HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

ECOTOXICOLOGICAL STUDIES OF CHEMICAL POLLUTANTS OF AGRICULTURAL ORIGIN ON AQUATIC ORGANISMS BELONGING TO LOWER TAXONOMIC GROUPS

THESES OF DOCTORAL (PhD) DISSERTATION

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1. SCIENTIFIC BACKGROUND AND OBJECTIVES

A significant part of veterinary and pesticide formulations exerts an increased load on our environment. After their use and application, the various active ingredients and formulating agents may enter the soil and reach groundwater levels, as well as appear in surface waters. The active ingredients and additives that appear in our environment can affect various non-target organisms. Possible toxic effects are highly affected by the water solubility and persistence of the chemical compounds and the potential formation of toxic metabolites. Among residues of chemical compounds that appear in environmental compartments, different active ingredients are brought to the fore from time to time. Due to their persistence, some chemical compounds remain detectable in environmental samples for extended periods, while other active ingredients appear in the environmental matrix due to excessive momentary use. Aquatic organisms are outstandingly exposed to water contaminants because their contact with xenobiotics in their aqueous habitat is unavoidable.

Nowadays, the appearance of pharmaceutical residues in surface waters is receiving increasing attention due to the potential biological effects of these substances. Antibacterial sulfonamides [e.g., sulfamethoxazole (SMX)] widely used in human and veterinary medicine are the active ingredients of several pharmaceutical formulations in Hungary as well. Due to their low sorption capacity, sulfonamides can rapidly reach groundwater levels and appear in surface water bodies. The effect of sulfonamides in different pharmaceuticals is enhanced by their combination with trimethoprim (TRI) in the formulations, due to the significant synergism between the two active ingredients.

Pesticide residues also represent remarkable environmental risks. Active ingredients of neonicotinoid type insecticides in extensive worldwide use have become ubiquitous contaminants by now. Pollinating insects are at particular risk by the use of neonicotinoids, therefore the application of these compounds

is severely restricted in the European Union (EU) particularly on flowering crops. Due to the physicochemical properties of neonicotinoids (e.g., water solubility, slight persistence) and to inappropriate plant protection technologies, neonicotinoids can emerge in surface waters where they may exert adverse effects on non-target organisms. The appearance of the globally market-leading, non-selective herbicide active ingredient and desiccant glyphosate (GLY) in surface waters is also a globally observed phenomenon nowadays, especially in areas where extensive cultivation of GLY-tolerant genetically modified crops supplemented with the increased use of formulations containing GLY or where desiccation is typical. Thus, the pollutant concentration of GLY can reach the order of 5200 μg/l.

Formulations applied in veterinary medicine and plant protection products contain various additives, including formulating agents besides the active ingredients. The primary purpose of adding formulating agents is to improve the efficacy and bioavailability of the formulation by increasing the solubility, adsorption, and uptake of the active ingredient. As formulation additives are inactive from the aspect of the required main biological effect of the pharmaceutical or pesticide, these additives have long been considered as inactive or inert components, therefore their authorization required simplified risk assessment compared to the active ingredients. However, in recent years, several studies proved the high individual toxicity of formulating agents, including POEA (a mixture of polyethoxylated tallow amines) used in the production of formulated preparations containing GLY as formulating agent, as well as the increased combined toxicity of the active ingredient and additives together in the formulations as compared to the individual toxicity of GLY. The assessment of the combined toxicity of active ingredients and additives is of utmost importance, as most of the active ingredients enter our environment in the form of mixtures.

The aims of my PhD work were the following:

- assessment of the sulfonamide type sulfadiazine, sulfaguanidine, sulfamethazine, SMX, and TRI veterinary and neonicotinoid type acetamiprid, clothianidin, thiacloprid, and thiamethoxam pesticide active ingredients for individual and combined toxicity between the active ingredients and additives used in their formulations on the immobilization of water fleas (*Daphnia magna*) and on the enzyme activity of glutathione-*S*-transferase;
- investigation of the individual and combined toxicity of the components of the GLY-based ROUNDUP CLASSIC and MEDALLON PREMIUM formulations on the immobilization of *D. magna* and the growth and photosynthetic activity of floating unicellular green (*Desmodesmus subspicatus*, *Pseudokirchneriella subcapitata*, *Scendesmus obtusiusculus*) and blue-green (*Anabaena flos-aquae*) algae;
- assessment of the effects of ROUNDUP CLASSIC and its components on the biomass of algal communities in biofilms grown under natural conditions in different Hungarian river and standing surface water bodies (River Danube, Lake Balaton, Lake Velencei), on the structure of biofilms, and on the composition of diatom (*Bacillariophyceae*) communities;
- study of the degradation of GLY in surface water samples originated from River Danube and Lake Balaton in the form of the pure active ingredient and formulated preparation (ROUNDUP CLASSIC) in the presence and absence of biofilms as well;
- investigation of the degradation of linear alkyl benzene sulfonates used as formulating agents in the formulation of neonicotinoids in aqueous media and in various experimental settings.

2. MATERIALS AND METHODS

The acute toxicity of the sulfonamide type sulfadiazine, sulfaguanidine, sulfamethazine, sulfamethoxazole (SMX), and trimethoprim (TRI) veterinary, neonicotinoid type acetamiprid (ACE), clothianidin (CLO), thiacloprid (TCL), thiamethoxam (TMX) as well as glyphosate (GLY) pesticide active ingredients was investigated individually and in combination with the additives found in their formulated preparations based on the results of the immobilizations tests performed on water fleas (*Daphnia magna*), less than 24 hours old, according to the OECD 202 guideline. In MOSPILAN 20 SG and ROUNDUP CLASSIC pesticide formulations, the quality and quantity of the formulating agents are also known, the combined toxicities of the active ingredients and the formulating agents were evaluated in pure mixtures of the neat components (at equivalent concentrations to their formulated product), in addition to the assessment of the individual toxicity of the formulating agents. Immobilization was the endpoint of the studies, so the number of mobile individuals was determined after 48 hours of exposure, and then the 48h EC_{50} values characterizing the toxicity were calculated. The individual and combined effects of the GLY-based ROUNDUP CLASSIC and MEDALLON PREMIUM formulations and their components were investigated on *D. magna* juveniles hatched from dormant eggs provided by the Daphtoxkit F test kit distributed by MicroBioTests as well.

The study of the individual and combined effects of the active ingredients SMX and TRI, as well as their formulated preparation SUMETROLIM, and the neonicotinoid-type formulation MOSPILAN 20 SG [active ingredient: ACE, formulating agent: linear alkylbenzene sulfonates (LAS)] was extended to the evaluation of the effects on glutathione-*S*-transferase (GST) activity also in *D. magna* individuals less than 24 hours old. The concentrations used in the enzyme activity assays corresponded to the 48h EC_{20} values originated from the acute immobilization tests. GST activity was characterized by the amount of the colorimetric product formed during the GST-catalyzed reaction between

glutathione and the applied substrate, the absorbance of the reaction product was determined at 340 nm. The specific enzyme activity of the samples was evaluated for protein content determined by the Bradford method. The effects of the treatments were evaluated by one-way analysis of variance using Tukey's test as a *post-hoc* test.

The individual and combined phytotoxicity of GLY-based ROUNDUP CLASSIC and MEDALLON PREMIUM formulations and their components were investigated in 72-hour algal growth inhibition tests according to the OECD 201 guideline. The growth inhibition tests were performed on three floating unicellular green algae (*Desmodesmus subspicatus*, *Pseudokirchneriella subcapitata*, *Scendesmus obtusiusculus*), while the phytotoxicity of GLY was determined on a filamentous blue-green algae (*Anabaena flos-aquae*) as well. Inhibition of algal growth was the endpoint of the tests. Upon exposure, the optical density (OD) and chlorophyll-a-content of the samples were measured, then the 72h EC_{50} values characterizing the toxicity were calculated based on measured data. The OD was determined with the use of a spectrophotometer at 750 nm. Chlorophyll-a-content after alcoholic extraction was determined spectrophotometrically at three different wavelengths (750, 666, and 653 nm).

The effects of ROUNDUP CLASSIC and its components on the photosynthetic activity of *P. subcapitata* were investigated in samples originated from algal growth inhibition assays based on the detection of laser-induced chlorophyll-a-fluorescence using a portable device called FluoroMeter Module. During the measurements after 10 minutes of dark adaptation, the samples were excited at 635 nm. Following excitation, the intensity of the fluorescent light emitted by the sample was instrumentally detected (690 and 730 nm). In our studies, changes in the Fv/Fp values correspondig to the photochemical efficiency of the PS II photochemical system and the vitality index (Rfd values) characterizing photosynthetic activity were investigated. The effects of the treatments were analysed using general linear models.

During our investigations on biofilms performed at the community level in 2015 and 2016, natural biofilms were grown on glass substrates fixed on a specially developed buoy structure in major domestic surface water bodies (2015: Lake Balaton, River Danube; 2016: River Danube, Lake Velencei) for 6 weeks under natural conditions. After biofilm colonization, the effects of ROUNDUP CLASSIC and its components on the biomass and composition of algal communities in biofilms, and the structure of biofilms were investigated under laboratory conditions in aquaria containing natural water originated from the location of biofilm colonization. The effects of GLY were studied in the form of the pure active ingredient and formulated preparation (ROUNDUP CLASSIC), where the investigated GLY concentration was 100 μg/l. The individual toxicity of POEA (a mixture of polyethoxylated tallow amines) was also evaluated in investigations performed in 2016. Throughout the study period, complete water exchange was performed weekly with water from the original locations, where the biofilm had been developed, during weekly water exchange readjustment of the investigated test concentrations was carried out as well.

The effects on the biomass and composition of the algal community in biofilms and on the structure of biofilms were characterized by the changes in species composition of algal communities (proportion of green, blue, and diatom algae species) and values characterizing the biomass of the algal community of the biofilms (the sum of chlorophyll-a data determined at different measurement points per algal group) detected by an algae torch instrument. Based on the measurements, the composition of the algal communities in control and treated biofilms were compared with a two-sample z-test, while the statistical evaluation of the effects on the algal biomass of the biofilms was performed using general linear models. Changes in the structure of biofilms as a result of the treatments were examined by scanning electron microscopy. In 2015, the experiments performed on biofilms were completed with the trait-based investigation of the effects of GLY on the composition of diatom communities in biofilms. The selected biological traits were the cell size [(1) < 99 μm^3, (2)

100–299 μm^3, (3) 300–599 μm^3, (4) 600–1499 μm^3, (5) > 1500 μm^3] and the ecological guilds [(1) low-profile, (2) high-profile, (3) motile, (4) planktic]. The distribution of various taxon groups was assessed by Fisher's exact test at a significance level of 5%.

The degradation of GLY was investigated in surface water samples originated from Lake Balaton and River Danube in the form of pure active ingredient and formulated preparation (ROUNDUP CLASSIC) as well. The study of GLY degradation was performed in the absence of biofilms with daily water sampling for one week. The initial concentration of GLY was 100 μg/l. The kinetics of GLY degradation was also determined in the presence of biofilms in the experimental system set up for testing of toxicity on biofilms, where water samples were also collected daily for the first week, then weekly before the performed water exchange and after the repeated adjustment of investigated GLY concentration. GLY concentration of the samples was analysed by high-performance liquid chromatography combined with fluorescent UV detection (HPLC-UV), but water samples with GLY content below the limit of detection of HPLC-UV (LOD: 5 μg/l) were subjected to liquid chromatography-tandem mass spectrometry.

The degradation of LAS used as formulating agents in the formulation of neonicotinoids was investigated in the form of pure detergents mixture ($C_{10–13}$) and formulated preparation (MOSPILAN 20 SG) was studied for 27 days in distilled water and surface water samples originated from River Danube. In our experiments, the kinetics of LAS degradation were also studied for 14 days in the presence of different neonicotinoid active ingredients (ACE, CLO, imidacloprid, TCL, and TMX), but only in surface water samples from River Danube. The initial concentration of LAS was 14,4 mg/l. After the adjustment of the experimental concentrations, water samples were collected 3, 6, 9, 12, and 24 hours later, and then in every 2-3 days. LAS concentration of the samples was analysed by HPLC-UV technique.

3. RESULTS

During my PhD work, among the investigated sulfonamide [sulfadiazine, sulfaguanidine (SGD), sulfamethazine (SMZ), sulfamethoxazole (SMX)] and diaminopirimidine [trimethoprim (TRI)] type veterinary active ingredients, SGD proved to be the most toxic, while SMZ, SMX, and TRI proved to be less toxic based on the results of *Daphnia magna* immobilization tests. Between the formulated veterinary drugs investigated, containing SMX and TRI, SUMETROLIM was more toxic than COTRIM E-ratiopharm. During the assessment of the combined effects of SMX and TRI investigated together at concentrations equivalent to SUMETROLIM, the individual toxicity of TRI was lower than the combined toxicity of the two active ingredients. In glutathione-*S*-transferase (GST) enzyme activity assays, the exposure to SMX, TRI, and SUMETROLIM resulted in increased enzymatic activity in the *D. magna* juveniles tested at concentrations corresponding to the 48h EC_{20} values originated from the acute immobilization tests. There was no difference in the individual toxicity of the two active ingredients based on the results of acute immobilization tests, however, in the enzyme assays, a higher level of induction of GST detoxification enzyme was caused by TRI compared to SMX.

Comparing the individual and combined toxicity of the investigated neonicotinoid acetamiprid (ACE), clothianidin (CLO), thiacloprid (TCL), and thiamethoxam (TMX) active ingredients and additives, the individual toxicity of ACE and CLO was lower, than that of their formulations, in contrast to TMX-based formulation, where the individual toxicity of the corresponding active ingredient was higher as compared to the combined effect. A strong synergistic effect was observed when the combined effect of ACE and linear alkyl benzene sulfonates (LAS) used as formulating agents was investigated in pure form (a mixture of neat ACE and LAS at equivalent concentrations to their formulated product MOSPILAN 20 SG), but reduced combined toxicity was observed for the

formulation containing other additives. High individual toxicity of LAS was demonstrated by the results compared to the toxicity of ACE individually. Based on the GST enzyme activity values determined for the 48h EC_{20} values originated from acute immobilization tests increased toxicity of ACE was observed: the exposure of the investigated ACE concentration (65 mg/l) resulted in a significant decrease in enzyme activity, in contrast to the results of acute immobilization tests, where even the highest dose (200 mg/l) caused only 10% immobility in the tested individuals. The high toxicity of LAS observed in acute immobilization tests was not corroborated by the enzyme activity assays at the investigated concentrations. Decreased enzymatic activity was demonstrated after the exposure to ACE alone and in the presence of LAS, while the formulating agent alone and the formulation had no effect on GST activity.

In the study of the individual and combined toxicity of the components of GLY-based formulations performed in acute immobilization tests with the use of our own laboratory *D. magna* colony and on juveniles provided by the Daphtoxkit F test kit distributed by MicroBioTests, the order of toxicity for both tested preparations (ROUNDUP CLASSIC, MEDALLON PREMIUM) and *D. magna* strains was as follows: active ingredient < formulations < formulating agents. In the algal growth inhibition tests on the investigated green algae species, the order of toxicity for GLY-based formulations and its components was mostly in line with the trend observed for *D. magna* on the basis of 72h EC_{50} values calculated from the result of optical density and chlorophyll-a-content measurements. In the study of phytotoxic effects, significant differences were found in the sensitivity of different algae species, even for species belonging to the same family. During the investigations of the effects of ROUNDUP CLASSIC and its components on the photosynthetic activity of *Pseudokirchneriella subcapitata*, the treatment of the algal cells with ROUNDUP CLASSIC resulted in a significant decrease of the Fv/Fp values corresponding to the photochemical efficiency of the PS II photochemical system, but only at the highest test concentration [POEA (a mixture of polyethoxylated tallow amines): 18,9 mg/l,

GLY: 50,6 mg/l]. The Fv/Fp values were not affected by the individual exposure of the components. The Rfd values indicated an increase in photosynthetic activity in the low concentration ranges, while at higher test concentrations, a decrease was observed upon exposure to the formulation and POEA.

Biofilms originated from River Danube and Lake Velencei adapted well to laboratory conditions, and a significant decrease in algal biomass was not observed in the untreated control during the experimental period. In contrast to biofilms from River Danube and Lake Velencei, a degradation of biofilms was detected under laboratory conditions in the control units originated from Lake Balaton. The effects of ROUNDUP CLASSIC and its components were demonstrated on the biomass of algal communities in domestic surface water biofilms, while an increase of algal biomass was detected as a result of GLY treatment in different years, yet only in some cases and after an initial decrease. The ROUNDUP CLASSIC treatment resulted in a decrease in the algal biomass of the exposed biofilms, except for the Danube data set from 2016, however, significantly slower increase in biomass was observed in this case as well. An increase in biomass values was caused by POEA treatment, however, in samples from Lake Velencei, similarly to GLY, only after an initial decrease. The realignment of algal communities was observed in the treated biofilms, as more sensitive species to the effects of treatments were replaced by more tolerant mainly filamentous green algae species, which can utilize the test substances as a source of nutrients. At the end of the experiment, increased production of extracellular, mucous exopolymers was observed in the treated biofilms compared to the control, mainly in the presence of POEA, which can be interpreted as an increased stress response in biofilms. Based on the result of the performed trait-based method using the examination of the biological characteristics, a significant change can be observed in the cell number ratio of the diatom communities over time in the control and treated groups during the analysis of cell size and ecological guild categories, in biofilms from Lake Balaton and River Danube as well. GLY treatment resulted in an increase in the

relative frequency of the smallest and largest cell size categories in biofilms originated from River Danube, however, the increase in the smallest cell size category was stronger in the treated biofilm samples from Lake Balaton as compared to the largest cell size category. The effects of GLY were detected on the relative frequency of ecological guild categories only in biofilms originated from River Danube, where an increase in the relative frequency of low-profile guilds and a decrease in the frequency of the motile guild were confirmed compared to the control.

A rather varying tendency of GLY degradation was observed in the form of the pure active ingredient and formulation as well as in the two natural water bodies. After the initial decrease of GLY concentration in water samples originated from River Danube, the concentration stagnated in both forms, while no change in the measured concentration was observed during the one-week study period in water samples originated from Lake Balaton. The concentration of GLY in aqueous medium is significantly affected by the presence of POEA, because in the samples collected after adjusting the GLY concentration, much higher concentrations were determined in the form of the formulation in the investigated surface water samples originated from River Danube and Lake Balaton, also in the presence of biofilms. The environmental concentration of GLY is highly affected by the presence of biofilms as well.

The rate of LAS degradation in the form of a pure detergent mixture significantly exceeded the rate of degradation in the form of MOSPILAN 20 SG both in distilled water and in water samples from River Danube. The DT_{50} values characterizing the degradation of the detergent mixture are much lower in the natural water sample than in distilled water, while there was no difference in DT_{50} values in the presence of ACE and other additives in the formulation MOSPILAN 20 SG. Based on our results, the degradation of LAS is significantly influenced by the physical, chemical, and biological properties of the aqueous medium, and the presence of certain neonicotinoid-type active ingredients (ACE, imidacloprid, TCL) and other additives in the formulations.

4. NEW SCIENTIFIC RESULTS

1. During the assessment of the effects of sulfamethoxazole and trimethoprim (TRI) investigated on *Daphnia magna* individually and together at concentrations equivalent to their formulation SUMETROLIM used in human and veterinary medicine, the individual acute toxicity of TRI is almost ten times lower than the observed combined toxicity of the pure mixture of the active ingredients, and the increased activity of the enzyme glutathione-*S*-transferase (GST) was demonstrated for the investigated active ingredients and formulation.

2. In the study of the acute toxicity of neonicotinoid formulations on the immobilization of *D. magna*, I was the first to prove that the combined toxicity of the additives and active ingredients found in clothianidin-based APACHE 50 WG and MOSPILAN 20 SG containing acetamiprid (ACE) formulations exceeds the individual toxicity of their active ingredients.

3. A strong synergistic effect was demonstrated on *D. magna* when the combined effect of ACE and linear alkyl benzene sulfonates (LAS) used as formulating agents was investigated in pure form (a mixture of neat ACE and LAS at equivalent concentrations to their formulated product MOSPILAN 20 SG), but reduced combined toxicity was observed for the formulation containing other additives. Decreased GST enzyme activity was proven during the exposure of ACE alone (65 mg/l) and its pure mixture with the formulating agent (7,4 mg/l ACE and 0,9 mg/l LAS). In addition, the degradation of LAS was significantly influenced by the physical, chemical, and biological properties of the aqueous medium, and may also be affected by the presence of neonicotinoid-type active

ingredients. ACE, imidacloprid, and thiacloprid active ingredients significantly increase the half-life of LAS.

4. In the study of the phytotoxicity of glyphosate (GLY)- based ROUNDUP CLASSIC formulation and its components, a hormetic effect on the photosynthetic activity of the *Pseudokirchneriella subcapitata* algae was demonstrated. A decrease in photosynthetic activity was proven at the higher test concentrations of POEA (a mixture of polyethoxylated tallow amines) (19,2 mg/l) and the formulation (11,6–50,6 GLY).

5. The effects of ROUNDUP CLASSIC formulation and its components (GLY: 100 µg/l; POEA: 37,4 µg/l) were proven even at concentrations much lower than those reported in the scientific literature on the biomass and composition of algal communities in biofilms grown under natural conditions in different Hungarian surface water bodies, and the effects were found site-specific. Based on the biological trait-based investigations of diatom communities in domestic surface water biofilms, the effects of GLY and its formulation on the relative frequency of diatoms in different cell size and ecological guild categories was demonstrated.

6. A rather varying tendency of GLY degradation was proven in the investigated surface waters. Based on our studies the environmental concentration of GLY is highly affected by the presence of POEA and biofilms as well.

7. CONLCUSIONS AND SUGGESTIONS

Our results indicate differences within each substance class in the acute toxicity of the investigated sulfonamide and neonicotinoid active ingredients in spite of the similarities in the chemical structures of the compounds in each group, which differences warn that great care must be taken when performing extrapolations (e.g., QSAR modeling) to estimate toxic effects based on common structural elements/similar structure. In the study of sulfamethoxazole and trimethoprim (TRI), there was no difference in the effects of the active ingredients in the immobilization tests, whereas significant differences were observed between their effects on glutathione-*S*-transferase enzyme activity. Consequently, in the authorization process for veterinary and plant protection products, it would be necessary to extend the scope of mandatory standard tests to more sensitive test methods and endpoints (e.g., enzyme activity tests, immunomodulatory effects), especially if the mechanism of action is to be investigated.

Significant differences in susceptibility of the studied algal species were observed in many cases, even for *Desmodesmus subspicatus* and *Scenedesmus obtusiusculus* species belonging to the same family (*Scenedesmaceae*), presumably due to differences in the morphology, cell biology, and physiology of different algal cells. Due to the significant variability in the results of standard tests, the implementation of the toxicity tests on several members of a given taxon is advised to be included in environmental risk assessment, where the species to be studied can be selected based on, inter alia, ecological relevance, and distribution or the sensitivity of the species. In the evaluation of phytotoxic effects, a rapid indication of the development of toxic effects allowed by the measurement of photosynthetic activity, which can be achieved without damaging the cells.

Although the individual toxicity values determined in our standard tests are higher compared to the average detectable environmental concentrations of

the investigated veterinary and neonicotinoid type active ingredients in surface waters, environmental risk assessment must be carried out in their case as well. The toxicity of residues in surface waters can be significantly affected by habitat-specific environmental conditions, and for certain specific point sources (e.g., after pharmacological treatment of aquaculture) the level of contamination can reach the order of mg/l. The investigated active ingredients are present in surface waters in relatively low concentrations but continuously, therefore the study of the effects of long-term chronic exposure is essential in environmental risk assessment. The toxicity values determined for glyphosate- (GLY) based formulations also remain below the average level of environmental contamination, however, in watercourses near treated areas, environmental concentrations may significantly increase after heavy rains.

Significant differences were observed in the individual and combined toxicity of the components of veterinary and plant protection products. The various additives used in the formulations cannot be considered as evidently inactive ingredients due to their physicochemical properties and their participation and role in various biological interactions, therefore their toxicological and ecotoxicological evaluation is essential during the environmental risk assessment of formulations applied in agricultural practice. Similarly to ROUNDUP CLASSIC containing a mixture of polyethoxylated tallow amines (POEA) as a formulating agent, in tests investigating the toxicity of the components of MEDALLON PREMIUM the investigated alkyl polyglucoside (APG) formulating agent proved to be the most toxic component. Due to the incriminatory scientific results, the use of POEA as a formulating agent in GLY-based formulations has been banned in the EU. Although the individual toxicity of APG on *D. magna* and algal test organisms is lower compared to the individual toxicity of POEA, it affects the toxicity of the formulation, so revision of GLY-based pesticide products formulated with APGs may also be necessary. Our results demonstrate and support the scientific suggestions, which require changes in official authorization and regulation, including strict

regulation of additives, future development of a common approach to the environmental risks of mixtures of different chemical compounds, moreover, the development and introduction of new effective guidelines for the detection of combined toxicity.

In the study of natural surface biofilms, uniform, statistically identical algal units were provided with the use of the special buoy structure developed for biofilm colonization, however, significant differences in the sensitivity of biofilms grown under different natural conditions were observed. The effects of the investigated GLY concentration (100 µg/l) were demonstrated on the evolution of the algal biomass, and the rearrangement of the composition of the algal communities was detected in the treated freshwater biofilms. Diatom communities in natural biofilms can be good indicators of GLY contamination in freshwaters, and compared to analysis at the level of species the use of a clearer data set is ensured by the biological trait-based analysis of diatoms when comparing trends and testing hypotheses. However, the standardization of studies performed on biofilms is difficult and thus less reproducible, based on their results more precise conclusions can be drawn for aquatic ecosystems. The advantage of ecotoxicological studies on biofilms is that the adverse effects of different pollutants can be investigated at a community level, and they better represent the natural processes in the environment than standard studies on single species, as not only individuals of a species but populations of different species are exposed to xenobiotics in environmental compartments. The effects of different populations on each other after exposures can be assessed as well in studies at a community level.

A rather varying tendency of GLY degradation was proven in the form of pure active ingredient and formulation, and in the two natural surface water bodies as well, mainly due to the physical (e.g., temperature, suspended solid particles) and chemical (e.g. pH, dissolved oxygen content) characteristics of different natural waters, and the differences in the composition of the microbial community. Based on our results, the environmental concentration of GLY is

highly affected by the presence of POEA and biofilms as well in the aquatic environment. The authorization and the assessment of environmental risks of pesticide active ingredients and their formulations are currently carried out based on DT_{50} values determined under laboratory conditions in tests performed in distilled water. However, based on the literature data and our results, the continuous review of the DT_{50} values used in the processes of authorization and environmental risk assessment and the determination of habitat-specific data are essential. The tendency of the environmental concentration of linear alkyl benzene sulfonates in surface waters also depends significantly on physical, chemical, and biological characteristics of the aqueous medium (e.g., the presence of suspended solid particles, the density and composition of the microbial community), and on the presence of certain neonicotinoid type active ingredients.

The results of my PhD work draw attention to the fact that in the authorization and risk assessment processes of veterinary drugs and plant protection products, mandatory ecotoxicological studies should be performed on several representatives of a given taxon. The combined toxicity of the formulations and the environmental fate of active ingredients are highly affected by formulating agents applied in the formulated preparations, therefore these additives cannot be classified as unequivocally inert components. The ecotoxicological evaluation of formulating agents individually and in combination with the active ingredients is essential during a reliable environmental risk assessment of formulations used in agricultural practice.

8. SCIENTIFIC PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

Publications in foreign languages in peer-reviewed, scientific journals:

Székács I., Fejes Á., **Klátyik Sz.**, Takács E., Patkó D., Pomóthy J., Mörtl M., Horváth R., Madarász E., Darvas B., Székács A. (2014): Environmental and toxicological impacts of glyphosate with its formulating adjuvant. *Int. J. Biol. Veter. Agric. Food Engineer.* **8**: 213-218.

Mörtl M., Kereki O., Darvas B., **Klátyik Sz.**, Vehovszky Á., Győri J., Székács A. (2016): Study on soil mobility of two neonicotinoid insecticides. *J. Chem.* **2016**: Article ID 4546584. *IF: 1,300*

Klátyik Sz., Bohus P., Darvas B., Székács A. (2017): Authorization and toxicity of veterinary drugs and plant protection products: residues of the active ingredients in food and feed and toxicity problems related to adjuvants. *Front. Vet. Sci.* **4**: 146. *IF: 2,164*

Klátyik Sz., Takács E., Mörtl M., Földi A., Trábert Zs., Ács É., Darvas B., Székács A. (2017): Dissipation of the herbicide active ingredient glyphosate in natural water samples in the presence of biofilms. *Int. J. Environ. Anal. Chem.* **97**: 901-921. *IF: 1,372*

Takács E., **Klátyik Sz.**, Mörtl M., Rácz G., Kovács K., Darvas B., Székács A. (2017): Effects of neonicotinoid insecticide formulations and their components on *Daphnia magna* – the role of active ingredients and co-formulants. *Int. J. Environ. Anal. Chem.* **97**: 885-900. *IF: 1,372*

Sági Gy., Bezsenyi A., Kovács K., **Klátyik Sz.**, Darvas B., Székács A., Mohácsi-Farkas Cs., Takács E., Wojnárovits L. (2018): Radiolysis of sulfonamide antibiotics in aqueous solution: Degradation efficiency and assessment of antibacterial activity, toxicity and biodegradability of products. *Sci. Total Environ.* **622-623**: 1009-1015. *IF: 4,900*

Mörtl M., Takács E., **Klátyik Sz.**, Székács A. (2019): Aquatic toxicity and loss of linear alkylbenzenesulfonates alone and in a neonicotinoid insecticide formulation in surface water. *Sci. Total Environ.* **652**: 780-787. *IF: 4,900*

Mörtl M., Vehovszky Á., **Klátyik Sz.**, Takács E., Győri J., Székács A. (2020): Neonicotinoids: spreading, translocation and aquatic toxicity. *Int. J. Environ. Public Health* **17**: 2006. *IF: 3,127*[1]

Zaller, J.G., Weber, M., Maderthaner, M., Gruber, E., Takács, E., Mörtl, M., **Klátyik, Sz.**, Győri, J., Römbke, J., Leisch, F., Spangl, B., Székács A. (2021): Effects of glyphosate-based herbicides and their active ingredients on earthworms, water infiltration and glyphosate leaching are influenced by soil properties. *Env. Sci. Eur.* **33**: 51. *IF: 5,394*[2]

Cumulative impact factor: 24,529

[1] average impact factor of the last 5 years
[2] average impact factor of the last 2 years

Abstracts published in a foreign language conference publication:

Székács I., Fejes Á., **Klátyik Sz.**, Takács E., Patkó D., Pomóthy J., Mörtl M., Horváth R., Madarász E., Darvas B., Székács A. (2014): Environmental and toxicological impacts of glyphosate with its formulating adjuvant. *Abs. International Conference on Agricultural and Environmental Systems* (Miami, FL, USA), p. 213.

Klátyik Sz., Földi A., Ács É., Cséffán T., Pasaréti Gy., Mörtl M., Takács E., Bohus P., Székács A., Darvas B. (2015): Reaction of biofilms (cyanobacterial, diatom and green algae) from Lake Balaton and River Danube to herbicide preparation Roundup and its components (glyphosate, POEA). *Proceedings of the 21st International Symposium on Analytical and Environmental Problems* (Szeged, Hungary), pp. 378-382.

Klátyik Sz., Mörtl M., Takács E., Székács A., Darvas B. (2016): Effects of neonicotinoid insecticide formulations and its components on *Daphnia magna*. *Abs. 9th European Conference on Pesticides and Related Organic Micropollutants in the environment és 15th Symposium on Chemistry and Fate of Modern Pesticides* (Santiago de Compostela, Spain), pp. 151-152.

Klátyik Sz., Trábert Zs., Ács É., Mörtl M., Takács E., Bohus P., Székács A., Darvas B. (2016): The effects of herbicide preparation Roundup and its componentrs (glyphosate, POEA) on the algal communities of natural biofilms. *Abs. 9th European Conference on Pesticides and Related Organic Micropollutants in the environment és 15th Symposium on Chemistry and Fate of Modern Pesticides* (Santiago de Compostela, Spain), pp. 395-396.

Klátyik Sz, Sági Gy, Takács E, Kovács K, Wojnárovits L, Takács E, Darvas B, Székács A (2017): Comparative evaluation of veterinary active ingredients and formulations. *Proceedings of the 23rd International Symposium on Analytical and Environmental Problems* (Szeged, Hungary), pp. 444-449.

Mörtl M., Takács E., **Klátyik Sz.**, Székács A. (2017): Determination of surfactants used in agrochemicals. *18th European Meeting on Environmental Chemistry (EMEC)* (Porto, Portugal), pp. 26-29.

Sági Gy., Bezsenyi A., Kovács K., **Klátyik Sz.**, Darvas B., Székács A., Wojnárovits L., Takács E. (2017): Changes in the biological degradability and toxicity of sulfonamide antibiotics in activated sludge and river water due to ionizing radiation treatment. *Programme and Abstracts - International Conference on Applications of Radiation Science and Technology (ICARST-2017)* (Vienna, Austria), p. 165.

Barócsi A., Lenk S., **Klátyik Sz.**, Takács E., Szarvas G., Gádoros P., Domján L., Kocsányi L., Székács A. (2018): Indication of algae density and polycyclic aromatic hydrocarbons in water quality assessment using sensitive fluorescent techniques at high dynamic range. 5. *5th Leaf Optics Workshop* (Ebernburg, Germany), pp. 5-6.

Gémes B., **Klátyik Sz.**, Lázár D., Kocsányi L., Takács E., Székács A. (2018): Comparison of four methods to estimate algal biomass in pesticide toxicity

assays. *Young Researchers' International Conference on Chemistry and Chemical Engineering* (Budapest, Hungary), p. 62.

Adányi N., Berki M., Kónya É., **Klátyik Sz.**, Lázár D., Gémes B., Csősz D., Lenk S., Barócsi A., Csőke L. T., Csákányi A., Domján L., Szarvas G., Kocsányi L., Székács A. (2019): Fluorescence instrumentation for rapid, *in situ* water quality assessment. *11th International Conference on Instrumental Methods of Analysis (IMA-2019)* (Athens, Greece), p. 151.

Csősz D., Lenk S., Barócsi A., Csőke L. T., **Klátyik Sz.**, Lázár D., Berki M., Adányi N., Csákányi A., Domján L., Szarvas G., Kocsányi L., Székács A. (2019): Sensitive fluorescence instrumentation for water quality assessment. *Conference Proceedings OSA Optical Sensors and Sensing Congress* (San Jose, CA, USA), p. 20.

Lázár D., Gémes B., **Klátyik Sz.**, Csősz D., Lenk S., Barócsi A., Kocsányi L., Adányi N., Takács E., Székács A. (2019): Fluorescence instrumentation for rapid, *in situ* water quality assessment. *11th International Conference on Instrumental Methods of Analysis (IMA-2019)* (Athens, Greece), p. 211.

Abstracts published in Hungarian conference publication:

Füleki L., **Klátyik Sz.**, Gyurcsó G., Bánáti H., Kardos C., Szternácsik K., Takács E., Magor Cs., Székács A., Darvas B. (2014): A Magyarországon 1962-2013 között gyomok ellen alkalmazott hatóanyagok [No 3]. *Abs. IV. Ökotoxikológiai Konferencia* (Budapest), pp. 11-12.

Klátyik Sz., Földi A., Ács É., Cséffán T., Pasaréti Gy., Mörtl M., Takács E., Bohus P., Székács A., Darvas B. (2015): Balatoni és dunai biofilmek (kék-, zöld-, kovaalga) reakciója Roundup készítményre és összetevőire (*glyphosate, POEA*). *Abs. V. Ökotoxikológiai Konferencia* (Budapest), pp. 17-19.

Ottucsák M., Takács E., **Klátyik Sz.**, Darvas B., Székács A. (2015): A környezet- és élemiszer-biztonsági kockázatok súlyozása a *glyphosate* gyomirtó-hatóanyag és adjuvánsai példáján. *Abs. V. Ökotoxikológiai Konferencia* (Budapest), pp. 28-29.

Klátyik Sz., Földi A., Trábert Zs., Pozderka V., Ács É., Cséffán T., Pasaréti Gy., Mörtl M., Takács E., Bohus P., Székács A., Darvas B. (2016): Roundup Classic gyomirtó szer és összetevőinek (*glyphosate*, *POEA*) hatása természetes biofilmek algaközösségeire. *Abs. VI. Ökotoxikológiai Konferencia* (Budapest), pp. 17-19.

Klátyik Sz., Mörtl M., Takács E., Székács A., Darvas B. (2016): Neonikotinoid rovarirtószer-hatóanyagok és -készítmények hatása nagy vízibolhán (*Daphnia magna*). *Abs. VI. Ökotoxikológiai Konferencia* (Budapest), pp. 19-20.

Klátyik Sz., Székács A., Darvas B., Mörtl M. (2016): A *glyphosate* gyomirtószer-hatóanyag lebomlása balatoni és dunai vízmintákban, valamint biofilmek jelenlétében. *Abs. VI. Ökotoxikológiai Konferencia* (Budapest), pp. 21-22.

Klátyik Sz., Földi A., Ács É., Takács E., Mörtl M., Székács A., Darvas, B. (2017): A *glyphosate* gyomirtószer-hatóanyag hatásai a vízi életközösségekre. *Abs. III. Parlamenti Nyílt Napok a GMO-król* (Budapest), pp. 65-66.

Klátyik Sz., Földi A., Ács É., Takács E., Mörtl M., Székács A., Darvas B. (2017): A *glyphosate*-tartalmú gyomirtószerek hatásai vízi életközösségekre. *Abs. VII. Ökotoxikológiai Konferencia* (Budapest), pp. 23-24.

Klátyik Sz., Sági Gy., Takács E., Kovács K., Wojnárovits L., Takács E., Darvas B., Székács A. (2017): Állatgyógyászatban alkalmazott készítmények és hatóanyagok összehasonlító vizsgálata. *Abs. VII. Ökotoxikológiai Konferencia* (Budapest), pp. 25-26.

Mörtl M., Takács E., **Klátyik Sz.**, Székács A. (2017): Növényvédő szerek tapadásfokozó adalékanyagainak meghatározása. *Program és előadás-összefoglalók – 60. Magyar Spektrokémiai Vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia* (Debrecen), p. 138.

Székács A., Mörtl M., Takács E., **Klátyik Sz.**, Gyurcsó G., Simon L., Oláh M., Darvas B. (2017): Növényvédő szerek és állatgyógyászati termékek adalékanyagainak meghatározása és ökotoxikológiai értékelése. *Program és előadás-összefoglalók – 60. Magyar Spektrokémiai Vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia* (Debrecen), p. 74.

Gémes B., **Klátyik Sz.**, Lázár D., Lenk S., Barócsi A., Kocsányi L., Takács E., Székács A. (2018): Toxicitási tesztekben alkalmazott algabiomassza-becslési módszerek összehasonlítása. *Abs. VIII. Ökotoxikológiai Konferencia* (Budapest), pp. 10-11.

Klátyik Sz., Mörtl M., Takács E., Székács A. (2018): A lineáris alkil-benzol-szulfonátok toxicitása és lebomlása felszíni vizekben önmagukban és neonikotinoidok jelenlétében. *Abs. VIII. Ökotoxikológiai Konferencia* (Budapest), pp. 12-13.

Lázár D., Gémes B., **Klátyik Sz.**, Csősz D., Lenk S., Barócsi A., Kocsányi L., Adányi N., Takács E., Székács A. (2019): Algadenzitás *in situ* meghatározása fluoreszcenciás módszerrel. *Abs. IX. Ökotoxikológiai Konferencia* (Budapest), pp. 14-15.

Lázár D., Gémes B., **Klátyik Sz.**, Csősz D., Lenk S., Barócsi A., Kocsányi L., Adányi N., Takács E., Székács A. (2019): Algadenzitás *in situ* meghatározása fluoreszcenciás módszerrel. *LXI. Hidrobiológus Napok - Új utak a hidrobiológiában* (Tihany) p. 21.