Hort4EUGreen

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SUSTAINABLE USE OF PESTICIDES AND THEIR RESIDUES MONITORING

Problematic issues of concern

Volume 4

University of Agronomic Sciences and Veterinary Medicine of Bucharest
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"Enhancing practical skills of horticulture specialists to better address the demands of the European Green Deal"

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Summary

This Training Module is intended to provide insight into some problematic issues of concern related to pesticides. The examples include glyphosate, the most often used pesticide worldwide and neonicotinoids tailored to the insect nervous systems, which are the most frequently used insecticide group. There are further problems related either to application or to mode of action. The goal of this course is to highlight to the different environmental problems, which may appear even in those cases, where the reduction of harmful environmental effects have been aimed. This module will probably develop the skills to understand complexity of environmental problems caused by pesticides.

Learning outcome descriptors

By the end of the Module, the trainee should be able to:

- Understanding the chemical and biological properties of glyphosate and neonicotinoids;
- Understanding the ecotoxicological impact of glyphosate and neonicotinoids and expand this knowledge to other pesticides that are issues of concern;
- Have an overview on residue monitoring techniques and risk assessment of the hazardous plant protection products;

General and transferable skills

1	Be able to evaluate scientific literature and studies related to glyphosate, neonicotinoids, and other pesticides;
2	Effectively communicating complex scientific information to diverse audiences, including farmers, policymakers, and the general public about glyphosate and neonicotinoids;
3	Understanding the legal and regulatory frameworks governing pesticide use;

Knowledge, understanding and professional skills

1	Detailed knowledge of the chemical structures and properties of glyphosate and neonicotinoids.
2	Understanding the modes of action, at the molecular level, of nicotinic acetylcholine receptors by neonicotinoids.
3	Understanding the acute and chronic toxicological effects of these pesticides on target and non-target organisms, including humans, beneficial insects, and aquatic life.
4	Skills in data collection, analysis, and interpretation to inform decision-making and policy about problematic active substnaces.

Unit 4.1 Glyphosate

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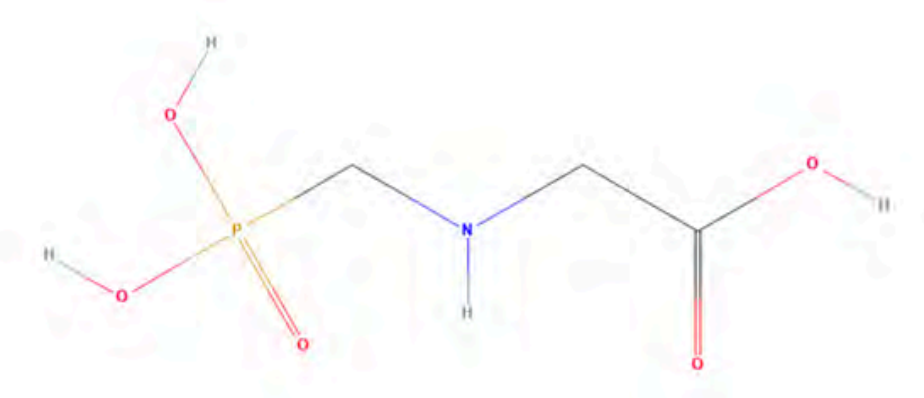
Ionuț Silviu Beia

Glyphosate, also known as N-(phosphonomethyl)glycine, was first introduced as a herbicide ingredient in 1971 and has since become the top-selling herbicide globally. In the 1980s, its patent was renewed due to new composition, achieved through acquisitions among major pesticide companies.



Molecular Formula

$C_3H_8NO_5P$

$HOOC-CH_2-NH-CH_2-PO(OH)_2$

Despite this extended patent protection, glyphosate became a generic compound in 1991 in many countries outside the US and its US patent expired in 2000. The use of genetically modified (GM) crops that are tolerant to glyphosate was introduced in the US in 1996, providing further protection and increasing its market dominance, securing its position as the

leading herbicide. For nearly 30 years, Monsanto Corporation held a favorable market position for its glyphosate-based herbicide due to its patent protection. Monsanto's leading glyphosate-based herbicide was the Roundup group, which mostly contained isopropylammonium or potassium salts of glyphosate.

When the patent protection expired outside the US in 1991, a 50% market drop appeared within 5 years for Monsanto. However, the introduction of glyphosate-tolerant (GT) GM crops after 1996 more than made up for the initial market losses, as Roundup could then be exclusively marketed as a product tied to Roundup Ready (RR) crops (soybean, cotton, maize, canola, alfalfa, and sugar beet). The market for glyphosate has been continuously growing worldwide since 1974. The use of glyphosate also increased in regions where GM crops were not cultivated. As of 2012, glyphosate represented 12% of the overall pesticide market and 13% of the synthetic pesticide market.

The global production capacity of glyphosate, estimated to be 1.1 million tons per year in 2012, rose along with the market boost of glyphosate. The majority of the production capacity has been established in China, which was capable to produce 826 thousand tons annually already in 2010. That means that China alone can currently to satisfy the entire world demand for glyphosate.

4.1.1 The authorisation status of glyphosate in the European Union

In addition to patent protection, the legal approval for the use of any pesticide active ingredient must be periodically renewed by national or international authorities when it is intended for agricultural use. Usually pesticides are reapproved for 10-year periods. In the EU, the last re-approval of glyphosate took place in 2017, but European Commission extended its authorization only for a 5-year period until 2022. Therefore, glyphosate is up for a new re-registration procedure. Before its last reapproval in 2017, glyphosate was scheduled for re-registration in 2013 with Germany serving as the lead evaluator (so-called rapporteur) and Slovakia as a co-evaluator. The re-approval received widespread attention due to the significant financial interests involved, as well as concerns over the environment and public health.

As for burning pesticide authorization problems in the European Union, the case involving the re-registration of the herbicide active ingredient glyphosate is probably the most divisive current social issue. A major factor of disagreement in the debate is that major assessment agencies formulated completely opposing opinions on this active ingredient and its formulated PPPs. The two major registration agencies the

European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA), as well as the US Environmental Protection Agency (US EPA) on the one hand, and the International Agency for Research on Cancer (IARC) on the other hand, announced their divergent assessments of the carcinogenicity status of this regulated agrochemical. Glyphosate was categorized by the US EPA as "not likely to be carcinogenic to humans" in Group E. EFSA and ECHA arrived at similar conclusions when they determined that glyphosate was "unlikely to pose a carcinogenic hazard". In contrast, IARC, an agency of the World Health Organization (WHO) of United Nations (UN) classified glyphosate into Group 2A, “probably carcinogenic to humans”.

The issue is rather controversial, yet sources behind the differences in the expert opinions by these agencies can be specified. A major difference is that while IARC considered potential cancer hazards in its classification, other agencies based their statements on calculated risks (and not hazards) of the agrochemical. Both positions can be justified: a hazard-based definition of carcinogenicity (IARC) calls attention to the biological effect that is likely to occur if someone is exposed to the substance, and this classification does not take the likelihood of the exposure to the chemical into

consideration. In contrast, a risk-based assessment (EFSA, ECHA, EPA) categorizes the substance with the probability of exposure taken into consideration.

Questions about the hazards and risks associated with this active ingredient's formulated herbicide preparations (see below) have divided scientific communities as well as official health and environmental authorities and organizations. The findings also raise important questions regarding risk assessment and product regulation. The diverging opinions indicate that hazard-based (IARC) and risk-based (EFSA) safety analyses may lead to completely opposing conclusions.

4.1.2 The role of co-formulants in the authorization status of glyphosate

Another source of diverging opinions has been the actual chemical identity or composition of the substance containing glyphosate that served as the basis of the biological classification: the pure active ingredient or the formulated PPP. Formulated PPPs may include various additives, such as surfactants, in addition to the active ingredient(s). These additives have traditionally been considered as "inert" or inactive components in relation to the primary biological effects of the formulation. This classification is based on the definition that any component responsible for the main biological effect is considered an active ingredient, not an additive. However, these so-called "inert" ingredients may have biologically or chemically active side-effects, which must also be taken into account in risk assessments and policy decisions.

It has been demonstrated that formulated glyphosate-based herbicide products, particularly those containing polyethoxylated tallowamine (POEA) as a formulant exert far stronger cytotoxic, mutagenic or endocrine-disrupting effects than glyphosate alone. In vitro cytotoxicity of the formulated glyphosate preparation Roundup® has been shown to be

orders of magnitude higher than that of glyphosate alone on various cell lines including human hematopoietic Raji cells (Epstein-Barr virus transformed human lymphocytes); diploid fin cell line from the Oriental weather loach Misgurnus anguillicaudatus (DIMF); human epithelial keratinocyte cells (HaCaT); murine stem cell-like neuroectodermal cells (NE-4C); human epithelial type 2 cells (Hep-2); human fibroblast cells (GM38); human fibrosarcoma cells (HT1080); primary neonate human umbilical vein endothelial cells (HUVEC); embryonic kidney cells (HEK293); murine osteoblast precursor cells (MC3T3-E1); human hepatoma cells (HepG2); human chorioplacental cells (JAr); human choriocarcinoma cells (JEG3). Cytotoxicity levels expressed as effective dosages of the given substance causing 50% mortality (ED50) are depicted on Figure 4.1.

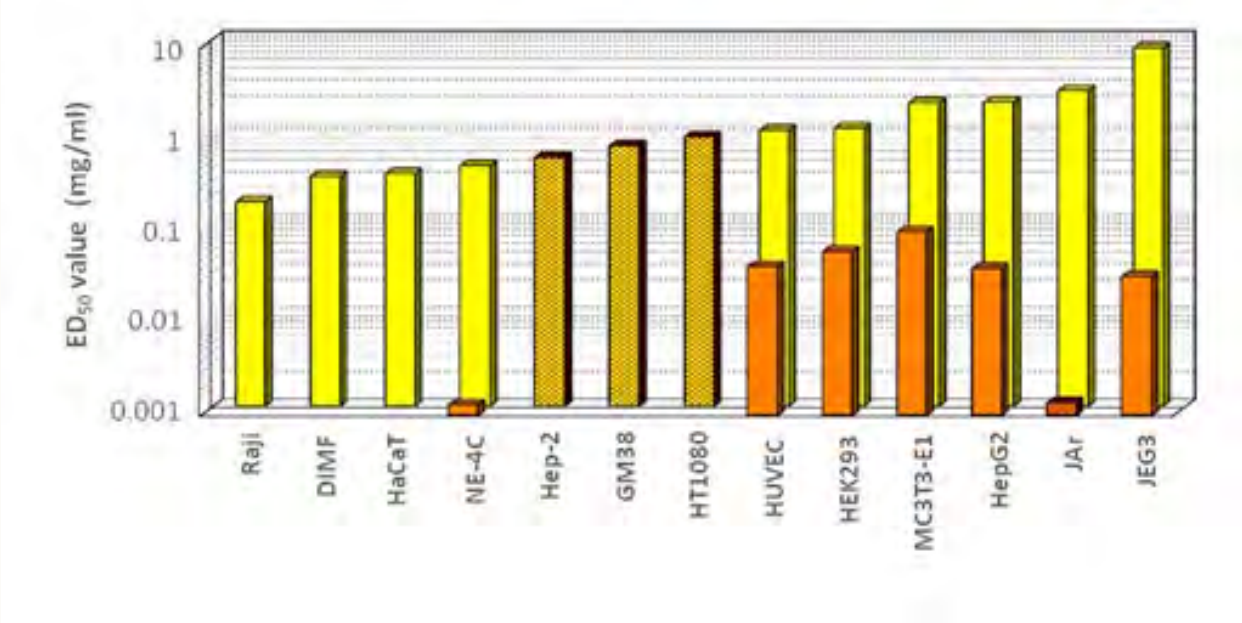


Figure 4.1. *In vitro* cytotoxicity of glyphosate (yellow and grid brown columns; plain yellow and grid brown column patterns indicating cytotoxicity detected by MTT test and mutagenicity tests, respectively) and its formulated preparation Roundup® containing polyethoxylated tallowamine (POEA) as a co-formulant (orange columns) on various cell lines. (For the names and origins of the cell lines, see the text above). Note that the formulation with POEA exerts substantially higher (29-260-fold) toxicity in all cases determined [Székács and Darvas, 2018].

4.1.3 The overall use of glyphosate and its emergence as a ubiquitous environmental water pollutant

After a steady annual increase of 4.9-7.2%, the yearly consumption of glyphosate worldwide was 825.8 million tons in 2014 already. Exact volume data are not openly available, but on the basis of the market size in US dollars, the annual growth of the market remained in the same range, and therefore, glyphosate use (agricultural and miscellaneous) exceeded 1 million tons annually in 2019, and could reach nearly 1.2 million tons in 2022, representing nearly half (approximately 45% in tons) of the overall pesticide market. This immense amount of a xenobiotic cannot be dumped into the environment with impunity.

Although the half-life (DT50) of glyphosate is rather short in water (28–91 days, if photodegradation is excluded), its decomposition can be much slower in soil (up to 142 days depending on edaphic and climatic conditions) and in sediments (up to 518 days under aerobic and 208 days under anaerobic conditions). Moreover, its main metabolite aminomethylphosphonic acid (AMPA) is more persistent in these matrices than glyphosate itself (e.g., its half-life is 76-240 days in soil). As a result of the immense and increasing release into the environment and under certain conditions slow

decomposition, glyphosate and AMPA became ubiquitous water contaminants worldwide. They have been found to enter surface waters in various regions, including the Americas (the US, Canada and Mexico, as well as Argentina and Brazil), especially in areas where GM (GT or RR) crops are grown. In the US, glyphosate levels in surface water have reached up to 5,200 ng/l. The concentration of glyphosate in surface waters in the EU is lower, but still commonly detected in regions like Germany, France, and the Mediterranean.

Figure 4.2. The maximal levels detected for glyphosate (shown as black numbers) and AMPA (shown as gray numbers in parentheses) in surface water and groundwater worldwide. Corresponding official limit values in drinking water (shown as red numbers and abbreviations) e.g., the Maximum Admissible Concentration (MAC) in the European Union, the Maximum Contaminant Level (MCL) in the US, and the Health-Based Guideline Value (HBGV) in Australia [Székács and Darvas, 2018].

Figure 4.2 shows the worldwide distribution of peak glyphosate residues in surface and drinking water, along with the maximum allowed concentrations of glyphosate in drinking water in different regions. The US allows for a maximum 7,000-fold higher pesticide residue levels in drinking water compared to the EU, with Australia being even more lenient. However, it is important to note that these residue levels only represent worst-case scenarios and do not reflect everyday situations.

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Unit 4.2 Neonicotinoids

Mária Mörtl, András Székács,
Liliana Bădulescu, Monica Badea

Sustainable use of pesticides (Directive 2009/128/EC) in the 21st century includes minimizing the environmental impact of agricultural production and preserving biological diversity as much as possible. In the field of insecticides, just as for other pesticide classes, the aims the newly developed active ingredients acting by novel modes of action represent are improved environmental features, on one hand, and the hope that these compounds will be effective against pests that has developed resistance to conventional long established and environmentally less benign insecticide classes, such as pyrethroids, organophosphates, carbamates, on the other hand. In addition, these new compounds must show high selectivity, particularly in regard to increasing environmental and human safety measures for regulatory approval.

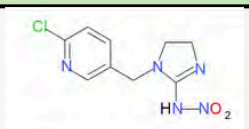
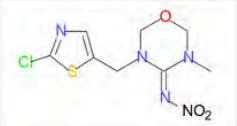
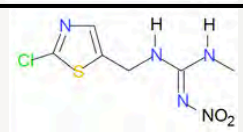
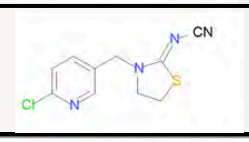
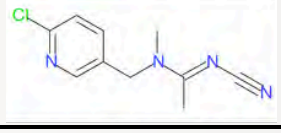
The introduction of neonicotinoids as a new class of insecticides aimed to meet these requirements, as they were regarded to act selectively by binding to the nicotinic acetylcholine receptor (nAChR) target site in the insect central nervous system.

4.2.1 The history of neonicotinoids, development of new ingredients

The natural alkaloid (S)-(−)-nicotine has long been proposed to be applied as a botanical insecticide, mostly used in the form of aqueous tobacco extracts. Disadvantages of this natural compound were that it had limited insecticidal efficacy, while being fairly toxic to mammals. Biochemical studies indicated that nicotine acts on insects in the central nervous system by affecting their neural system, binding to receptors specific for the main neuromodulator acetylcholine. Consequently, these nicotine-sensitive receptors have been termed nAChRs. Random screening and synthetic modifications, based on the models of the corresponding receptor–ligand binding domains within nAChRs lead to development of the new class of nAChR agonists. Neonicotinoid insecticides (Table 4.2.1.) act selectively as reversible agonists of the post-synaptic nAChRs of the insect central nervous system, but show either little or almost no binding affinity to mammalian nAChRs. High selectivity for insecticidal action is due to striking differences in functional architecture and binding sites of nAChRs. After step-by-step structural optimization of the chemical structure of nAChR-agonist, imidacloprid was the first registered active ingredient in this chemical family, introduced in 1991, followed by nitenpyram and acetamiprid in 1995, thiamethoxam in 1997, thiacloprid in 1999 and clothianidin in 2002.

Other active ingredients have also been developed, which are not authorized in the European Union. A research program has been started also in China to develop new active ingredients with a similar mode of action. Referring to their mode of action, this active ingredient family has been termed “neonicotinoids” (new nicotine-like substances). Their favorable safety profile, high target specificity, and versatility in application (foliar, seed treatment, soil drench, stem application) resulted in their rapid boom among pesticides, the market share of neonicotinoids in the total global market for these insecticides rose to 28.5% by 2011.

Table 4.2.1. The most important neonicotinoid insecticide active ingredients

Active ingredient CAS No.	Chemical structure	KOW, logP*	Water solu-bility (g/L)	DT50 values** soil (day)
imidacloprid 138261-41-3		0.57	0.51	28-1250
thiamethoxam 153719-23-4		-0.13 (25°C)	4.1	7-3001
clothianidin 210880-92-5		0.91	0.327	148-6931
thiacloprid 111988-49-9		1.26	0.185	3-74
acetamiprid 135410-20-7		0.8	4.25	31-450

* Octanol/water partition coefficient, **D. Goulson, J. Applied Ecology (2013)

Imidacloprid became generic (off-patent) in 2006, whereas patent protection for ingredients (thiacloprid, thiamethoxam, acetamiprid, clothianidin, etc.) expired in 2013. In the modern crop protection imidacloprid has become the most successful, highly efficacious, and best-selling insecticide worldwide until its restriction in the EU in 2013.

4.2.2 Application of neonicotinoids

Neonicotinoids are used on large field crops in different formulations, for example sprayed in orchard, applied as soil drench or granular formulations for turf / pasture. They were also extensively used as seed coating among others on rape (canola), cereals, maize, sunflower, and beet. Despite of the fact that only about 2% of the neonicotinoids applied as seed coating is absorbed by the crop, their application permits good systemic control of piercing-sucking insects in addition to their contact and stomach activity. This efficiency lead to the rapid growing in the seed treatment market, dominated by corn, soybean, cotton, and wheat, and among others coated barley, oilseed rape, sunflower, and vegetable seeds are also available. This increasing trend resulted for example in the fact that almost all maize seeds sold in the US were coated by neonicotinoids. Worthy of note that in some cases (e.g. in California) the planting of treated seeds is not considered as a pesticide application and it is not reported to the databases.

Compared to non-polar insecticides, commercial neonicotinoids usually have low lipophilicity (logP values) (See Table 4.2.1.) that allows uptake of these active ingredients followed by their spreading in the entire plant. Only a small portion of insecticide enters the plant, 0.5-2% of the seed coating material is lost as dust during the sowing, and 96% or more is adsorbed by the soil. Due to their high water solubility they usually do not remain there, but leach easily to waterways contaminating the surface water.

Dosages recommended for neonicotinoids were substantially lower than those of earlier applied neurotoxic insecticide active ingredients, but this indicates not only their effectiveness against the target pest, but implies higher toxicity to non-target species as well. Initially the recommended treatment rates were 0.25 mg clothianidin/kernel, but later typically 0.6-1.2 mg neonicotinoid per kernel was applied on maize. Dosages in seed coating, but also in other pesticide applications, are gradually growing as insect resistance develops against this class of insecticides, too.

After comparing the recommended doses for granule and spray applications to those for seed coating, it can be concluded that the dosages are very similar in all cases and result in practically the same environmental loads. The dosages of neonicotinoids used in seed coating are equivalent to 30–85 g active ingredient/ha (0.6–1.22 mg active ingredient/seed at 50–70 thousand (maize) plants/ha). The US EPA performed a comparable

computation, which was based on a maximum planting density of 86,500 maize seeds per hectare and treatment rates of 0.25 or 1.25 milligrams of active ingredient per kernel. The findings revealed that application rates for clothianidin ranged from 21.7 to 108.7 grams of active ingredient per hectare. The usual doses for spray and soil granule applications range from 20 to 70 grams of active ingredient per hectare (or 20-70 milligrams of active ingredient per liter at a volume of 1000 liters per hectare) and 110 grams of active ingredient per hectare (or 10 grams of active ingredient per kilogram at a rate of 11 kilograms per hectare), respectively. Seed coating is more advantageous in terms of pesticide usage only if multiple spray applications are required during the growing season (as the number of permitted spray applications is limited to two per season). The primary distinction between granules and coating materials versus spraying agents is that the former are in a solid form, resulting in higher concentrations of active ingredients in the local soil. Seed treatment significantly reduces surface exposure of the soil in comparison to in-furrow and surface applications. However, cases of contamination have also been detected with the use of seed coating.

4.2.3 Contamination of the environment, toxic effects

Due to their extended use and high mobility, neonicotinoids are frequently detected in the environment. Their contamination rates (incidence) of in surface water often reached 100%, and residue levels typically ranged below ng/L and some hundreds ng/L in different monitoring studies. Peak concentrations measured worldwide for surface and ground water are ranging from some ng/L to about 200 µg/L. According to the US Environmental Protection Agency guideline, the “Aquatic Life Benchmark” for thiamethoxam for invertebrate chronic (average) exposure is 0.05 µg/L, whereas for maximum value (acute exposure) 11 µg/L was set in 2016. Although the existing values recommended to aquatic ecosystems vary widely across countries, there is an increasing body of evidence that the occurrence and levels of neonicotinoids pose usually low acute risk, but high chronic risk to aquatic ecosystems. Neonicotinoids can exert adverse effects on numerous sensitive aquatic invertebrates at low concentrations below threshold levels (e.g. below 1 µg/L under acute exposure or 0.1 µg/L for chronic exposure). Aquatic insects show EC50 values below 0.1 mg/L, thus they are the most sensitive species among the aquatic invertebrates. Measured levels in aquatic environments induce sublethal effects on non-target and/or beneficial organisms as well.

Neonicotinoids were also frequently found in soils, some of them accumulated as they proved to be persistent (e.g. chlothianidin). Pesticide drift originated either from dust formed during the sowing of coated seeds or from spraying resulted in pollution of field margins adjacent to the treated agricultural fields. Contamination often occurs via soil, as these ingredients are systemic, they are uptaken by wild flowers and detected also in pollen and nectar. They were also uptaken by target crops especially from coated seeds, spread in the whole plant (translocation) and appeared occasionally at high levels in their guttation liquid as well. Their efficiency against the insects manifested also on non-target species, including pollinators, particularly honeybees. The corresponding toxicity values for bees are very low (e.g. oral LD50 4-5 ng/bee for thiamethoxam and clothianidin) and sublethal effect has to be also considered. First problems were observed in Germany in 2008, when using pneumatic planting machines the honey bees flying nearby were poisoned from the dust, which contained neonicotinoids from the coating material. Improvements in seed coating technology (e.g., application of polymers) and deflectors attached to pneumatic sowing equipment reduced abrasion and dust formation during planting. Non-target effects are not restricted to honey bees, but occur also on other pollinators (e.g. bumblebee), butterflies and aquatic insects that are negatively affected. Declines were detected in insectivorous bird populations upon this impact, but other birds may also be at risk. Although the toxicity

levels of neonicotinoids are much lower for vertebrates (toxicity index values being much higher) than to insects, yet vertebrates may also be exposed to lethal doses via consumption of seeds coated by neonicotinoids. Despite of the fact that seeds are covered by soil after sowing, some of drilled seeds (~0.5-1%) remain accessible to be eaten by birds or other animals. For example the LD50 value of clothianidin to the grey partridge is 5 mg, and if these birds each consume 5 maize seeds coated by 1 mg neonicotinid/kernel, then this exposure will kill half of the birds in the population. The same happens upon consumption of 6 beet seeds or 32 oilseed rape seeds. As a grey partridge eats approximately 60 maize seeds per day, severe bird poisoning incidents may happen even if only some of these seeds is coated. For terrestrial species e.g. earthworms morphological and behavioral changes were also observed. Concerning consumer safety, residues of neonicotinoids have often been detected in foods including honey. Taking into consideration that mammalian nAChRs show none to low affinity to these insecticides at concentrations in the micromolar range at the target site, the determined levels are usually of negligible or low risk.

4.2.4 Regulation of neonicotinoids nowadays

As a consequence of their enormous global technological and business success, and consequent wide-scale use, neonicotinoids appeared in surface water as ubiquitous pollutants, some of them showing persistence and accumulation in soils as well. Thus, it is justified to state that neonicotinoids have been overused by now, facilitated by their favorable mammalian safety characteristics and effectiveness against insect pests. The widely observed contamination due to overextended application represents a source of acute and/or chronic exposure of pollinators and other non-target species and it is considered as a risk to aquatic ecosystems. The occurrence of neonicotinoids and their potential toxic effects have triggered a global response, and many countries and governmental agencies are developing policies to mitigate the use and release of neonicotinoids in aquatic ecosystems. The recommended or acceptable concentration levels in water quality guidelines that are demonstrated to result in negligible risk are strictly decreasing. However, the acute values are sometimes limited to single species and multi-species are rarely considered. In addition, due to the lack of chronic data, they are often extrapolated from acute toxicity data, which may impede correct ecological risk assessment. Mixture toxicity caused by simultaneous exposure towards multiple toxicants (pesticides in this case) is not sufficiently taken into consideration.

The negative effects on non-target insect species, particularly for bees led to the restriction of the use of three of the most important neonicotinoids (clothianidin, imidacloprid and thiamethoxam) in the EU in 2013. The use and sale of seeds treated with plant protection products containing those active substances were prohibited to take the most cautious approach possible to protect bees. Following the restrictions on these three neonicotinoids in the EU in 2013, several countries (e.g. Romania, Bulgaria, Hungary) have repeatedly granted emergency authorisations and applied for multiple derogations on major crops since the entry into force of the restrictions. Alternatives would have been available to about one third of the products for which emergency authorizations were granted. Also in other countries, the farmers used unfortunately alternative seed treatments or more soil/foliar treatments in the first growing season after restrictions, despite of the fact that at least one non-chemical alternative method (e.g., microorganisms, semiochemicals, etc.) was available in most of the cases. Alternative insecticides to substitute neonicotinoids included other chemical insecticide groups, mostly pyrethroids, but also an organophosphate chlorpyrifos, until authorization of the latter expired in 2020. Based on the results reported in the scientific literature, application of these neonicotinoid active ingredients was banned under field conditions in the EU in 2018, and their use has been restricted to closed greenhouses. Manufacturer withdrew clothianidin from the EU renewal process, and the European

Commission decided not to renewthe approval of thiacloprid in 2020. After the prohibition of all outdoor uses of the three neonicotinoids and the non-renewal of approval of thiacloprid, ten EU countries have repeatedly granted emergency authorizations for their use in sugar beets.

The risk assessment survey process of thiacloprid by US EPA was voluntarily canceled by the registrant in 2014, whereas the dockets for all the neonicotinoid pesticides are open, as the planned completion has gradually bee shifted to 2024. The registration authority in Canada completely reversed their assessment in 2021, and concluded that some uses of thiamethoxam and clothianidin do not pose a risk to aquatic insects, while other uses do pose risks of concern. New mitigation measures were introduced for the uses that remain registered including revised label instructions such as reduced application rates, reduction in number of applications, and spray buffer zones. Acetamiprid remained authorized as it pose low risk to bees.

4.2.5 Conclusions

The Integrated Pest Management (IPM) fitness is of utmost importance nowadays, in particular, selectivity for beneficial and pollinator species has to be optimized. Damages can be lowered, but not prevented by appropriate selecting space and time for foliar applications against starting pest populations, when beneficial arthropods are still absent. Nevertheless, the prophylactic use of neonicotinoids as seed coating is incompatible with the general principles of IPM, as the crop protection measure is started much before the pest population dynamics would justify it and results in unavoidable chemical pressure on the environment regardless whether the pest population emerges or not. Thus, its benefit is doubtful as it unnecessary pollute the environment if the pest is present at low levels, and the costs have been shown usually not returned by the increased income of farmers. The costs are lower and the yields are higher when using an IPM approach (monitoring pest populations and spray only when it is unavoidably necessary) compared to those cases when neonicotinoids are used as seed coating. According to Directive 2009/128/EC (Article 14) Member States have to „take all necessary measures to promote low pesticide input pest management, giving wherever possible priority to non-chemical methods, so that professional users of pesticides switch to practices and products with the lowest risk to human

health and the environment.” Reactive use of chemicals is the last resort, which is based on monitoring and exceeding the threshold for the pest.

Due to the strict restrictions on clothianidin, imidacloprid and thiamethoxam in the EU, possible alternative pest management strategies should be introduced to eliminate neonicotinoid-based chemical pest control in cropping systems. Prophylactic use without on-site risk assessment (e.g. seed coating) has to be avoided, in order to prevent environmental loads and unnecessary exposure of non-target species. Insecticides are rarely needed to control early-season pests (e.g., in maize), because related crop loss is either low or can be largely eliminated by non-chemical and agroecological methods (e.g., crop rotation). The agrotechnological solutions require more knowledge about insect pests and include diverse protecting tools. The most common alternatives to chemical pest management are biological methods, which include attract-and-kill strategies (e.g., using microbial agents), mating disruption, application of natural insecticides or insect repellents (e.g., nettle extract or biological control with flowers grown on the field margins) and trap attractants (e.g., pheromones). Worthy of note that forecasts of heavy infestations may be simpler for those pests (e.g., pests with multi-year life cycles), where historical data are available, but valid prediction of damages might be more difficult if there is no

previous experience on temporal pest population dynamics (e.g. for new invasive species). As neonicotinoids replace the older insecticides in the global agriculture, their increasing global production and price erosion is likely to further accelerate their application intensity and to further facilitate the development of pest resistance. Therefore, the discovery and development of new nAChR agonists that overcome metabolic resistance is essential; however, mutations in nAChR subunits covering critical amino acids may reduce the efficacy of any new agonist showing a binding mode similar to neonicotinoids.

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Unit 4.3. Emerging methods based on unusual small molecular mass pesticide active ingredients

András Székács

Pesticide discovery has substantially slowed down during the last two decades, particularly if the number of active ingredients with new modes of action is considered. This is due to the stricter registration requirements, substantially increased development costs, and the spread of agricultural gene technology.

Due to the above reasons; increasing strictness of approvals, the rise in development costs, and the spread of GM technology, the intensity of active ingredient development has gradually declined worldwide. In fact, only multi-national giant companies have sufficient capital for the regulatory approval of new active ingredients. As a consequence main directions in pesticide development have become modified (listed below).

Development of pesticides with already known mode of action. This is the main development direction, which expands the range of active ingredients and can modify environmental properties, but does not promise a biochemical solution to the emerging resistance situation. Nonetheless, the number of pesticide active ingredients developed decreased from 124 in 1990-1999 to 40 in 2010-2019.

Development of herbicides with a new mode of action. This is the most expensive and business-wise the riskiest direction. In the area of herbicides, the last example of an active ingredient with a new mode of action, isoxaflutole, an inhibitor of the enzyme p-hydroxyphenylpyruvate dioxigenase, was introduced three decades ago, in 1992. Bayer announced in 2020 that it will introduce a new mode of action herbicide active ingredient to the market, but this is expected only by 2030. It is already known that they want to introduce the GM crops that are resistant to this new active ingredient, but since then, no more information has been made public about the substance.

Development of herbicides that act through RNA interference (RNAi). The discovery of RNA interference (RNAi), awarded by a Nobel Prize in medicine in 2006, initiated a range of applications that use double strain RNA (dsRNA) molecules as active ingredients, and deliver these oligoribonucleotides not in a host organisms, but as individual chemical compounds. This new chemical plant protection direction is based on the activation of natural RNA silencing biochemical pathways against dsRNA molecules, which involves the authorization and application of dsRNA molecules as active ingredients. Although the RNAi technology is mainly proposed for pest control, there have been advances in its application against weed pests, but the main limiting factors are the degradation of the active ingredient, dsRNA molecules, and the unknown environmental risks.

Thus, RNAi-based plant protection products are substances that utilize the RNA interference mechanism of plant cells for the protection against harmful insects or fungi. RNAi represents the blocking of harmful gene expression in plant cells. This plant protection strategy is devised in various forms including sprayable double stranded ribonucleic acid molecules directly applied to the plants to be protected. RNAi-based plant protection products are currently undergoing the approval process by authorities to prove their safety and efficacy before entering the market. Although there are still environmental and safety issues that need to be addressed, RNAi-based plant protection products offer significant opportunities for the development of plant protection. The major barrier to the direct application of dsRNA molecules is the high instability of these compounds, and attention has to be paid to side-effects attributable to the active ingredients or to the formulating agents used for their stabilization. The RNAi-based plant protection agents also have disadvantages, including:

(i) Environmental uncertainty: The effect of RNAi-based plant protection agents on the environment is still unknown and this can seriously affect environmental impacts.

(ii) Unintended effects: RNAi-based plant protection agents can affect not only targeted pests but also other unwanted insects, including community insects and the animals that consume these insects. (iii) Effectiveness: The effectiveness of RNAi-based plant protection agents is still in progress and

it is not guaranteed that they will always be effective against targeted pests.

(iv) Stability: Ribonucleic acid molecules are highly unstable under natural conditions, therefore, their stability can be provided only by special additives or formulants, often nanodisperse substances. This can create toxicity problems as stabilized ribonucleic acids may exert unintended side-effects, on the one hand, and the formulating additive (eventually nanomaterial) may exert its own toxicity to various non-target organisms.

(v) Food safety: The impact of RNAi-based plant protection agents on food safety and human health is still unknown and this raises serious environmental protection issues.

(vi) Prices: The price of RNAi-based plant protection agents is significantly higher than the price of traditional insecticides, and this can pose a significant burden on farmers. Despite these disadvantages, RNAi-based plant protection agents may offer significant opportunities for the development of plant protection, but the authorities must continuously monitor the effects to ensure safety and effectiveness. In addition, the authorization of these oligoribonucleotides as pharmaceuticals or pesticide active ingredients of raises additional environmental concerns as well.

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Maximum Residue Levels (MRLs) in EU Food Safety Regulation

A Maximum Residue Level (MRL) is defined in EU legislation as the highest concentration of a pesticide residue legally permitted in or on food or feed, when pesticides are applied in accordance with Good Agricultural Practice (GAP). MRLs aim to ensure that residues present in food at harvest and at marketing are safe for consumers, taking into account both agricultural practice and health protection standards.

Regulation (EC) No 396/2005 is the principal legislative instrument governing MRLs in the European Union. It establishes the overall regulatory framework for setting, reviewing, and enforcing MRLs for pesticides in or on food and feed of plant and animal origin, harmonising residue limits across Member States for products sold within the EU and imported from third countries.

MRL setting and regulatory process

MRL setting relies on several key components of data and assessment, including:

- residue field trials conducted according to Good Agricultural Practice;
- toxicological reference values, such as Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD), derived from toxicological studies;
- dietary exposure estimates, often calculated using the EFSA Pesticide Residue Intake Model (PRIMo), which quantifies dietary intake under both chronic and acute exposure scenarios.

EU legislation also outlines that, if a pesticide is not explicitly addressed by a specific MRL in the regulatory annexes or database, a default MRL of 0.01 mg/kg is generally applied, representing the typical analytical limit of quantification.

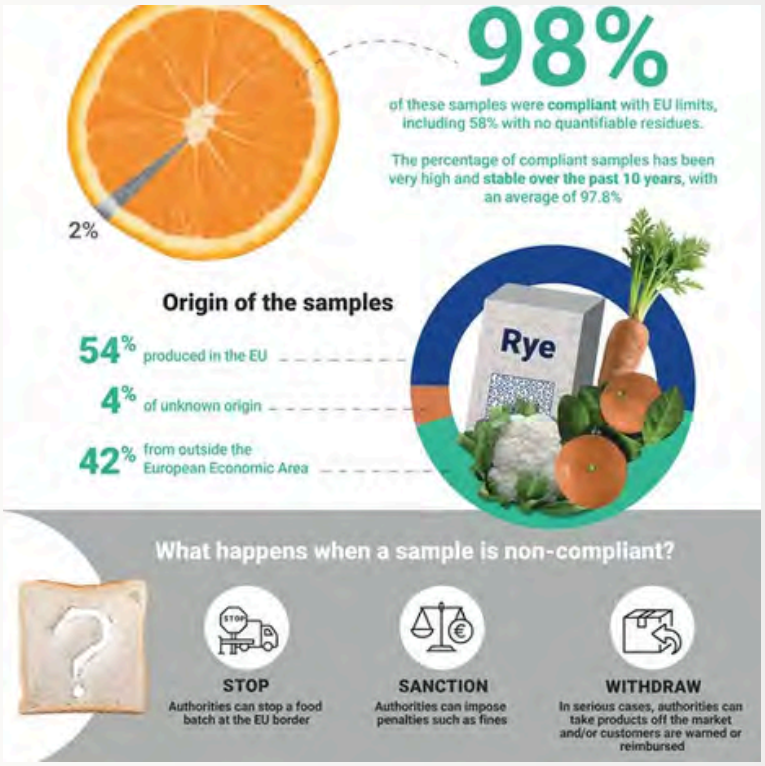
Role of EFSA reasoned opinions and MRL reviews

Under Article 12 of Regulation (EC) No 396/2005, EFSA periodically reviews the existing EU MRLs for active substances already included in the regulatory framework. For example, EFSA has conducted reviews of MRLs for substances such as glyphosate, phosmet, and others to ensure that current residue limits remain protective for consumer health based on updated toxicological and exposure data.

These reviews result in reasoned opinions published by EFSA and may lead to regulatory actions such as lowering, raising, or maintaining existing MRLs, taking into account new scientific evidence and data gaps. Reviews may be undertaken both for substances that remain approved and for those no longer authorised in the EU but with existing Codex-based or import-tolerance MRLs.

Monitoring and compliance

After MRLs are established, official monitoring programmes assess compliance. EFSA's annual reports on pesticide residues summarise findings across thousands of food samples analysed within EU Member States.



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Food authorities in EU countries monitor pesticide residues in food to ensure they do not exceed EU limits, and prevent health risks.
133 000 food samples were collected in the EU, Iceland and Norway in 2023 (the latest reporting year) covering food from all continents.
98% of these samples were compliant with EU limits, including 58% with no quantifiable residues. The percentage of compliant samples has been very high and stable over the past 10 years, with an average of 97.8%.

Origin of the samples

- 54% produced in the EU
- 4% of unknown origin
- 42% from outside of the European Economic Area

What happens when a sample is non-compliant?

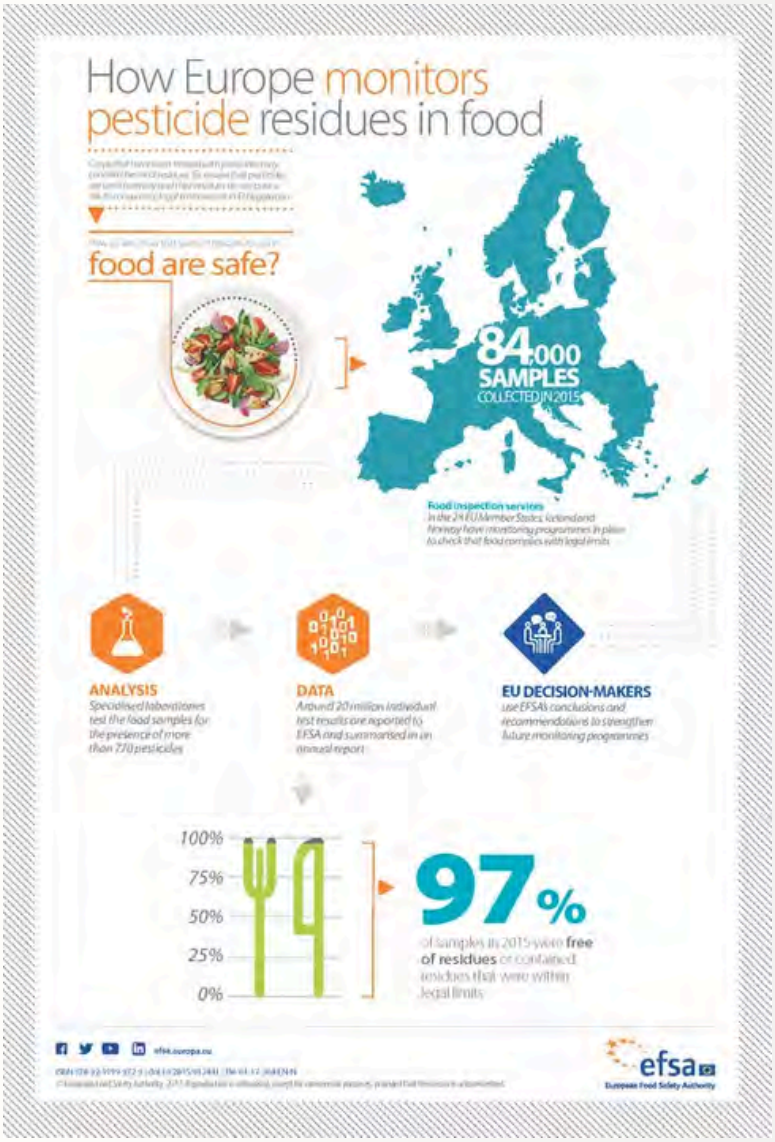
- STOP: Authorities can stop a food batch at the EU border
- SANCTION: Authorities can impose penalties such as fines
- WITHDRAW: In serious cases, authorities can take products off the market and/or customers are warned or reimbursed

Crops that have been treated with pesticides may contain chemical residues. To ensure that pesticides are used correctly and their residues do not pose a risk to consumers, legal limits are set in EU legislation.

How do we know that levels of residues found in food are safe?

84,000 samples collected in 2015.

Food inspection services in the 28 EU Member States, Iceland and Norway have monitoring programmes in place to check that food complies with legal limits.



Analysis

Specialised laboratories test the food samples for the presence of more than 770 pesticides.

Data

Around 20 million individual test results are reported to EFSA and summarised in an annual report.

EU decision-makers

EU decision-makers use EFSA's conclusions and recommendations to strengthen future monitoring programme.

97% of samples in 2015 were free of residues or contained residues that were within legal limits.

Source here

According to the 2023 EU report on pesticide residues in food, the majority of analysed samples complied with established MRLs, demonstrating that pesticide residue levels on the market generally remain within the legal and health-protective limits established under Regulation (EC) No 396/2005.

Such monitoring efforts also inform risk managers whether existing MRLs and agricultural practices continue to protect consumer health or require adjustments based on changing exposure patterns or analytical improvements.

Consumer dietary exposure and PRIMo

A central tool in the risk assessment of pesticide residues is the EFSA Pesticide Residue Intake Model (PRIMo), which quantifies potential dietary intake of pesticide residues across different foods and consumer populations. This model incorporates data from residue field trials, consumption patterns, and toxicological reference values to estimate both acute and chronic exposures.
PRIMo enables risk assessors to evaluate whether estimated consumer intakes exceed toxicological thresholds such as ADI or ARfD. If the model indicates that exposures are below health-based guidance values, the MRL may be considered safe from a consumer health perspective, supporting regulatory decisions in accordance with EU food safety objectives.

In addition to modelling tools, scientific literature and regulatory reporting, such as European Union reports on pesticide residues, provide empirical context showing trends in compliance and areas of potential concern. These integrated assessments form the basis for evidence-driven regulatory actions and the continuous refinement of MRLs through updates to Regulation (EC) No 396/2005 and associated database entries.

Appendix

Comparative regulatory status of major pesticide classes in the European Union

Application route	Environmental compartment affected	Transport mechanism	Persistence tendency	Typical metabolites
Foliar spraying	Air, soil, plant surface	Spray drift, volatilisation	Low–moderate	Polar degradation products
Seed coating	Soil, groundwater	Leaching, abrasion dust	Moderate–high	Parent compound residues
Soil incorporation	Soil, root zone	Adsorption, microbial transformation	High	Bound residues
Surface runoff	Surface waters	Hydrological transport	Variable	Hydrolysis products
Atmospheric deposition	Vegetation, water bodies	Long-range transport	Low	Oxidised derivatives

Toxicological endpoints used in pesticide risk assessment

Toxicological endpoint	Definition	Test system	Regulatory relevance	Main uncertainty sources
Acute toxicity (LD50)	Single-dose lethality	Rodent models	Classification and labelling	Species extrapolation
Chronic toxicity	Long-term adverse effects	Repeated-dose studies	Acceptable daily intake (ADI)	Exposure variability
Reproductive toxicity	Effects on fertility and development	Multigenerational studies	Risk management decisions	Dose–response uncertainty
Endocrine disruption	Hormonal system interference	In vivo/in vitro assays	Hazard-based cut-offs	Mechanistic ambiguity
Neurotoxicity	Nervous system impairment	Behavioural and biochemical tests	Worker and consumer safety	Developmental sensitivity
Ecotoxicity	Effects on non-target species	Pollinators, aquatic species	Environmental protection	Field-to-lab extrapolation

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Environmental pathways and contamination routes of pesticide residues

Pesticide class	Representative active substances	Primary mode of action	EFSA hazard focus	Current EU authorisation status	Main regulatory restrictions	Latest EFSA review (year)
Glyphosates	Glyphosate	Inhibition of EPSPS enzyme (shikimate pathway)	Carcinogenicity, endocrine effects, ecotoxicity	Restricted approval	Use limitations, monitoring of co-formulants	2023
Neonicotinoids	Imidacloprid, Thiamethoxam, Clothianidin	Nicotinic acetylcholine receptor agonists	Pollinator toxicity	Mostly banned	Outdoor use prohibited	2018–2021
Pyrethroids	Cypermethrin, Deltamethrin	Voltage-gated sodium channel disruption	Aquatic toxicity	Approved with restrictions	Buffer zones, application limits	2020–2022
Organophosphates	Chlorpyrifos (legacy)	Acetylcholinesterase inhibition	Neurotoxicity	Non-approved	Complete ban	2019
Carbamates	Carbaryl (legacy)	Acetylcholinesterase inhibition	Human toxicity	Non-approved	Complete ban	2018
SDHI fungicides	Boscalid, Fluopyram	Succinate dehydrogenase inhibition	Mitochondrial toxicity	Approved with review	Ongoing reassessment	2022
New low-MW actives	Various	Target-specific biochemical pathways	Case-specific	Conditional approval	Data gaps monitoring	2022–2024

Analytical methods for pesticide residue detection in plant matrices

Analytical technique	Target compounds	Typical LOD	Advantages	Limitations	Typical application
GC-MS	Volatile, semi-volatile pesticides	ng/kg	High selectivity	Thermal degradation	Legacy compounds
LC-MS/MS	Polar and non-volatile pesticides	ng/kg	High sensitivity	Matrix effects	Regulatory monitoring
HPLC-DAD	UV-active compounds	µg/kg	Robust, low cost	Lower specificity	Screening
HRMS	Unknown or emerging residues	ng/kg	Non-target screening	High cost	Research, surveillance


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