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Aquatic toxicity and loss of linear alkylbenzenesulfonates alone and in a neonicotinoid insecticide formulation in surface water

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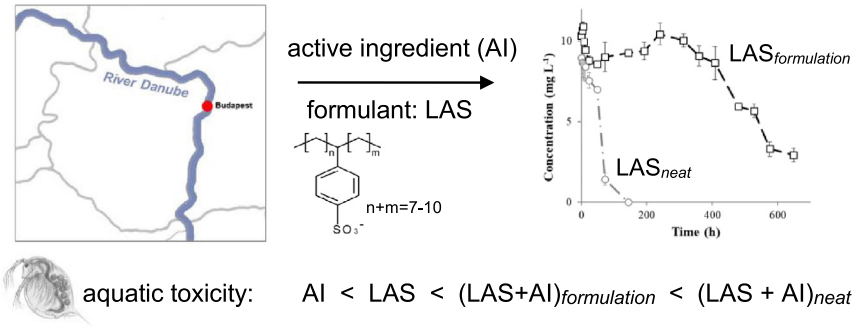
HIGHLIGHTS

- Loss rates of LASs were 4-fold faster in surface water than in distilled water.
- Loss rates of a LAS-formulated insecticide were slower than that of neat LASs.
- The loss of LASs is affected by certain neonicotinoid active ingredients.
- LASs as co-formulants exert aquatic toxicity on *Daphnia magna*.
- Synergistic aquatic toxicity occurred between LASs and acetamiprid.

GRAPHICAL ABSTRACT



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ABSTRACT

Substance losses of linear alkylbenzene sulfonates (LASs) in a neat surfactant mixture, in an insecticide formulation Mospilan 20 SG, and in solutions with different neonicotinoid active ingredients (AIs) was studied in distilled water and in surface water samples originated from River Danube. Analytical measurements were performed both by HPLC-UV and commercial ELISA methods. Loss rates of LASs were found different in these aqueous matrices, with decomposition rates higher for the neat surfactant mixture than for Mospilan 20 SG (nearly 2- and 9-fold in distilled water and in surface water from River Danube, respectively). Half-lives determined in surface water from River Danube were shown to be affected by the presence of neonicotinoid AIs thiacloprid > imidacloprid > acetamiprid (ACE), while clothianidin and thiamethoxam did not affect LAS decomposition. Aquatic toxicity of Mospilan 20 SG, along with that of its AI ACE and co-formulant LAS, as well as the mixture of ACE and LAS was also investigated in the 48-h acute immobilisation assay on the water flea (*Daphnia magna*) aquatic indicator organism. LAS appeared to be significantly (8-fold) more toxic in the *D. magna* test than ACE, and the toxicity of the formulated insecticide was found to be 1.3 and 19.6 times higher than explained by its AI and LAS content, respectively, indicating synergistic toxicity. The strongest synergy between ACE and LASs was observed, when the neat forms of the two substances were applied in combination at concentrations equivalent to those in Mospilan 20 SG.

1. Introduction

Surfactants in agrochemicals are used not only to improve the water solubility of pesticide active ingredients (AIs), but these adjuvants also improve spreading, penetration and thus, efficacy of the pesticides formulated with them (Castro et al., 2014). These formulation additives

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are often regarded as inert components, but have been proven in numerous cases to exert detrimental side-effects or modify the toxicity of the AIs. A recent and well-known example is the formulating agent for the herbicide AI glyphosate, polyethoxylated tallow amine (POEA), which is now banned in European Union (EU) (EU, 2017). Due to knowledge gained regarding unanticipated toxicities of formulating surfactants, authorisation and use of these agricultural additives will probably be more strictly regulated in the future.

Among anionic surfactants linear alkylbenzene sulfonates (LASs) are produced in the largest quantities worldwide and are employed not only in the households, but also in numerous agrochemical formulations as adjuvants (Castro et al., 2014). Environmental levels of LASs in surface waters are influenced mainly by the efficacy of communal wastewater facilities. Typical levels of LAS in environmental samples are summarized in the Supplement. Their environmental fate including degradation in different water purification systems, as well as levels in sludge and wastewaters were extensively studied (Mungray and Kumar, 2009). The removal of LASs under aerobic conditions is effective, and due to effective biodegradation only 10–20% of influent LAS is retained in the sludge and about 1% is released to surface waters. Half-lives (DT_{50}) of LASs in surface waters are low, usually only a few days (OECD SIDS, 2005) or even less. In parallel to biodegradation, adsorption to suspended solids in surface water is considerable because the LAS homologues, particularly in longer alkyl chain lengths, become readily adsorbed to the large size fraction (>11 μm) of particles that settle within a few hours (Sakai et al., 2017). A strong persistence of LASs was observed under anaerobic conditions (Lara-Martín et al., 2007), and removal in sewers ranges between 40 and 60% of the load of LASs. Thus, LASs accumulate in anaerobic sediments and sewage sludge, whereas their less hydrophobic degradation products, sulfophenyl carboxylic acids (SPCs) are found to remain predominantly in solution. SPCs were suspected of by-passing the drinking water purification processes, as they were detected in drinking water at levels between 1.6 and 3.3 μg L^{-1} (Eichhorn et al., 2002).

Our previous studies demonstrated the unanticipated toxicity of assumedly inert formulating agents of agrochemicals, seen for the components in formulated pesticides containing neonicotinoid insecticide AI (clothianidin, CLO) and herbicide AI glyphosate. Earlier we have investigated sulfonic acid surfactants in a CLO-based insecticide formulation, and found it to be more toxic to *Daphnia magna* Straus than CLO itself (Takács et al., 2017). Similarly, the presence of POEA was also found to affect the dissipation of glyphosate, and dissipation profiles differed in the investigated natural surface waters from River Danube and Lake Balaton (Klátyik et al., 2017).

In the present work, we focussed on the investigation of LAS surfactants and their decomposition in surface water. Loss of LASs was monitored alone and in an insecticide Mospilan 20 SG formulated with LASs and containing acetamiprid (ACE) as an AI. Loss rates of LASs were studied in the presence of other neonicotinoid insecticide AIs by using HPLC-UV instrumental and commercial enzyme-linked immunosorbent assay (ELISA) methods. The study was completed with ecotoxicity evaluation of ACE, LAS and their combination in pure forms and in Mospilan 20 SG insecticide formulation on the aquatic test organism *Daphnia magna*. Toxicity determined from the acute immobilisation tests on *D. magna* characterise the co-exposure and combined effects of AIs and adjuvants (e.g. surfactants).

2. Materials and methods

Pesticide formulation Mospilan 20 SG (Cheminova, Lemvig, Denmark), an ACE-based insecticide and Apache 50 WG® (Sumimoto Chemical Takeda Agro Co. Ltd., Tokyo, Japan) containing CLO were purchased from a local distributor. Composition and safety information on Mospilan 20 SG (Sumi Agro Hungary, 2014) are summarized in the Supplement (Table A.1). Analytical standards ACE, CLO, imidacloprid (IMI), thiacloprid (TCL), thiamethoxam (TMX), solvents, and a commercial mixture of sodium linear alkylbenzenesulfonates (LASs) were obtained from Sigma Aldrich Co. LLC (Darmstadt, Germany). This latter substance, also used as a formulating surfactant e.g., in Mospilan 20 SG, is a mixture containing 10–13 carbon atoms in the alkyl chain, therefore it is referred in the text as C_{10}-C_{13} alkylbenzenesulfonates or C_{10}-C_{13} LASs. The sodium salt of hexadecane-1-sulfonic acid (CAS 15015-81-3) was obtained from Merck (Darmstadt, Germany). Nonit, a commercial tank mixture additive containing 60% of dioctyl sulfosuccinate sodium salt was obtained from its local distributor, Arysta LifeScience Magyarország Kft. (Budapest, Hungary).

2.1. Analytical methods

LASs in Mospilan 20 SG and the corresponding C_{10}-C_{13} alkylbenzenesulfonates were analyzed by liquid chromatography using UV detection (HPLC-UV) performed on a Younglin YL9100 HPLC system equipped with a YL9150 autosampler (Younglin, Anyang, Korea). LASs were separated on an Acclaim Surfactant Plus column (Thermo Scientific, Waltham, MA, USA) (150 mm × 3.0 mm i.d., 3 μm) at 30 °C, and UV detector signals were recorded at λ = 225 nm, using the method recommended by the producer (Thermo Scientific, 2012), with slight modifications in the mobile phase pH and composition. The mobile phase flow rate was 0.6 mL min^{-1} with isocratic elution for 8.0 min (25:75 = A:B eluents, A = 100 mM ammonium acetate in water, pH = 5.0, B = acetonitrile). Samples of 20 μL volume were injected. Quantitative analysis was performed by external calibration. Further details of the method validation applied are given in the Supplement. For parallel detection of neonicotinoids, our method developed earlier (Mörtl et al., 2016) was used.

A commercial immunoanalytical test, a competitive ELISA was applied for detection of LASs at low levels, and results were also compared with those obtained by the HPLC-UV method. The Ecologiena® LAS ELISA kit was purchased from Abraxis LLC (Warminster, PA, USA, part number #520031). The procedure provided by manufacturer (Tokiwa Chemical Industries Co Ltd., Tokyo, Japan) was applied as follows. Samples were filtered and methanol was added to obtain a final methanol concentration of 10% (v/v). Standard solutions in the range of 0.02 mg L^{-1} to 1 mg L^{-1} were prepared in serial dilution. The Conjugate solution in buffer and then LAS standards or samples (100 μL of each) were transferred into each well of the uncoated microplate and incubated for 60 min at room temperature. In order to remove non-bonded LAS and LAS-enzyme conjugate, each well was rinsed four times with 300 μL of diluted washing solution. Then 100 μL of the chromophore reagent was added, incubated for 30 min, and the reaction was stopped by adding 100 μL of aqueous acid. Quantification was performed by reading the absorbance at 450 nm on an iEMS MF microplate reader (LabSystems, Helsinki, Finland). Signal background of natural surface water originated from River Danube as a matrix and cross-reactivities (CRs) with different sulfonates were also checked. Levels of sulfonates (Nonit, dioctyl sulfosuccinate sodium salt, Apache and hexadecane-1-sulfonic acid sodium salt) were 100 mg L^{-1}. Calibration curves recorded with kit standard, Mospilan 20 SG or C_{10}-C_{13} alkylbenzenesulfonates as well as curves for loss of LASs were compared.

2.2. Loss of LASs

The disappearance rates of LASs were monitored both by HPLC-UV and ELISA methods, the latter allowing analyte detection also below 1 mg L^{-1}. Degradation tests were carried out as described below, with initial concentrations of C_{10}-C_{13} alkylbenzenesulfonates of 14.4 and 10 mg L^{-1} in the HPLC-UV and ELISA method, respectively.

The HPLC-UV biodegradation tests were performed similarly to the method, described in a study on LAS degradation in Lake Dianchi (Wang et al., 2010). The water used in the present degradation study was collected from River Danube at Budapest (Hungary) on the day of

the commencement of the tests (see below). Water quality parameters at the time of the test were as follows: water temperature of 18 ± 2 °C, pH of 8.2 ± 0.2, dissolved oxygen of 10.6 ± 0.7 mg L^{-1}, resolvable phosphate of 0.06 ± 0.01 mg L^{-1}, total nitrogen content of 2.9 ± 0.4 mg L^{-1}, chlorophyll-*a* content of 0.017 ± 0,006 mg L^{-1}. Individual tests were conducted in triplicates, using Erlenmeyer flasks of 100 mL total capacity, each filled with 50 mL of previously shaken, not filtered river water containing algae and other suspended particles. Various amounts of LASs, Mospilan 20 SG or neonicotinoid AIs were then added. The flasks were not stoppered and the spiked solutions were kept under a chemical hood prevented from exposure to direct light during the experiments, as recommended in a LAS degradation study (Wang et al., 2010). In degradation experiments, 1 mL of water was withdrawn to analyze changes in LAS and neonicotinoid content. Prior to HPLC measurements, all samples were filtered through a 0.22 μm polytetrafluoroethylene syringe filter (Labex Ltd. Budapest, Hungary). Substance loss studies were performed with spiked distilled water samples in the same way with three independent replicates for every solution. ACE (or neonicotinoid) and LAS levels were measured after 3, 6, 9, 12 and 24 h post-spiking, then on every second or third day until the end of the experiments.

First the loss of LASs in Mospilan 20 SG and in the C_{10}-C_{13} alkylbenzenesulfonate mixture were studied in surface water samples collected from River Danube on the 8th August 2017 and parallel with that in distilled water. ACE and LAS levels were monitored until the end of the experiment (648 h, 27 days) and low levels of LAS were determined by ELISA. Sampling of water from River Danube was also carried out on the 30th of August, and the experiment with C_{10}-C_{13} alkylbenzenesulfonates was repeated. A parallel detection of ACE was performed not only with Mospilan 20 SG solutions in water collected from River Danube or distilled water, but also with those containing only ACE analytical standard at the same initial level as in Mospilan 20 SG solution (120 mg L^{-1}). Danube water was collected again on 20th September, and loss of C_{10}-C_{13} alkylbenzenesulfonates and LASs in Mospilan 20 SG were monitored again in spiked water collected from River Danube as well as in distilled water prior to ELISA measurements for 216 h.

Loss of LASs alone and in the presence of different neonicotinoid AIs in natural surface water originated from River Danube was also studied. C_{10}-C_{13} alkylbenzenesulfonates and neonicotinoid AIs were spiked into water on the day of sampling (6th September 2017) and levels were monitored for 336 h (14 days). The initial levels of C_{10}-C_{13} alkylbenzenesulfonates were 14.4 mg L^{-1} and 120, 134.5, 137.8, 136.2 and 157.2 mg L^{-1} for ACE, CLO, IMI, TCL and TMX, respectively. Parallel monitoring of LASs and neonicotinoid AI levels were performed in each solutions (see Section 3.3).

2.3. Determination of acute toxicity on Daphnia magna

The standard laboratory stock cultures of *D. magna* derived from LAB Research Kft. (Veszprém, Hungary) were maintained in reconstituted ISO test water according to the relevant ISO standard and OECD guideline (OECD, 2004; ISO, 2012). The sensitivity of the *D. magna* laboratory colony was verified with the reference substance (potassium dichromate, $K_2Cr_2O_7$) prior to testing and was proven to be appropriate (24-h EC_{50} = 0.7 mg L^{-1}) within the acceptable range of 0.6–2.1 mg L^{-1} based on the relevant standard protocol (OECD, 2004).

Ecotoxicity tests with *Daphnia* neonates (<24 h) were performed according to the OECD Test No. 202: *Daphnia* sp. acute immobilisation test (OECD, 2004). The duration of the test was 48 h. During the tests *D. magna* individuals were not fed additionally, and pH and temperature were continuously controlled to ensure the appropriate test conditions. Five concentrations of the test substance along with a control were investigated in four repetitions at each level. Twenty *D. magna* neonates were used for each concentration and the untreated control, divided into four groups with solution volumes of 10 mL for each. Test solutions were prepared by dissolving the test substances at known concentrations in aerated ISO standard water. Each test was repeated three times for each investigated test compound alone, in combination and in formulation as well. Immobilisation and mortality were checked at 24 and 48 h after the test initiation and was compared to the values observed in the untreated control groups (OECD, 2004). EC_{50} values were statistically determined at 48 h and calculated by statistical software ToxRat® (ToxRat Solutions GmbH, Alsdorf, Germany). The 48-h EC_{50} values calculated for the formulation Mospilan 20 SG were corrected with the nominal content of the active ingredient and the investigated surfactant as well on the basis of the material safety data sheet (see Table A.1).

3. Results and discussion

3.1. Determination of LASs by HPLC-UV and ELISA

LAS content was determined in a commercial C_{10}-C_{13} alkylbenzenesulfonate mixture and in an ACE-based insecticide Mospilan 20 SG by using a HPLC-UV method. The combined HPLC chromatograms of the C_{10}-C_{13} alkylbenzenesulfonate mixture and neat sodium dodecylbenzenesulfonate are shown in Fig. 1, indicating four major components (C_{10}, C_{11}, C_{12} and C_{13} homologues at retention times of 3.24, 3.56, 3.95 and 4.42 min, respectively) and the chemical identity of C_{12} alkylbenzenesulfonate demonstrated by analyzing the single compound ELISA standard sodium dodecylbenzenesulfonate a retention time of 3.99 min by the HPLC-UV method. Our results determined for Mospilan 20 SG (See Table A.2) were in accordance with literature data (OECD SIDS, 2005). Commercial LAS is exclusively manufactured as mixtures of C_{10} to C_{13} or C_{14} linear alkyl chain homologues, having average alkyl chain lengths ranging from $C_{11.3}$ to $C_{12.6}$, and the proportion of homologues below 10 and above 14 carbon unit is <1%. The distribution of the peak pattern of the four alkyl homologues (C_{10}-C_{13}) is slightly shifted towards the shorter homologues with progress of degradation. The degradation rate is increasing with the length of alkyl chain, as this lipophilic moiety is the subject to the initial microbial attack (OECD SIDS, 2005).

HPLC results were supported by corresponding data obtained by using the Ecologiena® LAS ELISA kit. Results were in accordance with the parallel HPLC measurements and showed a similar pattern as observed in earlier samples of surface water from River Danube. Details of the instrumental (HPLC-UV) and immunoanalytical (ELISA) determinations are described in the Supplement and calibration curves are shown in Fig. 2. CRs observed with other sulfonates (e.g. Apache and sodium hexadecane-1-sulfonate) were low (2.1 and 2.5%, respectively). According to the MSDS of Apache 50 WG® (Arysta, 2013), the formulated insecticide product consists of 50% CLO, >10% of calcinated diatomaceous earth (CAS 91053-39-3) and a complex mixture of alkanesulfonic acid derivatives (CAS 68937-98-4) as a dispersing agent. Homologue distribution and isomers in this anionic surfactant were characterised earlier by LC-MS (Takács et al., 2017).

3.2. Decomposition of LASs (alone or in Mospilan 20 SG) in distilled water and in samples collected from River Danube

LASs were determined and their loss (alone and in the presence of formulated insecticide Mospilan 20 SG) was characterised in distilled water and in surface water collected from River Danube. Curves for LAS homologue loss in C_{10}-C_{13} alkylbenzenesulfonates and in Mospilan 20 SG in distilled water and in surface water from River Danube are presented on Fig. 3 and summarized results are shown in Fig. 4. Decomposition rates were different as a) the level of the homologue containing the shortest alkyl chain (C_{10}) decreased slower compared to other homologues in each matrix; b) it depended on the matrix and also c) the presence of ACE in surface water from River Danube. DT_{50} values were found to be 58.7 ± 2.0 and 495 ± 32 h for LAS alone and in formulation

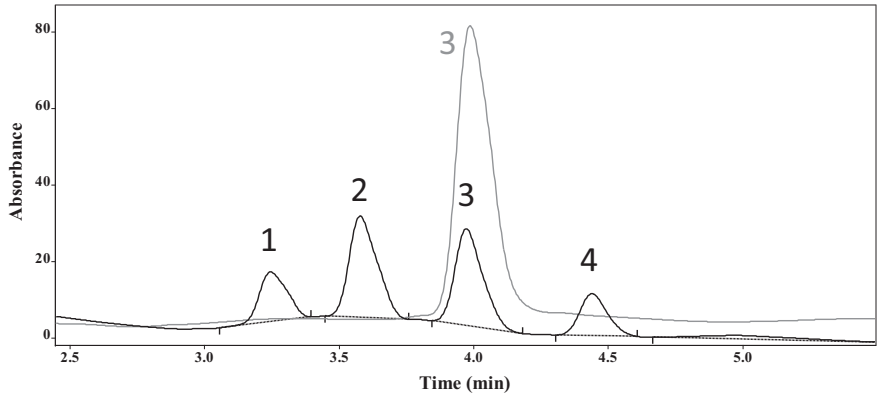

Fig. 1. HPLC-UV chromatogram of C_{10}-C_{13} alkylbenzenesulfonates (black) and the LAS standard sodium dodecylbenzenesulfonate (gray) provided to the ELISA kit. Individual LASs corresponding to peaks in the chromatogram: 1 – decylbenzenesulfonate, 2 – undecylbenzenesulfonate, 3 – dodecylbenzenesulfonate and 4 – tridecylbenzenesulfonate.

in surface water, respectively. Similar tendencies were obtained in distilled water for LASs alone and in Mospilan 20 SG, but some differences were observed between the corresponding DT_{50} values (215 ± 18 and 519 ± 32 h). The initial concentrations as well as the relative amount of the last homologue (C_{13}, RT = 4.4 min) was lower in surface water from River Danube than in distilled water, probably due to adsorption to colloidal particles in surface water.

To ensure reliability the experiment run in triplicates was repeated. Substance loss of both C_{10}-C_{13} alkylbenzenesulfonates and LAS homologues in Mospilan 20 SG were determined in distilled water and in surface water from River Danube by the ELISA method (Fig. 5) and compared with the parallel HPLC-UV detection. Although the ELISA investigation lasted only 216 h (and not 648 h as before), the same tendencies were observed (Fig. 4). During this period the level of LASs from Mospilan 20 SG remained constant, whereas concentration of C_{10}-C_{13} alkylbenzenesulfonates decreased by 27% in distilled water. A similar tendency was observed in surface water from River Danube as a matrix, but loss rates were higher probably due to higher microbial activity and nutrition content. The content of Mospilan 20 SG decreased by 15%, whereas C_{10}-C_{13} alkylbenzenesulfonates almost disappeared (see Fig. 5).

Despite the broad sample collection period at River Danube, results occurred to be similar. The DT_{50} values for C_{10}-C_{13} alkylbenzenesulfonates in surface water from River Danube were 58.7 ± 2.0 (8th August), 40.6 ± 3.1 (30th of August), 72.7 ± 1.8 (20th September) and 77.4 ± 13.5 h (6th September). Parallel detection of ACE showed no significant changes in River Danube as well as in distilled water during the experiment (648 h, 27 days). Exact initial concentrations in solutions containing either Mospilan 20 SG or ACE analytical standard ranged between 122.1 and 123.5 mg L^{-1}.

The observed slower decomposition rate of the homologue containing the shortest alkyl chain is in accordance with literature data, as microbial activity is known to be higher for homologues having longer alkyl moiety (OECD SIDS, 2005). Such microbial activity, promoted by biomass of plant or animal origin in the surface water medium (Federler and Ventullo, 1990; Belanger et al., 2002), is likely to be the reason of the increased decomposition rate that was observed in surface water from River Danube water relative to distilled water. The presence of ACE was also found to influence the loss rate probably due to its toxicity to the microorganisms responsible for LASs degradation in river water. DT_{50} values in surface water were found to be 58.7 ± 2.0 and 495 ± 32 h for LASs alone and in formulation, respectively. In contrast, no significant differences were determined between the corresponding DT_{50} values in distilled water. The relative amount of the homologue with the largest molecular weight (C_{13}, RT = 4.4 min) was found to be lower in surface water from River Danube compared to distilled water, probably due to adsorption to the colloidal particles.

Anaerobic degradation of LASs in coastal marine sediments was studied earlier (Lara-Martín et al., 2007). LAS levels remained constant during the initial 15-day period, but a notable decrease in LAS and increase in SPCs amounts was observed later in the blank and low level test samples (10 and 20 mg L^{-1}). Until the end of experiment (165 days) 66–79% of the initial LAS content disappeared. LAS degradation was slower at higher concentrations (50 mg L^{-1}) and only 13–14%

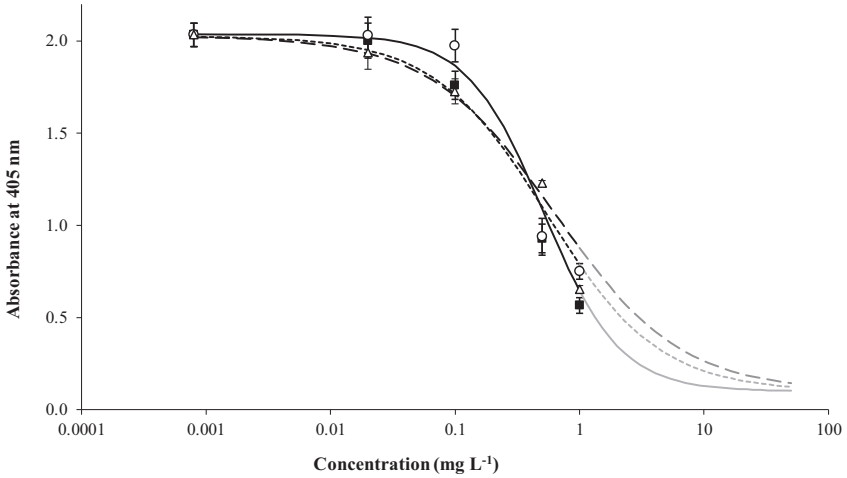

Fig. 2. Calibration curves measured with Mospilan 20 SG (△, dashed line), C_{10}-C_{13} alkylbenzenesulfonates (○, dotted line) and the LAS standard (■, solid line) in the Ecologiena® LAS ELISA kit. The sections of the calibration curves above the highest standard LAS concentration in the ELISA kit, 1 mg L^{-1} (indicated in gray) are extrapolations.

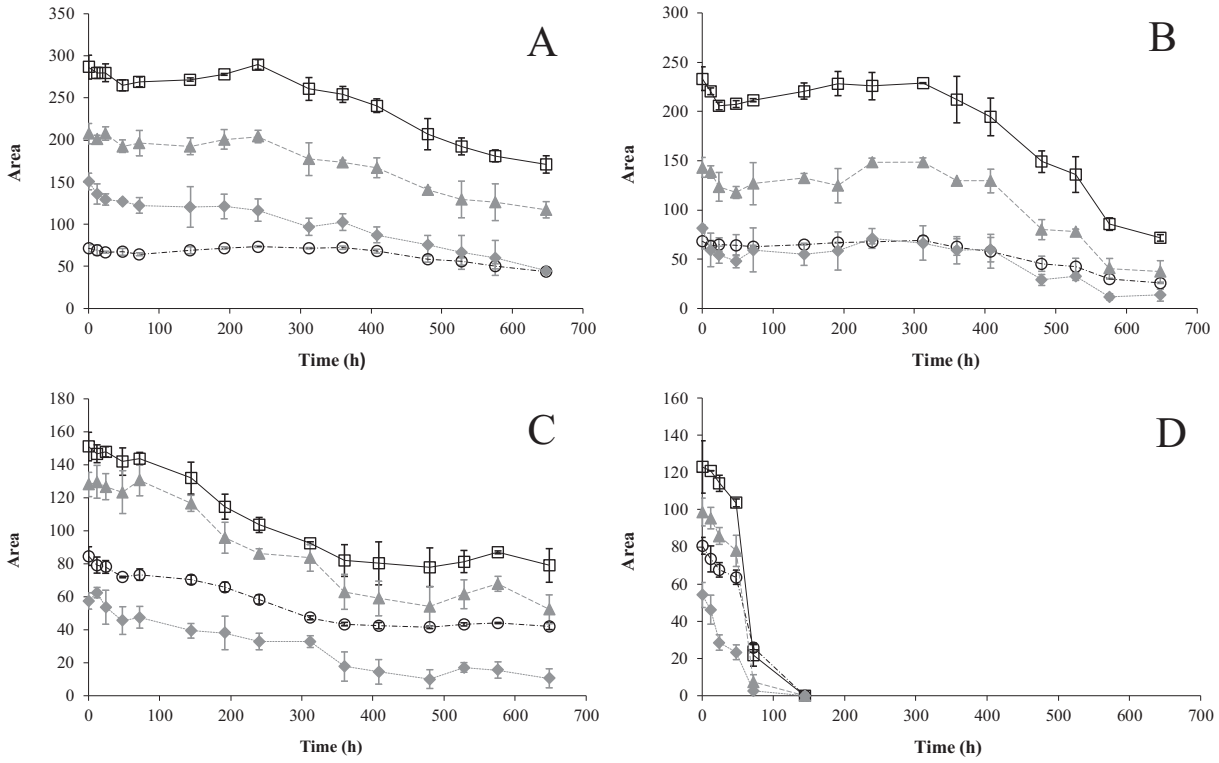

Fig. 3. Curves for loss of LAS homologues in Mospilan 20 SG in distilled water (A) and in surface water from River Danube (B) and for C_{10}-C_{13} alkylbenzenesulfonates (○ C10, □ C11, ▲ C12 and ♦ C13.) in distilled water (C) and in surface water from River Danube (D).

of the initial level underwent decomposition during the study. Reduced degradation indicates that LASs above 20 mg L^{-1} are toxic to sulfate-reducing bacteria, which were found to be the most abundant microorganism subgroup in the samples. The half-life for LASs under anoxic conditions in marine sediment was estimated to 90 days, despite of aerobic degradation values, which usually ranges from 1 to 4 days. If sulfate-reducing and methanogenic activities of microbial community are inhibited, even higher DT_{50} values (e.g. several years) should be expected (Lara-Martín et al., 2007).

3.3. Decomposition of LASs in surface water from River Danube in the presence of different neonicotinoid AIs

The decomposition of LASs was studied with C_{10}-C_{13} alkylbenzenesulfonates alone and in the presence of different neonicotinoid AIs (ACE, TMX, CLO, TCL and IMI) in surface water collected from River Danube. No significant differences were observed between the corresponding DT_{50} values for LASs alone and in the presence of TMX or CLO; a somewhat longer half-life was obtained for LAS with ACE; and the longest values were determined in the presence of TCL and IMI. The plots shown in Fig. 6 display loss of LASs measured, curves fitted by logistic (sigmoid) non-linear regression, and DT_{50} values were defined by the inflection points of these curves. Averages of DT_{50} values were found to be 77.4 ± 13.5, 75.5 ± 11.0 and 79.4 ± 12.3 h for LASs alone and in the presence of TMX or CLO, respectively. In the presence of ACE 107 ± 4 h were calculated from the curves, whereas nearly doubled values, 146 ± 5 and 139 ± 6 h were obtained for DT_{50} of LASs in the presence of TCL and IMI, respectively.

Parallel detection of neonicotinoid AIs showed no significant changes, except for a slight decrease in the level of TMX. During the experiment (336 h), the level of TMX decreased by 18.9% and 15.1%, when it was alone and together with C_{10}-C_{13} alkylbenzenesulfonates,

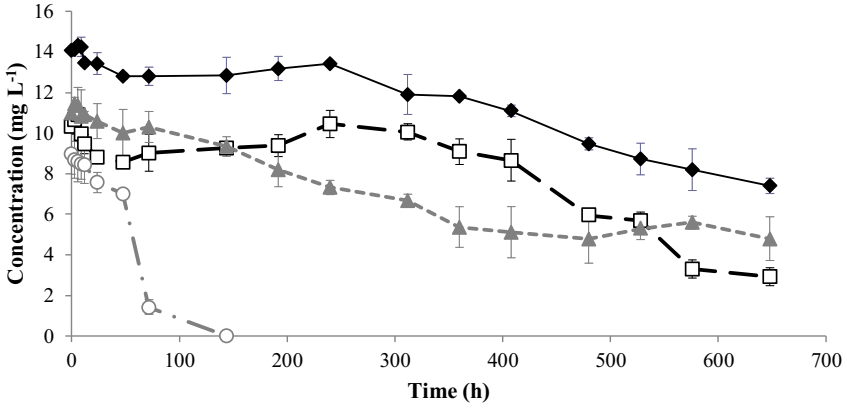

Fig. 4. Curves for loss of LASs (summarized) in C_{10}-C_{13} alkylbenzenesulfonates and Mospilan 20 SG in distilled water and in surface water from River Danube. ▲ LAS and ♦ Mospilan 20 SG in distilled water, ○ LAS and □ Mospilan 20 SG in surface water from River Danube.

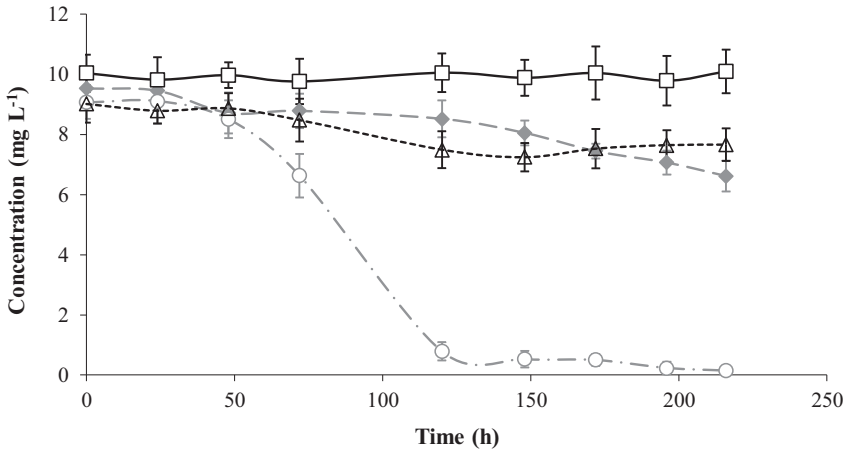

Fig. 5. Curves for loss of LASs (summarized) in C_{10}-C_{13} alkylbenzenesulfonates and Mospilan 20 SG in distilled water and in surface water from River Danube measured by ELISA. □ Mospilan 20 SG and ♦ LAS in distilled water, ○ LAS and Δ Mospilan 20 SG in surface water from River Danube.

respectively. The levels of other neonicotinoid AIs remained constant during the entire experiments. According to the Pesticide Properties Database (PPDB, 2018), neonicotinoids CLO, IMI and TMX are stable in water, while dissipation of ACE and TCL are moderately fast. The corresponding DT_{50} values are 40.3, 30, 30.6, 4.7, 8.5 days, respectively. Our results confirm this stability.

Formulating surfactants, added to pesticide products to facilitate the penetration capacity of the AIs, are also known to affect their environmental fate. In our earlier study (Klátyik et al., 2017), the presence of formulating agent POEA was found to strongly affect the dissipation of glyphosate from natural surface waters (River Danube and Lake Balaton) and to modify dissipation profiles as well. In the current study, an opposite trend, the presence of AIs affecting the decomposition rates of the surfactants, was observed. Loss rates of commercial LASs (C_{10}-C_{13} alkylbenzenesulfonates) in surface water from River Danube were found to decrease in the presence of different neonicotinoids (ACE, IMI and TCL). In contrast, disappearance times determined for LASs alone and in the presence of TMX or CLO occurred to be similar.

3.4. Acute individual and combined toxicity on Daphnia magna

The immobilisation tests on *D. magna* were carried out with ACE, LAS, with a combination of neat ACE and LAS, and with Mospilan 20 SG insecticide formulation containing 20.2% ACE and 2.4% LAS according to its material safety data sheets (MSDSs). Significant differences in the toxicological parameters were determined between individual and combined effects exerted by ACE and LAS. Results and calculated 48-h EC_{50} values are summarized in Table 1. The individual acute toxicity of ACE was significantly lower than that of LASs, since the AI caused 10% immobilisation in the treated groups at a concentration of 200 mg L^{-1}, while the calculated 48-h EC_{50} value for LASs was 13.03 ± 3.32 mg L^{-1}. Similarly, to our results Yamada et al. (1999) and the related MSDS reported low toxicity of ACE on *D. magna* (Table A.1), however the determined 48 h EC_{50} value for ACE in Daphnia test given in Pesticide Manual are for LC_{50} (24-h) >200 mg L^{-1}, and LC_{50} (48-h) 49.8 mg L^{-1} (MacBean, 2012). On the basis of our results acute toxic effect of ACE on *D. magna* was not determined above 200 mg L^{-1} in this study due to the low environmental relevance. The detected level of ACE in surface water was ranging from 0.000008 to 0.044 mg L^{-1} according to Morrissey et al. (2015) remained much below the tested concentration, in addition in our monitoring studies performed between May and June of 2017 the presence of ACE was not detected in surface water samples obtained from River Danube (Budapest, Hungary).

The high toxicity of the surfactants was proven earlier by several studies (Tsui and Chu, 2003; Mesnage et al., 2014; Székács et al., 2014) on *D. magna* as well (Brausch et al., 2007), however various coformulants (e.g. surfactants) used in formulated plant protection products have long been considered as inert ingredients in the aspect of the main biological effects of the pesticide formulation. The determined 48 h EC_{50} for LAS was similar to the value (48-h EC_{50} = 10.09 mg L^{-1}) reported by Jurado et al. (2011). The toxic effects of LAS and other surfactants (e.g. cetyl trimethyl ammonium chloride and alkyl ethoxylate) on several aquatic invertebrates (e.g. *Rhabditis* sp., *Gammarus* sp., and *D. magna*) were investigated by Lewis and

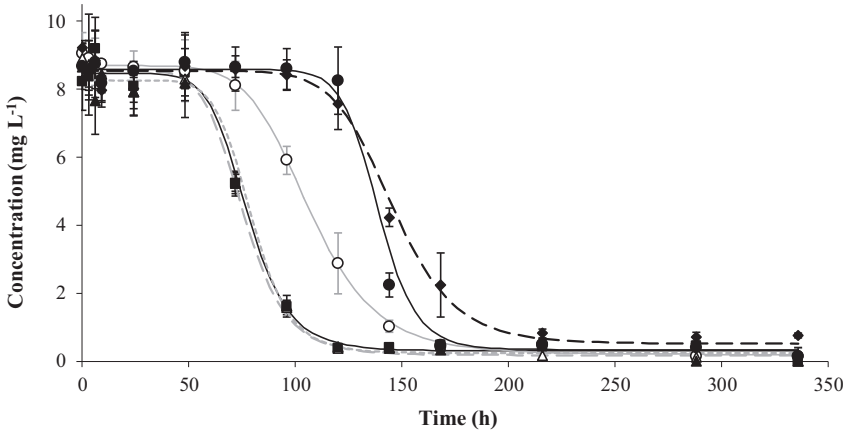

Fig. 6. Curves for loss of LASs alone (■) and in the presence of different neonicotinoids (○ ACE, △ TMX, ▲ CLO, ● IMI, ♦ TCL) measured in surface water from river Danube. Summarized concentrations of LAS homologues of commercial C_{10}-C_{13} alkylbenzenesulfonates are shown as a function of time.

Table 1
Results of acute toxicity assay on *Daphnia magna* immobilisation with analytical standard of ACE (active ingredient, AI), surfactant (LAS) and with formulated neonicotinoid Mospilan 20 SG.

	48-h EC_{50} (mg L^{-1})[a]					
	Min	Max	Avg.	±SD	Ratio of component (%)[b]	Ratio to Mospilan 20 SG (%)[c]
Individual toxicity						
ACE	>200[d]	–	–	–	100	388
LAS	11.2	18.0	13.0	3.32	100	215
Combined toxicity in pure form[e]						
ACE	1.47	3.58	2.22	1.18	89.4	4.91
LAS	0.18	0.45	0.28	0.15	10.6	7.38
Combined toxicity in Mospilan 20 SG[f]						
ACE	41.3	66.0	51.5	10.5	20.2	100
LAS	4.90	7.84	6.12	1.25	2.4	100

[a] Four repetitions (four beakers with five *D. magna* neonates each) were used for each concentration tested, the tests were repeated with each compound three times.
[b] Ratio of the component in the investigated test solution.
[c] Ratio of the 48-h EC_{50} values determined for the investigated components in Mospilan 20 SG and the corresponding 48-h EC_{50} values calculated for individual and combined toxicity tests.
[d] 200 mg L^{-1} concentration ACE caused immobilisation of 10% of the *D. magna* juveniles.
[e] The investigated components were investigated together in equivalent concentrations than in the formulated product Mospilan 20 SG.
[f] Average of 48-h EC_{50} values was 254.9 ± 51.9 mg L^{-1} for Mospilan 20 SG, EC_{50} values for components were calculated according to the MSDS.

Suprenant (1983). LAS was the less toxic investigated surfactant, while *D. magna* was the most sensitive to the tested surfactants. The determined 48 h EC_{50} for LAS on *D. magna* was 1.8–5.6 mg L^{-1} (Lewis and Suprenant, 1983). According to Verge et al. (2001) the acute toxicity of LASs was increased with the length of alkyl chain, while toxicity was decreased at lower molecular weight of LAS on *D. magna* (Verge et al., 2001). In addition, the acute toxicity of LAS on *D. magna* neonates was observed to be highly affected by the hardness of both the test water and the reconstituted water used for culturing (Maki and Bishop, 1979).

Evaluation of the combined toxicity of ACE and LASs in the formulation Mospilan 20 SG was higher, than the individual toxicity of the components. The corrected 48-h EC_{50} values for Mospilan 20 SG according to the content of ACE and LASs were 51.5 ± 10.5 and 6.12 ± 1.25 mg L^{-1}, respectively. In addition, when ACE and LAS were investigated in pure form (a mixture of neat ACE and LAS at equivalent concentrations to their formulated product Mospilan 20 SG), toxicity was even higher (48-h EC_{50} values were 2.22 ± 1.18 and 0.28 ± 0.15 mg L^{-1} for ACE and LAS, respectively). The observed increased combined toxicity of the two substances ACE and LASs indicate a strong synergistic effect between these compounds. In contrast, reduced combined toxicity in the formulated product (77.4% relative to that of the neat substances) is attributed to the presence of additional unknown additive(s) used in the formulation on the basis of the MSDS.

Similarly to the present study, possible response of additives (e.g. surfactants) for the enhanced or reduced toxic effect of AIs was proven in other studies on various indicator species in aquatic biosystems, especially algae, and for pesticides of other AIs e.g. the glyphosate-based herbicide formulations most widely evaluated (Székács et al., 2014; Defarge et al., 2016; Klátyik et al., 2017), dissipation of glyphosate monitored in the presence and absence of formulant POEA (Klátyik et al., 2017), as well as on neonicotinoid AIs and formulations (Takács et al., 2017). Earlier we have characterised the surfactant in a CLO-based insecticide formulation by LC-MS and the *D. magna* immobilisation test. The formulation containing sulfonic acids as additives was found to be 46.5 times more toxic than CLO itself on *D. magna* (Takács et al., 2017). The effect of LAS and herbicide AI atrazine was investigated on marine microalgae (Debelius et al., 2008), and EC_{50} values obtained in growth inhibition tests ranged between 0.776 and 1.84 mg L^{-1} for LAS. For atrazine significantly lower values were determined as it has a more severe contaminant effect than LAS itself. Toxic concentrations of LASs have been reported for different freshwater and marine microalgae varying between 0.1 and 100 mg L^{-1}. The wide range of toxic concentrations may be due to the use of non-standardized LAS mixtures with varying alkyl chain lengths and/or phenyl isomers distribution in the toxicity tests. Indeed, some authors reported homologue-specific 72-h IC_{50} values in the range of 1.4–13 and 0.18–1.2 mg L^{-1} for C_{11} and C_{13} homologues, respectively. Similarly, sigmoid curves were obtained for phytotoxicity (growth inhibition) as a function of C_{12}-LAS concentration (Renaud et al., 2011). In their study, IC_{50} values for tested marine microalgae were significantly different, and ranged from 0.5 to 2 mg L^{-1}. These values are below the levels applied in our study.

4. Conclusion

Loss rates of LASs were found to be strongly influenced by the aqueous matrix and to depend on LAS composition as well. Decomposition of LASs was more rapid in surface water from River Danube than in distilled water. The distribution of homologues was affected by a) different decomposition rates faster for homologues containing the longest alkyl chain in each matrix, and b) adsorption of the most hydrophobic homologue with the longest alkyl chain (C_{13}) more extended to the particles in surface water. The presence of different neonicotinoid AIs together with LASs in surface water from River Danube either had no effect on the loss of LASs (TMX, CLO) or affected the loss rates negatively (ACE, TCL, IMI). A commercial ELISA method was proven to be suitable for determination of low concentrations of LASs, thus could extend the detection range of the HPLC-UV method.

Significant differences were found between individual and combined effects of ACE and LASs on *D. magna*. A strong synergistic interaction was detected between ACE and LASs, which slightly decreased in the presence of other additives used in the formulation Mospilan 20 SG. Our results indicate that co-occurrence of the investigated AI and surfactant has significant effects on their combined toxicity and environmental fate as well, moreover, co-formulants (e.g. surfactants) cannot be classified as unequivocally inert components in the aspects of their (eco)toxicological evaluation.

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Appendix A. Supplementary data

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