

Herbicides in surface water bodies - behaviour, effects on aquatic organisms and risk assessment

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SUMMARY

Pesticides play a very important role in reducing losses and maintaining quality in crop production. Although positive effects of pesticide use are undeniable, adverse effects are frequent. This has led to a comprehensive reevaluation of the benefits of pesticide use and potential adverse effects on human health and the environment before placing them on the market. The fact that pesticides are designed to be toxic and are deliberately introduced into the environment, makes them a very important and strictly regulated group of pollutants. The most commonly used group of pesticides are herbicides, and their detection in surface water bodies has been repeatedly reported. In spite of being designed to be toxic to target species, adverse effects on other inhabitants of aquatic environments have also been observed. In order to prevent negative environmental effects, the registration process for active substances and plant protection products involves predictive environmental risk assessments (ERA). Reliable assessment of long-term effects on non-target species, natural populations and ecosystems is a priority and ERA process is constantly being improved.

Keywords: herbicides; water; aquatic organisms; toxicity; risk assessment

INTRODUCTION

Despite constant development of new technologies for sustainable crop production, the use of pesticides remains an essential tool in integrated pest management (Rice et al., 2007). Pesticides are biologically active substances designed to prevent, control or localize (repress) negative impact of pest organisms in crop production. At least two traits distinguish them from other chemicals: they are designed to be toxic (to target organisms) and they are deliberately introduced into the environment (Kim et al., 2017).

Worldwide annual consumption of pesticides is estimated to be 2.4 million tons (Mahmood et al., 2016;

Sharma et al., 2019), but the Food and Agriculture Organization of the United Nations reported that more than 4 million tons of pesticides per year was used during 2012-2017 (FAOSTAT, 2017). It is estimated that less than 1% of the applied pesticides reaches target organisms, while the rest ends up in the environment (Gavrilescu, 2005; Arias-Estévez et al., 2008; Ortiz-Hernández et al., 2013). Even though pesticides provide benefits for crop production, their use can lead to a serious adverse environmental impact (Mahmood et al., 2016). Once in the environment, the fate and behavior of pesticide substances depend on numerous physical, chemical and biological processes. These processes can be roughly grouped into three categories:

sorption, degradation and transportation. Sorption is the process of pesticide binding to mineral or organic soil particles. Major indicators of pesticide sorption potential are physicochemical properties of pesticide substances: water solubility, n-octanol-water partition coefficient (K_{ow}) and dissociation (Arias-Estévez et al., 2008; Wauchope et al., 2002). The degree of pesticide binding to soil stands in negative correlation with their solubility in water, i.e. it is positively correlated with K_{ow} (Wauchope et al., 2002). Pesticide degradation involves processes of physicochemical (hydrolysis, photolysis, etc.) and biological transformation into mostly less toxic or non-toxic and mineral compounds (Vargas, 1975; Wauchope et al., 2002; Solomon et al., 2014). The rate of degradation of pesticides is expressed as the half-life (DT_{50}) and it is defined as the time (days) needed for half of the initial pesticide concentration to disappear from soil or water by transformation (FAO, 2000). Pesticide transportation is the movement of substances in the environment by processes of volatilization, runoff, leaching and plant uptake. Processes of sorption and degradation have significant impact on pesticide transportation. Pesticide application in crops inevitably results in transport of a share of parent compounds and/or degradation products (metabolites) to surrounding nontarget plots (Arias-Estévez et al., 2008). It is estimated that nearly 2% of all pesticides applied in crop production end up in surface waters (Sauco et al., 2010). Their evaporation depends primarily on vapor pressure, so that pesticides with high vapor pressure consequently reach the air by evaporation from soil or other treated surfaces (Walker, 2008). Once in the environment, pesticide substances can reach nontarget organisms and affect them adversely (Rice et al., 2007). Although new technologies have developed less toxic substances that cause minimal negative effects on nontarget organisms, many substances lacking these properties are still in use (Gavrilescu, 2005; Arias-Estévez et al., 2008). In order to develop effective pest management strategies with minimal adverse impact on the environment and human health it is crucial to understand the fate and behavior of pesticides, and their effects on target and nontarget species (Rice et al., 2007). Before placing them on the market, all pesticide substances are thoroughly tested in order to minimize their potential negative impact on the ecosystem in a process of environmental risk assessment. Environmental risk assessment is relying on the results of existing standardized tests and models, but also includes constant upgrading based on scientific and expert knowledge.

HERBICIDES

Herbicides in surface water

Herbicides are the most commonly used pesticide group with a portion of about 40% in total annual worldwide consumption (Grube et al., 2012). Herbicides are primarily used in crop (agricultural) production, but significant amounts are also applied in forestry, for weed control in public areas (e.g. roadsides) and for maintaining parks, golf courts and other sports grounds (Solomon et al., 2014; Park et al., 2017). They reach waters by direct application intended to control aquatic weeds (channels, rice fields, etc.) or indirectly by herbicide transportation from treated areas (Solomon et al., 2014). Transport to surface and ground waters occurs mainly by leaching and runoff from agricultural fields (Wauchope et al., 2002; Gavrilescu, 2005; Botelho et al., 2012). Smaller amounts reach water bodies by drift, atmospheric deposition, washing of equipment and work clothes, spillage and leakage or irregular waste disposal (Fogg et al., 2003). In addition, users are quite often not aware of the risk that pesticide application carries for human health and the environment and fail to comply with all safety precautions and recommendations concerning the application method, thus only aggravating this problem (Damalas & Eleftherohorinos, 2011). A number of plant protection products need mitigation measures in order to reduce exposure of nontarget organisms and to ensure safe pesticide use. These measures include pesticide application at certain distance from surface water bodies in order to reduce direct exposure of the aquatic environment via spray drift. Pesticide transport via runoff and spray drift could be mitigated by introducing vegetative buffer strips. Additional recommended mitigation measures include: reduced tillage, hedgerows, drift reducing nozzles, edge of field bunds, artificial wetland/retention ponds, vegetated ditch and inter-row vegetated strips (Reichenberger et al., 2007; Boivin & Poulse, 2016; Ippolito & Fait, 2019). Inclusion of mitigation measures is demanded for the approval and placing on the market of some plant protection products. One of the challenges in managing the risk of pesticides is identification of locations that need mitigation measures (Di Guardo & Finizio, 2018). This also raises a question about the awareness of end users about these measures, and also about the tools and possibility of proper monitoring of their implementation. In truth, it is hard to verify if the application by end users is performed in compliance with recommendations of the appropriate authorities.

Pesticide monitoring programs have an important role in implementation and relevant advisories on the best mitigation measures in order to achieve protection goals (Knauer, 2016).

Pesticide residues in water affect its quality and, depending on concentration and retention time, can adversely affect the health of people, susceptible organisms and aquatic ecosystems (Carter, 2000; Rice et al., 2007). Due to irreversible changes detected in aquatic ecosystems worldwide, from the standpoint of environmental protection, preserving the quality of water resources is one of the greatest challenges (Botelho et al., 2012). Monitoring pesticide residues has been at the focus of a large number of researchers who have repeatedly found herbicides in surface and ground waters, and the most common compounds belonged to the triazine and anilide groups (Gašić et al., 2002; Cerejeira et al., 2003; Arias-Estévez et al., 2008; Dougherty et al., 2010; Reilly et al., 2012; De Liguoro et al., 2014; Köck-Schulmeyer et al., 2014). Monitoring studies generally differ regarding their sampling methods, frequency of sampling and special coverage. A recent study reported continuous monitoring of a large number of substances (Boye et al., 2019). The study is one of a kind, since it covers a time frame of over 15 years and an analysis of all EU-listed priority substances and almost all active substances registered for use in Sweden. In addition, it includes improvements in analytical methods that allow detection of substances present in traces (low limit of detection). The authors emphasized that the main contribution of their research was to capture all pesticide occurrence in surface water that would, in combination with targeting of intensive agricultural practices, reflect current agricultural management practices. Herbicides with high potentials to reach water bodies are characterized by moderate to high mobility through soil profile, persistence, solubility and moderate binding to organic matter. Given that agricultural areas are often located near creeks, rivers and lakes, the potential exposure of these ecosystems to herbicides is high (Botelho et al., 2012). Depending on physicochemical properties of the substances, upon reaching those water bodies, herbicidal substances can bind to suspended particles in the water column, accumulate in sediment, be absorbed by aquatic organisms or remain on the surface of water (hydrophobic substances). Further transport occurs through diffusion in water flows or by organisms uptake, and due to complex interactions within the food chain, the entire ecosystem may be affected (Figure 1) (Damalas & Eleftherohorinos, 2011; Botelho et al., 2012).

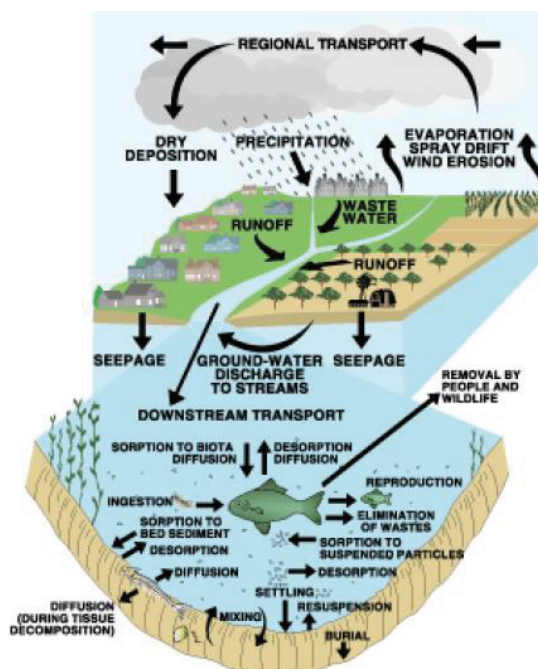


Figure 1. Pesticide movement in the hydrologic cycle including pesticide movement to and from sediment and aquatic biota within the stream (Iorio, 2008)

Effects of plant protection products

Plant protection products are mixtures of one or more active substances and a number of coformulants. The main role of coformulant addition is to increase product efficiency and stability, but also to achieve a number of other desirable properties, such as: reduction of pesticide deposition on non-target surfaces, increase the retention time on target organisms, better uptake and translocation (Knowles, 2005; Gašić & Orešković, 2006; Arias-Estévez et al., 2008; Solomon et al., 2014). The design of plant protection products can be diverse. Physical and chemical properties of pesticides generally determine their formulation type, but some active substances have such properties that allow them to be formulated in various ways. The selection of formulation type is very important as it determines the biological characteristics of the product, for example formulations containing soluble active substances have often better biological efficacy (Seaman, 1990). Over time, pesticide formulations have been improved in order to meet the strict requirements of regulatory authorities on the one hand, and high efficacy expectation on the other. A promising

direction of pesticide formulation improvements is the use of bioenhancing, environmentally friendly and nontoxic adjuvants (Wang & Liu, 2007; Gašić et al., 2012; Radivojević et al., 2016). The improvement of formulation types relies not only on replacement of toxic and nondegradable coformulants, but rather on advanced efficacy ensured through incorporation of the latest formulation technologies, such as: size reduction, increasing coverage of applied surface area, reduced wastage and dose rates with minimum pesticide residues (Hazra et al., 2017). Bearing in mind that pesticide active substances are used in mixtures with coformulants, rather than pure, it practically means that, in addition to active substance, all coformulants are also introduced to the environment, and their physical, chemical, toxicological and ecotoxicological properties are usually diverse. Some of them may have biological activity, could be toxic to humans, and could be chemically active. Some coformulants in pesticide formulations may exert environmental impact by interfering with active substance solubility, mobility, volatilization and so on. In that way they may affect pesticide distribution and persistence in the environment (Cox & Sorgan, 2006). Therefore, it is very important to make a proper selection of coformulants and direct their development towards formulations such as: suspension concentrates, microemulsions, granules, water dispersible granules, controlled release formulations, soluble concentrates, mixed formulations, etc. (Hazra & Purkait, 2019). In addition to the advancement of formulation process, ecofriendly, i.e. less toxic and readily biodegradable formulation products are being made. The active parts of such products are biological agents (e.g. *Bacillus* sp.) (Tanović et al., 2012; Hrustić et al., 2019) or naturally occurring substances (e.g. essential oils) (Tasiwal et al., 2009; Tanović et al., 2013).

Herbicide effects on aquatic organisms

In general, herbicides (except biocides) have low toxicity to animals, and these pollutants in the environment have been given little attention so far (Walker, 2008). Animals at higher trophic levels are dependent on plants, and perhaps the importance of plants has been overlooked, too. In addition to being a food source for many species, plants also provide habitat and shelter to a considerable number of species, so their removal from aquatic systems also indirectly affects animals (Cedergreen et al., 2004; Rosenkrantz et al., 2013; Solomon et al., 2014). Dying plants may deplete oxygen level and endanger the survival of other species.

This indirect impact on other species results from direct impact on plants, which mostly occurs in surface waters after deliberate use in order to control unwanted plant species in surface waters (Solomon et al., 2014). Adverse effects of herbicides on nontarget aquatic primary producers, both algae and plants, have been studied thoroughly (Knauer et al., 2008; Pereira et al., 2009; Dalton et al., 2013; Della Vecchia et al., 2016; Knežević et al., 2016; Nagai et al., 2016; Stevanović et al., 2013, 2019; Chamsi et al., 2019; Nagai, 2019). Arguments in favour of significant differences in sensitivity between floatant and rooted species have led to an extension of risk assessment framework for herbicides. This extension refers to the inclusion of additional testing with rooted macrophyte species (preferably *Myriophyllum*), in case of an apparent lack of sensitivity of *Lemna* sp. and algae to herbicides (e.g. $EC_{50} > 1$ mg/l) (EFSA, 2013). Literature reviewing regarding this topic indicates that better protection goals are reached by this decision since a universally most sensitive species does not exist (Turgut & Fomin, 2002; Teodorović et al., 2012; Tunić et al., 2015; Stevanović et al., 2016). Studies of herbicide effects on outdoor ponds found no direct adverse effect on the invertebrate community, but indicated that long-term changes in macrophyte populations could cause long-term adverse effects on invertebrate community structure (Burdett et al., 2001). Similar findings were reported by Hasenbein et al. (2017) in a study in which decrease in *Daphnia magna* abundance following herbicide application was determined. The authors concluded that the decline was a result of altered phytoplankton community structure. Disruption of population structures were well-documented by Brodman et al. (2010). In that study, constructed communities containing tiger salamander (*Ambystoma tigrinum*) larvae, three species of tadpoles (*Lithobates pipiens*, *L. clamitans* and *Anaxyrus americanus*) and naturally-occurring invertebrates were exposed to Accord, a glyphosate-based formulation. Higher mortality of salamander and one frog species (*L. clamitans*) were noted, while an increased survival of two other frog species was attributed to altered predator-prey relationships in the experimental pond communities. Also, a change in invertebrate community structure was registered. These results suggest that a direct impact on one species, leads to a series of indirect disturbances in other species and consequently affect the community as a whole. Herbicide concentrations in surface water are low and usually do not cause lethal outcome, but they lead to changes in biochemical parameters, hormone disbalance, histopathological changes, and to diverse morphological

and developmental disorders when early life stages are exposed. Juvenile and adult individuals may differ in their sensitivity. For instance, the chorion in early life stages of zebrafish (*Danio rerio*) can prevent the uptake of some substances, and so protect larvae (Braunbeck et al., 2015). Lower sensitivity of juveniles is sometimes related to metabolic pathways, i.e. the absence of their activity and inability to activate substances by metabolic transformation (Colombo et al., 1996). Fish acute toxicity tests are an integral part of hazard identification and risk assessment in the pesticide product registration process (Scholz et al., 2009; Braunbeck et al., 2015). Standard fish tests (acute and chronic) can provide information about toxic and reproductive effects or effects on individual growth, but they are not sensitive enough for assessment of physiological functions, and consequently many ecotoxicological effects can be underestimated (Weicher et al., 2017). Over the last decade, the fish embryo test (FET) has become an important tool for toxicity assessment of chemicals and wastewater. An important advantage of the FET test relies on its ability to measure developmental parameters and deformities, such as: spine, fin and craniofacial deformation, yolk sac and cardiac edema, heart rate and rhythm disorders, neurotoxicity and growth disturbance (Cook et al., 2005; Stehr et al., 2006; Domingues et al., 2011; Raftery et al., 2014; Pamanji et al., 2015). A strong positive correlation between the results obtained with standard acute and embryotoxicity tests with *D. rerio* has been demonstrated (Scholz et al., 2009; Belanger et al., 2013). Studies with early life stages have revealed atrazine's potential to feminize juvenile male frogs and fishes and its endocrine disruptive potential (Hayes et al., 2011; MacLoughlin et al., 2016; Hoskins & Boone, 2018). Lately, these assumptions have been reviewed because of a lack of firm evidence, so that some researchers have disputed them (Brain et al., 2018; Hanson et al., 2019).

Amphibians, compared to other groups of organisms, have not been sufficiently investigated. Nevertheless, the progressive decline of their natural populations has brought them into research focus. According to some estimates, one third of amphibian species are on the verge of extinction, and one of the reasons is the application of pesticides (Stuart et al., 2004; Hayes et al., 2006; McCallum, 2007; Mikó et al., 2017). Very permeable skin and dependence on water bodies during reproduction and crucial developmental stages make them very susceptible to contaminants. It is also noteworthy that most species lay eggs in shallow ponds of forest and agricultural areas where the content of

pesticides is usually higher, compared to large water bodies (Howe et al. 2004; Hayes et al. 2006; Kang et al. 2009). The negative impact of herbicides on growth, development and biochemical processes in different amphibian species has been repeatedly reported (Osano et al. 2002; Bonfanti et al. 2004; Howe et al. 2004; Hayes et al. 2006; McCoy et al., 2008; Oka et al., 2008; Kang et al., 2009; Lenkowski et al., 2010). For instance, glyphosate is one of the most commonly used herbicides, and effects of the active substance and its formulated products on nontarget aquatic species have been widely studied. Although glyphosate has low toxicity to aquatic organisms, the toxicity of formulated products varies. It has been proved that some coformulants are more toxic to many organisms than the active substance itself (Howe et al., 2004; Brodman et al., 2010; Janssens & Stoks, 2017; De Brito Rodrigues et al., 2019; Mesnage et al., 2019). Adverse effects on animals have been reported even for herbicidal substances that target the photosynthetic pathway (Mela et al., 2013; Wang et al., 2013; Stevanovic et al., 2017; Gaaied et al., 2019). Another difficulty is the variable toxicity of active substances and formulated products. Toxicity increase in formulated products may be avoided by proper use of formulation technology. De Andrade et al. (2019) studied the effects of a slow release formulation of atrazine on the freshwater teleost *Prochilodus lineatus*. The nanoencapsulated type of formulation was less toxic than the active substance, indicating that new technologies based on gradual release of active substance are environmentally more acceptable. Assessments of pesticide effects on aquatic organisms include all members of the aquatic environment: primary producers, invertebrates and vertebrates, and all scenarios from short- to long-term exposure. Although new species and models are being included and developed for effect assessment processes, according to a current regulation (EC, 2013a; b), inclusion of amphibians in this process is not mandatory, at least for now.

ENVIRONMENTAL RISK ASSESSMENT

By definition, environmental risk assessment (ERA) is a process of evaluation of the likelihood that adverse environmental effects may occur or are occurring as a result of exposure to one or more stressors (US EPA, 1992). From a regulatory point of view, environmental risk assessment enables the assessment of the probability and magnitude of adverse effects of chemical and physical agents on

populations, communities and the ecosystems. Given the number of different stressors, but also the number of different species in nature, the basic task of ERA is to extrapolate the results of ecotoxicological tests to higher levels of biological organization. Based on the nature of the process under review, environmental risk assessment can be predictive – when assessing potential effects of substances before they are placed on the market, or retrospective – when assessing the environmental effects of chemicals already present in the environment. Predictive ecological risk assessment is one of the basic procedures in the control and sustainable use of plant protection products, biocides and industrial chemicals to prevent unacceptable risk to populations of non-target species and ecosystems. It relies on the results of standardized ecotoxicological testing on selected, representative species of aquatic and terrestrial environments to determine the effects that a chemical would cause if it enters an ecological system. Retrospective risk assessment is an assessment of the state of the environment and the association of observed environmental effects with past/current exposure to a stressor. Retrospective ERA deals with the assessment of effects of a chemical already in the ecosystem, and it therefore may be more accurate and reliable than predictive risk assessment (Suter II, 2006).

Environmental risk assessment is a very complex process which can be divided into four categories (van Leeuwen & Hermens, 2001):

- hazard identification,
- effect assessment (assessment of concentration/dose – response/effect ratio),
- exposure assessment, and
- risk characterization.

Hazard identification involves the determination of intrinsic harmful properties that substances have, i.e. determination of the potential to cause adverse effects under certain exposure conditions. It involves the collection and evaluation of data on the types of effects of different chemicals and conditions of exposure, which may lead to adverse effects, i.e. it refers to the likelihood of damage resulting from exposure (van Leeuwen & Hermens, 2001).

Effect assessment is an assessment of the relationship between dose/concentration and onset and severity of the effect. It involves characterizing the quantitative relationship between the degree of exposure and the occurrence of adverse effects. Effect assessment uses data from laboratory and field testing and various epidemiological ecosystem studies. Assessment of

environmental effects begins with an assessment of effects, followed by an analysis of environmental response (identification of effects caused by changing stressor levels) and linking the effects to the ecologically relevant goals. Most information regarding the dose-response ratio refers to effects on a specific species, since information on dose dependency in populations, communities or ecosystems is difficult to measure due to their pronounced complexity (Newman & Unger, 2003; Foudoulakis, 2006). The dose-response ratio can be expressed in a variety of ways: as a function of intensity (dose, concentration), temporal (mean lethal dose/concentration for 24, 48, 72, 96 h) or spatial distribution. ERA is built based on the information on medium lethal (LC_{50}), effective/inhibitory concentrations (EC_{50}/IC_{50}) or maximum concentrations with no observed (adverse) effect (NOEC/NOAEL) (Beyer et al., 2014; FOCUS, 2015; Papadakis et al., 2015). The recommendation of the European Food Safety Authority (EFSA) is to determine concentrations causing adverse effects in 10% of a population (EC_{10}) in addition to NOEC values, as NOEC may depend on the experimental design, while EC_{10} is calculated on the basis of a dose-dependent curve (Azimonti et al., 2015).

Exposure assessment is the prediction or measurement of the temporal and spatial distribution of chemicals and their interaction with an environmental component of importance. The distribution of stressors covers transportation pathways from the source (location of its release in nature) to the site where it will exert an impact on the ecological system. This is the most unreliable step in the risk assessment process due to lacking information on the emission of chemicals. Differences in abiotic factors, such as meteorological factors (temperature, humidity, wind speed, precipitation), hydrological, geological (soil types), but also abiotic (differences in ecosystem structure and function) also contribute to the unreliability of this ERA step (van Leeuwen & Hermens, 2001). When assessing exposure, it is very important to consider the concentration, length and frequency of exposure. Exposure length refers to the time period during which a community is exposed to a stressor present at a concentration higher than the sensitivity threshold (Newman & Unger, 2003).

Risk characterization, the last step of risk assessment, covers the assessment of the nature, frequency and intensity of adverse effects likely to occur in environmental compartments under defined exposure conditions. It integrates all information obtained in previous steps and predicts on that basis the frequency, nature and magnitude of risk. Clearly defined protection

objectives contribute to accurate and reliable risk characterization (van Leeuwen & Hermens, 2001).

The predicted environmental concentration (PEC) is modeled based on pesticide behavior, fate and application (EFSA, 2013). Under EU Regulation 1107/2009, pesticide registration procedures permit the use of mathematical models to derive PEC values. For this purpose, the European Commission has set up a FOCUS Surface Water Working Group (FORum for Coordination of pesticide fate models and their Use), whose main objective is to establish procedures and models for calculating PEC values in surface waters (FOCUS, 2015). The FOCUS_{sw} (sw - surface water) program predicts PEC values for four levels of risk assessment based on defined scenarios relevant to agricultural production, properties of active substances, and application time, method and dose. The first two levels of risk assessment represent the worst case scenarios, while modeling for the third and fourth levels of risk assessment takes into account the fate of a substance in the environment, providing more realistic predicted values (EFSA, 2004). A number of researchers have pointed out that detected concentrations of some pesticides (insecticides and fungicides) in surface waters are higher than PEC values obtained by modeling in FOCUS, so a revision of this methodology is expected in the near future (Knäbel et al., 2012, 2014, 2016; Pereira et al., 2017; EFSA, 2018).

According to a guidance document on aquatic ecotoxicology, the acceptability of risk is defined by TER values (toxicity-exposure ratio) derived from the ratio of toxicity (LC₅₀, EC₁₀, EC₅₀, IC₅₀, NOEC) and exposure levels (PEC), and it allows quantitative assessment of acute and chronic risk. The defined threshold or trigger value for each group of organisms is then compared with TER values (for acute tests the threshold is 100

and for chronic 10). When a TER value is higher than the prescribed limit value or threshold, it is considered that there is no risk of adverse effect and consequently a higher level of risk assessment is not required; if the TER is lower than the threshold, the risk is unacceptable (EC, 2002).

For the purpose of harmonizing risk assessment for aquatic organisms with Regulation 1107/2009 and new scientific knowledge, EFSA has revised this document and published a guidance for tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013). According to this guideline, risk is assessed by determining the regulatory acceptable concentrations (RAC), values that are the ratio of toxicity and defined assessment factors (AF) (Table 1). If the RAC value is greater than the PEC value, the risk that a test substance is posing to the environment is unacceptable.

The guidance on tiered risk assessment of plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) recommends an inclusion of amphibians in risk assessment, but it only refers to the growth stages in aquatic environment, while terrestrial stages will be covered by a risk assessment guide for amphibians and reptiles. This guidance is still under revision and, based on scientific opinion on pesticide risk assessment for amphibians and reptiles (EFSA, 2018), it will cover all development stages, both aquatic and terrestrial. The frog embryo teratogenesis assay with *Xenopus laevis* is one of the few standardized tests for testing toxicity to amphibians. Although the scientific community emphasizes whole-life-cycle tests as the most representative (LAGDA test with *Xenopus tropicalis* is recommended), a combination of results obtained in assays for aquatic and terrestrial stages should not be neglected.

Table 1. Mandatory assays for herbicide risk assessment in surface waters and a brief overview

| Test organism | Assay | Exposure | Endpoint | AF (Trigger value) |
|----------------|------------|-----------|--|--------------------|
| Fish | Acute | 96 h | LC ₅₀ | 100 |
| | Chronic | 21 days | NOEC | 10 |
| | | | EC ₁₀ | 10 |
| Daphnids | Acute | 48 h | EC ₅₀ | 100 |
| | Chronic | 21 days | EC ₁₀ , NOEC | 10 |
| Algae | Short term | 72-120 h | E _b C ₅₀ E _r C ₅₀ | 10 |
| Aquatic plants | Short term | 7-14 days | E _b C ₅₀ E _r C ₅₀ | 10 |

r – growth rate IC₅₀

b – yield IC₅₀

The revised guidance (EFSA, 2013) is focused on the tiered structure of risk assessment and defines effect assessments by levels, while the methodology for exposure assessment remains unchanged (FOCUS). The tiered structure involves four levels of assessment, so that effects assessment goes from simple (laboratory tests) to complex (higher levels of environmental reality) experimental designs (Figure 2).

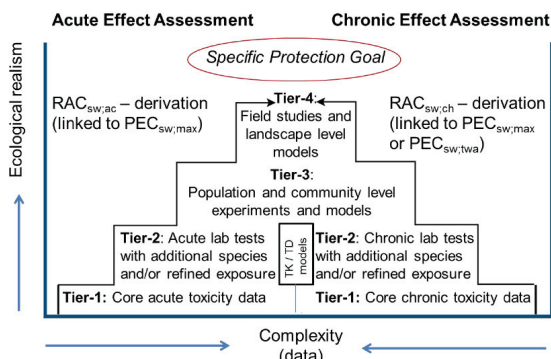


Figure 2. Schematic presentation of the tiered approach to acute (left) and chronic (right) effect assessments of plant protection products (EFSA, 2013).

Acute and chronic effect/risk assessments are mandatory in the registration process for plant protection products. Tier 1 and 2 effect assessments are estimated on the basis of single species laboratory toxicity tests, but in order to better address the risk of time-varying exposures, tier 2 estimates may be complemented with toxicokinetic/toxicodynamic (TK/TD) models. Tier 3 (population- and community-level experiments and models) and tier 4 (field- and landscape-level models) may involve a combination of experimental data and modeling to assess population and community level responses within a relevant spatio-temporal framework. All these models need to be properly tested and consistent with the required quality criteria. In accordance with effect assessment schemes, the regulatory acceptable concentrations should be determined and compared with predicted environmental concentrations in surface waters (PEC_{sw}). First tier RAC values are based on standard toxicity test results, while the second tier uses the results of standard and additional laboratory single species tests to obtain the geomean or species sensitivity distribution (SSD) or the results of tests with additional species and refined exposure. The SSD method can be used when a minimum of required data is provided – eight toxicity data for primary producers and invertebrates, or six for fish and/or amphibians. Based on the obtained

HC₅ values (hazard concentration for 5% of exposed species) and the corrected AF, the RAC can be calculated. Furthermore, risk assessment is done by comparing RAC with PEC values. If the minimum required data for SSD is not available, the geomean approach applies. Determination of the third tier RAC values are based on the results of micro and/or mesocosm studies.

Pesticide active substances are strictly regulated and predictive ERA applies to them, as well as to plant protection products, so that their adverse effects are evaluated prior to placing them on the market. In the European Union, placing on the market is defined by Regulation 1107/2009 (EC, 2009a), and in Serbia by Zakon o sredstvima za zaštitu bilja (2009). Plant protection product risk mitigation policy for human health and the environment is defined by EU Directive 2009/128/EC (EC, 2009b).

Prior to placing them on the market, active substances are thoroughly tested for toxicological, ecotoxicological, physicochemical and other properties, and this process is governed in the EU by Regulation 283/2013 (EC, 2013a), and in Serbia by a Regulation on the content and method of handling of documentation for the evaluation of active substances, i.e. the basic substance, and methods for testing the active substance (Pravilnik, 2012a). These assays are used to determine possible adverse effects on humans and non-target organisms, pollution of surface or groundwaters due to leaching, runoff, drift, etc. Placing on the market of plant protection products is governed in the European Union by Regulation 284/2013 (EC, 2013b), and in Serbia by a Regulation on the content and method of handling documentation for evaluation of plant protection products and methods for testing products for plant protection (Pravilnik, 2012b). Based on this Regulation, detailed studies of the effects on aquatic organisms shall be carried out for products whose toxic properties cannot be predicted on the basis of properties of their active substances, if the intended application is on water surfaces, or if there is no information on a similar product and extrapolation is not possible.

Pesticide risk assessment is an integral part of the pesticide registration process. Namely, the results of all assays required for the registration process are consolidated and considered in the process of environmental risk assessment. The process includes testing of aquatic organisms (algae, plants, invertebrates, fish), terrestrial vertebrates (birds and mammals), bees and other useful arthropods, nontarget meso- and micro-fauna (e.g. earthworms), nontarget terrestrial plants and soil microorganisms with the purpose of

predicting possible negative effects of a stressor on nontarget organisms and their natural populations or environmental impact under defined conditions (EFSA, 2013).

EU Regulation 1107/2009 defines that interactions between an active substance, a protective agent (protectant), a synergist and a coformulant must be taken into account when evaluating and authorizing plant protection products. Also, requirements for plant protection products registration, specified in Regulation 284/2013, include all information on potentially unacceptable effects on the environment, plants and plant products, as well as known or expected cumulative and synergistic effects.

For the assessment of mixtures (also for plant protection products), the mixture assessment approach may be applied to a specific ingredient, depending on the available toxicity data. Regarding component-based approaches, dose-response data for specific toxic effects of a single compound is required. The combined effects in mixtures may be additive, decreasing (antagonism, inhibition) or increasing (synergism, potentiation) (EFSA, 2015; Quignot et al., 2015).

It often happens during the process of pesticide registration that toxic properties of a preparation are equalized with the toxic properties of its active substance, so that ecological risk assessment for that active substance also applies to the preparation without assessing the properties of coformulants used in the formulated preparation (Mesnage et al., 2014). This is especially important when there is a number of different formulations based on the same active substance, i.e. when different formulations contain different sets of coformulants. Their toxic properties can vary greatly, which certainly affects the toxic properties of those formulations, so their effects on non-target species and the environment can also be very diverse. It is clear that risk assessment based on the active substance is not always sufficiently reliable when it comes to the effects and toxicity of plant protection products formulated differently. On the other hand, the action of one or more active substances is very difficult to predict; differences in the expression of individual effects in relation to the effect of mixture, as well as the phenomenon of potentiating, antagonistic, synergistic or additive effects are the subject of research by numerous authors (Kortenkamp et al., 2009; Beyer et al., 2014; Kortenkamp, 2014). Finally, once a plant protection product is placed on the market, pesticide monitoring is the only indicator of its proper use and accurate risk assessment. Monitoring data could be beneficial for

the development and improvement of test models for predictive pesticide behavior in the environment.

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Herbicidi u površinskim vodama – ponašanje, efekti na akvatične organizme i procena rizika

REZIME

Porast broja stanovnika dovodi do konstantne potrebe za povećanjem produktivnosti biljne proizvodnje, a veoma važnu ulogu u postizanju tog cilja imaju pesticidi, hemijska ili biološka sredstva za zaštitu bilja. Pored nesporno pozitivnih mogu imati i neželjene efekte, a ulažu se veliki napor kako bi se, pre stavljanja u promet, izvršila pravilna procena koristi koju nam upotreba pesticida donosi i potencijalnih neželjenih efekata na čoveka i životnu sredinu. Najmanje dve osobine ih odvajaju od drugih supstanci – dizajnirani su da budu toksični i ciljano se unose u životnu sredinu, pa je stoga njihov promet striktno zakonski regulisan. Najčešće korišćena grupa pesticida su herbicidi, a njihova detekcija u površinskim vodama veoma je česta. Iako su dizajnirani da utiču na ometanje razvoja primarnih producenata, neželjena dejstva registrovana su i na drugim konstituentima vodene životne sredine. U cilju sprečavanja negativnih ekoloških efekata, process registracije aktivnih supstanci i sredstava za zaštitu bilje obuhvata preventivnu ekološku procenu (ERA – Environmental risk assessment). Pouzdana procena dugotajnih efekata u životnoj sredini na neciljne vrste organizama, prirodne populacije i ekosisteme je osnovni zadatak ERA-e.

Ključne reči: herbicidi; voda; akvatični organizmi; toksičnost; procena rizika