SESSION 10

HERBICIDES IN THE ENVIRONMENT: EXPOSURE, CONSEQUENCES AND RISK ASSESSMENT – PART 2

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Biodiversity, herbicides and non-target plants

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ABSTRACT

Herbicides provide a useful tool for the farmer, grower and vegetation manager. However, they are capable of affecting non-target plants. Non-target plants may be those outside the target area, or those within the target area of conservation concern or whose control has untoward effects on biological diversity. A number of farmland birds, invertebrates and plants have shown population declines in Europe; changes in agriculture, including herbicides, are implicated. Whilst a better understanding of the impacts of weed control on biological diversity is needed, the new challenge is the development of more ecologically sustainable production, incorporating the maintenance of some weed species within crops. The first-generation genetically modified herbicide-tolerant (GMHT) crops seem unlikely to provide the required flexibility of management. For success, greater selectivity of herbicide chemistry is indicated, together with a range of risk avoidance approaches.

INTRODUCTION

Herbicides are an essential part of the farmer and grower's equipment for crop management. In addition, herbicides can play a useful role in vegetation management in a variety of non-crop situations, ranging from industrial areas to amenity sites (Marshall, 1994) and even nature reserves and conservation areas. For example, herbicides may be an essential part of control strategies for alien invasive species, such as giant hogweed (*Heracleum mantegazzianum*). Nevertheless, a range of environmental problems, including residues in water, has focussed attention on the regulatory process and the impact of herbicides in the environment. There have been a number of recent developments in approaches to risk assessment and risk avoidance for non-target effects of herbicides (Breeze *et al*., 1999). This paper reviews the definition of non-target plants, the use of herbicides and assesses the impacts of herbicides on non-targets and biological diversity. The implications of improved understanding of functional biodiversity and of developments in new technologies are discussed. Finally, a number of requirements for the future approval and use of herbicides are proposed.

DEFINING NON-TARGET PLANTS

The movement of herbicide away from the application area will bring it into contact with plants that are by definition non-targets. This "off-field" movement may be due to droplet drift, vapour movement, leaching and erosion, as well as inappropriate disposal. An extremely wide range of plant species (the national flora) is potentially at risk to such movement. Approaches to risk assessment and risk avoidance in the UK have been reviewed by Marshall *et al.* (2001). Advances in non-target risk assessment have also been made in Europe and North America, aimed at assessing the risks to off-field flora particularly from drift events (Hewitt, 2000).

There are also within-field non-target plants that need consideration. There are two very different scenarios where herbicides are used. In most situations, a herbicide is deployed to control all the plant species present except the single crop species. In the non-crop situation, either all species are targets for total weed control, or there is a single target species and all others present are non-targets. This is a simplification, as herbicide selectivities vary and the target group necessarily may be wider. Likewise, within a crop, there may be a number of unsown plant species present forming a weed assemblage. As many of these species reduce yield, or affect harvesting, storage or crop quality, farmers regard them all as weeds worthy of removal. Nevertheless, amongst these non-crop species, there may be both target and non-target species for weed control. A number of rare weed species, such as broad-leaved cudweed (*Filago pyramidata*), are subject to conservation effort and some are included within UK Biodiversity Action Plans (BAPs), the response to the Rio Convention on Biological Diversity (Anon, 1994). These may be regarded as non-target species. Of greater significance, as they are commoner and often have significant biomass, there is a suite of species that might be targets at higher density, but may be non-targets at low population levels for biodiversity reasons. There are a number of species that are almost invariably targets for control, usually because of their competitive ability, such as wild-oat (*Avena fatua*). The consideration of non-target species within the application area brings a number of potential complications to the regulatory process and to practical management. However, against the environmental background of significant declines in farmland wildlife across Western Europe, this is a challenge to be faced.

HERBICIDE IMPACTS AND NON-TARGET EFFECTS

Agricultural and horticultural habitats do not occur in isolation in the landscape. Field systems occur as mosaics of crop and non-crop habitat (Marshall, 1988) and may be refuges for many plant and animal species. Whilst most species associated with non-crop areas do not commonly pose serious threats to adjacent crops (Marshall, 1989), these areas may be important for the conservation of biological diversity in agricultural landscapes, particularly as production methods have intensified. Extensive studies of land use change and their ecological consequences also indicate that botanical diversity is continuing to decline (Haines-Young *et al.*, 2000). Whilst the causal effects are not agreed, they are most likely to be eutrophication and disturbance. Agricultural practices, including fertiliser and herbicide applications, are implicated (Kleijn & Snoeijing, 1997).

Within agricultural systems, there have been significant declines in both population sizes and ranges of common birds in the UK (Fuller *et al.*, 1995). Likewise, there have been significant declines in some taxa of invertebrates found within fields (Aebischer, 1991). The idea that arable fields are "ecological deserts" is ill founded, as there is a range of plant and animal species specifically adapted to the habitat, for example the cornfield flowers.

Individual plant species can be affected directly by a herbicide. As part of a plant community made up of many species, a plant species can also be affected indirectly following herbicide contamination. This can be mediated by competition between species, or by affecting plant recruitment (vegetative or from seed), or by affecting herbivore pressure or symbionts. Determining the effects of herbicides on plant communities is not straightforward (Cousens *et al.*, 1988). Susceptibility of plants to herbicides is not a constant characteristic, as application variables interact with plant variables.

Non-target effects of herbicides may be caused when materials reach situations beyond the target application area and/or reach species not intended to be affected growing within the target area. The direct adverse effects of herbicides can range from outright death of a plant or population, through minor effects, to enhanced growth. The spectrum of direct effects on individuals is matched by a spectrum of indirect effects on associated fauna and flora. Direct effects on plants can appear to be insignificant, for example, reduced flowering. However, such impacts may be of major significance to species where seed production is the key element of the regenerative cycle of the plant. Effects on germination and early recruitment of plant species are believed to be of particular importance at a growth stage that is particularly susceptible to pesticides. Non-target effects may have subtle effects on plant community composition, mediated by plant competition or by effects on the water and chemical environment in the rhizosphere.

It is unclear how important the non-target effects of herbicides are. For example, it is unknown if repeated drift events, or mixtures of herbicides at low doses, can have sub lethal effects on plant recruitment. The “off-field” movements from herbicide application are likely to be the most common cause of non-target effects (Breeze *et al.*, 1999). These can result from droplet drift, mist, solid and vapour movement. Of these drift forms, droplet movement is by far the most important and common form. Following application, pesticides may also undergo secondary redistribution with a risk of non-target effects, if pesticide concentrations are high enough.

BIODIVERSITY AND ECOSYSTEM FUNCTION

The reasons for the conservation of biodiversity are moral, aesthetic, social and economic. We steward other organisms for their intrinsic value and because species may be of benefit to human society and have economic value. A culture that encourages respect for wildlife is preferable to one that does not. Biodiversity can be easily lost but is difficult to regain, particularly if species are driven to extinction. Biodiversity, including genetic diversity, may provide economic benefits. Even at the level of landscape, biodiversity may influence tourism and sense of place. Perhaps of greatest concern is that biodiversity has a role in the function of ecosystems (Tilman *et al.*, 1996). Erosion of diversity may thus ultimately result in damage to ecosystem function.

Plants are key components of terrestrial ecosystems, providing the primary production upon which food chains are built. Different plant parts provide a range of resources for associated fauna (Figure. 1). Leaves and stems may be browsed, while pollen and nectar provide resources for pollinating insects. Fruits and seeds are important food for a large number of organisms. Plants have other functions as well as providing food for herbivores. They provide cover, reproduction sites and structure within habitats. Plants also form a substrate for bacteria, fungi etc., both above ground and in the soil.

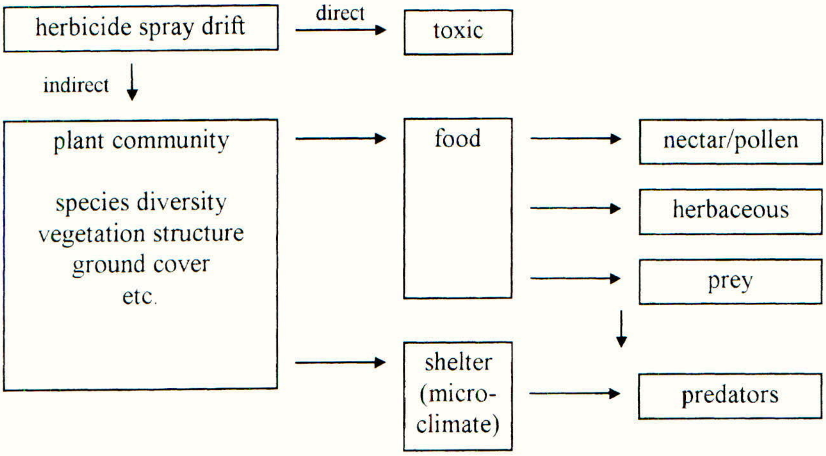

Figure 1. Potential ecological effects of herbicide spray drift on invertebrates – from Breeze *et al.*, (1999)

Even non-crop plants or weeds may play a role in the function of the ecosystem and in supporting many other species. As an example, the grey partridge (*Perdix perdix*) requires insects as chick food during the first ten weeks of rearing. Many of these insects are associated with annual dicotyledonous weeds in cereal crops in the UK. Adult partridges also feed on plants, particularly within arable crops. Management of the crop with pesticides and herbicides is therefore likely to have had a major impact on partridge populations, explaining the major declines in population of this bird species in the twentieth century (Potts, 1991).

Interactions between weed diversity and biodiversity

A comparison of herbicide-treated and untreated plots in the headlands of winter cereal fields in southern England (Moreby & Southway, 1999) has clearly demonstrated that untreated plots had greater weed density and diversity and significantly higher numbers of many invertebrate taxa, notably those that are important in the diet of farmland birds. Studies of the insects associated with soybean in Iowa, USA, indicate that weedier fields have generally higher insect densities. Weed management in herbicide-resistant soybean generally gave fewer insects (Buckelew *et al.*, 2000). The effects were indirect, mediated through the weed flora. Several initiatives, notably for integrated crop management, indicate there are implications for biological diversity within fields from different approaches to weed control. The protection of the farmers' investment and avoidance of risk have been the driving forces for efficient weed control in the past. However, an emerging new paradigm is to match crop production with conservation of biological resources (Paoletti *et al.*, 1992) and the development of more sustainable systems. This may require the maintenance of some weeds within fields.

NEW TECHNOLOGIES FOR WEED MANAGEMENT

Genetically modified herbicide-tolerant (GMHT) crops

The introduction and testing of GMHT crops, whilst widely accepted in North America, has been opposed by many interest groups in Europe. Current work on the field-scale evaluation of the biodiversity impacts of these crops in the UK is examining the likely impact of modified herbicide use within the crop (Firbank *et al.*, 1999). The first generation of GMHT crops are engineered for tolerance to broad-spectrum herbicides, notably glyphosate and glufosinate. These *may* allow greater flexibility in weed management, but there may be effects on biodiversity as a result.

Watkinson *et al.* (2000) simulated the effects of the introduction of GMHT crops on weed populations and the consequences for seed-eating birds, using fat-hen as the model weed. They predicted that weed populations might be reduced to low levels or practically eradicated, depending on the exact form of management. Consequent effects on the local use of fields by birds might be severe, because such reductions represent a major loss of food resources. Buckelew *et al.* (2000) have shown that herbicide-resistant soybean crops tend to have lower insect population densities, associated with fewer weeds.

Whilst it may be argued that GMHT crops offer the opportunity to delay weed control, some crops, most notably maize, are particularly susceptible to early weed competition. Such crops are likely to be treated with herbicide around the time of crop emergence to eliminate weeds early in the life of the crop. The technology offers reduced risk to the farmer, with opportunities for repeated application, should this become necessary. Environmentally, the technology offers the possibility of clean crops and thus adverse biodiversity effects, as well as the unknown, if low, possibility of gene transfer to wild relatives. Nevertheless, it must be accepted that in the developing world, where weeds are the primary source of crop loss, this first-generation technology may have an important role.

Integrated weed management

Approaches to weed management over recent years have taken an holistic view of the crop rotation as a whole, rather than simply in single crops, as part of integrated crop management (ICM). ICM considers fertiliser use, targeted pesticide use, alternative control techniques, forecasting and modelling, as well as crop rotation (Jordan & Hutcheon, 1995). Economic pressures have also forced farmers and growers to consider the number of herbicide applications made and the dose of active ingredients used. Reduced dose applications have become common. Within ICM, the manipulation of crop architecture, tillage regimes, mechanical weed control, allelopathy, mulching, biological control may all contribute to "integrated weed management".

However, "*devising integrated weed management strategies that address a diversity of weed species with a diversity of life history traits is difficult*" (Mortensen *et al.*, 2000). A sound understanding of species, population and community ecology can contribute to weed management. Advances include population equilibria, density-dependent effects, crop competition models and integration with herbicide dose-response studies.

RISK MANAGEMENT

Risk management needs to address herbicide susceptibility and exposure. Exposure can be most easily manipulated, though susceptibility may be influenced, for example by protectants. The key to risk avoidance must be in targeting only those plant species or populations that require control. This means that precision in chemistry, *i.e.* selectivity of herbicide, and precision of application, *i.e.* only to the target plants, offers the most robust way forward. Aspects of dose, formulation, application timing and application technology may be usefully modified within a sound weed forecasting and decision-support framework. There may nevertheless be opportunities for spatial approaches to biodiversity maintenance. For example, conservation headlands, in which limited pesticide applications are made to the outside 6m or 12m of crop, allow sufficient weeds and invertebrates to survive for grey partridge populations to switch from decline to increase (Rands & Sotherton, 1987).

NEW DIRECTIONS FOR HERBICIDE USE AND WEED BIODIVERSITY

Ecologically, there is a requirement for greater specificity of herbicide action for minimising environmental and non-target effects. This runs against the trend for more broad-spectrum products produced by manufacturers. In order to cover the high costs of product development, manufacturers require products that will sell into global markets. This has resulted in herbicides with wide weed spectra coming to market, with more selective products rarely being commercialised. Greater herbicide selectivity is not without practical and financial difficulties. The inertia of commercial development could only be mobilised by legislative and regulatory requirements, possibly backed up by redirected farm support to growers. In addition, there could be difficulties if there are insufficient product options, e.g. herbicide resistance. Nevertheless, there could be opportunities for specialist market development, if agricultural support is redirected from production to environmental support. Non-crop vegetation management could provide a diversity of niche markets.

Clearly, where selectivity in chemistry is limited, there are opportunities for achieving selectivity by exploiting application technology and spatial methods, as well as manipulating crop phenology and growth characteristics. Further work on the opportunities for arable biodiversity areas, such as conservation headlands, is required.

Under the regulatory regimes for pesticides, there is a need to consider non-target, indirect effects that occur within the target crop area. This will require testing on a wider range of plant species representative of the diverse flora of arable and horticultural fields.

Current integrated weed management programmes might be further developed and modified to maintain adequate populations of the most important weed species for biodiversity, while controlling the most damaging. There is some possibility of relaxing weed control either rotationally or in limited areas of fields. Nevertheless, the major constraint is that the most fecund and often the most competitive weed species respond best to reduced control. Therefore, relaxed weed control would need to be managed carefully to allow the less common and less competitive species to increase, while controlling the competitive species. This may indicate a new approach to weed management, with the explicit aim of maintaining specific weed assemblages. These might be more traditional assemblages that were common 100 years ago, or tailored to maintaining beneficial invertebrate species, or for biodiversity more generally. An understanding of the selection pressures applied by management, including the use of herbicides, and their effects on diversity, ranging from genetic to community levels, is needed.

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Terrestrial non-target plant testing and assessment; the conservative nature of the process

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ABSTRACT

Terrestrial non-target plant testing and assessment is an emerging topic in Europe, as it was not previously discussed under the framework of Directive 91/414/EEC in the European Union. Test methods and assessment techniques are under development and an evaluation of the conservative nature of the study design, the exposure assessment and the safety goals need to be carefully considered before implementation. Non-target plants are defined as plants outside of the agricultural unit (i.e., the treatment area, plus a defined area around the treatment area used for agriculture). For non-herbicides, safety can generally be addressed using data generated from plant safety screens and efficacy work done during the development of the product. For herbicides, draft OECD regulatory test methods exist for both soil and foliar exposure assessments. The draft test methods use a suite of domesticated species to indicate the range of response (e.g., two to three orders of magnitude in range, typically) which may be expected for other species not included in the test. This approach makes use of readily available species with well-defined growth characteristics that allow determination of reliable metrics (e.g., visual effects and biomass), and end-points (e.g., EC_{50}). Because the end-points are based on sub-lethal effects and not lethality, and the study design is biased towards a "worst-case" scenario, the approach provides a very conservative estimate of phytotoxicity. These data combined with a conservative estimate of exposure, allow for a very conservative estimate of risk to non-target plants.

INTRODUCTION

Several activities have been ongoing on both the international and national level to address safety to non-target plants. On a national level, Germany has recently included non-target plant risk assessments as part of their National requirements (Füll *et al.*, 1999). And, the European Commission has suggested the addition of terrestrial plant data as part of the dossier package for all crop protection products (CPPs) in their recent Guidance on Terrestrial Ecotoxicology (European Commission, 08.07.2000).

In the area of testing, the Organization for Economic Cooperation and Development (OECD) has been working to revise Technical Guideline 208, for the testing of Terrestrial Plants, Growth Test. The main purpose of the revision is to modify the current guideline to allow for the testing of crop protection products (CPPs) (OECD, 2000).

The European and Mediterranean Plant Protection Organization (EPPO) has developed a proposed risk assessment scheme using the data generated in evaluating product phytotoxicity leading to an estimate of potential risk (EPPO, 2000).

Exposure is predicted using the drift data described by Ganzelmeier *et al.*, (1995).

The purpose of this paper is:

- to briefly review several of these on-going activities,
- to identify those factors in the current glasshouse/laboratory test design which contribute the highest levels of conservatism to the evaluation of plant safety,
- to quantify what contribution each factor may contribute to the conservative nature of the assessment and uncertainty in a determination of the level of safety afforded non-target plants and,
- to demonstrate that current tests are performed in a conservative fashion, such that in combination with a conservative estimate of exposure a very conservative estimate of risk to non-target plants (NTPs) is attained.
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BRIEF REVIEW OF TERRESTRIAL NON-TARGET PLANT TEST METHODS

For non-herbicides, data available from screening and efficacy studies generated by companies to address plant safety are being used to address product safety. Additionally, under Annex III, Section 6.6.1 through 6.6.3 of 91/414/EEC, data are generated to address safety to crops and the subsequent rotational crops for which a product is intended (EU Commission, 27 July 1993). This approach has been used successfully for insecticides and fungicides in general, and for herbicides to address in-field plant safety.

In screening studies, plants are sprayed at the maximum application rate of the product at plant growth stages typical of product use and assessed for visual injury. Since different companies may use different techniques and different rating systems to develop these data, the OECD has proposed guidance (OECD, 2000; Annex III) on what information should be supplied by the registrant and how the data can be normalized to provide uniformity in the hazard assessment.

For herbicides, two regulatory methods are being proposed to assess effects (OECD, 2000). One method assesses effects to seedlings via exposure through the soil, while the other assesses effects to young plants (two to four leave stage) via exposure through the foliage. In most cases, exposure via the foliage produces higher sensitivity, and for regulatory purposes, these data, rather than soil exposure data and seedling emergence, have been primarily used in Germany. There may be exceptions to this general rule, and in cases where the product may show pre-emergence soil activity, tests using soil exposure and seedling emergence may be conducted preferentially.

The test duration is between 14 and 21 days, depending upon the species and growth of the control group. Six species, 2 monocotyledon and 4 dicotyledon species, from the list of species shown in Table 1 (OECD, 2000) are used. The species used in these tests are intended to provide a range of response, similar to other ecotoxicological tests and not to act as taxonomic surrogates. Therefore, several species that are known to be sensitive to the herbicide are tested, as well as a tolerant species. In this fashion, inter-species response may vary as much as a factor of 1000-fold plus, from the most sensitive to the most tolerant species tested.

At the end of the test, the plants are assessed for visual injury (e.g., chlorosis, leaf curling, shoot height, etc.) and biomass (fresh or dry weight). Since a plant species is tested at several concentrations, an EC_{50} and/or EC_{25} are determined. The most sensitive species end-point is then used for the safety assessment.

Table 1. List of species recommended for use in plant tests

Family	Species	Common names
DICOTYLEDONAE		
Chenopodiaceae	*Beta vulgaris*	Sugar beet
Compositae (Asteraceae)	*Lactuca sativa*	Lettuce
Cruciferae (Brassicaceae)	*Brassica alba*	Mustard
Cruciferae (Brassicaceae)	*Brassica campestris* var. *chinensis*	Chinese cabbage
Cruciferae (Brassicaceae)	*Brassica napus*	Oilseed rape
Cruciferae (Brassicaceae)	*Brassica oleracea*	Cabbage
Cruciferae (Brassicaceae)	*Brassica rapa*	Turnip
Cruciferae (Brassicaceae)	*Lepidium sativum*	Garden cress
Cruciferae (Brassicaceae)	*Raphanus sativus*	Radish
Cucurbitaceae	*Cucumis sativa*	Cucumber
Leguminosae (Fabaceae)	*Glycine max (G. soja)*	Soybean
Leguminosae (Fabaceae)	*Phaseolus aureus*	Mung bean
Leguminosae (Fabaceae)	*Pisum sativum*	Pea
Leguminosae (Fabaceae)	*Trifolium ornithopodioides*	Fenugreek/Birdsfoot trefoil
Leguminosae (Fabaceae)	*Trifolium pratense*	Red Clover
Leguminosae (Fabaceae)	*Vicia sativa*	Vetch
Solanaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae (Apiaceae)	*Daucus carota*	Carrot
MONOCOTYLEDONAE		
Gramineae (Poaceae)	*Avena sativa*	Oats
Gramineae (Poaceae)	*Hordeum vulgare*	Barley
Gramineae (Poaceae)	*Lolium perenne*	Perennial ryegrass
Gramineae (Poaceae)	*Oryza sativa*	Rice
Gramineae (Poaceae)	*Secale cereale*	Rye
Gramineae (Poaceae)	*Secale viridis*	Rye
Gramineae (Poaceae)	*Sorghum bicolor*	Grain sorghum
Gramineae (Poaceae)	*Sorghum vulgare*	Shattercane
Gramineae (Poaceae)	*Triticum aestivum*	Wheat
Gramineae (Poaceae)	*Zea mays*	Corn
Liliaceae (Amarylladaceae)	*Allium cepa*	Onion

THE CONSERVATIVE NATURE OF TERRESTRIAL NON-TARGET PLANT EFFECTS TESTING

Key in any assessment, is the reliability of the data and the uncertainty which may exist in extrapolating laboratory data to the environment. In conducting non-target plant tests in the glasshouse/laboratory, there are numerous factors that make this test very conservative in nature and subsequently the assessment as well. The factors to consider and the contribution each factor may contribute to an overly conservative estimation of effects in the environment are as follows (GCPF NTP Work Group, 2001) and are summarized in Table 3. Overall, a 100 to 6000 over estimate of effects is expected based on current test methods.

Exposure (spray drift versus drench application)

Non-target plant testing is conducted to assess the safety of crop protection products (CPP) to plants growing outside the agricultural unit (*i.e.*, the treatment area, plus some small area around the field (EPPO, 2000). However, there is a significant discrepancy between the exposure used in the glasshouse test and potential exposure in the real world via spray drift. In the glasshouse study, plants are treated using some form of sprayer that normally simulates overhead hydraulic spraying as provided by a field tractor spray and utilises normal application spray volumes – approximately 200 L/ha.

Although a range of active ingredient dose rates is tested, no variation in spray volume is used. For example, if the predicted spray drift in the field for ground applications were estimated to be 1% of the application rate, a predicted spray drift of 2L/ha would be expected. It is possible therefore that the greenhouse testing procedure provides for a worse case situation whereby the use of higher spray volumes in the glasshouse results in better spray coverage and therefore an overestimate of activity which may be due to drift. Limited data (GCPF NTP Work Group, 2001) indicate that by using reduced volumes to simulate drift injury can be over estimated using standard high volume techniques by a factor of 2 to 10. More research is needed to develop an understanding of the relationship between plant response from high volume exposures versus drift exposures.

Comparison of lethal and non-lethal effects

While the EC_{25} or EC_{50} may be used to assess plant safety, a 50 or 25% effect does not mean that plant survival will be impacted. Using available regulatory data, a determination of the ratios between an EC_{25}, EC_{50} and EC_{80} was made. The slope was determined and an estimated treatment rate necessary to produce mortality (e.g., LC_{50}) versus a transient effect (EC_{50}) (GCPF NTP Work Group, 2001). This comparison was made for both seedling emergence studies and vegetative vigour studies (Table 2) indicating that the EC_{80}/EC_{25} ratio is between 10 and 20. The EC_{80}/EC_{50} ratio as well as the EC_{50}/EC_{25} ratio for these endpoints is about 3.

These results indicate that if the EC_{80} is representative of a lethal effect, the safety provided between a regulatory evaluation end-point (e.g., EC_{50}) and the lethal effect level can be as large as a factor of 10 to 20.

Table 2. Comparison of EC_{25}, EC_{50} and EC_{80} (lethality estimate) for several products

Seedling Emergence

Endpoint	EC_{80}/EC_{25}	No. of Chem.	EC_{80}/EC_{50}	No. of Chem.	EC_{50}/EC_{25}	No. of Chem.
Survival	31	2	2.2	2	9.9	3
Visual	9.9	4	3.2	4	2.5	4
Emergence	3.9	1	2.1	1	1.9	1
Plant Ht	24	13	5.4	14	3.3	15
Plant Wt	12	14	3.2	14	3.1	14
Mean	16.2		3.2		4.1	

Table 2. Continued

Vegetative Vigour

Endpoint	EC_{80}/EC_{25}		EC_{80}/EC_{50}		EC_{50}/EC_{25}	
Survival	4.6	6	2.2	6	2.1	6
Visual	8	6	2.9	6	2.3	6
Emergence						
Plant Ht	10	18	3.2	18	2.7	18
Plant Wt	9.6	23	3	23	2.7	23
Mean	8.1		2.8		2.5	

Effect of soil pasteurization on non-target plant test results

Soil Pasteurization is sometimes used by researchers, *in lieu* of fungicide seed treatments, to reduce the potential for soil- or water-borne pathogens to cause bacterial, fungal or viral infections of plant seedlings resulting in either mortality or damping-off effects of the test plants. While this may have less of an effect on the results of a vegetative vigour study where test material exposure to the plant is through the foliage, it can have significant effects on plant responses observed in the soil emergence study.

For those test materials which are degraded primarily by microbial or extra-cellular enzyme degradation mechanisms, the observed plant responses can be overly conservative, especially if plant exposure at a given soil concentration must be prolonged to produce the observed effect. Therefore, using un-Pasteurized soil could reduce the level of effect by a factor that is related to the rate of product bio-degradation, but a fungicide may be required to prevent pathogenic effects.

Greenhouse versus field effects

Various studies have shown that greenhouse-grown plants are more susceptible to herbicide injury than plants grown in the field, i.e., a higher application rate is required to cause injury to field grown plants (Fletcher, *et al.*, 1990; De Ruiter *et al.*, 1994; GCPF NTP Work Group 2001). The difference in susceptibility has been attributed to physical and metabolic differences between plants raised in the greenhouse and field, differences in dissipation/degradation characteristics of the product in greenhouse versus field conditions, plant age and structure, cuticle thickness, and other factors. Based on these studies an over estimate can range from 2 to 30 fold (GCPF NTP Work Group, 2001).

Decreasing sensitivity to herbicides based on increasing plant age/size

Regulatory testing requires the use of an early plant growth stage. This, in part, is because smaller plants allow for uniform coverage of the test plants with the spray solution, provide reproducible plant growth stages, allow for rapid production of plants for testing, test a growth stage sensitive to the CPPs and represent the worst-case condition (Brandt, 2000). Several studies (Klingaman *et al.*, 1992; Blackshaw, 1991; Wicks *et al.*, 1997; Rosales-Robles *et al.*, 1999) have shown that differences in plant age compared to very early growth stages can account for a 3- to 5-fold higher sensitivity in younger plants.

Table 3. Summary of factors contributing to the conservative nature of non-target plant tests

Test component	Factor
Exposure (drench in test versus drift in field)	Sophisticated tests to evaluate this are limited, but early indications suggest that a study performed using drift type exposure (patchy exposure of mainly the upper plant parts) exhibits half the level of effect as a study where there is thorough coverage of the complete plant. A factor of 2 or more.
Non-lethal (EC_{25}) versus lethal (EC_{80}) end-point	In going from an EC_{25} to an EC_{80}, an 8- (mean for vegetative vigour tests) to 16-(mean of seedling emergence tests) fold higher rate is needed. However, an EC_{80} is not equivalent to a lethal dose. It's justified to suppose a factor of 10 to 20 for the difference between the observed non-lethal endpoint and a lethal endpoint as used for all other groups of organisms in basic risk assessments for ecotox.
Greenhouse versus field	Between 3- and 30-fold, in order for the same level of effect shown in the greenhouse to be observed in the field.
Plant age	Between 3- and 5-fold less sensitive at later plant growth stages.
Total range of factors	180 to 6000

Inter-species differences

It is generally assumed that an uncertainty factor must be attached in any assessment due to differences in species and the question of whether on not the most sensitive species has been tested. However, based on a review of 11 herbicides, representing 9 different chemistries and 8 modes of action, it was demonstrated that use of the most sensitive crop species from regulatory tests provides an adequate margin of protection for all of the other non-crop species tested with that herbicide (McKelvey, *et al.*, 2001).

As such, the regulatory tests conducted using crop species provides an indication of the range of response that could occur in the field on non-target species. Additionally, using the current approach suggests that an uncertainty factor of 1 can be used to provide an adequate level of protection in performing a risk assessment. A typical case for one product for both pre-emergence and post-emergence tests is shown in Figures 1 and 2.

EXPOSURE

Risk is a function of both hazard and exposure and the more important component of risk assessment is exposure assessment as it can be modified by changes in how the product is used.

Any risk assessment proposal needs to focus on the exposure assessment. For terrestrial plants, there is no currently accepted EU method of exposure estimation, however, the EPPO risk assessment (EPPO, 2001) proposes to use the data generated by Ganzelmeier *et al* (1995) or the data by Rautman (2000) which takes into account drift reduction technology.

As mentioned earlier, consideration of the type of foliar exposure used in the laboratory versus the type of exposure that a plant may encounter (i.e., drift) needs to be considered in higher tiers of a risk assessment. Additionally, it needs to be considered that every application will not necessarily drift off-target and interception by the three dimensional nature of plants will diminish the amount of CPP potentially drifting much faster with distance than is predicted by the Ganzelmeier or Rautman exposure tables. These factors will add to the conservatism of the risk assessment.

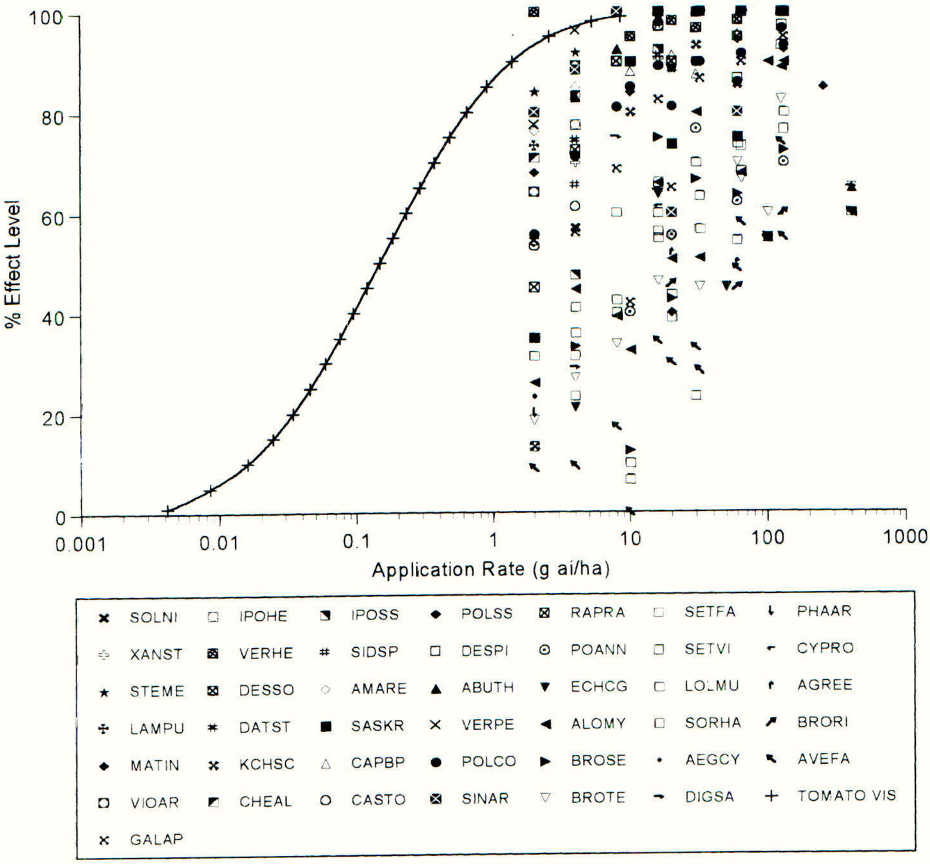

Figure 1. Pre-emergence data comparison for a sulfonylurea herbicide between the response for the most sensitive regulatory species (line) and several non-domesticated plant species (symbols)

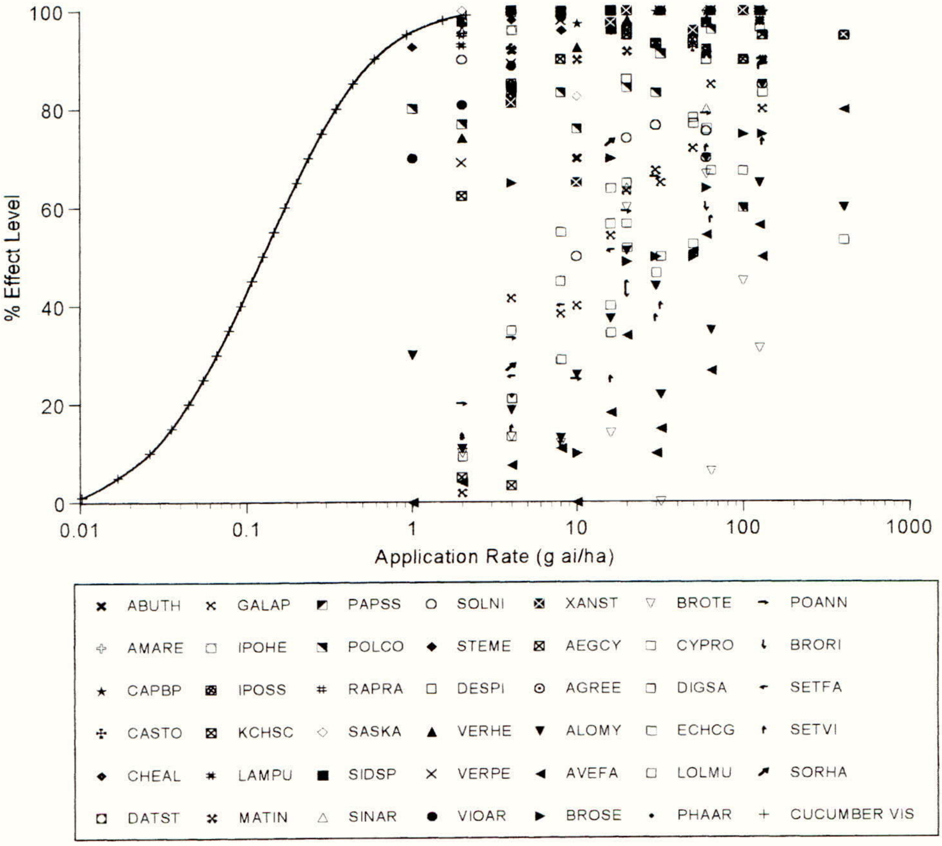

Figure 2. Post-emergence data comparison for a sulfonylurea herbicide between the response for the most sensitive regulatory species (line) and several non-domesticated plant species (symbols)

CONCLUSIONS

Proposed terrestrial non-target plant tests are designed to be conservative in nature, and it is estimated that the effects observed in laboratory tests versus the field will be overly conservative by a factor of 100 to 6000 depending upon the product. A comparison of sensitivities for several typical domesticated species used for proposed regulatory tests to non-domesticated species indicates that the most sensitive regulatory species from those tests is as sensitive as any of the non-domesticated species tested. This comparison plus the conservative test design and the assumptions used in the exposure assessment suggests that an uncertainty factor of one or less should provide adequate protection to non-target plants.

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