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Agrochemical residues in waterways: A review of new tools and their use in water quality

Summary

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Agrochemical residues in waterways: A review of new tools and their use in water quality monitoring.

Summary

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Introduction

Managing the effects of agrochemicals in Victorian waterways requires far more information than we (as scientists and/or managers) can afford to directly measure for all the places and all the times, and all the agrochemicals of interest. Strategies and/or tools are therefore required to focus monitoring and risk assessment programs in a cost-effective manner, and to predict agrochemical concentrations and effects in locations that have never been directly assessed (or inadequately assessed).

To assess the risk of agrochemicals to aquatic ecosystems, information is required on the environmental fate of particular chemicals, their concentrations in the environment (exposures) and toxicity to aquatic organisms (as illustrated by the ecological risk assessment framework shown in Figure 1). The overall ecological risk can then by determined based on the general principle that risk is a function of toxicity and exposure (or likelihood of an adverse effect).

A large number of chemical sampling methods and analytical tools are available to assist with data generation for all levels of the risk assessment framework. Sampling methods range from traditional spot sampling, through newer automated grab sampling, to time integrated passive sampling. The chemical analytical tools available range from classical chromatographic/mass spectrometric techniques, to indicative bioassays and enzyme immunoassays, to automated in-situ gene-on-a-chip technology.

The various models and tools available could aid in the assessment of agrochemical risks to waterways in Victoria. However, prior to adoption, their strengths and weaknesses needs to be assessed. This publication provides a summary of our review of new tools for the monitoring of agrochemicals in water and sediments, including their advantages and limitations.

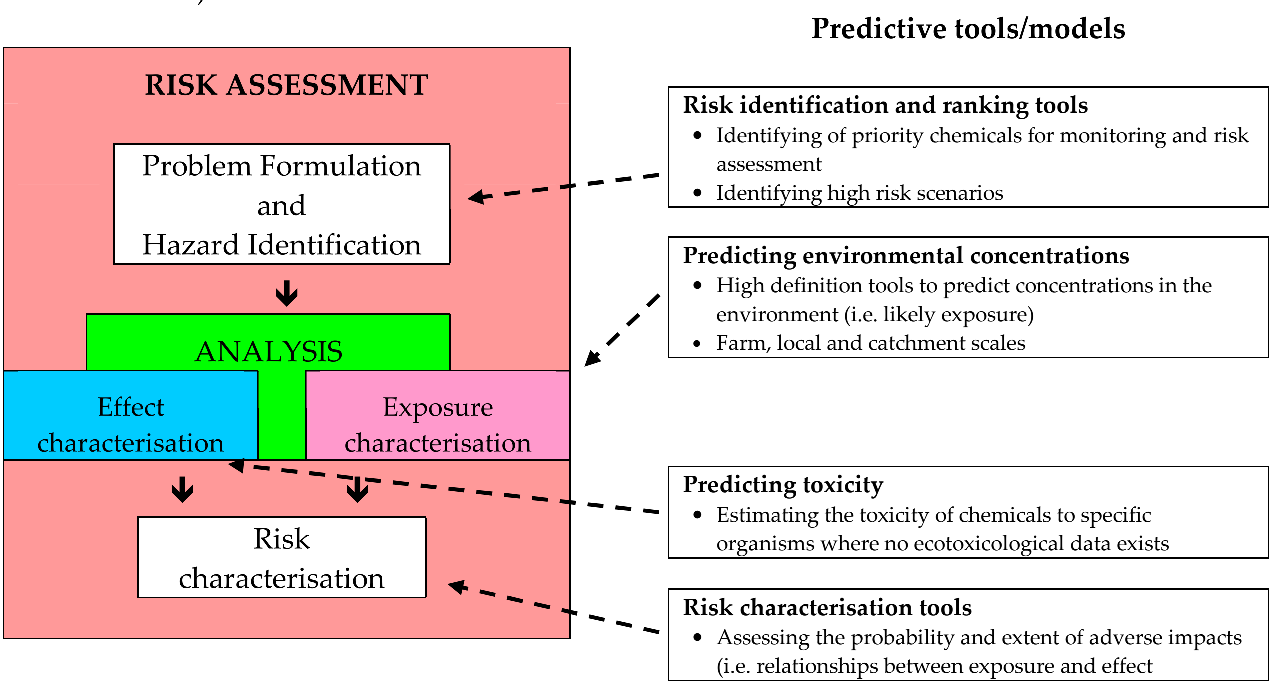

Figure 1 *The ecological risk assessment framework and the main types of predictive tools/models available to assist with the various levels of risk assessment*

Agrochemical monitoring tools

The perception (often correct) is that measurement of agrochemicals in waters and soils is expensive, which inhibits monitoring, resulting in increased risk to agriculture and the wider, natural environment.

A range of alternative tools is available for water quality monitoring which may improve this situation. The new tools can be classified on the basis of their relationship between the sampling and analytical processes.[1,2]

- **In-situ methods** generally involve the use of a sensing device immersed in a water body, with data collected automatically and either recorded for later retrieval or transmitted to a distant groundstation for interpretation.
- In-situ monitoring tools generally use spectroscopic or electrochemical techniques to acquire continuous data. This technology is most mature for the measurement of pH, temperature, conductivity, dissolved oxygen, turbidity and chlorophyll.[3] Some biological early warning systems can also be placed in situ.

- **On-line methods** use analytical instruments located near to the waterway being studied. Sampling is generally automated, and both discrete and continuous measurement of the sample is possible, depending on the analytical method employed.
- Some biological early warning systems, such as the mussel monitor, Daphnia toximeter, and algal monitors, can be installed on-line, to provide qualitative water quality information.[3]
- On-line methods permit frequent and rapid sampling and analysis, can be used to monitor remote or sensitive locations, but because of their cost, require secure, powered sites for their application.

- **Off-line methods** are the most commonly used methods to monitor pesticides. They require that a sample of water is collected at specified intervals in the field and transported to the analytical system. Typically, this means the sample is taken to a laboratory for analysis by classical chromatographic and mass spectrometry techniques.
- By using accredited laboratories and methods, stakeholders have a high degree of confidence in the results obtained. However, there can be less confidence associated with sampling procedures, since infrequent sampling at an often limited number of sampling points may not provide a representative picture of water quality, especially when contaminants show spatial and temporal variation.

Figure 2. DPI Queenscliff NATA accredited water quality laboratory.

Sampling for agrochemicals in the aquatic environment

Sampling can be defined as the process of selecting a portion of the environment under investigation small enough in volume to be transported conveniently to the laboratory, and small enough to be conveniently handled within the laboratory, while still being representative of the environment under investigation.[2]

- Sampling is an integral part of the analytical process, but typically represents the main contribution to error in the whole analytical process.

Spot samples

Spot (or grab) samples are commonly used to characterise residues in surface waters.

- The advantage is that the matrix itself is analysed and concentrations can be easily related to guideline or trigger values.
- The disadvantage of grab samples is that they may miss a peak if they are taken too infrequently (although this depends on the hydrology of the waters being sampled).[2]

Figure 3. Grab sampling in action

Integrative sampling with passive samples

A 'passive sampler' can be defined as a device that is able to acquire a sample from discrete location without the active media transport induced by pumping or purge techniques.[4]

- Most passive samplers are based upon membrane extraction technology, and typically consist of a receiving phase with high affinity for organic contaminants, separated from the aquatic environment by a diffusion limiting membrane.

Passive sampling has some advantages (A) and disadvantages (D) in that:

A. The samplers need little attention apart from deployment and collection.
A. The samplers also integrate and average exposures over time, thus enabling identification of events that may be missed by grab sampling.
D. The concentration data from the sampler are not directly compatible to water concentrations without extensive calibration, and few calibration factors are available.
D. Some pesticides may be missed, as they are not readily portioned into the sampler. That said,

One way to use passive integrative samplers is as a screening tool to identify which pesticides are found in a waterway, thus targeting analytes for further analysis by statistically valid grab sampling regimes.

- Passive samplers could also be used to check for the presence of unexpected residues that may not be part of routine screening via grab samples.

Rapid monitoring of agrochemicals in water with immunoassays

Immunoassays have potential utility as a method for the rapid mapping of contaminant levels across large areas, to identify gradients within river systems, and optimise the design of monitoring networks.

Immunoassays work on two basic principles, namely:

- That there is a highly specific and sensitive reaction between target chemical (the antigen) with biologically-derived antibodies.
- The extent of the antigen-antibody reaction can be quantified by a colorimetric test.

These principles have lead to the development of enzyme-linked immunosorbent assays (ELISA), which combine the unique specificity of immunoassay with the high sensitivity of detection of an enzymic marker

Immunoassay tests are fairly simple to perform, and can be done in a field or laboratory setting.

- In the field, immunoassay tests are done in individual test tubes and using a hand held spectrophotometer.
- For the laboratory, the most popular format is a 96-well plate with a microplate reader.

Immunoassays are potentially very useful additions to water quality investigations, *but* their primary function is to provide a qualitative to semi-quantitative screening to detect the presence or absence of a targeted chemical or chemical family. [6,7]

Immunochemical methods have some advantages (A) and disadvantages (D), such as:

A. Simplicity of operation and rapidity of determinations.
A. Simple and often non-destructive sample preparation.
A. Relatively low cost single analyte determinations.
A. General reliability without need for very expensive equipment.

D. Narrow specificity and the effect of matrix components (immunoassay tests may give false positives, i.e., detection by immunoassay, but not by laboratory analysis).
D. Some immunoassays will react with all compounds in a particular group, rather than individual compounds within the group, e.g. all triazines, rather than just simazine, or just atrazine.
D. Many test kits for pesticides have detection limits higher than those available through classical GC or LC techniques, and hence higher than water quality guideline or trigger values.
D. If more than two or three immunoassay tests are performed for different compounds in one water sample, the cost savings rapidly diminishes if all of the compounds are available from an accredited laboratory in one analytical suite.

Immunoassay kits are currently available for a large number of agrochemicals, including atrazine/triazines, cyanazine/triazines, alachlor/acid amides, metolachlor/acid amides.

Monitoring with biological methods.

One of the challenges for scientists and waterways managers is to derive threshold concentrations for contaminants below which biodiversity and the functional attributes of natural systems are protected.

- In other words, what concentration is dangerous to the ecosystem in question?

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality provide trigger values that indicate the potential toxicity of a range of chemicals to aquatic organisms, and which can be used to assist with characterising the likely ecological effects.[8]

- But, water quality trigger values have only been prescribed for 28 agrochemicals, due to a lack of toxicity data.

There are now a number of standardised biological tests offered by a range of agencies around the world, and although using a standard species helps achieve comparable results, there are some specific criticisms often raised by stakeholders:

- Are the standard species representative of, and appropriate for Australian conditions?
- Test organisms are often in-bred to some degree, so do test groups adequately encompass the genetic variability found in wild populations?
- Test organisms are often picked to be as uniform as possible, so do test groups adequately reflect the variation in organisms' response produced by age, sex, nutritional status, reproductive status, genetics, or interactions with other organisms, e.g. predators, prey and parasites?
- Most aquatic toxicology tests are conducted using single chemicals at concentrations significantly higher than those found in the natural environment, so how relevant are they to the lower concentrations of chemicals in complex mixtures wild organisms are exposed to?
- Most tests are short term and use mortality as the only end point, so how relevant are they to the long term and developmental impacts chemicals can elicit on aquatic organisms?

How can we predict what concentration of agrochemical is harmful to a waterway if there is little, or no, toxicity data for local species?

- Cautiously use overseas data. Chemical sensitivity does vary between species, but it is the taxonomic composition of species used in the assessment of risk that is most significant, not the habitat and geographical distribution of the species.
- Use a species sensitivity distribution (SSD), a statistical distribution estimated from a sample of toxicity data and visualised as a cumulative distribution function.[9]

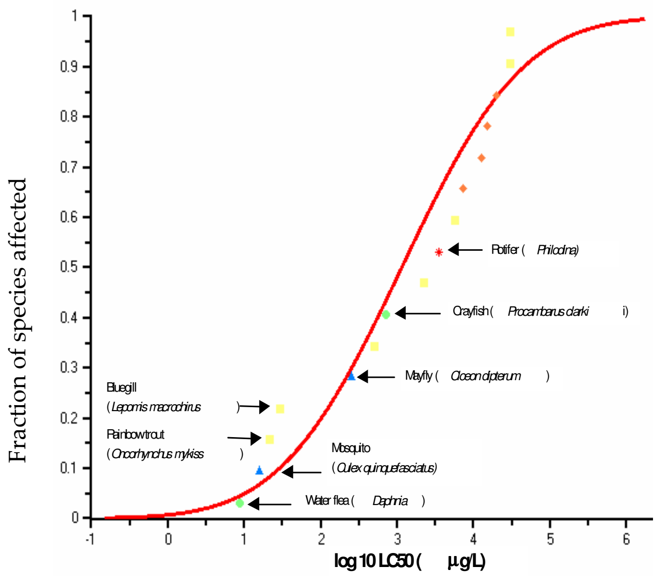

Figure 4. SSD for methidathion based on available acute toxicity data

Rapid biological monitoring methods.

The need to bioanalyse a large number of samples in a relatively short period of time has led to the development of fast, miniaturised tests for toxicity called microbiotests. Microbiotests use the specific responses displayed by unicellular or multicellular organisms when exposed to the sample to provide a measure of sample toxicity. [10]

Microbiotests have a number of advantages over standard bioassays, such as:

- Relatively low cost per sample
- Ability to operate with small sample volumes
- No need to culture test organisms, since many are stored in cryptobiotic form, e.g. rotifers as cysts, crustaceans as resting eggs
- Ability to work with several samples at once
- Short response time
- Good repeatability and reproducibility
- Tests can be replicated under controlled laboratory conditions

Many of the tests that have been developed use indicator organisms from either plants (algae and vascular plants) or animals (crustaceans, rotifers and protozoans). A number of commercial systems are now available, including:

- Algaltoxkit F, using the algae *Selenastrum capricornutum*
- Daphtoxkit F magna and Daphtoxkit F pulex, using the cladoceran *Daphnia magna* and *Daphnia pulex*, respectively
- Ceriodaphtoxkit F using the cladoceran *Ceriodaphnia dubia*
- Thamnotoxkit F using the crustacean *Thamnocephalus platyurus*
- Rototoxkit F and Rototoxkit F short-chronic tests using the rotifer *Brachionus calyciflorus*
- Protoxkit F using the protozoan *Tetrahymena thermophila*
- Ostracodtoxkit F using the crustacean *Heterocypris incongruens*

A much smaller number of commercial systems are also available for the assessment of toxicity in marine or estuarine systems, including:

- Rototoxkit M using the rotifer *Brachionus plicatilis*
- Artotoxkit M using the crustacean *Artemia franciscana*

A number of commercial systems are now available that measure global toxicity, genotoxicity, or cytotoxicity. These systems are based on whole luminescent or fluorescent organisms, or isolated biochemical systems:

- Microtox assay, based on the natural luminescence of the marine bacterium *Vibrio fischeri*, this assay has been standardised

Biosensors have been developed for in-situ monitoring of water quality and for detecting potential toxicity. Two main types of biosensor are available:

- Biocatalysis-based biosensors that use enzymes, with a response produced from product formation, reagent decrease or reaction inhibition
- Bioaffinity-based biosensors that use the interaction between genetically-engineered microorganisms, DNA, antibodies or proteins with a given analyte

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