Recent developments in non-target terrestrial plant test protocols and risk assessment.

P Ashby
Pesticides Safety Directorate, Mallard House, Kings Pool, 3 Peasholme Green, York YO1 7PX, UK
Email: paul.ashby@psd.defra.gsi.gov.uk

ABSTRACT

The main points of the draft revised OECD 208 Terrestrial (Non–target) Plant Test and the draft EPPO Decision-Making Scheme for Non-target Plants are discussed. The current approach to non-target plant risk assessments is outlined. Some outstanding areas of concern are identified. The potential need to consider indirect effects arising from the removal of plants from the agro-ecosystem and of the 'in-crop' effects is highlighted. Ongoing research to better establish the level of concern is discussed.

INTRODUCTION

Within the EU the risk to the main areas of wildlife from the commercial use of plant protection products (hereafter referred to as pesticides) is assessed under Directive 91/414/EEC. For non-target plants, Section 8.6, Annex II of the Directive lays down the following requirement for applicants:

"A summary of the available data from preliminary tests used to assess the biological activity and dose range finding, whether positive or negative, which may provide information with respect to possible impacts on other non-target species, both flora and fauna, must be provided, together with a critical assessment as to its relevance to potential impact on non-target species."

Due to the variation in the methods used in preliminary plant toxicity testing and the lack of a clear reason as to why they should be done, risk assessments for flora have lacked the detailed consideration given to other areas. Within the EU the term 'flora' is generally interpreted as meaning terrestrial non-crop species (either mono- or di-cotyledons). For pesticides the risks to crop plants and aquatic plants are considered separately in the EU and are excluded from further consideration in this paper.

Within the UK there is increasing concern over the possibility of indirect effects arising from the removal of non-crop plant species from arable areas (i.e. Campbell *et al.* 1997), and over the wider issue of biodiversity and sustainability of modern agriculture. A joint proposal between the US Environmental Protection Agency and the Canadian Pest Management Regulatory Agency to harmonise non-target plant toxicity testing under NAFTA was considered by the Scientific Advisory Panel (SAP) in June 2001. The SAP agreed that the non-target plant testing scheme needed to be improved, but could not reach consensus on a number of key issues (The Weekly Report of the US EPA Office of Pesticides Programs (for week ending 13 July)).

Thus the need to develop standardised test protocols and risk assessment schemes to allow a more refined assessment of the risk to non-target plants posed by the use of pesticides is now greater than ever. This paper sets out some recent developments in these areas.

REVISION OF OECD GUIDELINE 208 (Terrestrial (Non-target plant test)

The need to globally harmonise plant toxicity testing and for revision of the Guideline 208 (1984) has been acknowledged by OECD. Following meetings in 1997 and 1999, a draft version of the revised guideline was produced in July 2000. The Guideline serves for general chemicals as well as pesticides. Hence the use to which the results will be put needs to be fully understood before testing is undertaken. The main points of the revised Guideline are highlighted below.

Guideline 208 now consists of two protocols:

- Part A, a seedling emergence and growth test in which the test compound is incorporated into the growing medium, and

- Part B, a vegetative growth test in which young plants are oversprayed with the test compound.

The vegetative growth test was developed primarily for pesticides as spray drift is considered to be a major route of exposure for foliar applied compounds.

The issues of number and type of species tested were major and prolonged areas of discussion. Testing of up to 10 species is proposed. Annex 2 of OECD 208 provides a list of recommended test species; these are all crop species. Traditionally screening studies for herbicidal activity have used representatives of the main crop types.

Concern has been expressed as to the representativeness of these species for non-crop species. Boutin & Rogers (2000) in their analysis of two Canadian and US EPA data sets conclude that there is no consistent pattern in the available data. In separate studies using 5 common herbicides and 15 test species (8 dicots + 7 monocots), 'selectivity factors' >44,000 have been estimated based on ED50 values (Pestemer 1999). Thus, given the current knowledge base, the likelihood of selecting representative species suitable for all pesticides seems low. OECD 208 does make the important statement "The list may be extended to include non-crop species if a suitable seed source is provided...". [As part of the OECD discussion Boutin (Environment Canada) produced a list of 35 non-crop species which have been tested and for which suitable seed sources are known.]

The two new OECD 208 guidelines will not address all potential concerns. For example, they do not address the issue of potential effects on reproduction or of repeat applications. Without modification they are not suitable for testing compounds whose main activity is via the vapour phase.

DRAFT EPPO DECISION-MAKING SCHEME FOR THE ENVIRONMENTAL RISK ASSESSMENT OF PLANT PROTECTION PRODUCTS (Chapter 13. Non-target plants)

Following several years of discussion and changes in panel membership, a draft scheme has recently been produced (October 2000). The key points of the draft scheme are highlighted below:

Definition of "non-target" area

The scheme is concerned with the assessment of the risk in the "off-crop" area. Field margins of 1 m and 3 m are assumed for arable and orchard crops respectively. Initial risk categorisation is based on predicted environmental concentrations (PEC) at these distances.

Selection of toxicity endpoint

A number of potential endpoints exist for plants; seedling germination, biomass (fresh or dry weight shoot weight or shoot height) and visual stress (chlorosis, mortality, developmental abnormalities). For risk assessment it is proposed that the toxicity endpoint used should be the most sensitive one measured for each species. It is also proposed that the 50% effect value (EC50) should be used in the initial risk assessment.

The main reason for this is that it will be based on the most sensitive of the sub-lethal effects obtained from glasshouse studies (*i.e.* OECD 208), which are assumed to overestimate toxicity compared to naturally exposed field grown plants of the same species. Furthermore, the natural variability in responses of plants, particularly if non-crop species are tested, is considered too large to justify using lower effect values such as NOECs or EC5s.

Selection of species

Estimations of the number of species for which testing is required to establish a reliable estimation of the range of sensitivity vary, but figures in excess of 30 species have been quoted (Breeze *et al.* 1999). Given the number of species potentially exposed this is not surprising, but if data for such numbers of plants species were required then it would be disproportionately higher than for other areas (i.e. aquatics, birds). For herbicides, which it is reasonable to assume pose the highest risk to non-target plants, there is often other valuable information, which can be taken into account. For such products specific label claims of activity are made; in some countries (*i.e.* UK) these claims must be supported by efficacy field data. Thus there exists a body of evidence, which identifies some of the more sensitive non-crop species. This information can be used to focus a more detailed laboratory dose response testing regime on these or closely related species. This principle underpins the draft EPPO scheme. Results from tests on such species can then form the basis of a risk assessment. For herbicidally active compounds dose response testing for at least 6 species is proposed.

Calculation of toxicity endpoint for use in decision making

Where acceptable EC50 values for 6 species are available a statistical approach based on the distribution of the EC50 values derived from the OECD tests is proposed in order to determine a calculated toxicity value (*i.e.* HD5). Thus the scheme differs from classical deterministic risk assessments, where an uncertainty factor (typically 10 or 100) is applied to the lowest observed endpoint. However, for plants there is currently no substantive body of data to support this approach. Validation of this step is likely to be required before the scheme can be accepted.

Routes of exposure

The calculated toxicity value is then compared with the appropriate exposure estimate to derive an Exposure:Toxicity Ratio. The routes of exposure considered are spray drift, run-off and gaseous transport. Aerial drift of herbicides is known to cause impacts on plants in areas close to the point of application. This route of exposure is considered to represent the main route of exposure for plants outside of the treated area. The predicted exposure level for each route of exposure is to be obtained from the relevant EPPO Chapter. For spray drift, the exposure value will come from the EPPO Air Scheme (this is likely to be taken from the published BBA spray drift data set (www.bba.de)). For gaseous transport, it is unlikely that the EPPO Air Scheme will be able to produce a value in the short term, hence for compounds which are expected to pose a risk via volatilisation non standard tests/scenarios will be required. The EPPO soil scheme should provide a run-off PEC. All exposure scenarios in the scheme may be defined as "off-crop".

Refinement of risk

The susceptibility of plants to pesticides may be affected by many variables (Marshall 2001 this publication). The scheme acknowledges this and suggests some possible refinement options including; more detailed consideration of the dose response data, more realistic exposure scenarios, testing of less sensitive growth stages (if appropriate to the intended use), consideration of importance of seedbank for sensitive species and use of higher tier studies (**i.e**. semi-field studies). Experience in the conduct and evaluation of semi-field studies is however very limited and such studies should only be conducted once the overall object has been clearly identified.

FUTURE DEVELOPMENTS

The proposed EPPO Decision-making scheme provides a basis for categorising the risk to non-target plants ('Negligible', 'Low', 'Medium' or 'High'). As such it does not attempt to define the 'acceptability' of the risk identified; the final decision on which will, in the foreseeable future, rest with individual countries. In defining 'acceptability' regulators must address the challenge of clearly defining the overall protection goal; this has yet to be done.

Currently risk assessments for non-target plants are limited to the 'off crop' area and tend to be rather qualitative. This situation has arisen because of the general belief that all non-crop plants within the cropped area have the potential to significantly reduce yield and/or cause contamination of seed lots. A reflection of this can be seen in the current UK approach, which for highly active compounds (*i.e.* some sulfonylurea herbicides), consists of the use of advisory label warnings such as

"Take extreme care to avoid drift onto nearby plants"

In contrast to the restrictions which can be applied to the use of certain pesticides near surface waters, there are no specific non-target terrestrial plant buffer zones in the UK. Where data are available to indicate phytotoxicity to non-target plants at distance from the point of application, authorisation has been refused in the UK.

The well publicised reductions in populations of some arable bird species, the demise of certain arable plants and the potential introduction of crops tolerant to broad spectrum herbicides has meant that the view that the cropped area should be free of all non-crop plants is being increasingly challenged (Marshall 2001 this publication). In response to such concerns over the sustainability of modern agriculture, the UK has begun to ask the questions which species of non-target plants are present, and what role do they play, in the agro-ecosystem?

A MAFF commissioned desk study by Breeze *et al.* 1999, identified a number of the more common non-crop plant species associated with agricultural systems. This study also identified some possible associations between these species and some invertebrates and birds. This work has recently been updated by Marshall *et al.* 2001. Existing evidence indicates that certain species *i.e.* blackgrass (*Alopecurus myosuroides*), winter wild oat (*Avena fatua*) and common cleavers (*Galium aparine*) are of such high competitive ability that there is limited opportunity to reduce the high levels of control currently used. However, for other species of far lower competitive ability, the need for consistently high levels of control is more questionable.

The limited available evidence suggests that some plant species which may be important for invertebrates and birds are those which pose less of a threat to agricultural production. Further research is underway to establish whether for some species a balance between weed control and biodiversity can be found (P Lutman BBSRC Rothamsted *pers comm*).

Evidence of the extent to which the use of herbicides *per se* may have impacted on the long-term diversity of non-crop plant species in arable areas is contradictory. Surveys in West Sussex (England) appear to show limited effects of herbicide usage on arable weed populations in cereal fields over the period 1970 to 1995 (Ewald 1999). For the following reasons these results are questionable; surveys conducted at approximately the same time of year, assessed presence/absence only, started after herbicide usage was already well established.

In contrast, claims of increases in plant diversity in organic compared to conventional production fields have been made in Germany, Denmark and Sweden, although again the impact of herbicides cannot be accurately judged. There are a number of other factors, which are considered to play an important role in the diversity of arable weeds. Several authors conclude that the current floristic composition of arable areas is dominated by a relatively small number of species better suited to high nutrient levels. Removal or restrictions on herbicide usage may thus result in the increased dominance of a small number of the more competitive species and not achieve any significant increase in biodiversity. Cropping regime is also considered to be another important factor. The potential scale of changing cropping practice is highlighted by the major reduction in the area of spring barley from 44.7% to 10% of total arable area which occurred in the UK between 1974 and 1998 (based on published MAFF Pesticide Usage Survey Data).

Whilst the evidence that the use of herbicides *per se* is adversely affecting the long term diversity of plants in arable areas is not conclusive, the use of such compounds is likely to have a major impact on their short term abundance (Breeze *et al.* 1999). For associated species *i.e.* phytophagous insects and insectivorous/seed eating birds this potential short-term loss of habitat/food supply may have important implications.

Thus the potential for indirect effects of herbicides is an area which requires further detailed consideration. The Department for Environment, Food and Rural Affairs (DEFRA) has taken over from MAFF the responsibility for a major 5 year research project 'Assessing the indirect effects of pesticides on birds.' (Commission No PN0925). This project will produce a framework for the assessment of the indirect effects of pesticides on birds reflecting the causal chain of pesticide effects on resources, the effects of resources of bird performance and the effects of performance on bird populations. The framework will be tested by expanding ongoing studies on 11 farmland bird species and large-scale replicated field experiments. The study will provide a basis for the decision as to whether indirect effects are substantial enough to warrant regulatory action and an assessment of the extent to which current risk assessment methods provide protection against potential indirect effects.

If future research does identify certain plants with important ecological roles then a potential refinement of the EPPO approach to species selection could be to require specific testing on such species, or their close relatives. It is acknowledged that if several countries were to adopt such an approach it could result in the need to supply and evaluate data on numerous different species. This situation would place a heavy burden on both agrochemical companies and regulators alike and, if possible it should be avoided.

However, this serves to highlight one of the main problems with non-target plant risk assessments i.e. the lack of a robust toxicity database on which to make a judgement as to the representativeness of different species. Indeed, Boutin & Rogers (2000) considered this aspect so important as to conclude, "an improved database on phytotoxicity is a pre-requisite to refine the risk assessment of pesticide effects on non-target plants." Taken in isolation this is a valid statement. However, it is unlikely in the short term that such a comprehensive data set of sufficient quality will be available. The proposed EPPO scheme therefore represents a pragmatic compromise between the increasing pressure to address the issue and the current lack of detailed knowledge.

The recognition of ecologically important plant species currently considered as being 'weeds' would require some consideration of the 'in-crop' risk. Such a development would require a new approach to risk assessment and risk management techniques. If this scenario does arise, then the challenge of protecting/encouraging such species, whilst not unduly compromising the ability to control pernicious weeds, is one which will require the combined efforts of researchers, agrochemical companies, pesticide regulators, environmental policy makers and field based advisory services.

CONCLUSION

The proposed revision of OECD Guideline 208 provides protocols suitable for testing the phytotoxicity of the majority of pesticides. Such harmonisation of testing lays the foundation for the proposed EPPO decision-making scheme. Current risk assessments for non-target plants are focused on the potential for effects in the 'off-crop' area. Concern over the sustainability of some modern intensive agricultural practices is currently challenging the basis of this. If it is deemed necessary to assess the risk to non-crop plants in the 'in-crop' area, a whole new approach to risk assessment and risk mitigation will be required and a clear overall protection goal for non-target plants will need to be defined. The pesticide regulatory process provides a potential route via which appropriate phytotoxicity data can be demanded. However, potential risk management options for non-target plants will need careful consideration and a multi-disciplined approach if the desired objectives are to be achieved.

REFERENCES

Boutin C, Rogers C A (2000). Pattern of sensitivity of plant species to various herbicides – an analysis with two databases. *Ecotoxicology*, **9**: 155-271

Breeze V G, Marshall E J P, Hart A, Vickery J A, Crocker J, Walters K, Packer J, Kendall D, Fowbert J, and Hodkinson D (1999). *Assessing pesticide risks to non-target terrestrial plants: A desk study. Commissioned by MAFF Pesticide Safety Directorate* (Project No PN0923)

Campbell L H, Avery M I, Donald P, Evans A D, Green R E, & Wilson J D (1997). A review of the indirect effects of pesticides on birds. *JNCC Report* No **227**. Joint Nature Conservation Committee. Peterborough.

Council Directive, (1991). Council Directive 91/414/EEC of July 1991 concerning the placement of plant protection products on the market. *Off. J. Eur. Commun.*, No. **L230,** July, 1-32 (as amended and adapted)

European and Mediterranean Plant protection Organisation and Council of Europe: Decision-making scheme for the environmental risk assessment of plant protection products. *Proposal for Chapter 13 Non-Target Plants* (draft October 2000)

Ewald J A, Aebischer N J (1999). Pesticide use, avian food resources and bird densities in Sussex. JNCC Report No **296**. Joint Nature Conservation Committee. Peterborough.

Marshall E J P, Brown V, Boatman N, Lutman P, Squire G (2001). *The impact of herbicides on weed abundance and biodiversity. Commissioned by DEFRA Pesticide Safety Directorate* (Project No PN0940)

Organisation for Economic Co-operation and Development (draft July 2000). Proposal for revision of Guideline 208 Terrestrial (Non Target) Plant Testing: (Part A) Seedling Emergence and Seedling Growth Test and (Part B) Vegetative Vigour Test.

Pestemer W, Zwerger P (1999). Application of a standardised bioassay to estimate the phytotoxic effects of frequently used herbicides on non-target plants. *Proceedings of the XI Symposium Pesticides Chemistry*, Cremona Italy pp 762-770.

Assessing the potential risks of herbicides to non-target aquatic plants

S J Maund, R Grade, J F H Cole, J Davies
Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK
Email: steve.maund@syngenta.com

ABSTRACT

The current preliminary risk assessment scheme for non-target aquatic plants in the EU is described. Reviews of laboratory and field data have demonstrated that under most circumstances, the current study requirements and risk assessment procedures for herbicides should afford reasonable protection for non-target aquatic plants (and other non-target aquatic organisms) in the field. However, where concerns are identified (either through triggering or because of regulatory concerns about inadequacies of standard studies for certain modes of action), higher-tier studies and risk assessment procedures are needed. The approaches described by the HARAP workshop provide a suitable framework for developing higher-tier studies, and some examples of potential approaches for aquatic plants are reviewed. Effective implementation of higher-tier aquatic non-target plant risk assessment will require the development of clear protection goals. Ideally, these goals should be based on ecological information about the aquatic plant assemblages that are associated with agro-ecosystems. A number of initiatives are underway that may enable such risk assessment procedures to be developed in the future.

INTRODUCTION

Assessing the impacts of herbicides on aquatic plants can be a complex matter. On the one hand, they may be a target organism. On the other hand, protection of certain aquatic plants may be a key goal, for example rare, threatened or endangered species. Among lists of threatened and endangered plant species, aquatic and wetland plants are often well-represented, possibly due to habitat declines and land/water management practices. Furthermore, it is important to attend to the functional role of aquatic plants in aquatic ecosystems. Plants are of key importance for their role in primary production and community metabolism. Less obviously, but perhaps of equal importance, they also provide substrates and habitat or micronutrients for other organisms. What is more (but less commonly considered), the presence of aquatic plants may have a profound influence on the fate and distribution of pesticides in the aquatic ecosystem. For these reasons, aquatic plants are beginning to receive more attention in pesticide regulation.

The preliminary risk assessment process for aquatic plants is well-established and generally appears to be effective at identifying low risk compounds. However, for compounds which fail the preliminary assessment, whilst there are a range of options available for higher-tier studies, methodologies are far from standardised and implementation of such data into risk assessment is still under discussion. In this paper, current risk assessment procedures in the EU are discussed, and potential higher-tier approaches are described.

PRELIMINARY RISK ASSESSMENT FOR AQUATIC PLANTS

In the EU risk assessment scheme under 91/414/EEC, all active ingredients must be tested for effects on the growth of a green alga (usually *Pseudokirchneriella subcapitata* previously known as *Selenastrum capricornutum*). For herbicides, an additional algal species is required (the blue-green *Anabaena flos-aquae* is suggested), as well as studies on the floating pond weed *Lemna* sp. (usually the species used are *L. gibba* or *L. minor*). In some cases, where regulatory authorities are concerned that the specific mode of action of the compound is not covered (e.g. if the mode of action is specific to dicotyledonous plants, considering that *Lemna* is a monocot) other studies may be needed. In such cases, the draft EU Guidance Document on Aquatic Ecotoxicology recommends that data from terrestrial plant studies may also be useful for evaluating selectivity. Such data are also relevant for assessing potential risks to emergent (also called semi-aquatic) plants. In some cases, tests with other species (e.g., *Myriophyllum* or *Glyceria* sp.) have been requested by certain authorities, although as yet there are no harmonised guidelines for such studies (see below). This is usually only required if it is anticipated that the standard test species will not be sensitive to the mode of action of the compound. The effect concentrations from these studies (usually 72 or 96 h EC50s for algae, and 7-14 d EC50 for *Lemna*) are then compared to the relevant exposure concentrations, and if the resulting toxicity exposure ratio is less than 10, higher-tier assessments are required.

VALIDITY OF THE PRELIMINARY RISK ASSESSMENT SCHEME

A number of authors (e.g. Fairchild *et al.*, 1998; Peterson *et al.*, 1994) have suggested that testing schemes for aquatic plants may need to be extended because comparison of toxicity endpoints for various herbicides with different algal and macrophyte species do not show consistent results (*i.e.*, no one species is consistently the most sensitive). Selecting 'sensitive' species for toxicity testing is a long-recognised problem (Cairns, 1986). A counter-balancing consideration, though, is that for routine regulatory testing purposes it is essential that test methods involve organisms which can be readily cultured in the laboratory, are reproducible, and are cost-effective. At present, such methodologies for a much broader range of species are limited.

Whilst the conclusion that no one species can ever be the most sensitive is incontrovertible, it also perhaps misses the key point of species selection for risk assessment. This is that species are selected for risk assessment purposes as indicator organisms, not as surrogates. The principle aim of preliminary risk assessment scheme is to identify compounds which present low risks to aquatic plants. So the fundamental question should not be whether the species tested are always the most sensitive, but whether the risk assessment process using the standard species affords adequate protection. What we really need to know is whether the toxicity data that are generated, in combination with an uncertainty factor, are protective of effects seen under field conditions (additionally of course there is the consideration of the likelihood of the exposure concentration that is used in the risk assessment actually occurring from normal uses).

It has generally been assumed in the EU that the lower tiers are conservative, because of the combination of the worst-case nature of the exposure estimates and the sensitivity of the toxicity test endpoints used, combined with the use of a safety factor. For the EU risk assessment scheme, a recent comprehensive review of the latter two assumptions has been made by Brock *et al.* (2000) using laboratory and field studies published in the literature. For herbicides, studies were reviewed on compounds with a wide range of modes of action (photosynthesis inhibition, auxin simulating, and 'other' growth inhibition mechanisms). Generally, they found that the EU risk assessment criteria (based on laboratory toxicity data) were protective of the effects observed in the field. The one exception to this was auxin simulating herbicides, which were not particularly toxic to algae or *Lemna*, but did have some effects on other macrophyte species in the field. The conclusions of the study are encouraging and suggest that in most cases, the proposed scheme will be effective at identifying safe compounds.

OPTIONS FOR HIGHER-TIER STUDIES

If a compound fails the preliminary risk assessment, there are two options for further refinement. Firstly, it may be appropriate to refine the exposure concentrations. Previously in Europe, there have not been many options to do this, but under the new FOCUS surface water scheme, a series of steps will be available with which to refine exposure estimates. Alternatively, it may be appropriate to refine effect concentrations by performing further ecotoxicological studies. Guidance on the conduct of higher-tier aquatic studies was developed at the HARAP workshop (Campbell *et al.*, 1999). In this guidance, there are a number of options for assessing higher-tier risks, and these fall in to several categories:

- Interrogation of core data,
- Additional species testing,
- Modified exposure studies,
- Indoor and/or outdoor micro- and mesocosm studies.

Each of these study areas has potential application for higher-tier assessments of aquatic plants, and are discussed further below.

Interrogation of core data

If higher-tier assessments are triggered, the first point to establish is what is known about mode of action and therefore likely species affected. Valuable information on this can be gathered from reviewing data from terrestrial plant studies (where a range of monocots and dicots are studied) or from data from herbicide efficacy screens. These data may then also be used to refine the risk assessment, particularly if the major route of entry for the herbicide is determined to be spray drift.

A second consideration is what the critical endpoint of the studies are that have triggered the concern. It is important to consider what the likely environmental consequences of the measured effects will be. For example, in algal studies, compound may be algistatic (*i.e.* they limit growth but do not kill algal cells) or algitoxic (resulting in cell death) at concentrations relevant to the predicted environmental concentration. The former has potential consequences for recovery, and aids the design of any necessary higher-tier studies.

Additional species testing

There is a wide range of algal species which can be used to evaluate relative sensitivity (see Lewis (1995) for a review of methods and relative sensitivity data). Reviews of published methods for testing aquatic macrophytes have been produced by Freemark & Boutin (1994) and Lewis (1995). Until recently, the use of submerged plant species in toxicity tests has been limited by the difficulty of generating algae-free cultures. Work by Roshon has led to production of a draft American Society of Testing and Materials guidance for *Myriophyllum sibiricum*. Additionally, there are few cited laboratory methods for emergent species (Davies *et al.*, 1999). However, none of these proposed tests have been validated under a regulatory testing framework. Whilst development of standard, harmonised methods for macrophytes is a clear need for the future, validation of any new test is critical before it can be implemented as a regulatory requirement. Furthermore, there will need to be a clear understanding of how data so developed will be used in the risk assessment process (e.g., the ecological relevance of the various endpoints measured).

At present, comparatively little is known about the relative sensitivity of macrophyte species. Although much data have been published on effects of herbicides on aquatic plants, studies have often been conducted with a view to controlling nuisance species, where aquatic plants are the target species. Consequently, data are difficult to compare due to the use of different methods. A few authors have attempted to make comparisons in species sensitivity, (Davies *et al.*, 1999; Fairchild *et al.*, 1998), but clearly relative sensitivity will depend on the mode-of-action of the compound and the route of exposure of the pesticide.

Many endpoints have been proposed including root and shoot dry weight, root and shoot height, side shoot production, chlorophyll content, photosynthetic rates and enzyme activities such as peroxidase. Measurements of dry weight and biomass are more easily interpreted while measurements of chlorophyll content, photosynthetic rates and enzyme activities are more prone to sampling variation and low-dose enhancement. Thus data can be very difficult to interpret in terms of detrimental effects on a population. In particular, photosynthetic inhibitors like isoproturon have been reported to stimulate chlorophyll content while having no visible effect on biomass (J Davies, unpublished data). Further studies are needed to establish the link between effects at the sub-organism level to effects at the individual level, with linkages of these to effects at the population and community level being a necessary longer-term goal.

Modified exposure and recovery studies

One option for refining effects concentrations is to modify the exposure conditions in the toxicity test. Two approaches to this have been developed. The first is where the exposure concentration in the test vessel can be modified using a variable dosing system e.g., for algae (Grade *et al.*, 2000). Flow-through methods are mentioned for *Lemna* in OECD draft guideline and have also been published for other rooted macrophytes (Steinberg & Coonrod, 1994). Alternatively, it is possible to modify the exposure by adding sediment to the test system, where it is anticipated that the test compound will be dissipated more rapidly in the presence of sediment e.g. for algae (Shillabeer *et al.*, 2000). Similar approaches would be also possible for macrophytes.

Micro- and mesocosm studies

Algae and aquatic macrophyte have been studied extensively in micro- and mesocosm studies. There have been a number of review of such studies, and the reader is again referred to the reviews of Lewis (1995) and Brock *et al.* (2000). The considerations that apply to micro- and mesocosm studies on aquatic fauna also translate in most part to studies on flora, and recommendations for conduct and interpretation can be found in the HARAP (Campbell *et al.*, 1999) and CLASSIC (Heger *et al.*, in press) workshop proceedings. Indeed, even in small microcosms, it is possible to study assemblages of macrophytes that are reasonably representative of natural systems. Williams *et al.* (in press) have found for example that in 1 m^3 outdoor microcosms, the assemblage composition of submerged macrophytes was similar to that found in natural ponds.

FURTHER CONSIDERATIONS FOR HIGHER-TIER RISK ASSESSMENT

The paper so far has focused mostly on the methods that are available for higher-tier aquatic plant assessment. In relation to developing a higher-tier risk assessment scheme, this comes at the problem from the wrong direction. The key need for further development of aquatic plant risk assessment is a fundamental review of risk assessment goals for aquatic plants. As in other areas of ecological risk assessment, a frequently unanswered question is "what are we trying to protect?" This is a particularly difficult question to answer for most pesticides, because they are designed to kill organisms (or at least their close relatives) that under other circumstances we may want to protect. However, in order to produce a rational and cost-effective risk assessment procedure, it is a question that must be tackled. This also leads on to the perennial question of "what is an unacceptable impact?"

Perhaps one of the first steps in trying to answer this difficult question is to know which species of aquatic plants are associated with the water bodies in agroecosystems, and to understand their life-history (e.g., when and how quickly they grow, their reproductive rate, etc.). This information would help in formulating appropriate experiments to assess for potential impacts, and also enable the development of suitable risk assessment paradigms. A number of projects are underway at the moment which may offer potential in this direction in the future. For example, the UK Pesticide Safety Directorate is currently funding a project which will develop scenarios for aquatic ecosystems in the UK agricultural landscape. Information on the floral assemblages associated with these ecosystems will be gathered. In addition, the Freshwater Biological Association in collaboration with the Ponds Conservation Trust have initiated a project called Freshwater Life (www.freshwaterlife.org) which will gather together information on the life-history and taxonomy of aquatic flora and fauna. Furthermore, the National Biodiversity Network in the UK will be collating distribution maps for British macrophyte species (www.nbn.org.uk). Similar intiatives are also underway in other EU countries, so the potential for better informed risk assessment procedures in the future is increasing.

REFERENCES

Brock T C M; Lahr J; van den Brink P J (2000). *Ecological risks of pesticides in freshwater ecosystems. Part 1: Herbicides.* Alterra-Rapport 088. 124 pp., Alterra Green World Research, Wageningen, Netherlands.

Cairns J (1986). The myth of the most sensitive species. *Bioscience* **36**: 670-672

Campbell P J; Arnold D J; Brock T C M; Grandy N J; Heger W; Heimbach F; Maund S J; Streloke M (1999). *Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides.* SETAC-Europe Press.

Davies J; Pitchford H F; Newman J R; Greaves M P (1999). Toxicity tests for assessment of pesticide effects on aquatic plants. *Proceedings of the Brighton Crop Protection Conference – Weeds 1999*, 717-722.

Fairchild J F; Ruessler D S; Carlson A R (1998). Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor and metolachlor. *Environmental Toxicology and Chemistry*, **17**: 1830-1834.

Freemark K; Boutin C (1994). Nontarget-plant risk assessment for pesticide registration. *Environmental Management* **18**: 841-854.

Grade R, Gonzalez-Valero J; Höcht P; Pfeifle V (2000). A higher tier flow through toxicity test with the green alga *Selenastrum capricornutum*. *The Science of Total Environment* **247:** 355-361.

Heger W; Brock T C M; Giddings J M; Heimbach F; Maund S J; Norman S; Ratte H-T; Schäfers C; Streloke M (in press). *Guidance document on Community Level Aquatic System Studies - Interpretation Criteria*. SETAC Press.

Lewis M A (1995). Algae and Vascular Plant Tests. In: *Fundamentals of Aquatic Toxicology*, ed/ G M Rand. pp. 135-170. Taylor & Francis, Washington DC.

Peterson H G; Boutin C; Martin P A; Freemark K E; Ruecker N J; Moody M J (1994). Aquatic phytotoxicity of 23 pesticides applied at expected environmental concentrations. *Aquatic Toxicology* **28**: 275-292.

Shillabeer N; Smyth DV; Tattersfield L (2000). Higher tier risk assessment of agrochemicals, incorporating sediment into algal test systems. *Proceedings of the BCPC Conference – Pests and Diseases 2000*, 359-364.

Steinberg S L; Coonrod H S (1994). Oxidation of the root zone by aquatic plants growing in gravel-nutrient solution culture. *Journal of Environmental Quality*, **23**: 907-913.

Williams P; Whitfield M; Biggs J; Corfield A; Walker D; Fox G; Henegan P; Jepson P; Maund S J; Sherratt T N and Shillabeer N (in press). How realistic are mesocosms? - a comparison of the biota of mesocosms and natural ponds. *Environmental Toxicology and Chemistry*.