in seed coating [1,2]. Due to their systemic properties in plants, they are absorbed and transported to all parts and tissues [3,4]. These ingredients affect the central nervous system of insects as agonist of the nicotinic acetylcholine receptors (nAChRs), resulting in nervous stimulation at low concentrations, and receptor blockage, paralysis and death at higher concentrations. The toxicity of neonicotinoids in vertebrates is lower due to weaker binding

CONTACT Mária Mörtl m.mortl@cfri.hu

†This paper was presented at the 15th Symposium on Chemistry and Fate of Modern Pesticides, Santiago de Compostela (Spain), 4th–7th October 2016

© 2017 Informa UK Limited, trading as Taylor & Francis Group

of these chemicals to the α-subunit of their nACh receptors, which is different from the subunit found in invertebrates; and due to additional differences in properties and structure of these subunits between insects and vertebrates [5–7]. Nonetheless, neonicotinoids exert serious adverse effects on the ecosystem and non-target organisms. Pollinators are among the most endangered species by the application of systemic neonicotinoid formulations. Uptake and excretion of neonicotinoids by the plant transport system [1,8] exert high risk to pollinators especially in flowering crops. Therefore, the application of several neonicotinoids (imidacloprid – IMI; thiamethoxam – TMX; clothianidin – CLO) was banned or restricted in the European Union in 2013 [9], but the reassessment of neonicotinoid active ingredients (AIs) has not yet been completed.

Due to their high water solubility, neonicotinoids can reach surface waters and adversely affect the aquatic ecosystems and non-target organisms [10–12]. As they are often detected in surface water, mitigation of their risk and protection of aquatic habitats is also of high importance. Contamination rates in surface water samples and concentrations of neonicotinoids are variable [13]. High detection frequencies were observed in US stream waters (CLO >75%, TMX >47%, IMI >23%) [14]. The concentration of IMI was consistently reported at low μg L^{-1}, levels of CLO and TMX are often unreported and/or unknown [13]. However, water samples collected from wetlands in Canada contained CLO and/or TMX at peak concentrations of 3.11 and 1.49 μg L^{-1}, respectively, found in 16–91% of the samples [15]. Drifting of contaminated dust during sowing of coated seeds also raises contamination levels of puddles in corn fields. Neonicotinoids were found in all water samples collected from these fields, and their concentrations ranged from 0.01 to 63 μg L^{-1} [16]. Residues of CLO and TMX were detected at levels the level up to 43.60 μg L^{-1} (mean = 2.28 μg L^{-1}) and 16.50 μg L^{-1} (mean = 1.12 μg L^{-1}) in 100% and 98.7% of surface water samples, respectively, collected within or around of maize fields in Ontario [17].

Pesticide formulations applied in chemical plant protection contain various co-formulants, beside their AIs. Co-formulants have long been considered as inert/inactive ingredients, therefore, their authorisation requires simplified risk assessment compared to the AIs, according to current legislation [18]. However, 'inertness' of the additives can be considered only from the aspect of the main effect of the plant protection product (PPP). In this context, additives cannot exert main activity *per definitionem*, as otherwise they would be considered as AIs. Inertness, however, cannot be postulated from the aspect of side-effects, as substances used as additives are as prone to reveal unintended adverse side-effects, as the AIs. Differential toxicity of PPPs compared to their AIs have been demonstrated for several formulated pesticide products, including herbicides, fungicides and insecticides [19], raising attention to the fact that the toxicity characteristics of the AIs are far not sufficient for the risk assessment of the PPP. Several studies proved additive, synergistic or antagonistic effects between the AI and the additives in pesticide formulations, and toxicity has been confirmed for various additives (e.g. surfactants) [20–22]. Thus, in order to perform appropriate environmental risk assessment of formulations used in agriculture, the toxicological evaluation of surfactants and other co-formulants is essential.

Determination of neonicotinoids is generally performed by liquid chromatography (LC) [23,24], but quantification and qualification of surfactants used in different pesticide formulations is a more complex issue. Conventional analytical methods are usually

insufficient to detect surfactants from different classes. Aromatic surfactants can be determined by using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, but lack of chromophores allows their determination only after chemical modification with an UV absorbing moiety [25]. The complexity of surfactant mixtures often makes quantification uncertain. Sufficient resolution of peaks can be achieved easier by using a gas chromatographic (GC) method, but analysis of ionic surfactants requires more complicated sample pre-treatment. For example, alcohol sulphates have to be hydrolysed to alcohols and converted to volatile silyl derivatives prior to analysis [26]. Therefore, LC methods coupled to mass spectrometry (MS) became the most frequently used technique for the analysis of surfactants. However, characterisation and quantification present a number of challenges, if target compounds are either unknown or reference materials are commercially not available. In addition, even if surfactants are identified, the oligomer distribution may vary among formulations, as reported for glyphosate formulations containing polyethoxylated tallow amine (POEA) [27]. Furthermore, the environmental fate may also affect homologue distribution patterns. Unsaturated tallow moieties in POEA are more prone to degradation, than saturated tallow homologues, and the degradation rate depends on the number of ethoxylate units as well [28]. Widespread occurrence of POEA on agricultural soils was reported from the USA [28]. The first multiple-class analytical method for surfactants, suitable for environmental monitoring purposes, was published only recently [29].

Daphnia magna (Straus, 1820; *Cladocera, Daphniidae*) is a proper indicator of the disadvantageous effects of environmental contaminants in natural waters due to its outstanding sensitivity to changes in water quality. However, according to the results of earlier toxicity studies, this species does not occur to be particularly sensitive to neonicotinoids in the immobilisation test: reported 48-hr EC_{50} values, summarised by Anderson *et al*. (see Appendix, ref [13].) are in the range of 6.029–56.6, 67.6–119 and >106 mg L^{-1} for IMI, CLO and TMX, respectively [11–13,30–32]. In addition, surfactants and other additives may have adverse effects on *D. magna* [22]. Ecotoxicity of IMI to aquatic organisms clearly showed that crustaceans and insects represent sensitive species groups [33]. On the basis of acute and chronic toxicity ranking of individual species among crustaceans, *D. magna* was found to be the least sensitive. Despite of the similar feeding strategies and life-forms, even closely related species within a given taxon show significantly higher in sensitivity (e.g. *Ceriodaphnia dubia, Gammarus pulex* and *Gammarus roeseli*). As toxicity to *D. magna* were over one order of magnitude lower than to other species, this species was not included in the ecotoxicity characterisation of IMI [33], but was considered in other studies, e.g. by Hayasaka *et al*. [30] indicating significant differences in the sensitivity among *Daphnia* species (*D. magna, D. pulex, C. dubia and C. reticulata*). In addition, substantial differences among various strains of *D. magna* in sensitivity to xenobiotocs have also been reported [34]. According to 48-hr EC_{50} values of CLO, the most sensitive species was *C. dubia* (1.7 mg L^{-1}), while *D. magna* appeared to be 40 times less sensitive (67.6 mg L^{-1}) [30]. Nonetheless, *D. magna* is a widely applied indicator organism for aquatic toxicity studies, and is the preferred test species according to the ISO 6341 and OECD guidelines [35–37].

The aim of the study was to investigate and compare the potentially adverse acute effects of three neonicotinoid AIs, thiacloprid (TCL), TMX and CLO, as well as their formulations on *D. magna* to explore the role of surfactants in the toxicity, and to

demonstrate that the toxicity of the AIs may not be sufficient alone to explain toxicity characteristics of the formulations. For this purposes, we have determined the surfactant composition in formulations, and analytically verified the levels of exposure of *D. magna* by determining AI content in the test solutions as well. *D. magna* was chosen as a test organism in this study due to being a standard organism in ecotoxicology, easy maintenance under laboratory conditions, short life cycle, low requirements about culture and test medium volumes, as well as information available about the genetic and epigenetic background of its sensitivity.

2. Experimental

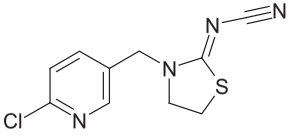
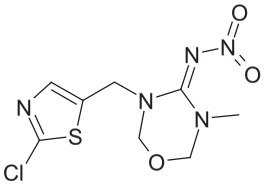
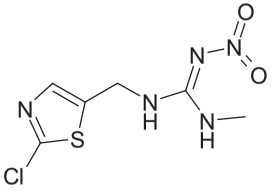
2.1 *Standards and reagents*

Analytical standards of CLO, TCL and TMX were obtained from Sigma-Aldrich Co. LLC (Darmstadt, Germany). All standards were of ≥97.5% purity. Sodium salt of hexadecane-1-sulphonic acid (CAS 15015-81-3) of synthesis grade purity (≥95%) was obtained from Merck (Darmstadt, Germany). Neonicotinoid formulations Actara SC® (Syngenta Inc., Basel, Switzerland), Apache 50 WG® (Sumimoto Chemical Takeda Agro Co. Ltd., Tokyo, Japan) and Calypso 480 SC® (Bayer CropScience AG, Leverkusen, Germany) were purchased from local distributors. Their composition according to material safety data sheets (MSDSs) are summarised in Table 1 and some other characteristics of the formulated neonicotinoid PPPs are in Table 2. Acetonitrile (ACN), methanol (MeOH) and ammonium acetate used for HPLC analyses were obtained by Sigma Aldrich Co. LLC.

2.2 *HPLC analysis*

Exact ingredient contents were checked in test solutions prepared from the AIs and also from formulated pesticides. For establishing analytical standard curves with the three neonicotinoids TCL, TMX and CLO, stock solutions at a concentration of 1.0 g L^{-1} were prepared in methanol from each compound. This stock solution was diluted to the required standard concentrations. AI content was determined for each AI in the most concentrated aqueous solution (170, 622 and 340 mg L^{-1} for TCL, TMX and CLO, respectively) used in the test similarly to PPPs. Analytical standards, as well as PPPs were weighted, and dissolved in volumetric flasks to 100 mL in ISO Test water (see Section 2.3.). Masses of Actara, Apache and Calypso were 402.0, 15.89 and 21.76 mg, respectively. These stock solutions were diluted 100-fold for Actara and 10-fold for the other two solutions and filtered prior to determination of ingredient content. Three independent dilutions were measured, and their concentrations were calculated on the basis of calibration curves obtained with analytical standards of the given AI. Analyses of neonicotinoids were performed on a Younglin YL9100® HPLC system equipped with a YL9150 autosampler according to the method published earlier [41]. Briefly, compounds were separated on a C_{18} column (150 mm × 4.6 mm i.d., 5 µm) at 40°C. UV detector signals were recorded at λ = 252 and 269 nm. Eluent flow rate was 1.0 mL min −1 with isocratic elution for 10 min (70:30 = A:B eluents, A = 90% water:10% MeOH, B = MeOH). External calibration was used for quantitation.

Table 1. Composition of the formulated neonicotinoid PPPs studied [38–40].

Formulation	AI[a]	Concentration (%)[b]	Additive[c]	Concentration (%)[b]
Calypso 480 SC	Thiacloprid (TCL) (CAS 111988-49-9) 	40.4	Benzisothiazolinone (CAS 2634-33-5)	0.01–0.05
			glycerine (CAS 56-81-5)	10
Actara 240 SC	thiamethoxam (TMX) (CAS 153719-23-4) 	21.6	Ethoxylated polyarylphenol phosphate ester (CAS 90093-37-1)	1–5
			Tristyrylphenol ethoxylates, polyethylene glycol mono(tristyrylphenyl)ether, ethoxylated polyarylphenol, $(C_2H_4O)_nC_{30}H_{30}O$ (CAS 99734-09-5)	1–5
			Ethoxylated lignosulphonic acid, sodium salts (CAS 68611-14-3)	1–5
Apache 50WG	clothianidin (CLO) (CAS 210880-92-5) 	50	Calcinated diatomaceous earth (CAS 91053-39-3)	≥10
			$C_{14\text{-}18}$-alkanehydroxy-, $C_{12\text{-}20}$-alkapolyene-, $C_{14\text{-}18}$-alkene- and $C_{12\text{-}20}$-alkenehydroxysulphonic acids, sodium salts (CAS 68937-98-4)	≥10

[a]Active ingredient. Chemical Abstracts Registry Numbers (CAS Reg No.) are shown under the substance name in parentheses.
[b]Percentage mass/mass concentration as indicated on the material safety data sheet (MSDS) of the product.
[c]Additives, including surfactants, used for formulation of the pesticide product. Chemical Abstracts Registry Numbers (CAS Reg No.) are shown under the substance name in parentheses.

Table 2. Active ingredient (AI) content and other characteristics of the formulated neonicotinoid PPPs studied.

Formulation	AI	Concentration[a]		Density	Water solubility[b]	Environ. hazard[c]
		(g L^{-1})	(m/m%)[d]	(g L^{-1})	(g L^{-1})	
Calypso 480 SC	Thiacloprid (TCL)	480 (534.5±28.8)	40.4 (45.0±2.4)	1190	0.185	H400, H410
Actara 240 SC	Thiamethoxam (TMX)	240 (250.4±7.2)	21.6 (22.5±0.7)	1110–1150	4.100	H351, H400, H410
Apache 50 WG	Clothianidin (CLO)	n.a.[e]	50.0 (42.9±2.5)	n.a.[e]	0.340	H400, H410

[a]Nominal concentration shown as indicated on the material safety data sheet (MSDS) of the product. Numbers in parentheses below the nominal values show concentrations determined by instrumental (HPLC) analysis.
[b]Water solubility of active ingredient (AI)
[c]Environmental hazards indicated on the material safety data sheets (MSDSs) of the products [38–40]. According to the EU Regulation 1272/2008, categories are: H400 – very toxic to aquatic life; H410 – very toxic to aquatic life with long lasting effects; H351 – suspected carcinogen
[d]Percentage mass/mass concentration
[e] n.a.: does not apply to solid granules

Analytical separation of surfactants or formulated pesticide samples was performed by using an Agilent 1200 liquid chromatograph equipped with a Thermo Scientific Acclaim®Surfactant Plus column (150 mm × 3.0 mm i.d., 3.0 µm) (Thermo Fisher Scientific Inc., Waltham, USA). Each compound was detected and identified with MS coupled to LC. MS experiments were performed by an Agilent 6410 triple quadrupole tandem mass spectrometer with an electrospray ionisation source. First, compounds were separated by using 10 mM ammonium acetate, pH = 5.0 (A)/ACN (B) eluent system. The initial eluent constitution was 60% A and 40% B, after 5 min B was increased to 85%, and it was maintained from 15 min until the end. LC-separations were performed at 25°C at a flow rate of 0.25 mL min^{-1} and the injection volume was 3 µL. The MS parameters were as follows: gas temperature 300°C; gas flow 12 L min^{-1}; nebuliser 30 psi; capillary voltage 3000 V. Full-scan mode was selected in negative ionisation mode measuring the mass/charge ratios (*m/z*) between 50 and 800. Later separations were made by using higher buffer concentration (100 mM). The initial eluent 30% B was gradually increased to 95% B in 10 min, maintained until 15 min and returned to 30% B at 16 min. The flow rate was set to 0.4 mL min^{-1}, and 10 µL was injected. Accordingly, higher nebuliser gas temperature and pressure were applied (350°C; 50 psi). The data were collected in full-scan negative-ion mode at a range of 50–400 *m/z*.

Because of the similarity of the homologues, it was assumed that each sulphonate gives a similar instrument response. This allows to convert relative intensities to concentrations, or to estimate the level of a particular compound in the surfactant mixture. External calibration was obtained with 5 standard solutions prepared from hexadecane-1-sulphonic acid sodium salt in the range of concentrations between 1.25 and 50 mg L^{-1}. Calibration solutions were prepared from a stock solution by diluting it in acetonitrile:buffer = 50:50 (v/v). All samples were filtered through a 0.45 or 0.22 µm polytetrafluoroethylene syringe filter (Phenomenex) prior to HPLC analysis.

2.3 *Toxicity testing*

The standard laboratory colony of *D. magna*, originated from LAB Research Kft. (Veszprém, Hungary), was cultured in reconstituted water prepared according to ISO

6341, as follows. Distilled water was supplemented with 294 mg of $CaCl_2 \cdot 2\ H_2O$, 123.25 mg $MgSO_4 \cdot 7\ H_2O$, 64.75 mg $NaHCO_3$ and 5.75 mg KCl per liter. It is also called ISO Test water by the standard and OECD 202 guideline [35,37]. Half of the culture media was changed weekly, while the entire culture media was changed monthly. *D. magna* were fed four times a week with algal suspension (originated unicellular green algae *Pseudokirchneriella subcapitata* in batch-culture with Z8 growth medium applied under controlled laboratory conditions (T = 20 ± 2 °C, continuous illumination) [42]. The algal suspension was centrifuged (30 min, 3000 rpm) and resuspended in ISO Test water three times prior to feeding. *D. magna* individuals were maintained under 16-hr light and 8-hr dark periods and at 20 ± 2°C temperature. Before each test period, the sensitivity of *D. magna* was checked and considered proper according to the standard protocol, providing a 24-hr EC_{50} of potassium dichromate ($K_2Cr_2O_7$) (0.7 mg L^{-1}) within the acceptable range of 0.6–2.1 mg L^{-1} [35].

The acute immobilisation tests on *D. magna* were conducted on the basis of OECD Test No. 202 [35]. During the tests *Daphnia* juveniles aged less than 24 hr were used and exposed to the test substances. Therefore, 24 hrs prior to testing, adult *D. magna* were separated and juveniles being produced overnight were used in toxicity testing. The test duration was 48 hrs. Aerated reconstituted ISO test water was used in the tests. The pH value of the solutions remained between the acceptable range of 6–9 (7.5 ± 1.5) during the experiments. The temperature was kept between 18 and 22°C (20 ± 2°C), with 16-hr light and 8-hr dark photoperiods. There was no feeding of the test organisms during the test. Test containers were 15 mL beakers with a solution volume of 10 mL. The test solutions were ISO standard water with known concentrations of neonicotinoid AIs and formulations. In each test, five concentrations of the investigated compound along with a control were used in four replicates at each level. Five *D. magna* juveniles were placed in each beaker using a pipette at test initiation. Altogether 24 beakers (four replicates per concentration and control) and 120 *D. magna* juveniles were used in a test, and each test was carried out three times. Triplicate tests were performed for each (3) compound alone and in formulation. Immobilisation was recorded upon 24 and 48 hrs of exposure, and was compared to the untreated control values. *Daphnia* juveniles were considered to be immobilised, if they were unable to swim within 15 s, after gentle agitation of the test vessels (even if they could still move their antennae) [35]. The criteria of the chosen test were verified. In the control groups, immobilisation did not exceed the acceptable 10%. EC_{50} values were statistically determined at 48 hr and calculated by statistical software ToxRat® (ToxRat Solutions GmbH, Alsdorf, Germany). HPLC analysis for determination of AI content of different formulations applied in the test was performed according to our method published earlier [41]. The 48-hr EC_{50} values were calculated using the measured concentrations of AIs ($EC_{50[AI]}$).

3. Results and discussion

3.1 *Chemical analysis of the AIs in the formulated pesticides studied*

All three analytes were determined from HPLC peak areas at the corresponding retention times with excellent linear calibration characteristics (calibration curves shown in Figure 1). Thus, for quantification of target compounds, peak areas determined for TCL

and TMX at 252 nm and for CLO at 269 nm were used. (The UV absorbance maxima were very similar for TCL and TMX.) The linear regression values of external calibration curves were 0.9998, 0.9994 and 0.9996, and the slopes were 78.27, 67.13 and 73.85 for TCL, CLO and TMX, respectively. Peak purity was checked by ratios of signal intensities (peak areas) recorded at 252 nm and at 269 nm. These values for standard solutions were 2.559, 1.685 and 0.809 for TCL, TMX and CLO, respectively. Relative standard deviations (SDs) established for different concentration levels for three parallel injections were between 0.71% and 1.93%.

To assure that the intended amounts of the AIs were applied, AI content was determined for each AI in the most concentrated aqueous solution. Levels were 167.2 ± 4.5, 629 ± 12.1 and 329 ± 8.4 mg L^{-1} containing nominally 170, 622 and 340 mg L^{-1} of TCL, TMX and CLO, respectively. Similarly 97.9 ± 5.9, 905.9 ± 24.2 and 68.1 ± 3.4 mg L^{-1} AI were measured for solutions containing 217.6, 4020 and 158.9 mg

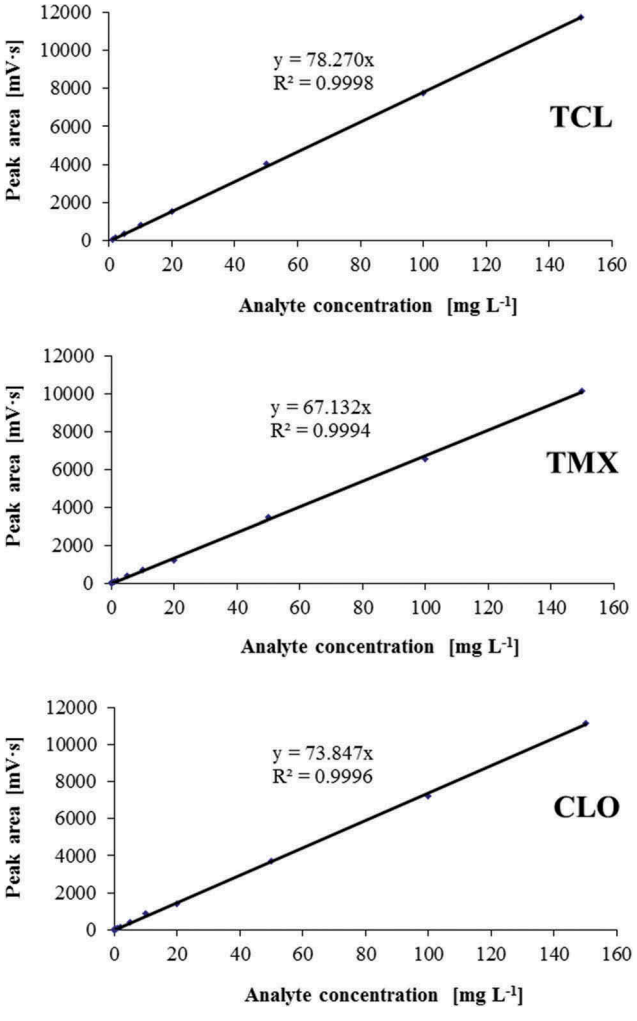


Figure 1. Calibration curves for TCL and TMX determined at 252 nm and CLO determined at 269 nm.

L^{-1} of Calypso 480 SC®, Actara 240 SC® and Apache 50 WG®, respectively. Corresponding nominal concentrations of the AIs were 87.9 mg L^{-1} for TCL, 869 mg L^{-1} for TMX and 79.5 mg L^{-1} for CLO, representing 111 ± 6%, 104 ± 3% and 86 ± 5% recoveries for AIs. These stock solutions were applied in the toxicity experiment.

3.2. *Chemical analysis of the surfactants in the formulated pesticides studied*

In some cases, scarce information is available from the manufacturer about the nature and amount of surfactants or other additives applied in different PPP. On the MSDS of Calypso 480 SC®, 40.4% TCL, benzisothiazolinone (CAS 2634-33-5) preservative (0.01–0.05%) or glycerine (CAS 56-81-5) 10% are indicated as hazardous ingredients [38]. According to its MSDS, printed in 2015, Actara 240 SC® contains 21.6% TMX and three different formulating agents, including Soprophor 3D33 (CAS 90093-37-1) surfactant (ethoxylated polyarylphenol phosphate ester) at a concentration of 1–5% (see Table 1) [39]. (It may be noted that a decade earlier the product was formulated with Soprophor 3D33 alone [39].) The two additional surfactants are ethoxylated polyarylphenol, $(C_2H_4O)_nC_{30}H_{30}O$ (CAS 99734-09-5) and ethoxylated lignosulphonic acid sodium salts (CAS 68611-14-3). These common names of the surfactants denote complex mixtures of individual components encumbering the characterisation and quantitative determination of surfactants. The situation is the same for Apache 50 WG®. According to its MSDS [40], it consists of 50% CLO, more than 10% of calcinated diatomaceous earth (CAS 91053-39-3) and a mixture of alkanesulphonic acid derivatives (CAS 68937-98-4) as a dispersing agent. This loosely defined anionic surfactant is prepared from olefins of various carbon chain lengths by sulphonation. Other additives are not listed. As indicated in Table 1, not only the constitution, but also the concentrations of the additives are indefinite. Calypso 480 SC® does not contain any polymer surfactant and analysing Actara 240 SC®, we could not detect any of the polymers indicated by the LC method applied. The latter formulation contains hydrophobic surfactants and probably that group of compounds irreversibly bind to the solid phase of the Acclaim surfactant column.

In case of Apache 50 WG®, the mixture of long-chain sulphonates was detected by using LC-MS. Proposed structures and relative peak intensities are listed in Table 3. Homologues were eluted separately from the Acclaim surfactant column, but only partial separation of isomers was achieved using buffer at low concentration (10 mM). In the extracted ion chromatograms appeared double, triple or even more peaks belonging to the different (groups of) isomers (Figure 2). Better resolution of peaks was reported by using UPLC [43], however, not all peaks were baseline separated. Isomers containing a sulphonate group in an internal position of the linear alkyl chain eluted earlier compared to the isomer where the sulphonate group was connected to the second carbon atom (external). Higher buffer concentration (100 mM) in the eluent decreased the retention times (Figure 3) as it was expected for anionic surfactants on Acclaim surfactant column, and later eluting components appeared in the chromatogram (See m/z = 331 and 349). Simultaneously, the efficiency of the separation somewhat decreased, but peaks of isomers belonging to the same homologue did not appear in a single peak. Sulphonic acids belong to strong acids, thus, sulphonates are present in an ionic form, and pH of the mobile phase has insignificant effect on the charge state of the surfactant. However, it is important to adjust the pH, as the charge state of surface

Table 3. Retention time (Rt), peak intensity and estimated concentration of surfactant homologues found in APACHE 50 WG (500 mg L^{-1}).

Peak	$[M-H]^-$	Rt (min)	Proposed chemical formula	Peak intensity[a]	Concentration (mg L^{-1})[b]
1	275	12.48, 12.69	$C_{14}H_{27}SO_3$	3,703,311	1.40
2	289	10.80, 11.84	$C_{15}H_{29}SO_3$	319,375	0.12
3	291	10.79, 11.19, 11.51	$C_{15}H_{31}SO_3$	185,389	0.07
4	293	10.88, 11.48, 12.29	$HOC_{14}H_{28}SO_3$	1,381,118	0.52
5	303	13.78, 13.96, 14.20	$C_{16}H_{31}SO_3$	6,025,149	2.29
6	305	13.77, 13.98, 14.22	$C_{16}H_{33}SO_3$	467,285	0.18
7	307	11.61, 12.38,	$HOC_{15}H_{30}SO_3$	49,347	0.02
8	317	12.10, 12.91	$C_{17}H_{33}SO_3$	124,985	0.05
9	321	12.36, 12.94, 13.58	$HOC_{16}H_{32}SO_3$	1,278,501	0.48
10	331	15.46, 16.40	$C_{18}H_{35}SO_3$	25,327,962	9.61
11	333	16.42	$C_{18}H_{37}SO_3$	2,526,888	0.96
12	349	13.90, 15.01, 15.90	$HOC_{18}H_{36}SO_3$	8,584,022	3.26

[a]Summarised peak intensities of extractive ion chromatograms for homologues
[b]As linear hexadecane-1-sulphonic acid in the 500 mg L^{-1} APACHE solution

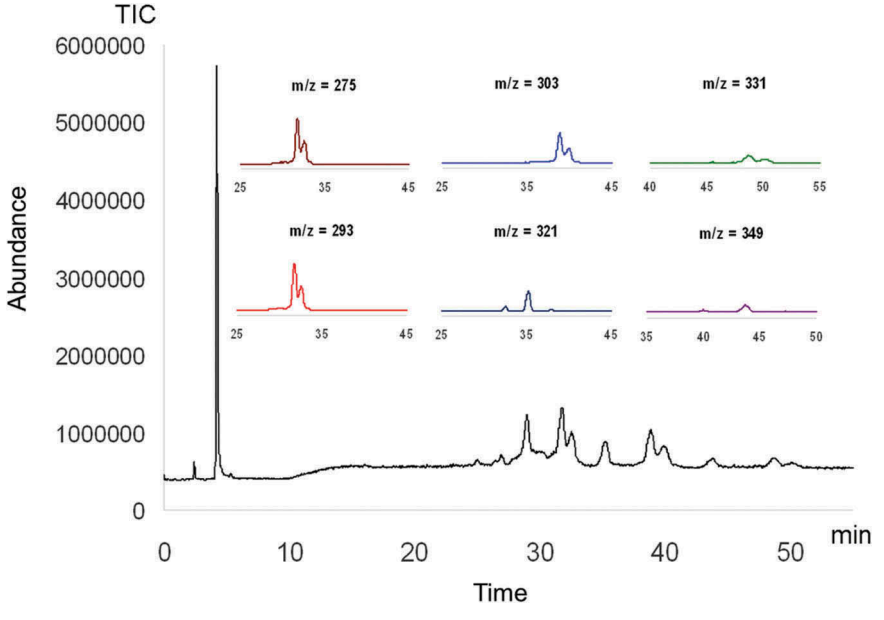


Figure 2. Chromatogram (total ion count, TIC) recorded by applying low buffer concentration (10 mM) and extracted ion chromatograms.

silanol groups in the stationary phase is pH dependent. Alkaline pH results in a lower retention of anionic compounds. Because there were no standards available for each homologue, it was assumed that every homologue gives identical instrument response. This practical assumption was used for a similar analysis of POEA by Tush *et al*. [28]. Linear hexadecane-1-sulphonic acid sodium salt was not present in APACHE sample, and it eluted later (14.65 min) than components of C_{16} sulphonates in APACHE formulation (see line 6 in Table 3), indicating that these compounds are secondary sulphonates rather than the corresponding primary isomer. A commercial sulphonate mixture studied by Baena-Nogueras *et al*. [43] consisted of C_{14}, C_{15}, C_{16}, C_{17}, whereas homologue distribution observed in APACHE formulation was different. For quantitative determination of

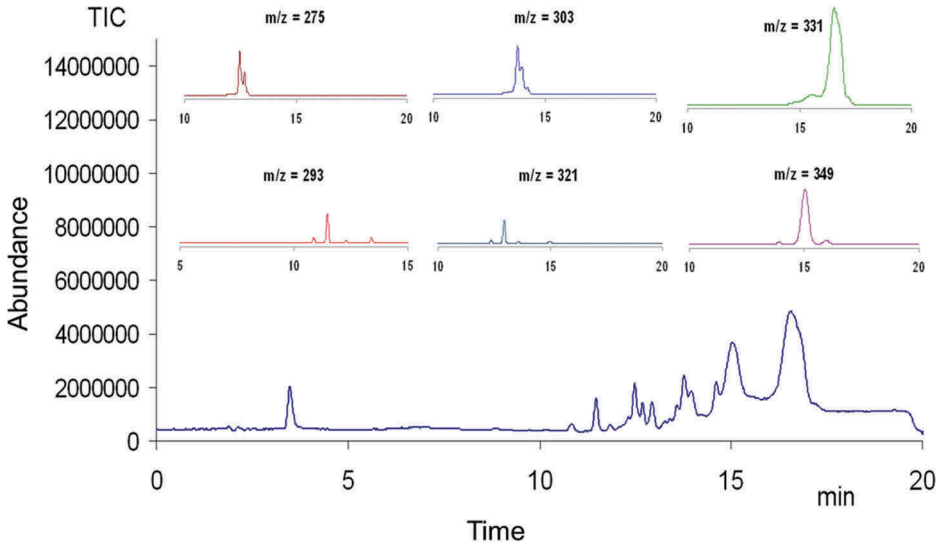


Figure 3. Chromatogram (total ion count, TIC) recorded by applying high buffer concentration (100 mM) and extracted ion chromatograms.

sulphonates, we used the linear calibration curve obtained for linear hexadecane-1-sulphonic acid. The estimated concentration of the homologues were calculated by their extracted ion intensities ($[M\text{-}H]^-$) related to that of linear hexadecane-1-sulphonic acid (Table 3). Uncertainty in the results caused by matrix effects or derived from the inhomogeneity of formulated pesticide was not considered. In this way the amounts of surfactants were about two-fold lower than expected. Nonetheless, linear hexadecane-1-sulphonic acid is only a characteristic component for quantification of the substance group. Standard addition of the actual components in the surfactant product at their true proportions would probably provide more precise results, than quantification with linear hexadecane-1-sulphonic acid, yet certified reference material for this alkylsulphonate mixture is not available commercially.

3.3. *Results of toxicity testing*

On the basis of the acute immobilisation tests on *D. magna*, significant differences were observed in the toxicity of the neonicotinoid AIs and their formulations tested. Among the examined AIs, TCL was the most toxic (EC_{50} = 5–13.5 mg L^{-1}), followed by TMX (EC_{50} = 93–159 mg L^{-1}) and CLO (EC_{50} > 340 mg L^{-1}) (Table 4). CLO at the concentration of 236 mg L^{-1} resulted in 25% mortality, while at its water solubility limit, 340 mg L^{-1} caused immobilisation of 38.3% of the *D. magna* juveniles. The extrapolated 48-hr EC_{50} value of 531.4 mg L^{-1} above water solubility limit of CLO was calculated on the basis of the linear part of the sigmoid dose–response curve. CLO is a metabolite of TMX, as cleavage of the oxadiazine ring in TMX forms CLO as insecticidal active metabolite in insects and plant tissues [6]. This conversion results in lower toxicity to *D. magna*. In case of CLO the 48-hr EC_{50} values obtained from the acute toxicity tests on *D. magna* were significantly higher compared to the results of other research groups (Hayasaka *et al.*

Table 4. Results of acute *Daphnia* immobilisation tests with analytical standards of the active ingredients (AIs) and with formulated neonicotinoid PPPs of different active ingredients (AIs).

		48-hr EC_{50} (mg L^{-1})					
Formulation	AI	Min	Max	Avg.	±SD	%SD	PPP/AI ratio[b]
Thiacloprid standard	TCL	5.0	13.5	10.1	4.5	34%	
Thiamethoxam standard	TMX	93.0	159.0	126	46.7	37%	
Clothianidin standard	CLO	>340	–	531.4[c]	–	–	
Calypso 480 SC	TCL	17.38	39.67	27.0	9.45	35%	2.668
Actara 240 SC	TMX	165.71	307.58	226.72	68.21	30%	1.799
Apache 50 WG	CLO	7.18	14.19	11.43	3.74	33%	0.022[d]

[a]Four repetitions (four beakers with 5 *D. magna* juveniles each) were used for each concentration tested, the tests were repeated with each compound three times.
[b]Ratio of the 48-hr EC_{50} values determined for a formulated plant protection product (PPP) and the corresponding AI
[c]Extrapolated value above water solubility limit of CLO, on the basis of the linear part of the sigmoid dose–response curve determined. At the water solubility limit, 0.340 g L^{-1} concentration CLO caused immobilisation of 38.3% of the *D. magna* juveniles.
[d]Estimated ratio on the basis of the extrapolated EC_{50} of CLO (see also footnote [c]).

67.56 mg L^{-1}), the United States Environmental Protection Agency (US EPA; 119 mg L^{-1}) and the toxicity value found in IUPAC database (>40 mg L^{-1}) [13,30,31,44]. The calculated 48-hr EC_{50} values of TCL on the basis of our experiments (5–13.5 mg L^{-1}), were 6–17-fold lower compared to the toxicity values listed by the Food and Agriculture Organisation of the United Nations (FAO; >85.1 mg L^{-1}) and in the IUPAC database (85.1 mg L^{-1}) [44,45]. The toxicity of TMX appeared to be in accordance with the values that were reported by Anderson *et al*. (>106 mg L^{-1}) and with the ones listed in the IUPAC database (>100 mg L^{-1}) [13,45]. This is not extraordinary, as differential sensitivity of various D. magna strains to organic micropollutants have been evidenced in the scientific literature [34].

The average 48-hr EC_{50} values determined for the investigated formulations was different compared to the toxicity values indicated on the MSDS of the given formulations. The average acute toxicity values of the investigated formulations were 2.2–3.2-fold higher than the reported values on the basis of the MSDSs [38–40]. In contrast to the toxicity of AIs, investigated formulation Apache 50 WG®, (containing 50% CLO) proved to be the most toxic ($EC_{50[AI]}$ = 11.43 ± 3.74 mg L^{-1}) Calypso 480 SC®, (containing 48% TCL) was less toxic ($EC_{50[AI]}$ = 27 ± 9.45 mg L^{-1}) while the toxicity of Actara 240 SC®, (containing 24% TMX) was the lowest ($EC_{50[AI]}$ 226.72 ± 68.2 mg L^{-1}) for the applied aquatic test organism (Table 4). The toxicity of AIs TCL and TMX were 2.7 and 1.8 times higher than their formulations investigated, respectively, while Apache 50 WG® was found to be 46.5 times more toxic that explained by its AI, CLO. Probably the applied formulating agents are responsible for the enhanced adverse effect for Apache 50 WG®, and possibly also for the reduction in the toxicity of Calypso 480 SC® and Actara SC®, relative to their AIs. This is in accordance with scientific data and with our previous results on glyphosate-based herbicide formulations, where significantly higher toxicity was detected in case of the investigated formulating agents, compared to the AI and other formulations as well [22,46], in agreement with Brausch *et al*., who reported acute sub-lethal effects of the non-ionic formulating adjuvant POEA on *D. magna* e.g. growth inhibition at concentrations between 100 and 500 µg L^{-1} for all investigated POEA formulations [47]. The toxicity of the surfactants significantly depends on their molecular characteristics. The acute toxicity of the linear alkylbenzene sulphonates (LASs) on *D. magna* increased with the alkyl chain length or homolog

molecular weight [48]. The increase of the chain length of alcohol ethoxylates decreased, acute toxicity on *D. magna* [49,50]. On the basis of the Ecotox database of US EPA, sodium α-olefinsulphonate (sodium C_{14}-C_{16}) is moderately toxic to aquatic invertebrates, in case of *C. dubia* the lowest reported 48-hr EC_{50} was 4.5 mg L^{-1}. Due to their low toxicity and biodegradability, chronic effects of sodium α-olefinsulphonates (sodium C_{14}–C_{16}) as inert ingredients in pesticide formulations to are less likely to occur on aquatic species [51].

Values and percentages of SD observed in our acute toxicity tests on *D. magna*, although relatively high, fall in the range of literature data regarding the 'repeatability' of the assay at intra-laboratory level or the 'reproducibility' at inter-laboratory level investigated [52–54]. Moreover, the acute toxicity values of neonicotinoids appear to have more variation than those of other insecticides, at least when tested on bees [55].

4. Conclusion

Neonicotinoid-based insecticides and their AIs can affect adversely aquatic non-target organisms in unforeseen ways. As indicated by our results, formulating agents can enhance the toxicity of CLO or reduce the toxic effects of TCL and TMX in their formulated products. Consequently formulating agents applied in agrochemicals cannot be considered as unequivocally inactive ingredients from ecotoxicological and toxicological aspects. Due to difficulties in determination of surfactants in the environment, little is known about their current contamination rates and levels and thus effects on the diversity of ecosystems remain unknown. Therefore, it is necessary to develop reliable analytical methods, integrated regulation and a prerequisite system regarding qualitative and quantitative determination, application, classification and labelling of co-formulants. Our approach indicates substantial effects of the formulating agents on the ecotoxicity of the neonicotinoid PPPs studied, even though it results in slightly underestimated concentrations for sulphonic acid homologues. Further improvements will render the method useful not only in supporting ecotoxicity studies, but also in monitoring studies.

Acknowledgement

The authors thank to Csilla Magor and Judit Juracsek for their technical support.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Hungarian Scientific Research Fund [OTKA K109865 and OTKA K112978].

ORCID

Szandra Klátyik http://orcid.org/0000-0001-9566-6179
András Székács http://orcid.org/0000-0001-5816-3775

References

[1] D. Goulson, J. Appl. Ecol **50**, 977 (2013). doi:10.1111/1365-2664.12111.

[2] P. Jeschke, R. Nauen, M. Schindler and A. Elbert, J. Agric. Food Chem **59**, 2897 (2011). doi:10.1021/jf101303g.

[3] R.H. Bromilow and K. Chamberlain, *In Plant Contamination: Modeling and Simulation of Organic Chemical Processes*, edited by S. Trapp and J.C. Mc Farlane (CRC Press, Boca Raton, 1995). ISBN: 9781566700788.

[4] J.-M. Bonmantin, C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E.A.D. Mitchell, D.A. Noome, N. Simon-Delso and A. Tapparo, Environ. Sci. Pollut. Res. **22**, 35 (2014). doi:10.1007/s11356-014-3332-7.

[5] M. Tomizawa and J.E. Casida, Annu. Rev. Pharmacol. Toxicol. **45,** 247 (2005). doi:10.1146/annurev.pharmtox.45.120403.095930.

[6] N. Simon-Delso, V. Amaral-Rogers, L.P. Belzunces, J.M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D.W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C.H. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E.A.D. Mitchell, C.A. Morrissey, D.A. Noome, L. Pisa, J. Settele, J.D. Stark, A. Tapparo, H. Van Dyck, J. Van Praagh, J.P. Van Der Sluijs, P.R. Whitehorn and M. Wiemers, Environ. Sci. Pollut. Res. **22**, 5 (2015). doi:10.1007/s11356-014-3470-y.

[7] K. Matsuda, S.D. Buckingham, J.C. Freeman, M.D. Squire, H.A. Baylis and D.B. Sattelle, Br. J. Pharmacol **123**, 518 (1998). doi:10.1038/sj.bjp.0701618.

[8] V. Girolami, L. Mazzon, A. Squartini, N. Mori, M. Marzaro, A. Di Bernardo, M. Greatti, C. Giorio and A. Tapparo, J. Econ. Entomol. **102**, 1808 (2009). doi:10.1603/029.102.0511.

[9] European Parliament and Council, Offic. J. Eur. Comm. **L139**, 12 (2013). doi: 10.3000/19770677.L_2013.139.eng.

[10] Á. Vehovszky, A. Farkas, A. Ács, O. Stoliar, A. Székács, M. Mörtl and J. Győri, Aquat. Toxicol. **167**, 172 (2015). doi:10.1007/s10661-016-5432-y.

[11] T. Tišler, A. Jemec, B. Mozetič and P. Trebše, Chemosphere **76**, 907 (2009). doi:10.1016/j.chemosphere.2009.05.002.

[12] F. Sánchez-Bayo, K. Goka and D. Hayasaka, Front. Environ. Sci. **4**, 1 (2016). doi:10.3389/fenvs.2016.00071.

[13] J.C. Anderson, C. Dubetz and V.P. Palace, Sci. Total. Environ. **505**, 409 (2015). doi:10.1016/j.scitotenv.2014.09.090.

[14] M.L. Hladik, D.W. Kolpin and K.M. Kuivila, Environ. Pollut. **193**, 189 (2014). doi:10.1016/j.envpol.2014.06.033.

[15] A.R. Main, J.V. Headley, K.M. Peru, N.L. Michel, A.J. Cessna and C.A. Morrissey, PLoS One **9**, e92821 (2014). doi:10.1371/journal.pone.0092821.

[16] O. Samson-Robert, G. Labrie, M. Chagnon and V. Fournier, PLoS One **9**, e108443 (2014). doi:10.1371/journal.pone.0108443.

[17] A. Schaafsma, V. Limay-Rios, T. Baute, J. Smith and Y. Xue, PLoS One **10**, e0118139 (2015). doi:10.1371/journal.pone.0118139.

[18] European Parliament and Council, Offic. J. Eur. Comm. **L104**, 1 (2004). ISSN: 1725-2555.

[19] R. Mesnage, N. Defarge, J.P. De Vendomois and G.E. Seralini, Food Chem. Toxicol **84**, 133 (2015). doi:10.1016/j.fct.2015.08.012.

[20] C. Cox and M. Surgan, Environ. Environ. Health Persp. **114**, 1803 (2006). doi:10.2307/4119588.

[21] R. Mesnage, B. Bernay and G.-E. Seralini, Toxicol. **313**, 122 (2013). doi:10.1016/j.tox.2012.09.006.

[22] I. Székács, Á. Fejes, Sz. Klátyik, E. Takács, D. Patkó, J. Pomóthy, M. Mörtl, R. Horváth, E. Madarász, B. Darvas and A. Székács, J. Biol. Vet. Agric. Food Eng. **87**, 213 (2014). ISSN: 2010-376X.

[23] K.P. Yáñez, J.L. Bernal, M.J. Nozal, M.T. Martín and J. Bernal, J. Chromatogr. A **1285**, 110 (2013). doi:10.1016/j.chroma.2013.02.032.

[24] W. Xie, C. Han, Y. Qian, H. Ding, X. Chen and J. Xi, J. Chromatogr. A **1218**, 4426 (2011). doi:10.1016/j.chroma.2011.05.026.

[25] M. Beneito-Cambra, L. Ripoll-Seguer, J.M. Herrero-Martínez, E.F. Simó-Alfonso and G. Ramis-Ramos, J. Chromatogr. A **1218**, 8511 (2011). doi:10.1016/j.chroma.2011.09.059.

[26] C. Fernández-Ramos, O. Ballesteros, R. Blanc, A. Zafra-Gómez, I. Jiménez-Díaz, A. Navalón and J.L. Vílchez, Talanta **98**, 166 (2012). doi:10.1016/j.talanta.2012.06.065.
[27] D. Tush, K.A. Loftin and M.T. Meyer, J. Chromat. A **1319**, 80 (2013). doi:10.1016/j.chroma.2013.10.032.
[28] D. Tush and M.T. Meyer, Environ. Sci. Technol. **50**, 5781 (2016). doi:10.1021/acs.est.6b00965.
[29] A. Bergé, B. Giroud, L. Wiest, B. Domenjoud, A. Gonzalez-Ospina and E. Vulliet, J. Chromatogr. A **1450**, 64 (2016). doi:10.1016/j.chroma.2016.04.078.
[30] D. Hayasaka, K. Suzuki, T. Nomura, M. Nishiyama, T. Nagai, F. Sánchez-Bayo and K. Goka, J. Pestic. Sci **38**, 44 (2013). doi:10.1584/jpestics.D12-061.
[31] United States Environmental Protection (US-EPA), Pesticide fact sheet, 2003 <https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-044309_30-May-03.pdf> [accessed 28.July.2017].
[32] Syngenta Material Safety Data Sheets of CRUISER® 5FS <http://www.syngenta-us.com/currentmsds.aspx?productId=721> (2015) [accessed 28.July.2017].
[33] C.E. Smit, C.J.A.M. Posthuma-Doodeman, P.L.A. Van Vlaardingen and F.M.W. De Jong, Hum. Ecol. Risk Assess. **21**, 1608 (2015). doi:10.1080/10807039.2014.964071.
[34] H. Toumi, M. Boumaiza, M. Millet, C.M. Radetski, B.I. Camara, V. Felten and J.F. Ferard, J. Environ. Sci. Health. B **50**, 34 (2015). doi:10.1080/03601234.2015.965617.
[35] Organisation for Economic Co-operation and Development, *Test No. 202: Daphnia Sp. Acute Immobilisation Test*, (OECD Publishing, Paris, 2004). p. 12.
[36] Organisation for Economic Co-operation and Development, *Test No. 211: Daphnia Magna Reproduction Test* (OECD Publishing, Paris, 2012), p. 25.
[37] International Organization for Standardization, *ISO 6341. Water Quality - Determination of the Inhibition of the Mobility of Daphnia Magna Straus (Cladocera, Crustacea) - Acute Toxicity Test*, Third ed. (International Organization for Standardization, Geneva, 2012), p. 22.
[38] Bayer CropScience Material Safety Data Sheets of Calypso 480 SC® <https://www.cropscience.bayer.ca/~/media/Bayer%20CropScience/Country-Canada-Internet/Products/Calypso/calypso_msds.ashx> (2011); <http://agro.bayer.co.hu/WEBSET_DOWNLOADS/82/msdsCalypso.pdf> (2014) [accessed 28.July.2017].
[39] Syngenta Material Safety Data Sheets of Actara 240SC ®. <41.164.31.82:8080/TRU-CAPE/Actara%20240%20SC.pdf> (2005); <https://www.syngenta.hu/product/crop-protection/rovarolo-szer/actara-sc> (2015) [accessed 28.July.2017].
[40] Arysta LifeScience Material Safety Data Sheet of Apache 50WG® <https://www.agrofert.cz/downloads/bezpecnostni-listy-agrochemikalie-sk/Apache%2050%20WG%20SK.pdf> (2013) [accessed 28.July.2017].
[41] M. Mörtl, O. Kereki, B. Darvas, Sz. Klátyik, Á. Vehovszky, J. Győri and A. Székács, J. Chem. 2016, 4546584. doi:10.1155/2016/4546584
[42] Scandinavian Culture Collection for Algae and Protozoa, Z8. <http://www.sccap.dk/media/freshwater/7.asp> [accessed 28.July.2017].
[43] R.M. Baena-Nogueras, E. González-Mazo and P.A. Lara-Martín, Environ. Pollut. **176**, 151 (2013). doi:10.1016/j.envpol.2013.01.020.
[44] International Union of Pure and Applied Chemistry, The Pesticide Properties Database, 2017 <http://sitem.herts.ac.uk/aeru/iupac/atoz.htm> [accessed 28.July.2017].
[45] Food and Agriculture Organization of the United Nations, FAO Specifications and Evaluations for Agricultiral Pesticides, 2010 <http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Thiacloprid_2010.pdf> [accessed 28.July.2017].
[46] N. Defarge, E. Takács, V.L. Lozano, R. Mesnage, J.S. De Vendômois, G.-E. Séralini and A. Székács, Int. J. Environ. Res. Public Health **31**, 264 (2016). doi:10.3390/ijerph13030264.
[47] J.M. Brausch, B. Beall and P.N. Smith, Bull. Environ. Contam. Toxicol. **78**, 510 (2007). doi:10.1007/s00128-007-9091-0.
[48] C. Verge, A. Moreno, J. Bravo and J.L. Berna, Chemosphere **44**, 1749 (2000). doi:10.1016/s0045-6535(00)00574-9.
[49] A.W. Maki and W.E. Bishop, Arch. Environ. Contam. Toxicol. **8**, 599 (1979). doi:10.1007/bf01055040.

[50] D.C. Wong, P.B. Dorn and E.Y. Chai, Environ. Toxicol. Chem. **16**, 1970 (1997). doi:10.1002/etc.5620160929.
[51] United States Environmental Protection (US-EPA), 2006 <https://www.epa.gov/sites/production/files/2015-04/documents/olenfinsulfonate.pdf> [accessed 28.July.2017].
[52] B.R. Parkurst, W. Warren-Hicks and L.E. Noel, Environ. Toxicol. Chem. **11**, 771 (1992). doi:10.1002/etc.5620110607.
[53] P.A. Lewis and W.B. Horning, Environ. Toxicol. Chem. **10**, 1351 (1991). doi:10.1002/etc.5620101014.
[54] G. Persoone, R. Baudo, M. Cotman, C. Blaise, K.C. Thompson, M. Moreira Santos, B. Vollat, A. Torokne and T. Han, Knowl. Manag. Aquat. Ecosyst. **393**, 01 (2009). doi:10.1051/kmae/2009012.
[55] F.D. Rinkevich, J.W. Margotta, J.M. Pittman, R.G. Danka, M.R. Tarver, J.A. Ottea and K.B. Healy, PLoS One **10**, e0139841 (2015). doi:10.1371/journal.pone.0139841.