Factors affecting risk assessment of pesticides in water bodies: a review

Abstract
Pesticides pose a significant threat to the environment in terms of toxic effects. However, pesticides toxicity is shaped by exposure factors and ecosystem characteristics. Such factors determine whether and in what extent the pesticide’s toxic potential will be actually expressed. Evaluation of the impact of such factors upon the pesticides toxic potential in a given environment is the task of risk assessment. The present study reviews the factors affecting the source, application pattern, load and availability of pesticides to water bodies, determining in this way the exposure status. Further on new trends in toxicity testing e.g. tiered and tailor made methodologies as well as the enhancement of the predictive principle in pesticides risk assessment are highlighted. Finally deterministic and probabilistic approaches in pesticide risk characterization are discussed.

Keywords: pesticide, toxicity, exposure, risk assessment

Abbreviations: ERA, ecological risk assessment; GAP, good agricultural practice; EFSA, European Food Safety Authority; ARID, acute reference dose; USEPA, United States Environmental Protection Agency; K_{oc}, adsorption coefficient; MECs, measured environmental concentrations; LD_{50}, lethal dose; PNEC, predicted no effect concentration; RQ, risk quotient; LOC, level of concern

Introduction
The potential of a chemical substance to cause one or more adverse effects to biological systems defines its toxicity. Effects, in terms of quality and quantity are supposed to be identical under standard conditions (toxicity assays and tests under strictly defined protocols). These effects could be expressed in different life organization levels, e.g. molecular, biochemical, physiological or even behavioral levels. Yet, under different ecosystems the potency of these effects depends on factors like exposure duration, intensity and distribution, organisms and hence population susceptibility, vulnerability of biota concerned, temporal or spatial emission of the toxic agent as well as nature of the emissions sources (point or non-point sources). Thus different ecosystems exhibit different vulnerability to contaminants due to their dependence on factors mentioned above. Hence a case per case study of the probability for a contaminant to demonstrate unacceptable adverse ecological effects upon a defined ecosystem, e.g. ecological risk assessment has grown in significance the last ten years. Ecological risk assessment process is accordingly based on exposure data in abiotic or biotic elements of a certain ecosystem as well as on effects caused by such an exposure upon the ecosystem.

Pesticides are chemical substances that are deliberately applied mainly in agro ecosystems but reach natural ecosystems through precipitation, drift and runoff. Due to often unavoidable interactions between natural ecosystems and human activities (Figure 1) they pose a profound risk to public health (Figure 1).

Figure 1 Indirect impact on public health due to pesticides environmental implications.
In this case pesticides function as primary stress factors to ecosystems which in turn act as secondary stressors to humans. However since uncertainty about the outcome is always present assessment of the evolving risk should be conducted in most cases. Pesticide residues derive from widespread point (commercial sites) as well as non point sources and cannot be controlled at any step after their application. Moreover as they appear in mixtures their potency as well as their toxicity is often underestimated (synergetic effects). Pesticide active ingredients are either dissolved in water bodies (hydrophilic substances) or are particle bound (potential for particle bound transport). Due to this fact pesticide residues transport in aquatic ecosystems does not follow the normal distribution. Therefore pesticide Ecological Risk Assessment (ERA) though fully justified is often complicated and a case per se.

ERA of pesticide residues in surface waters basically comprises assessment of factors affecting exposure, adverse effects (toxicity) and ecosystem characteristics.iii Ecosystem itself (study object of Eco toxicology) and not just single organisms (study object of Environmental Toxicology) is the ultimate investigation object of ERA.

The present review aims to

a. Present the main factors affecting pesticides exposure characterization in the ecological risk assessment process.

b. To describe factors and critical parameters in toxicity characterization and give a brief analysis of the toxic potential of each of the main pesticide classes (herbicides, fungicides, and insecticides) found in water bodies.

c. To highlight the risk characterization process followed in cases of pesticides ecological risk assessment.

**Exposure characterization of pesticides**

Water bodies are reservoirs having water the whole year or at least over a long period of the year. Consequently they include surface waters as well as groundwater bodies. It is obvious that risk assessment due to pesticide residues is applicable to both categories referring either to the respective ecosystems elements or to humans since both surface as well as ground water bodies serve as water drinking sources. Pesticides reach water bodies through drift, runoff or drain flow. Actually drift is the most studied way of contamination of water bodies.2 ERA of a pesticide is actually the assessment of the probability for the expression of its toxic potential upon ecosystems. Basic questions to be answered are: a. which are the factors to be considered in order to assess this probability with a decent certainty and b. Quantification of this probability itself.3 The answer to first question refers to the substance availability in the relevant environmental departments which generally follows a two-phase process.4 Source, initial concentration in water, route of the substance, spatial and temporal distribution and finally available concentrations constitute the exposure characteristics of a pesticide active substance in a defined ecosystem. Recently simulation models predicting the substance distribution in the ecosystem under study have been developed.5 Main factors affecting the pesticides availability to biological systems and thus shaping the expression of their toxic potential are as follows:

**Application scheme of pesticides in agro ecosystems**

Any factor (dose / concentration, application frequency, label restrictions oversight) leading to deviations from Good Agricultural Practice (GAP) could enhance their toxic expression in aquatic ecosystems. Special constituents of the application scheme are following factors:

i. **Single or multi-application scheme:** Repeated applications can have a different final risk assessment result than a single application. Repeated or pulsed application schemes may increase the selection pressure for tolerant individuals resulting to a different assessment decision compared to a single application assessment.6 Herbicides are as a rule applied once, while insecticides and especially fungicides are often applied repeatedly. This is a rule especially for fungicides applied preventively. An ecosystem has usually the ability to recover from a pesticide stress factor. Repeated exposures to pesticide reduce the chance for the ecosystem to recover.7

ii. **Direct application or indirect exposure:** Direct pesticide application on water surfaces is conducted for vector control, while indirect exposure takes place mainly through runoff, drift or precipitation. In the first case pesticide loads are significantly bigger than indirect exposure.

iii. **Buffer zones between site applied and bystanders:** Although guidelines for setting up and calculating buffer zones when applying pesticides (especially fumigants) are now quite strict this is not always the case in some countries. This fact increases the probability of contamination of adjacent zones. In all cases proximity of the application site to the adjacent water body should be taken into account.

**Fate of the parent compound in the environment**

The pesticide parent active ingredient is normally degraded in water in less hydrophobic and as a rule less toxic transformation products. Yet, these products alone or combined are in no case to be considered as non toxic. Such product can be produced not only through the natural environmental processes but also through water treatment processes such as water purification and disinfection. Therefore approaches have been developed for assessing ecological risk.8 Risk assessment processes of pesticide metabolites in human diet have lately developed to a rather significant matter of discussion in European Food Safety Authority (EFSA).9 The Agency discussed possibilities of applying the concept of Threshold of Toxicological Concern and proposed assessment schemes for chronic and acute dietary risk of pesticide metabolites, using the TTC approach. This approach has as ultimate target to define generic threshold levels for human health for all chemicals and is utilizing in a way the Acute Reference Dose (ARID) approach.10

**Volatility of the substance**

Only very few pesticides exhibit volatility over 10^-mm Hg, the limit over which pesticides are considered as hazardous air pollutants.11

**Solubility in water**

Water soluble substances are easily and very effectively transported in water. US Environmental Protection Agency (USEPA) sets the water solubility value of 3mg/L as the limit over which a substance is considered as potential water pollutant. Yet, this is not to be considered as 100% applicable since substances with water solubility less than 3mg/L have been found in groundwater.
Adsorption to soil particles

Water soluble substances generally exhibit a reduced potential for soil adsorption. On the contrary non polar (lipophilic substances) bind to soil particles according (among other factors) to their adsorption coefficient ($K_{oc}$) which is substance (physicochemical properties) and soil properties (organic content, colloidal nature, pH) dependable.

Dissipation of the substance

This is usually expressed through the DT$_{50}$ of the substance and contributes in combination with the $K_{oc}$ to the leaching potential through the GUS (Groundwater Ubiquity Score) index: GUS=$\log (DT_{50})x[4 - \log (K_{oc})]$. This is an empirical index and serves only as potential indicator since environmental conditions are not considered. GUS values greater than 2.8 indicate that that leaching of the substance is probable, while this is not likely to happen with GUS values less than 1.8. Intermediate values point to a limited leaching probability.

In detail following scheme is applied:14 GUS value and leaching probability: <0.1 extremely low; 0.1-1.0 very low; 1.0-2.0 low; 2.0-3.0 moderate; 3.0-4.0 high; >4.0 very high. However it should be noted that dissipation in groundwater compared to dissipation in surface waters follows a totally different pattern due to different environmental conditions, absence of photolysis and lower hydrolysis rate in comparison to soil surface.14,15 Apart from physicochemical properties dependence pesticide leaching in groundwater is often favored by preferential flow of water and solutes through soil and rock materials.16,17

SCI-grow index

This index is a screening model used by USEPA18 for assessing pesticide concentrations in vulnerable ground water. It takes into account environmental properties of the substance such as fate, application frequency as well as eventually existing small-scale prospective ground-water monitoring studies. In this aspect it complements the GUS index.

Estimated Environmental Concentrations or Expected Environmental Concentrations are estimates produced by either mathematical models or through a program of monitoring (field sampling) indicating the pesticide concentrations to which non-target areas and consequently biota using them as habitat are exposed. Input information includes pesticide use pattern given in the label as well as data concerning fate of the parent substance in the respective environment. In general, USEPA uses a tiered approach to estimate EECs, using the GENEEC2 as model, which utilizes the worst case scenario estimating EEC in water from sites that are highly vulnerable to runoff or leaching. Other more fine tuned models (e.g., PRZM-EXAMS) are used if higher accuracy is needed.19 In a lot of cases EECs are characterized as Predicted Environmental Concentrations or PEC values. If risk assessment is of short term interest Measured Environmental Concentrations (MECs) e.g., actual concentrations can be used. EECs’ advantage is the long term predictive value, while evolving uncertainty should be properly managed. The EEC of pesticides is then related to the EC$_{50}$ (concentration causing a 50% reduction in a chosen toxicity endpoint for a given aquatic test organism). At present, if only a few species are involved, the application of an assessment factor is suggested due to intraspecific differences in pesticide sensitivity.

Toxicity characterization

As mentioned before, the target of pesticides ERA today is the holistic approach which should result to assessment of effects to ecosystem and not merely to single organisms. This target is structured to following objectives:

i. Choice of endpoints covering in the best possible way life levels, growth stages and substances.

ii. The quickest and cheapest possible toxicity characterization.

iii. Experimentation with the least possible number of animals.

iv. Experimentation should be facilitated by the most efficient digital equipment, e.g. modeling.

v. It is evident (Figure 2) that through implementation of above principles losses in certainty of results are unavoidable. Therefore proposed degree of certainty is an important planning element in the whole procedure.

![Figure 2](image-url)
Standardized tests in vivo with single organisms aiming to assess pesticides environmental toxicity evaluate effects like mortality, immobilization, reproduction, growth and fecundity. It should be noted that according to OECD guidelines these tests are conducted provided that the preliminary mortality assay yields an LD₅₀ value less than 100mg/L.

Tests in vitro usually precede those in vivo and follow the QSARs evaluation. According to European legislation these tests contribute to a better understanding (e.g. mode and mechanisms of action) of the adverse effects and if necessary are being crosschecked with tests in vivo. Most of these tests have not proved to be scientifically valid for pesticides toxicity evaluation. Yet some of them, e.g., Microtox Test, acetyl-cholinesterase inhibition test (organophosphates), aldehyde dehydrogenase inhibition test (atrazine, captafol, carbendazim, diuron, maneb, methalochlor) have been successfully used.²³,²⁴

New trends in pesticides toxicity assessment in Europe are expressed by the integrated approach which is envisaged in the REACH regulation. This approach is supposed to contribute to a balance between reduction in experimentation cost and the growing uncertainty. REACH regulation actually imposes a unified toxicity evaluation on the ground of preceded separate assessments of impact upon environment and human health taking into account the predictive values of QSARs. In this sense toxicity evaluation tends more and more to actual risk evaluation.

In USA the integrated approach in pesticide toxicity assessment is also strongly supported by EPA. Thus more assessment endpoints combined with more life stages of organisms in less but better targeted tests are proposed. At the same time the Agency favors a more active involvement of toxicity mechanisms as well as pharmacokinetics in pesticides toxicity assessment.

On the other hand, aiming to a further cost reduction, the use of acute toxicity tests in such a way that toxicity threshold values (normally deriving from chronic toxicity tests) can evolve, is strongly encouraged.²² Economic data are constantly incorporated in the integrated approach by means of new simulation models.²⁵ Yet while using such models, a very high degree of complexity leading to unacceptable uncertainties should be avoided.

Beyond the integrated approach, development and use of alternative evaluation methods is at stake. This trend known as the 3R (replace-reduce-refine) effort targets the reduction of the adverse effects and if necessary are being crosschecked with tests in vivo. Most of these tests have not proved to be scientifically valid for pesticides toxicity evaluation. Yet some of them, e.g., Microtox Test, acetyl-cholinesterase inhibition test (organophosphates), aldehyde dehydrogenase inhibition test (atrazine, captafol, carbendazim, diuron, maneb, methalochlor) have been successfully used.²³,²⁴

Assessment endpoints selection

Final assessment endpoint selection is a crucial factor for the validity of risk characterization. There are three main criteria which lead to the correct choice. Endpoints should be of undisputable relevance to the ecosystem under consideration. Ecosystem components analysis is often needed to clarify the entities as well the attributes that are really critical to the specific ecosystem. Susceptibility of the entities to the stressor(s) under investigation is also a criterion which should be seriously considered. Often there is significant impact of factors like age, duration of exposure and endpoint selection on assay sensitivity.²¹ To assess pesticide impact on aquatic or mixed ecosystems (aquatic plus terrestrial) the most susceptible species would for example constitute a good assessment endpoint provided they are relative to the ecosystem under study. Crustaceans are very often among the most susceptible species in such ecosystems.²² Species which really represent the ecosystem have to be included in the selection. An additional issue is vulnerability of the entities to be chosen. Entities may be susceptible but for different reasons out of stressors reach. Still choosing the most susceptible and most vulnerable entities would not be the right choice unless relevance to the management goal is absolutely secured. In case of ecological risk assessments that address the ecosystem as a whole, more than one entities need to be included in the endpoints choice. Researchers often use organisms representing at least three life levels to obtain threshold LD(C₉₀) or No Observed Adverse Effects Level/Concentration (NOAEL(C)) values for the most susceptible representatives of the ecosystem under assessment. For populations, communities and ecosystems assessment a methodology which takes into account various interacting components of the system under study has been proposed. Namely the use of approaches like mean strain measurement, state-space analysis, and non metric clustering for analysis of standardized aquatic microcosm data sets has been discussed.²³ Apart from enhancing the predictive power of the assessment conclusion, these methods are expected to facilitate the choice of assessment as well as measurement endpoints in such environments.Endpoints also have to fit to the stressors nature. For example herbicide residues in water bodies have primarily to be assessed on aquatic plants since these are the most susceptible among other biota to herbicides. USEPA has identified aquatic eco toxicity benchmarks values concerning fish, aquatic invertebrates, vascular and nonvascular aquatic plants from risk assessments developed for individual pesticides.²⁴ These benchmarks could be among other things useful in the primary phase of risk assessment (problem formulation) where endpoints selection is applicable. On the other hand for complementary identification of certain pesticide group’s selection of specific biomarkers could be useful. Thus fish generally present an excellent tool for testing brain acetylcholinesterase inhibition due to organophosphate contamination.²⁶

Citation: Vassiliou G. Factors affecting risk assessment of pesticides in water bodies: a review. MOJ Toxicol. 2016;2(1):26–32. DOI: 10.15406/mojt.2016.02.00030
Factors affecting risk assessment of pesticides in water bodies: a review

However one should not disregard the fact that other non-scientific factors should be considered for the right selection of final endpoints. In many instances sociological, economic and political implications are of undisputed value to the final endpoints selection decision. It is often the case that such factors determine the level of unacceptable risk much more than pure scientific ones.1-5

Measurement endpoints selection

Measurement endpoints are “Measurable responses to a stressor that are related to the valued characteristic chosen as the assessment endpoints”. Properly selected measurement endpoints are used to quantify risk addressed to the assessment endpoints. Such responses include various yet absolutely measurable attributes of the respective endpoint(s). This can include specific measurements of receptor health, population indices, measurements of exposure, or direct measures of eco toxicological effects.6-8 Measurement endpoints can measure exposure, effects or ecosystem and receptor characteristics.

According to USEPA Guidelines for Ecological Risk Assessment following definitions are valid:

Measures of Exposure: “Measures of stressor existence and movement in the environment and their contact or co-occurrence with the Assessment Endpoint or its surrogate.”

Measures of Effect: “Measurable changes in an attribute of an Assessment Endpoint or its surrogate in response to a stressor to which it is exposed”.

Measures of ecosystem and receptor characteristics: “Measures of ecosystem characteristics that influence the behavior, life history, and distribution of populations or individuals in a community that may be adversely affected by contaminant exposure”.

Referring to pesticides in water bodies, measures of exposure are their actual or estimated concentrations in surface waters or groundwater. Since such residues mostly originate from agricultural use (through drift or runoff) their concentration follows a seasonal application pattern. Therefore in many cases maximum values are used over average or median values, particularly if acute risk assessment is aimed. On the other hand low concentrations are useful for assessing sub lethal effects over time. American Society for Testing and Materials has issued a guide aiming to facilitate endpoints selection.9-10

Measures of effects are usually critical doses or concentrations which either produce a certain effect on organisms, populations, communities or ecosystems or define a critical threshold of risk. Such measures of effects for aquatic environments (LC50, EC50, and NOAEC values) are practically provided by toxicity studies. A main difference between risk characterization and toxicity characterization is that in risk characterization process these values along with assessment endpoints, exposure and given ecosystem characteristics are used as data input for mathematical models. Although the output values from these models lie within a more realistic range concerning the concrete ecosystem to be assessed compared to values resulting from toxicity assays they are inferior in terms of certainty of results.

Risk evaluation

According to USEPA10 there are mainly two approaches in evaluating pesticides risk in ecosystems, namely the deterministic and the probabilistic approach.

The deterministic approach uses actually the point “double benchmark concept”. Namely a point measured or estimated concentration of a pesticide in water (usually EEC or PEC) is compared to an aquatic life benchmark. Such benchmarks are usually point estimates of effect concentrations such as LC50, EC50 or NOAEC values corresponding to selected assessment endpoints and assessment targets (e.g., acute or chronic risk).

Regarding acute aquatic risk assessment a certain LC50 value which usually corresponds to the most susceptible organism among the most relevant in the ecosystem under study is taken as benchmark. A chronic aquatic risk assessment requires normally a suitable NOAEC value as benchmark. In cases of lack of scientific evidence for long term toxicity an LC50 value corresponding to the most susceptible representative species and properly extrapolated through an assessment factor (usually 0.1 to 0.001) is applied. This benchmark is the so called Predicted No Effect Concentration (PNEC). The ratio EEC / PNEC is the Risk Quotient (RQ). Concerning the presence of more than one pesticide in a certain aquatic ecosystem USEPA suggests the additive model, the actual RQ being the sum of all substances RQs. This of course is much more valid in cases of pesticides with the same or similar modes of action. Risk Quotients constitute a second benchmark of Level of Concern (LOC). LOCs are threshold values for risk concern set by USEPA for each “risk situation”. Regarding aquatic risk four “risk situations” have been established, namely acute high risk, acute restricted use, acute endangered species and chronic risk with LOCs 0.5, 0.1, 0.05 and 1.0 respectively The deterministic approach has been used extensively for screening purposes of pesticides in surface waters.11-13 Yet it seems that the RQ approach enables incorporation of refined environmental exposures to the classical tier 1 numerical ranking RQ model.14

The probabilistic approach yields a range of values defining also their distribution. It can actually be applied either by incorporating either the entire stressor-response relationship or variability in exposure and/or effects. It is actually a comparison between stressor and exposure response curves. The outcome of such an approach is not merely a low or high risk statement (RQ approach) but an examination of many exposure-effects scenarios with predictive value and certainty calculation. By means of this approach a prediction of the most probable impact upon the ecosystem is made available. This approach can also predict incremental changes in exposures necessary to limit risk to a desired level. Especially for pesticides this method can answer to the question “which would be the appropriate application dose reduction in order to achieve a desirable reduction of risk?” a question which the RQ approach cannot answer. Yet a disadvantage of the method is that secondary effects which might evolve through inconsistencies between available measurement endpoints and ecosystem under study are not considered, unless an appropriate planning has been done in the problem formulation and analysis phases.

Conclusion

Pesticides are chemical substances and as such are likely to express their toxic potential in the environment. This likelihood depends on exposure, toxicity and environmental characteristics. However there are issues as application pattern, selectivity mechanisms, synergistic or antagonistic action and non point source origin which differentiate pesticides from the other industrial chemical substances. These properties should be taken seriously into account when pesticide risk is to be assessed.

New trends in toxicity testing tend to modify the classical approach to a risk assessment procedure. Hence tiered and tailor made testing is recently applied, while QSARs and use of biomarkers are encouraged as complementary measures. Modeling is today a significant tool in pesticide risk assessment with parallel efforts to decrease cost and uncertainty of results.

Citation: Vassiliou G. Factors affecting risk assessment of pesticides in water bodies: a review. MOJ Toxicol. 2016;2(1):26–32. DOI: 10.15406/mojt.2016.02.00030

Copyright: ©2016 Vassiliou
Acknowledgements
None.

Conflict of interest
The author declares no conflict of interest.

References
1. EPA. Guidelines for Ecological Risk Assessment. Federal Register; 1998. 63:26846–26924.
2. FOCUS. Landscape and Mitigation Factors. In: Aquatic Risk Assessment. Detailed Technical Reviews. Report of the FOCUS Working Group on Landscape 35 and Mitigation Factors in Ecological Risk Assessment. EC Document Reference 36. 2007. 2:436.
3. Van der Werf, Hayo MG. Assessing the impact of pesticides on the environment. Agriculture, Ecosystems & Environment. 1996;60(2–3):81–96.
4. Connell P, Laim P, Richardson B, et al. Introduction to ecotoxicology. Blackwell Science Ltd; 1999. 170 p.
5. Wetzel A. Environmental Bio monitoring: sensitivity and reliability in PAH–contaminated soil. In: Environmental Bio monitoring: The Biotecnology Ecotoxicology Interface. In: James M Lynch, Alan Wiseman, editors. Cambridge University Press; 1998. 299 p.
6. Reinert KH, Giddings JM, Judd L. Effects analysis of time–varying or repeated exposures in aquatic ecological risk assessment of agrochemicals. Environ Toxicol Chem. 2002;21(9):1977–1992.
7. Cooper CM. Biological Effects of Agriculturally Derived Surface Water Pollutants on Aquatic Systems—A Review. Journal of Environmental Quality. 1993;22(3):402–408.
8. EPA Calculating Buffer Zones. A Guide for Applicators | Pesticides | US EPA. 2013.
9. Escher BI, Fenner K. Recent advances in environmental risk assessment of transformation products. Environmental science & technology. 2011;45(9):3835–3847.
10. EFSA. Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment 1. EFSA Journal. 2012;10:2799.
11. The threshold of toxicological concern concept and its use in agrochemical and chemical regulation.
12. Kegley SE, Hill BR, Orme S, et al. PAV Pesticide Database, Pesticide Action Network. North America; 2014.
13. Dickey P. Guide to the City of San Francisco’s Reduced–Risk Pesticide List. Water. 2007;2–7.
14. Lowey M, Kirs V, Carvajal L, G, et al. Groundwater contamination by azinphos methyl in the Northern Patagonic Region (Argentina). Science of The Total Environment. 1999;225:211–218.
15. Nancy M Trautmann, Keith S Porter, Robert J Wagenet. PSEP: Fact sheets: Pesticides and Groundwater: A Guide for the Pesticide User. 2013.
16. Hallberg GR. Pesticides pollution of groundwater in the humid United States. Agriculture, Ecosystems & Environment. 1989;26(1–34):299–367.
17. Vryzas Z, Papadakis EN, Vassiliou G, et al. Occurrence of pesticides in transboundary aquifers of North–eastern Greece. Sci total environ. 2012;441:41–48.
18. EPA SCI–GROW Description/ Pesticides/ US EPA.
19. US EPA. O. of P. P. Technical Overview of Ecological Risk Assessment / Analysis Phase: Exposure Characterization | Pesticides | US EPA.
20. Commission E. Environment Directorate General Reach in brief. Regulation. 2007:1–19.
21. European Parliament. E C REACH Regulation. 2007;3:1–438.
22. Moriarty F. Ecotoxicology. In: The study of Pollutants in Ecosystems. 3rd ed. Academic Press; 1999. 347 p.
23. Qureshi A Ansar, Bullich AA, Don IL. Microtox toxicity test systems—Where they stand today. In: Microscale Testing in Aquatic Toxicology: Advances, Techniques, and Practice. In: Wells PG, Lee K, Blaise C, editors. USA: CRC Press; 1998. p. 185–218.
24. Obst U, Wessler A, Wiegand–Rosinus M. Enzyme Inhibition for Examination of Toxic Effects in Aquatic Systems. In: Microscale Testing in Aquatic Toxicology: Advances, Techniques and Practice. In: Wells PG, Lee K, Blaise C, editors. USA: CRC Press; 1998. p. 77–94.
25. EPA. Office of Pesticide Programs Strategic Direction for New Testing and Assessment Approaches for Pesticides. 2012.
26. Levitan L, Merwin I, Kovach J. Assessing the relative environmental impacts of agricultural pesticides: the quest for a holistic method. Agriculture, Ecosystems & Environment. 1995;53(3):153–168.
27. Sun X, Zhou Q, Ren W, et al. Spatial and temporal distribution of acethochlor in sediments and riparian soils of the Songhua River Basin in northeastern China. J Environ Sci. 2011;23(10):1684–1690.
28. Vryzas Z, Vassililou G, Alexoudis C, et al. Spatial and temporal distribution of pesticide residues in surface waters in northeastern Greece. Water research. 2009;43(1):1–10.
29. Racke KD. Release of pesticides into the environment and initial concentrations in soil, water, and plants. Pure Appl Chem. 2003;75(11–12):1905–1916.
30. Naddy RB, Johnson KA, Klaine SJ. Response of Daphnia magna to pulsed exposures of chlorpyrifos. Environmental Toxicology. 2000;19(2):423–431.
31. Bridges TS, Farrar JD. The influence of worm age, duration of exposure and endpoint selection on bioassay sensitivity for Neanthes arenacoeodontata (Annelida:Polychaeta). Environmental Toxicology and Chemistry. 1997;16(8):1650–1658.
32. Munn MD, Giliom RJ. Pesticide Toxicity Index for Freshwater Aquatic Organisms; Geological Survey, Water–Resources Investigations. Report 01–4077. 2001.
33. Palma G, Sanchez A, Olave Y, et al. Pesticide levels in surface waters in an agricultural–forestry basin in Southern Chile. Chemosphere. 2004;57:763–770.
34. Landis WG, Matthews RA, Matthews GB, et al. Application of multivariate techniques to endpoint determination, selection and evaluation in ecological risk assessment. Environmental Toxicology and Chemistry. 1994;13(12):1917–1927.
35. US EPA, O. of P. P. Office of Pesticide Programs’ Aquatic Life Benchmarks | Pesticides | US EPA. 2013.
36. Obst U, Wessler A, Wiegand–Rosinus M. Enzyme Inhibition for Examination of Toxic Effects in Aquatic Systems. In: Microscale Testing in Aquatic Toxicology: Advances, Techniques and Practice. In: Wells PG, Lee K, Blaise C, editors. USA: CRC Press; 1998. p. 77–94.
37. Strange EM, Lipton J, Beltman D, et al. Scientific and societal considerations in selecting assessment endpoints for environmental decision making. Scientific World Journal. 2002;2 (Suppl 1):12–20.
38. Checkai RT, Johnson MS, Hawkins MS. Selection of Assessment and Measurement Endpoints for Ecological Risk Assessment. Technical Document SFIM–AEC–ER–TR–2002(18, ARMY BIOLOGICAL TECHNICAL ASSISTANCE GROUP, U.S; 2002.
39. ASTM E1848 –96. Standard Guide for Selecting and Using Ecological Endpoints for Contaminated Sites. 2008.
40. US EPA. O. of P. P. Technical Overview of Ecological Risk Assessment / Risk Characterization | Pesticides | US EPA.

41. Hela DG, Lambropoulou DA, Konstantinou IK, et al. Environmental Monitoring and Ecological Risk Assessment For Pesticide Contamination and effects in Lake Pamvotis, Northwestern Greece. *Environ Toxicol Chem.* 2005;24(6):1548–1556.

42. Qu C S, Chen W, Bi J, et al. Ecological risk assessment of pesticide residues in Taihu Lake wetland, China. *Ecological Modeling.* 2011;222(2):287–292.

43. Karimi F, Moattar F, Farschchi P, et al. Ecological Risk Assessment of Agricultural Pesticides throughout the Shadegan Wetland, Iran. *Journal of Agricultural Science.* 2012;4(5):109–116.

44. Peterson RK. Comparing ecological risks of pesticides: the utility of a Risk Quotient ranking approach across refinements of exposure. *Pest manag sci.* 2006;62(1):46–56.