SESSION 5A

ECOTOXICOLOGY: SCIENTIFIC BACKGROUND AND CHALLENGES FOR REGULATION OF CROP PROTECTION PRODUCTS

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Environmental risk assessment of plant protection products – issues and challenges facing the regulatory community within the European Union.

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ABSTRACT

The paper outlines the current environmental regulatory procedure in the EU and highlights key challenges that face the assessment of plant protection products. In particular it highlights that further work is required to try and understand the regulatory trigger values used and how to use additional data appropriately. It also highlights challenges regarding qualitative and quantitative approaches to risk assessment.

INTRODUCTION

The risk that a plant protection product poses to the environment is assessed as part of the regulatory procedure. This assessment uses data from a wide range of exposure and effects studies. The output from these studies is then combined in an agreed risk assessment process to determine the risk. The risk is then compared to regulatory trigger values and depending on whether or not the trigger value is breached determines if further work is required before a decision can be made as to whether or not the product can be used. Whilst the data requirements and risk assessment process have been established for many years, science does not standstill, nor do people's concerns regarding the potential impact of plant protection products on the environment. In addition there are many issues with the current data requirements and risk assessment process that raises many challenges for the regulatory process. This paper will outline the background to the current regulatory procedure as well as highlight some of the key challenges that are facing the regulatory process.

BACKGROUND TO THE ENVIRONMENTAL ASSESSMENT OF PLANT PROTECTION PRODUCTS

Currently in the European Union pesticides are assessed via the EU Directive 91/414/EEC (see Anon, 1991). This Directive, and the associated Annexes, covers the risk to the operator, consumer and the environment. Annex II of 91/414/EEC outlines what data are required on the active substance, whilst Annex III of 91/414/EEC outlines the data required for the associated product. Annex VI, or the Uniform Principles, outlines, amongst other issues, the decision making criteria that need to be considered prior to an active substance being placed on Annex I and the associated product being authorised for use.

The risk to the environment covers both the fate and behaviour of an active substance and associated product (i.e. exposure) as well as possible effects of the active substance and/or product on non-target organisms (i.e. toxicity). Non-target organisms considered under 91/414/EEC include the following:- birds, mammals, aquatic life (including fish, aquatic invertebrates, algae and aquatic plants), non-target arthropods, honeybees, earthworms, soil

macro-invertebrates, soil microbial processes and terrestrial non-target plants. For further details see Anon (1991).

The above data are used to carry out regulatory assessments. These assessments are conducted in a standardised way to ensure that comparable decisions are made between active substances and associated products. Further information on the assessment processes used is provided in the introduction to Section 10 of Annex III. For example, reference is made to EPPO/Council of Europe schemes for environmental risk assessment (OEPP/EPPO, 1993). Further information is also provided in the associated guidance documents. For example, SANCO/4145/2000 (Anon, 2002a) provides details on how to carry out a risk assessment for birds and mammals as well as how to refine the risk should this be necessary. In a similar fashion, the exposure estimates are determined in a consistent manner using agreed models. For example, in determining whether groundwater may be contaminated by either the active substance and/or its' associated metabolites the FOCUS groundwater model is used. Likewise, the FOCUS surface water model is used to provide a range of 'predicted environmental concentrations' (PECs) in streams, ditches and ponds that are then used in the ecotoxicological risk assessment. (For further details on FOCUS see FOCUS, 1997 & 2001.)

Data on the toxicity and exposure are combined to determine the risk. In determining the risk, *usually* a single point estimate of toxicity is compared to a single point estimate for exposure. This results in either a 'toxicity:exposure ratio' (TER) or 'hazard quotient' (HQ) which is then compared to a regulatory trigger value in the Uniform Principles of 91/414/EEC (Council Directive 94/43/EC). If the resulting TER or HQ is within the regulatory trigger value, the risk is deemed to be 'acceptable'. For example if the acute TER for birds, as determined according to SANCO/4145/2000, is greater than 10, then the risk is considered to be 'acceptable'. It should be noted that the trigger values quoted in Annex VI, or the Uniform Principles, are, with the exception of the honeybee HQ of 50, arbitrary. Putting this another way, whilst the trigger values are used to indicate 'acceptability' there is no indication of what level of protection is being afforded by these trigger values. For example, there is no indication of the protection level, in terms of number of species protected, provided by acute data on one bird species and an uncertainty factor on 10.

If, as a result of the above risk assessment, the relevant trigger value is breached then, according to Annex VI of 91/414/EEC, no authorisation can be granted ***'unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs after use of the plant protection product according to the proposed conditions of use.'*** (see Anon, 1991). Once the 'unless' clause is invoked further work in the form of an 'appropriate risk assessment', is usually required to demonstrate that the risk is 'acceptable'. This 'appropriate risk assessment' usually takes the form of further information on either the toxicity of the compound or the exposure of non-target organisms to the compound. Determining whether 'no unacceptable impacts occur' is extremely difficult and requires a definition of what an 'acceptable impact' actually is.

In determining whether the risk from this 'higher tier', or 'refined', risk assessment is acceptable, requires an interaction between several areas as 'acceptability' and is a combination of social, economic, political and scientific issues. In determining acceptability there should be an interaction between decision-takers, policy-makers, scientific advisers and stakeholder representatives (Anon, 2000). Table 1 provides the definitions of these functions, whilst Table 2 provides an indication as to what organisation or individuals fall

within each category. Table 1 and 2 are purely included to provide 'working definitions' of these terms and provide an illustration as to who in the EU system fits into what category.

Table 1: Definition of the four functions involved in the regulatory process.

Function	Definition
Decision-taker	A person with the authority to take a policy decision. This may be a government Minister, or a person or body with the delegated authority to take a decision in the name of a Minister;
Policy-maker	A person or organisation charged with assisting a decision-taker in reaching a decision by providing policy analysis, generating policy options, or by conducting risk assessment (policy has been interpreted to include regulation)
Scientific adviser	A person or organisation responsible for providing scientific input to 'policy-making' or 'decision-taking'. This includes both scientists expert in narrow disciplines relevant to the problem in question, and more broadly-based scientists able to integrate several disciplines, and those within and outside the civil service
Stakeholder representative	A person or organisation representing the interests and opinions of a group such as farmers, industry and non-government organizations with an interest in the outcome of a particular policy decision. The section can also include members of the public.

Table 2: Characterisation of decision-taker, policy-maker, scientific adviser or stakeholder representatives.

Title	Organisation/individual
Decision-taker	Commission and Member States (MS) at the Standing Committee on the Food Chain and Animal Health (Pesticides Legislation) (SCFA) or Council.
Policy-maker	Individual MSs regulatory organisations and associated Ministries and Departments, Commission services
Scientific adviser	European Food Safety Authority (inc. EPCO process and the Panel on plant health, plant protection products and their residues (PPR)), scientific specialists from individual MSs.
Stakeholder representative	Non-governmental organisations, conservation organisations, European Crop Protection Association, farmers and growers etc.

The above provides a very brief outline of scientific data, the risk assessment process as well as the regulatory procedure, as currently practiced in the EU. Within these areas there are many challenges, some of which are considered further below.

CHALLENGES TO THE REGULATORY PROCESS

There are many challenges to the regulatory process, some are political, some economic and others social; however the ones we are interested in this paper are scientific ones. Having said that, as will be seen, some of the scientific challenges are inextricably linked to social,

political and economic challenges. Outlined below are some of the key challenges currently facing the environmental assessment of plant protection products.

Trigger values and uncertainty factors – what do they mean?

As was stated above, the trigger values used in the environmental risk assessment of plant protection products are arbitrary and on their own have no real scientific meaning. They are meant to cover uncertainties in the risk assessment and hence provide a certain level of protection; however the level of protection has never been determined as part of the regulatory process (Anon, 2002a, section 5). Whilst this is not an issue for the first tier, it can become a problem for the refined risk assessment.

For example, in trying to refine the risk assessment the following could be produced from a higher tier assessment: '1% of fish species have an LC_{50} less than the 90^{th} centile 'predicted environmental concentration' (PEC)'. Whilst this output makes sense, what is not known is how this compares with the first tier assessment. A comparison to the protection level afforded by the first tier is essential to ensure that decisions are comparable and not more or less protective. Therefore, in order to make use of this output appropriately, we need to know the protection level afforded by the first tier risk assessment where an uncertainty factor of 100 is applied to the more sensitive of two standard fish species. This latter issue applies to other areas as well.

The challenge facing regulation is to try and determine the protection level afforded by the first tier data set and the standard uncertainty factors stated in Annex VI of 91/414/EEC.

Guidance on how to refine the risk assessment

Refining the risk through extra data

There are currently several official guidance documents (see Anon, 2002a; Anon, 2002b; Anon, 2002c) and other documents produced within the EU plant protection product review process (e.g. opinions of the Panel on plant health, plant protection products and their residues [PPR] of the European Food Safety Agency [EFSA]) aimed at helping both Notifiers and Regulators carry out a risk assessment. Whilst these provide invaluable information on what can be done to refine the risk assessment, they do not necessarily provide the whole answer. For example, the outputs from the HARAP and CLASSIC workshops (see Campbell *et al.*, 1999 & Gidding *et al.*, 2002) provide information on what can be done to refine the risk to aquatic life. However, there is a lack of information on how to interpret a mesocosm study and in particular what constitutes an 'acceptable' study, i.e. there is lack of information on the appropriate diversity and abundance of invertebrates and algae. Hence it is extremely difficult from a regulatory point of view to determine whether the study is acceptable. It is very easy to discount a study due to either a 'low' diversity of species or 'low' abundance of key species, however what constitutes 'low' is not defined. This lack of guidance on what constitutes an acceptable study is in contrast to that available for lower tier studies where the guideline provides information on every aspect of how to conduct the study.

In the EU guidance document on birds and mammals (Anon, 2002a) there is much information on how to carry out a first tier risk assessment, including possible indicator species. However, there is no information on what indicator species should be used for

refined risk assessment. There is also a lack of information on how to refine the ecological elements of the risk assessment, i.e. the proportion of different food types in the diet (PD) and the proportion of food obtained from the treated area (PT).

The issues highlighted above are not intended in any way to be criticisms of the guidance documents but merely highlight the state of knowledge regarding these key issues. The challenge, therefore, is to try and address these issues via research. For example it should be possible to determine the appropriate diversity and abundance of invertebrates that should be present in a mesocosm, likewise it should be possible to determine appropriate focal bird species for a range of crops in Europe as well as the proportion of food obtained from those crops (PT) as well as the proportion of different food types obtained from the treated areas (PD).

It should be noted that whilst the above is long and potentially expensive wish list, it is generic data which once collected can be used for all assessments. With this in mind PSD/Defra has funded work to address the questions related to focal species, PT and PD. Links to some of this work can be found at http://www.pesticides.gov.uk/approvals.asp?id=713

In addition to individual Member States carrying out research to address these points, it should be possible for Industry and Regulators to work together to try and gather data on these generic issues.

Using higher tier data

Once higher tier data, as outlined above, are available, the next challenge is how to use it appropriately. This is best illustrated by the following hypothetical example:

> *The first tier assessment assumes that the entire daily intake of food is obtained from the treated area, i.e. PT = 1. However, data are available from radio-tracking studies in relevant habitats that indicates that 90% of blue tits obtain 65% or less of their daily food intake from the treated crop and 50% of blue tits obtain 5% or less of their daily food intake from the treated crop. Therefore, it should be possible to reduce PT from the default of 1 to either 0.65 if you want to protect 90% of the blue tit population or 0.05 if you want to protect 50% of the blue tit population.*

The key question from the above assessment is – what should the PT be reduced to and why? The answer to this question is not a scientific one, but a policy or political one and relates to what is the overall protection goal of the environmental risk assessment, i.e. what is the level of protection wanted? It should be noted that the above output may not be of immediate use to a decision-taker and hence further work may be required to link this output to the protection goal of the decision-taker.

This issue flags up the importance of effective communication between scientific or risk assessor and the decision-taker.

Qualitative versus quantitative risk assessment

At the first tier, the environmental risk assessment process gives a pass or fail output. If the product and associated use passes, then that is deemed to be 'acceptable'. If however the trigger value is breached the risk has to be refined. The current output from a refined risk assessment tends to be qualitative in nature or related to arbitrary TER values.

It is not known whether a meaningful output from a refined risk assessment is really that meaningful to decision-takers as it does not provide an indication of the potential size of an impact, the frequency of the impact and how certain the assessor is about it. Therefore, there is a challenge for scientific, or risk, assessors to ensure that the output from the risk assessment is as useful as possible for decision-takers. To this end there are two initiatives focused at producing more quantitative outputs to risk assessment, one is a UK based research project funded by PSD/Defra called WEBFRAM, whilst the other is an EU funded project called EUFRAM. Details of these are provided below.

WEBFRAM is a suite of research projects aimed at developing a model framework that incorporates both variability and uncertainty into the assessment of risk to non-target species. This work is designed to:-

- improve the realism of regulatory risk assessments,
- be in line with current regulatory risk assessment methodologies,
- cover the breadth of the current regulatory assessment, (i.e. birds and mammals, aquatic life, above and below ground invertebrates),
- review and recommend the most appropriate existing mathematical/statistical approaches to addressing uncertainty and variability,
- develop a generic model framework for the assessment of non-target risks and
- develop a model that is fully web integrated and accessible via a browser interface to allow access to all stakeholders.

The suite of projects started in 2003 and will run until 2007 and includes modules on bird and mammal risk assessment, aquatic risk assessment, above ground arthropods and below ground invertebrates. There is also an overarching project that is tasked with developing the web interface.

EUFRAM is an EU project funded under the fifth framework (QLK5 - CT 2002 01346). The main work of the EUFRAM project will be done by a core partnership of 27 organizations from government, industry and academia. The main output will be a framework document that should provide basic guidance for scientific or risk assessors. This framework document should have been developed in consultation with potential end-users from Regulatory authorities and Notifiers. Further information on the above can be found at www.eufram.com.

It is hoped that the above initiatives will provide a quantitative output to an environmental risk assessment that deals with both variability and uncertainty and hence provides an indication of how big, how often and how sure.

CONCLUSION

The above highlights several challenges that face the regulatory process – some, if not all, can be addressed via more generic data on key issues and better communication between scientific or risk assessor and decision-takers. One challenge not discussed above is one that currently falls outside the regulatory process, that of indirect effects and effects on wider biodiversity. The current risk assessment is predominantly focused on direct effects, i.e. it assesses the direct toxic effects of the compound and does not consider indirect effects, or effects between trophic levels. For example, an insecticide applied to cereals may be of low toxicity to birds but high toxicity to invertebrates. The impact at the field level may be that there is no direct risk to birds, but there is a predicted impact on non-target arthropods. The impact on non-target arthropods is deemed to be 'acceptable' as it passes the ESCORT 2 (Candolfi *et al*, 2001) criteria that 're-colonisation... be demonstrated within one year'. Therefore, the overall view of the product would be that it is 'acceptable' and the risk to the environment would be low. However, the temporary removal of non-target arthropods could result in a decrease in food availability for chicks and hence there could be an effect on chick survival. This effect has been illustrated by work on the grey partridge and the yellowhammer (see Potts, 1986 and http://www2.defra.gov.uk/research/project_data/More.asp?I=PN0925&SCOPE=0&M=PSA&V=NR%3A080). This issue has been considered by the Pesticides Safety Directorate and the Advisory Committee on Pesticides in the UK (see http://www. pesticides.gov.uk/acp.asp?id =1397 for details). This debate is also ongoing in Europe. The challenge here is how this issue should be dealt with – should it be addressed within the regulatory process, in its widest definition, or should it be dealt with via other means?

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Protecting the environment at the edge of the field – risk assessment strategies for terrestrial non-target plants in the EU

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ABSTRACT

The paper provides a short introduction into the rationale for assessing the risk from pesticide use for terrestrial non-target plants. The current EU assessment scheme is explained and upcoming developments as well as open requirements are outlined. A lack of methods for obtaining information on complex effects on non-target plants and plant communities under standardised conditions is identified. To fill this gap in the assessment scheme, a research project has been initiated that is aimed at setting up artificial communities of wild non-crop plants as testing systems. First intermediate results of this project are presented.

INTRODUCTION

Why is it necessary to assess the impact of pesticides on non-target plants?

Photoautotrophic organisms, ranging from algae to lichens, mosses and ferns to higher plants constitute the basis of life on earth. Being the primary producers of energy for food chains in ecosystems, they determine the amount of living mass (other organisms) that an ecosystem can support. Beyond that nutritional function, plants serve as shelter for many forms of wildlife populations (Martin, 1951; Hartman *et al.*, 1981). Depending on landscape and soil properties, climatic conditions and further parameters like anthropogenic use patterns of a site, plants form typical communities, which further determine the composition of arthropod, bird and small mammal communities in that habitat. Any effect exerted to individual plant species or groups of species within that plant community might thus result in significant effects on structure and function of the ecosystem on the whole.

Pesticides (this term used here as a synonym for both plant protection products and their active ingredients) are designed to strongly affect certain species of living organisms and will thus significantly alter the agro-ecosystems in which they are used. Owing to their transfer to non-target areas during and after application, their effects are, however, not limited to the treatment areas. This results in a high potential for impacting individual non-target plants, plant communities, and ecosystem function and structure. Such impacts can occur as immediate and obvious damage or as changes that manifest themselves over hours, days or weeks following exposure. Although it may be difficult to distinguish pesticide-related effects on plant biocenoses in field-border structures from other effects related to agricultural activities (e.g. fertilizers), indications that non-target plants can actually be affected by pesticide use are documented in the literature (Kleijn & Snoeijing, 1997). Effects of sub-

lethal herbicide doses on non-target plants are influenced by a diversity of factors (Follak & Hurle, 2002) and may affect physiology, growth, reproduction or competitiveness of the plants (Obrigawitch *et al.*, 1998, Marrs 1992, Dunker *et al.*, 2002). For example, application of herbicides was correlated with a shift from dicotyledonous to monocotyledonous plants in field border structures (Marrs & Frost, 1997; de Snoo & van der Poll, 1999). This type of effect was considered responsible also for shifts in the arthropod community in those structures like the decrease of pollinators (Roß-Nickoll *et al.*, 2004). On the other hand, sub-lethal effects of herbicides are often observed only temporarily, because the plants possess the ability to recover (Zwerger & Pestemer, 2000).

It is laid down in Council Directive 91/414/EEC (Anon., 1991) that a pesticide may only be authorised in the EU if it can be proven that 'it has no unacceptable influence on the environment, having particular regard to the following considerations: – its fate and distribution in the environment, particularly contamination of water including drinking water and groundwater; – its impact on non-target species'. The protection goal does include all environmental compartments and biocenoses (except, of course, the target organisms to be controlled by the use of the plant protection product); this is clearly reflected in the definition of the term 'environment' in Directive 91/414/EEC, which comprises 'water, air, land, wild species of fauna and flora, and any interrelationship between them, as well as any relationship with living organisms'.

How is the standard assessment currently done?

The data requirements for testing the toxicity of active ingredients and formulated plant protection products for non-target organisms are defined in Annexes II and III of Council Directive 91/414/EEC, following, in principle, a tiered approach. In the first tier, a limited number of species, serving as surrogate species for the respective entire biocenoses, are tested for their response to the chemical under standardised laboratory conditions. According to the uniform principles in Annex VI of the Directive, these endpoints are combined with estimated or modelled exposure concentrations to yield risk quotients as the basis for decision-making. To account for numerous uncertainties, e.g. regarding the actual representativeness of test species, the appropriateness of observed biological endpoints, the extrapolation from laboratory to field conditions etc., safety factors need to be considered. Higher-tier tests and/or exposure modelling under more realistic conditions may be performed in order to reduce some of these uncertainties and to refine the risk assessment.

Currently, terrestrial plants are mentioned in the text of the data requirements only in a non-specific manner under point IIA-8.6 'Effects on other non-target organisms (flora and fauna) believed to be at risk' and point IIIA-10.8 'Available data from biological primary screening in summary form'. Th e current text of Annex VI regarding ecotoxicology contains no reference to terrestrial non-target plants. To overcome this regulatory gap, procedures for assessing the effect of plant protection products on terrestrial non-target plants were first developed on a national level (e.g. in Germany: Füll *et al.*, 2000). An EU-harmonised approach was introduced with the 'Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC' (Anon., 2002).

The tiered approach starts with screening data for at least 6 species from different taxa tested at the highest nominal application rate. These data should be supplemented by further information on efficacy, selectivity, phytotoxicity, etc. included in the biological dossier or

obtained from other relevant sources. If a potential risk is identified, Tier 2 requires that dose-response bioassays are conducted according to either the current OECD Guideline 208 (OECD 1984) or the US EPA OPPTS 850.4100 and OPPTS 850.4150 (US EPA, 1996a, b). For herbicides and plant growth regulators, the assessment might start directly at Tier 2. The exposure assessment for the calculation of Toxicity Exposure Ratio (TER) values is in principle based on the spray drift values provided by Ganzelmeier *et al.* (1995) or other distribution models where relevant.

It is important to notice at this stage that the test species according to the current OECD Guideline 208 as well as to the EPA Guidelines comprise only crop plants, since the potential damage to neighbouring crops is an older concern than the potential impact on non-crop plants in the non-target area. In the context of environmental risk assessment, this testing concept raises several concerns, which have been summarised by Davy *et al.* (2001) as follows:

- Currently 10 terrestrial crop plant species are tested and used to represent the highly diverse terrestrial plant species (>30,000) that exist in the environment.
- Crop plants serve as surrogates for non-crop or native plant species.
- Annual plants serve as surrogates for perennial or woody plants.
- Early growth toxicity serves to predict reproduction and survival.
- Laboratory results are extrapolated to field conditions.
- The most sensitive plant stage(s) of growth is tested.
- The measurement endpoints do not consider the mode of action.
- The relevance of biochemical and/or physiological sub-lethal effects is unknown.

Which options are currently available for a refined risk assessment?

The number of at least six tested plant species is relatively high in comparison to the standard number of tested species in other assessment areas. Therefore, the Guidance Document on Terrestrial Ecotoxicology offers the possibility of a probabilistic risk assessment based on a species sensitivity distribution (SSD) already in Tier 2. However, the statement that 'if the ED_{50} for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable' is considered not fully appropriate in current assessment practice. There is in fact a reduction of uncertainty with respect to individual susceptibilities of plant species, but other important sources of uncertainty remain, e.g., extrapolation from laboratory to field conditions, effects on reproduction or effects on the plant community. Therefore, judging from current knowledge, the application of a safety factor is normally still required for probabilistic plant risk assessments based on SSDs.

Besides the possibility of a probabilistic risk assessment, only semi-field or field assays are discussed in the Guidance Document on Terrestrial Ecotoxicology as a potential refinement approach. The observation of effects on natural plant communities under field conditions (effects on plant abundance and biomass production at different field exposure levels) is in fact considered to address many of the uncertainties related to standard testing, as named above. However, it is already mentioned in the Guidance Document that such studies are not only time-consuming and expensive, but also are not standardised. Having just the background data from standard seedling emergence and vegetative vigour testing with individual plants available, there is only a limited basis for a scientifically sound assessment of possible effects on plant communities under complex field exposure conditions. With respect to the transferability of observed effects between different plant communities, more

information on sub-lethal effects, recovery and the impact of competition under standardised conditions is required than currently available. The experience with terrestrial plant risk assessment over recent years has confirmed that field or semi-field testing cannot be considered a practical approach for refining the risk assessment under the current conditions.

How is the assessment approach to be further developed?

Further development of the approach for assessing the risk to terrestrial non-target plants takes place on different levels at the moment. In the course of the upcoming revision of Council Directive 91/414/EEC, also the data requirements in Annexes II and III and the assessment and decision criteria in Annex VI are currently being revised. The new version of Annexes II and III will contain specific data requirements for testing the effects on terrestrial plants, thereby considering in principle the tiered approach as set out in the Guidance Document on Terrestrial Ecotoxicology. Similarly, a new Annex VI will contain the assessment and decision-making scheme from that Guidance Document. This is to be understood as the necessary legal fixation of the current assessment practice with some minor amendments.

With respect to actual testing, the new Annexes II and III make reference to the respective OECD Guidelines 208 (revised from old Guideline 208, now addressing only seedling emergence and growth) and 227 (new guideline, addressing vegetative vigour). Both guidelines are currently available as drafts from September 2003. One major development as compared to the old version of the OECD Guideline 208 is the inclusion of possible non-crop species in the testing scheme.

As is obvious from the discussion above, the revision of the Annexes of Council Directive 91/414/EEC and of the OECD test guidelines will provide the legal basis for performing risk assessments according to currently agreed standards, but will only in part address the gaps and shortcomings of those. The extension of the catalogue of potential test species addresses one of the above mentioned main concerns with regard to the current assessment practice. However, a need is still seen for extending also the catalogue of testing methods, in order to gain access to further information that is necessary for evaluating aspects like competition and recovery within the risk assessment.

The paper of Davy *et al.* (2001) addressed to the US EPA and the Canadian Pest Management Regulatory Agency suggests a comprehensive testing and assessment scheme. Amendments as compared to current practice include the kind and number of tested species, considering non-crop plants as well as additional plant families, further endpoints for sub-lethal and reproductive effects, multi-species testing and a complex 4-tiered assessment scheme. All those amendments are based on a scientific rationale that can in principle be supported also by EU authorities. However, considering the general structure of EU data requirements, it is deemed more feasible on an EU level to address some key issues first, in order to fill the gap between standard testing and field testing and enable scientifically sound higher-tier risk assessments. In that context, the German UBA has sponsored a research project that was aimed at three of those key issues; the inclusion of relevant non-crop plants in the assessment scheme and their testing in artificial communities to assess recovery effects under more realistic but still standardised conditions. In the following text, some intermediate results of that research project shall be presented.

SELECTION OF SPECIES FOR TESTING

The central aim of the research project 'Development of an extended method for assessing the risk to terrestrial plants exposed to plant protection products and their active ingredients' is to set up artificial communities of wild non-crop plants as testing systems. In order to identify appropriate plant species, a great number of germination and growth tests had to be performed. According to information from the literature on the occurrence of plants in field-border structures in Germany, a list of 1091 species had been compiled. For at least 231 species, seeds were commercially available; so they could be tested in the greenhouse. Seeds were not pre-treated and the conditions for germination and seedling growth (substrate, nutrient and water supply, temperature and light regime) were the same for all species, in order to achieve a maximum of standardization. Nevertheless, adaptation of germination conditions should always be considered if a certain species should be strongly required in the test system but will not germinate well under standard conditions. The main applicability criterion was set to 50 % germination in a three-replicate test and was passed by 69 species from 15 botanical families, 54 of those species being perennial. An overview of the species distribution over the botanical families is given in Table 1.

Table 1: Overview over botanical families with species fulfilling the criterion of minimum 50 % germination

Family	Number of species
Apiaceae (Umbelliferae)	1
Asteraceae (Compositae)	18
Brassicaceae (Cruciferae)	2
Campanulaceae	2
Caryophyllaceae	9
Dipsacaceae	1
Fabaceae	6
Lamiaceae (Labiatae)	3
Linaceae	1
Plantaginaceae	3
Poaceae (Gramineae)	18
Polygonaceae	1
Rubiaceae	1
Scrophulariaceae	2
Valerianaceae	1

For informative reasons, these data were also cross-checked against Annex 3 of the revised OECD Guideline 208 and the new Guideline 227, which both contain a list of potential test non-crop species selected from literature. As regards the selection of species for actual testing, the guideline suggests the following criteria:

- accessibility to characterised test species,
- plant is amenable to testing in the laboratory, and reproducibility within and across testing facilities,
- plant uniformity,

- their distribution, abundance and taxonomic representation suggest broad coverage of the plant kingdom,
- they are sensitive to many toxic compounds and have been used to some extent in previous bioassays (their use in herbicide bioassays, heavy metal screening, salinity and mineral stress tests and allelopathy studies indicates sensitivity to a wide variety of stressors), and
- they are compatible with the environmental growth conditions and time constraints of the test method;
- they meet the performance criteria of the test.

Of the 52 species contained in the OECD Annex 3, 20 had also been tested in the research project. For 7 species, germination was assessed at least 3 times and maximum germination showed a deviation of <25 % from the OECD data. For 6 further species, agreement was less satisfactory, but it has to be considered that 2 of these species were assessed for germination success only once. For 3 species, no agreement was found, whereas 4 species could not be compared owing to missing OECD germination data (Table 2).

Table 2: Semi-quantitative comparison of germination success between own experiments and OECD data (+ <25 % deviation; O >25 %, <50 % deviation; – >50 % deviation; ? no data; * high experimental variation)

Biological name	Common name	Botanical family	Agreement
Leontodon hispidus	Big hawkbit	Asteraceae	+
Agrostis capillaris/tenuis?	Common bentgrass	Poaceae	+
Centaurea cyanus	Cornflower	Asteraceae	+
Lotus corniculatus	Bird's-foot trefoil	Fabaceae	+
Festuca pratensis	Fescue	Poaceae	+
Digitalis purpurea	Foxglove	Scrophulariaceae	+
Centaurea nigra	Black knapweed	Asteraceae	+
Lychnis flos-cuculi	Ragged robin	Caryophyllaceae	O
Cynosurus cristatus	Dog's-tail grass	Poaceae	O
Trifolium pratense	Red clover	Fabaceae	O
Prunella vulgaris	Self-heal	Lamiaceae	O
Torilis japonica	Japanese hedge-parsley	Apiaceae	O
Anagallis arvensis	Scarlet pimpernel	Primulaceae	O
Hypericum perforatum ang.	Common St. John`s wort	Hypericaceae	–
Geum urbanum	Yellow avens	Rosaceae	–
Phleum pratense	Timothy	Poaceae	–
Bromus tectorum	Downy brome	Poaceae	?
Ranunculus acris	Common buttercup	Ranunculaceae	?
Galium mollugo	Hedge bedstraw	Rubiaceae	?
Cardamine pratensis	Cuckoo flower	Brassicaceae	*

TESTING OF PLANTS IN COMMUNITIES

As described above, the UBA-sponsored research project 'Development of an extended method for assessing the risk to terrestrial plants exposed to plant protection products and their active ingredients' was initiated to fill a gap in the current assessment scheme. Whereas standard tests for seedling emergence and growth and for vegetative vigour are available for obtaining reproducible results on short-term effects for single species (dose-response relationship: e.g. NOEC, EC_{50}), no agreed procedure exists as yet for evaluating under standardised conditions long-term effects of pesticides in general or the impact of inter-plant competition on such effects and on recovery.

The chosen approach for observing long-term effects in this research project was to establish at least one artificial plant community with selected wild species representing the relevant functions of the ecosystem at risk (marginal vegetation of non-target areas). The following requirements for such a test system had to be taken into consideration:

- Reproducibility
- Standardization
- Statistical evaluation
- Representativeness for non-target plants in Germany
- Seeds commercially available
- Good seedling emergence
- Homogeneous growth of plants

In a starter experiment, a community was formed by putting closely together pots planted with *Lotus corniculatus*, *Cardamine pratensis*, *Crepis biennis*, *Dianthus armeria* and *Cynosurus cristatus* (each plant in its own pot, i.e. no competition in the root zone). The combined plants were treated with the herbicide glyphosate at 0 to 70 % of the maximum recommended application rate and phytotoxicity, shoot length and fresh weight were assessed in 14-day intervals. For control purposes, each plant species was treated similarly also in individual culture.

A clear effect of the community versus monospecies assay was visible in the untreated controls, the fresh weight of all species (except *C. biennis*) being only half as high but the height unchanged as compared to the respective individual plants alone.

Plants in individual culture were sensitive against glyphosate in the following order: *L. corniculatus* = *C. biennis* < *D. armeria* < *C. pratensis* = *C. cristatis*. In the plant community, *L. corniculatus* proved to be significantly less sensitive than all other species and shows better development than its competitors with increasing herbicide rates. This is predominantly related to fresh weight, whereas the height remains below the values from the control, i.e. growth takes places near the soil surface and is associated with strong ramification. Overall, sensitivity in the community is also lower than in individual culture for *C. biennis*, but less pronounced as for *L. corniculatus*. No change of sensitivity was observed for *D. armeria*, whereas *C. pratensis* and *C. cristatis* reacted with greater sensitivity in the community, due to increased pressure of competition.

With respect to recovery, it can be concluded from the overall results at later stages that *L. corniculatus* shows better recovery relative to the control than in individual culture. Competition did not affect recovery of *C. biennis*, but exerted significant effects on the

recovery of *C. pratensis* and *C. cristatis*, which is in line with their increased 'initial' sensitivity against glyphosate in the plant community. Figure 1 clearly shows the growth promotion of *L. corniculatus* as compared to the control in the treated plant community at higher glyphosate levels.

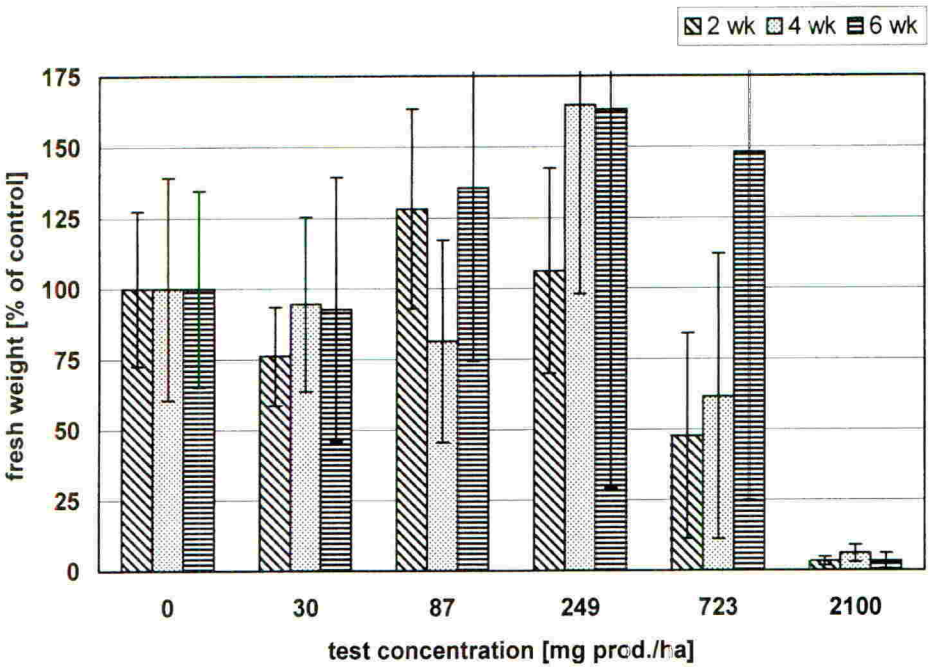

Figure 1: Development of fresh weight of *Lotus corniculatus* in a 5-plant community after treatment with different rates of glyphosate

CONCLUSION

Taking into account the screening data on germination success of wild plant seeds and their comparison with the data from Annex 3 of the OECD Guidelines 208 and 227, it can be concluded that a sufficient number of non-crop plant species fulfilling the OECD criteria are in principle available for setting up different artificial plant communities as test systems. The achieved selection covers, among other parameters, different families of mono- and dicotyledonous plants as well as different morphologies, two factors considered as important when assessing effects of plant protection products on plant communities and plant recovery after application.

A general applicability of the system for assessing complex effects of pesticides on non-target plants is already indicated by the results of a starter experiment with a community of 5 non-crop plants treated with glyphosate. Although wild plant species have a broader genetic variability than crop plants, statistically valid results can be obtained under standardised test conditions. The implementation of competition and recovery better reflects realistic

conditions. This can be realised with acceptable effort. At present, the work is focused on a test system for assessing effects on vegetative vigour, because of high variations observed for seedling emergence (time and rate) of non-crop plants.

With respect to non-target-plant risk assessment within the EU, a gap exists between standard testing according to the OECD Guidelines 208 and 227 and higher-tier testing under semi-field or field conditions. The introduced new test system has shown some potential for filling this gap by providing data on the impact of competition on long-term effects and recovery of plants under standardised conditions. This is considered a significant contribution to a more realistic but still protective risk assessment for terrestrial non-target plants as part of terrestrial biocenoses.

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The scientific issues & challenges of ecotoxicology testing for the regulation of pesticides – an industry perspective of current EU bird and mammal risk assessment scheme (SANCO/4145/2000).

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ABSTRACT

Ecological risk assessment for pesticides has been developing rapidly over recent years, particularly in the area of birds and mammals in Europe. The pace of change has led to some issues and it is important that the risk assessment methods are evaluated to see if the objectives are being met efficiently. Estimates suggest that 80% of products are failing the first tier of bird and mammal risk assessments carried out according to the latest guidance, SANCO/4145/2000 (Anon., 2002). The majority of these failures are for the long-term risk assessment. For the same compounds, this represents about twice as many failing compared to the EPPO risk assessment scheme (1992). The relative increase in conservatism shown by SANCO compared to EPPO for acute, short term and long term-risk is x 2, x 4 and x 10, respectively. As a consequence of this, the risk was compared to the most complete bird field study database available (Mineau, 2002) for those compounds where dermal and inhalation routes of exposure we not expected to influence the outcome. A 17-fold safety (or uncertainty) factor was observed when the acute Toxicity Exposure Ratio (TER) threshold of 10, derived from the acute oral LD_{50}, was compared to the lowest 90^{th} percentile of TER's for pesticides, using application rates where effects were observed in field studies. This factor increases to 25 when the Hazardous dose 5% (HD_{50}) was used. While the structure of the SANCO daily dietary dose model is scientifically sound some of the assumptions are overly conservative. The increased failure rate has resulted in the need for refinements which can be costly in terms of animal welfare and resource. Refinements, through better understanding of the exposure to focal species, are encouraged in the SANCO risk assessment scheme, but the regulatory outcome is uncertain because it is not yet known how they will be interpreted by European regulators. Both EPPO and SANCO schemes evaluate risk through oral exposure. There is a case for simply evaluating the potential for additional exposure through dermal and inhalation routes in order to determine if they are significant and requiring further attention or can be ignored. When this is done it is proposed that the protection levels for oral exposure be re-evaluated to provide a more efficient screen.

INTRODUCTION

Bird and mammal risk assessment started with Kenaga's paper in 1972 when he applied a simple daily dietary dose model for wildlife using estimates of food consumption from Nice (1938) and Kenleigh (1969). The next significant step forward was the European Plant Protection Organisation (EPPO, 1992) using estimates of field metabolic rates to provide

allometric dry weight estimates of food consumption (Nagy, 1987) for acute risk assessment only. Short and Long-term risk Toxicity Exposure Ratio's (TER's) were derived from toxicity and exposure expressed as dietary concentrations. The next major change was the introduction of the Guidance Document on Risk Assessment for Birds and Mammal under Council Directive 91/414/EEC by the European Commission (SANCO 4145/2000) in September 2002, which will be referred to hereafter as SANCO. SANCO has applied the daily dietary dose model (also called the Estimated Theoretical Exposure model) to acute, short and long term risk assessment and further refined the estimates of food consumption by taking account of the calorific content and assimilation efficiency of food types (Crocker *et al*, 2002). One of the benefits of this model is the ability to evaluate quantitatively those parameters which are most likely to influence exposure by incorporating knowledge of focal species and their ecology.

ETE/DDD Model (SANCO 4145/2000)

ETE or DDD = FIR/bw * C * PD * PT*AV

Where:

ETE = Estimated theoretical exposure.
DDD = Daily Dietary Dose
FIR = Food intake rate (g fresh weight food / day).
bw = Body weight of indicator bird or mammal (g).
C = Concentration of active substance in fresh diet (ppm).
PT = Fraction of diet obtained in treated area (between 0 and 1).
PD = Fraction of the food type (e.g. small insects) in diet (between 0 and 1).
AV = Avoidance factor (1 = no avoidance, 0 = complete avoidance).

SANCO recommends that realistic worst-case exposure of Plant Protection Products to small indicator species is assumed for the Tier 1 risk assessment, and as a result this provides conservative Tier 1 TER values. However, the net result of the SANCO procedure is that fewer test substances pass the first tier TER thresholds for concluding low risk. On the assumption that most products in commercial use present low risk to birds and mammals, which would be supported by data from the UK Wildlife Incident Investigation Scheme, this would appear to be an inefficient process for screening out potentially harmful products. Thus under the current SANCO risk assessment scheme a lot of valuable resource is potentially wasted on further evaluating low risk products, which would be better utilised on the evaluation of potential higher-risk products.

Therefore, whilst the new revised SANCO Bird and Mammal Risk assessment scheme is scientifically sound in principle, it would appear that it has not been calibrated correctly and is overly conservative, i.e. it does not adequately separate out true potential high risk products from low risk products. This results in the demand for more and more studies, many of which will be further investigating a 'theoretical risk' identified by a potentially overly conservative model rather than a true potential risk to wildlife.

This paper therefore sets out to demonstrate the increased conservatism associated with the new SANCO (2002) scheme in comparison with the former EPPO (1992) scheme and also to investigate the predictions of the SANCO scheme with field study effects analysed by Mineau (2002).

Comparison of toxicity exposure ratios (TERs) calculated using SANCO (2002) with EPPO (1992)

Most of the current pesticide registrations in Europe are based on the EPPO (1992) procedures for bird and mammal risk assessment. In the SANCO scheme, one of the major sources of conservatism comes from estimates of Food Ingestion Rate (FIR) lowering toxicity values relative to increased exposure values. This is illustrated below in Table 1 for a 10 g insectivorous bird using an application rate of 1 kg/ha. Data are typical hypothetical examples for illustrative purposes only. The SANCO endpoints for short- and long-term are calculated by multiplying EPPO endpoint with FIR/bw.

Table 1. Illustration of the change in the value of the toxicity endpoint

Toxicity test	EPPO Endpoint	FIR/bw	SANCO endpoint
Acute oral LD_{50}	100 mg/kg bw	NA	100 mg/kg bw
Short term dietary LC_{50}	500 mg/kg in diet	0.25	125 mg/kg bw/day
Long term dietary NOEL	50 mg/kg in diet	0.10	5 mg/kg bw/day

SANCO endpoints for short and long term risk assessment are now consistent with the acute endpoint in that they are expressed as estimates of daily dietary dose (mg/kg bw/day). These toxicological endpoints are used in Tier 1 risk assessments conducted according to the EPPO and SANCO schemes in Tables 2 and 3 respectively.

Table 2. Illustration of the risk (TER) using the old EPPO (1992) risk assessment procedure for a 10 g insectivorous bird.

	FIR/bw	C (mg/kg on food)	ETE (mg/kg bw /day)	Toxicity (mg/kg bw /day)	TER
Acute	0.97	29	28	100	3.6
Short term	NA	29	NA	500	17
Long term	NA	29	NA	50	1.7

Table 3. Illustration of the risk (TER) using the new SANCO (2002) risk assessment procedure for a 10 g insectivorous bird and the difference in the TER values for both schemes

	FIR/bw	C (mg/kg on food)	ETE (mg/kg bw /day)	Toxicity (mg/kg bw /day)	TER	TEREPPO /TER SANCO
Acute	1.04	52	54	100	1.9	x 2
Short term	1.04	29	30	125	4.2	x 4
Long term	1.04	29	30	5	0.17	x 10

As illustrated above, SANCO is more conservative than EPPO for short and long term risk assessments primarily because the toxicity and exposure units used in the EPPO (1992) scheme were expressed as concentrations in the diet and not estimates of daily dietary dose.

For greater realism, risk assessments for 20 Syngenta registered products (9 herbicides, 6 fungicides and 5 insecticides), all from different chemical groups, were evaluated at Tier 1 under both risk assessment schemes for risk to a 10 g insectivorous bird. The results are presented in Table 4 below.

Table 4. Percentage of compounds failing EPPO and SANCO risk assessments

Scheme	Failure Rates (%)		
	Acute	Short term	Long term
EPPO	15	10	40
SANCO	25	10	80

It is clear that the highest failure rates occurred with the long-term risk assessment and the SANCO scheme results in a doubling of the failure rate for this assessment. It should be noted that for those that failed, further study and refined risk assessment concluded low risk. All pesticides that failed the acute or short term risk also failed the long term risk assessment. This analysis is consistent with the industry as a whole where failure rates quoted at Tier 1 range from 50 to 80%. The risk assessment refinements necessary to overcome these failures include repeating the bird reproduction studies where NOEL's were maximum doses no longer useful for risk assessment; conducting time series residue trials on bird food items and making field observations to identify focal species, the proportion of time they spend foraging in crops and their diet.

Comparison of TER ratios for SANCO (2002) with field study effects analysed by Mineau (2002).

A realistic way to measure the 'conservatism' or 'protection level' for a risk assessment procedure like SANCO is to compare the estimates of risk (TER's) with field observations of effects. It is recognised that while wildlife incident data is a valuable check to see if harmful products slip through the regulatory procedures, it can underestimate the magnitude of incidents. However, field study data can probably be used to make the best comparisons. Mineau (2002) compared the toxic potential of pesticide cholinesterase inhibitors (using the HD_5 as the acute oral toxicity endpoint) with an analysis of impact reported in field studies. Mineau's paper is probably the most comprehensive review of field studies available. Impact scores were 1. no impact; 2. sub-lethal effects; 3. low mortality and 4. high mortality. Mineau concluded that dermal exposure may influence the effects observed in the field studies and should be taken into account in future avian risk assessments.

As a consequence of these conclusions, Mineau's data have been reanalysed and compare the impact scores with SANCO TER's for acute toxicity to a 10 g insectivorous bird using both LD_{50} and HD_5 values. LD_{50} values were the lowest values in the Pesticide Manual (Tomlin, 2003) and HD_5 (5^{th} percentile of the distribution of LD_{50} for all birds) from Mineau *et al* (2001). This was only done for compounds where there is unlikely to be any additional impact through dermal exposure by removing chemicals where the Toxic Potential plus the

Dermal Toxicity Index (TP and DTI), as reported by Mineau (2002), exceed the toxic potential (TP only) by a factor of 2. The field trial details used in the reanalysis (crop, rate and impact etc) were reported in Mineau *et al* (2001) as a link to the Environmental Toxicology and Chemistry web site (SETAC Supplemental Data Archive, Item ETC-21`-07-001:http://etc.allenpress.com). The field study database comprised more than agricultural crops; those generally described as grassland, cereals, leafy crops and orchards were retained for re-analysis while those generally described as forest and aquatic/wetland were rejected. The distribution of TER's together with the median and 90th percentile for products identified as having 1. no impact; 2. sub-lethal effects; 3. low mortality and 4. high mortality are presented in Figure 1. The current safety factor (TER = 10 for acute risk using the lowest LD_{50} and 1 using the HD_5), indicated by vertical solid line, is based on SANCO and the Impact Score based safety factor, indicated by a dotted line, is set at the 90th percentile of the Mineau Impact Score 2 (sub-lethal effects).

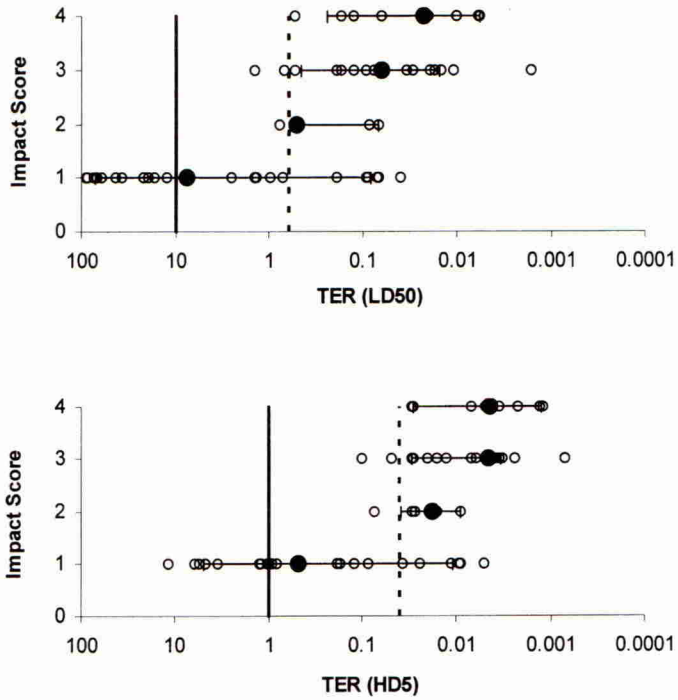

Figure 1. SANCO Toxicity Exposure Ratios (TERs) for avian acute toxicity, field observations of pesticide impact (Impact Scores) from Mineau et al. (2001) based on the LD_{50} and the HD_5. Y axis show Impact Scores where 1 = no impact, 2 = sub-lethal effects, 3 = low mortality and 4 = high mortality. Solid vertical line: the current safety factor (SANCO). Dotted vertical line: the Impact Score-based safety factor is set at the 90th percentile of Mineau *et al.*'s Impact Score 2. Open circles: observation from Mineau *et al.* (2001), filled circles: the median of observations from Mineau *et al.* (2001), error bars (horizontal) = 10th and 90th percentiles.

When the SANCO low risk threshold of 10, for acute risk using the lowest LD_{50}, is expressed as a ratio of the 90th percentile of TER's, calculated from Mineau's Impact 2 field studies, we get a value of 17, representing overprotection by this factor. Similarly, if a low risk threshold of 1, for acute risk using the HD^5, is also expressed as a ratio of the 90th percentile of TER's, calculated from Mineau's Impact 2 field studies, we get a value of 25. These ratio's indicate the degree of overprotection and will from now on be referred to as Protection Factors.

DISCUSSION

The protection factors of 17 and 25 for the LD_{50} and HD_5 above only relate to the acute risk assessment where parental toxicity results in sub-lethal effects and mortality. It is more difficult, if not currently impossible, to make the same comparison for long-term assessments because incident scheme and field study data are inadequate. However, comparison between EPPO and SANCO schemes (Table 4) indicates that the long-term assessment is more conservative than the acute and it is reasonable to expect protection levels for the long-term risk assessment to be even higher than for acute.

Conservatism in the SANCO scheme comes from several sources. For example, in the acute risk assessment these include the high allometric daily energetic expenditure for some small focal species, which when converted directly to a food intake rate without accounting for animal behaviour, can result in estimates of daily food consumption of greater than the animals bodyweight. When this is considered along with the acute oral toxicity endpoint measured for an instantaneous gavage dose, the source of the conservatism becomes clearer. Daily food intake is rarely ingested instantaneously. Clearly where the daily food intake is greater than the animals bodyweight, this will represent several times the capacity of the crop/stomach. Twelve hours represents a more realistic daily feeding period for most species leaving time for metabolism and excretion to prevent body burdens from reaching threshold levels in critical organs. While there are examples of gorge feeding where a crop/stomach may be filled within minutes to hours, this occurs in limited circumstances only when surplus food is available, i.e. a spill of treated seed.

The 50th and 90th percentile for values used in the SANCO model maybe reasonable on their own and when used to generate a joint probability distribution for exposure in a probabilistic risk assessment. However, they are quite conservative when multiplied together in a deterministic risk assessment, Cullen (1994).

There are legitimate reasons for using safety factors (or uncertainty factors) in risk assessment for screening to quickly register 'low risk' products and identify other products for further evaluation. Safety factors need to be based upon known uncertainty, for example differences in species sensitivity and ecology. In SANCO, Tier 1 safety factors (10 for acute and short-term; 5 for long-term) are only applied for differences in sensitivity betw een species and because the endpoint is a median lethal dose (not a NOEL). Ecological factors, such as PD and PT, are set to a default and extreme value of 1. The above analysis indicates that these safety factors need to be re-evaluated for a more efficient but still robustly protective risk assessment procedure.

As a consequence of frequently falling below TER thresholds, Industry has to routinely make refinements to its risk assessments. These refinements are focused on exposure, though it is

sometimes necessary to conduct additional toxicity studies. These may be very costly in terms of resource and animal welfare (or both), respectively. There are many circumstances where the bird reproduction NOEL is the maximum concentration tested. This was once sufficient for an assessment according to EPPO but is no longer so using the SANCO scheme and as a consequence tests may have to be repeated at higher dietary concentrations. In other situations it may be necessary to conduct field residue trials to understand the time course for exposure of individual products on bird food items. Producing robust measurements of ecological factors like PT (proportion of treated food in diet) and PD (proportion of different food types in the diet) requires considerable investment. There is spatial and temporal uncertainty concerning the relevance of information collected for focal species in one region and year. By including these ecological factors in the SANCO daily dietary dose model it is implied that regulatory authorities are able to interpret and accept these data as refinements in risk assessment. It is important that both regulators and Industry work together to agree a consistent procedure for identifying focal species, representative crops and appropriate bio-geographical zones within Europe. A consistent procedure needs to be agreed for the acceptance of ecological refinements which lead to refined estimates of exposure.

Both EPPO and SANCO risk assessment schemes have focused upon quantifying oral exposure on the basis that it is the major source of exposure for the majority of products and is believed to have been the source of exposure leading to most incidents in the field. However, it must be recognised that dermal and possibly inhalation exposure may significantly contribute to exposure for some products and that a simple realistic screening model is required to determine if significant exposure can be ruled out or more detailed evaluation is necessary. Once such a screen is in place, there is a very strong case for lowering the Tier 1 protection levels in the SANCO scheme as currently written.

CONCLUSIONS

The SANCO model needs to be re-calibrated to reduce false positives so that science and valuable resources are focused on products with real potential wildlife issues.

There are legitimate reasons for using safety factors in risk assessment for screening to quickly register 'low risk' products and identify other products for further evaluation. This is clearly not working at present.

Despite the SANCO risk assessment model being scientifically sound the above analysis indicates that the 'protection' level is too high resulting in unnecessary additional testing.

Further research is needed to confirm the assumptions in the model and to develop a consistent procedure agreed for the acceptance of ecological refinements which lead to refined estimates of exposure. This is particularly important since we cannot currently readily improve the ability to reduce PT and PD factors from worst case default factors due to the difficulties regulatory authorities have extrapolating from available focal species data to the bird community.

With a simple screening model in place for dermal and inhalation exposure and a better understanding of the uncertainties, there is a very strong case for lowering the Tier 1 protection levels in SANCO.

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The conservative nature of testing for ecological risk assessment of plant protection products with reference to terrestrial non-target plants

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ABSTRACT

This paper deals with testing and risk assessment of terrestrial non-target plants conducted in Europe. Focus is given to the numerous conservative aspects of the plant test (e.g., non-lethal end-points), the exposure assessment and subsequently the risk assessment as conducted under the current European Union registration requirements for plant protection products. An evaluation of the adequacy of these regulatory methods to the protection of wild species is also provided.

INTRODUCTION

In general, the safety afforded wild-species based on regulatory test methods and risk assessment is under constant scrutiny as to its adequacy. This paper takes an in depth look at one component of the total safety assessment conducted for any plant protection product (PPP) before it can be sold, terrestrial non-target plants.

Several activities have been ongoing on both the international and national level to address safety to terrestrial non-target plants (TNTPs) and the use of PPPs. On a national level, Germany requires a terrestrial non-target plant risk assessments as part of their National requirements (Füll *et al.*, 1999) and other European Union Member States are also beginning to request them. A requirement for testing of PPPs on TNTPs is now also a required part of dossier preparation in the EU as described in the Guidance on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final; 17 October 2002).

In the area of testing, the Organization for Economic Cooperation and Development (OECD) has recently revised Technical Guideline 208, for the testing of Terrestrial Plants, and has developed two test guidelines (TGs); TG 208, Terrestrial Plant Test, Seedling Emergence and Seedling Growth Test, and a new TG 227, Terrestrial Plant Test, Vegetative Vigour Test. The main purpose of the revision and new guideline is to modify the current 208 guideline to allow for both the testing of industrial chemicals and the testing of PPPs (OECD, 2005).

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 how 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 TNTPs is attained.

BRIEF REVIEW OF TERRESTRIAL NON-TARGET PLANT TEST METHODS

For non-herbicides, data available from screening and efficacy studies generated by PPP manufacturing companies are being used to address safety to TNTPs. 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 these 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 rating systems to develop these data, the data are normalized based on a scale of 1 to 10 or 1 to 100 to provide uniformity in the hazard assessment.

For herbicides, two regulatory test guidelines are being proposed to assess effects (OECD, 2005). One method assesses effects to seedlings via exposure through the soil, while the other assesses effects to young plants (two to four leaf 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 of the regulatory tests is between 14 and 21 days, depending upon the species and growth of the control group. A minimum of six species, 2 monocotyledon and 4 dicotyledon species, from the list of species shown in Table 1 (OECD, 2005) are used. Although other species (including wild species) could be used, these species in the table are preferred due to the availability of reliable seed stocks, knowledge of how to grow them in a reproducible fashion (both intra- and inter- laboratory wise), and their ability to produce reliable end-points. The species 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) and an ER_{50} or ER_{25} is determined, where appropriate. 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
MAGNOLIIDAE (Dicotyledons)		
Chenopodiaceae	*Beta vulgaris*	Sugar beet
Compositae (Asteraceae)	*Helianthus annuus*	Sunflower
Compositae (Asteraceae)	*Lactuca sativa*	Lettuce
Cruciferae (Brassicaceae)	*Sinapus alba*	White 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)	*Phaseolus vulgaris*	Dwarf bean, French bean, Garden bean
Leguminosae (Fabaceae)	*Pisum sativum*	Pea
Leguminosae (Fabaceae)	*Trigonella foenum-graecum*	Fenugreek
Leguminosae (Fabaceae)	*Trifolium corniculatus*	Birdsfoot trefoil
Leguminosae (Fabaceae)	*Trifolium pratense*	Red Clover
Leguminosae (Fabaceae)	*Vicia sativa*	Vetch
Linaceae	*Linum usitatissimum*	Flax
Polygonaceae	*Fagopyrum esculentum*	Buckwheat
Solanaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae (Apiaceae)	*Daucus carota*	Carrot
LILIIDAE (Monocotyledons)		
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)	*Sorghum bicolor*	Grain sorghum, 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

The key in any risk 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 risk assessment as well. The factors to consider, and the contribution each may give, to an overly conservative estimation of effects in the environment were discussed at a meeting of the GCPF (now CLI) NTP Working Group and presented to the US EPA Scientific Advisory Panel on non-target plants in 2001. These still hold true and are presented below and summarized in Table 3. Overall, a 180 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 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 ER_{25} or ER_{50} may be used to assess plant safety, a 25 or 50% effect does not mean that plant survival will be impacted. Using available regulatory data, a determination of the ratios between an ER_{25}, ER_{50} and ER_{80} was made. The slope was determined and an estimated treatment rate necessary to produce mortality (e.g., ER_{50}) versus a transient effect (ER_{50}) (GCPF NTP Work Group, 2001). This comparison was made for both seedling emergence studies and vegetative vigour studies (Tables 2a and 2b) indicating that the ER_{80}/ER_{25} ratio is between 10 and 20. The ER_{80}/ER_{50} ratio as well as the ER_{50}/ER_{25} ratio for these endpoints is about 3.

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

Table 2a. Comparison of Seedling Emergence ER_{25}, ER_{50} and ER_{80} (Lethality Estimate) for Several Products

Endpoint	ER_{80}/ER_{25}	No. of Chem.	ER_{80}/ER_{50}	No. of Chem.	ER_{50}/ER_{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 2b. Comparison of Vegetative Vigour ER_{25}, ER_{50} and ER_{80} (Lethality Estimate) for Several Products

Endpoint	ER_{80}/ER_{25}	No. of Chem.	ER_{80}/ER_{50}	No. of Chem.	ER_{50}/ER_{25}	No. of Chem.
Survival	4.6	6	2.2	6	2.1	6
Visual	8.0	6	2.9	6	2.3	6
Plant Ht	10	18	3.2	18	2.7	18
Plant Wt	9.6	23	3.0	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. It also serves as a means to reduce the number of weeds intruding into the study, requiring hand weeding. 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 an antifungal treatment of some kind 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). This same "hardening" of plants is observed every year by gardeners when they transfer greenhouse reared seedlings to the outdoors.

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. This is very

important from a risk assessment stand-point, as any stand of plants in the field will be comprised of plants of different age and different sensitivities. Subsequently, while some plants may be affected by an off-target exposure event, not all will and these differences will assist in the long term survival of the species.

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., 2002). 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 Figure 1.

EXPOSURE

Risk is a function of both toxicity and exposure. It is the exposure assessment that determines whether a risk exists and, in part, what might be done to mitigate a potentially unacceptable risk by mitigation of exposure. Any risk assessment proposal needs to focus on the exposure assessment. For terrestrial non-target plant assessments in Europe, the spray drift data of Ganzelmeier *et al* (1995) and the data of Rautman *et al*. (2000) are used.

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 PPP potentially drifting much farther with distance than is predicted by the Ganzelmeier or Rautman exposure tables. These factors will add to the conservatism of the risk assessment. Potential risks can also generally be managed by the use of spray drift reduction technology and/or buffers.

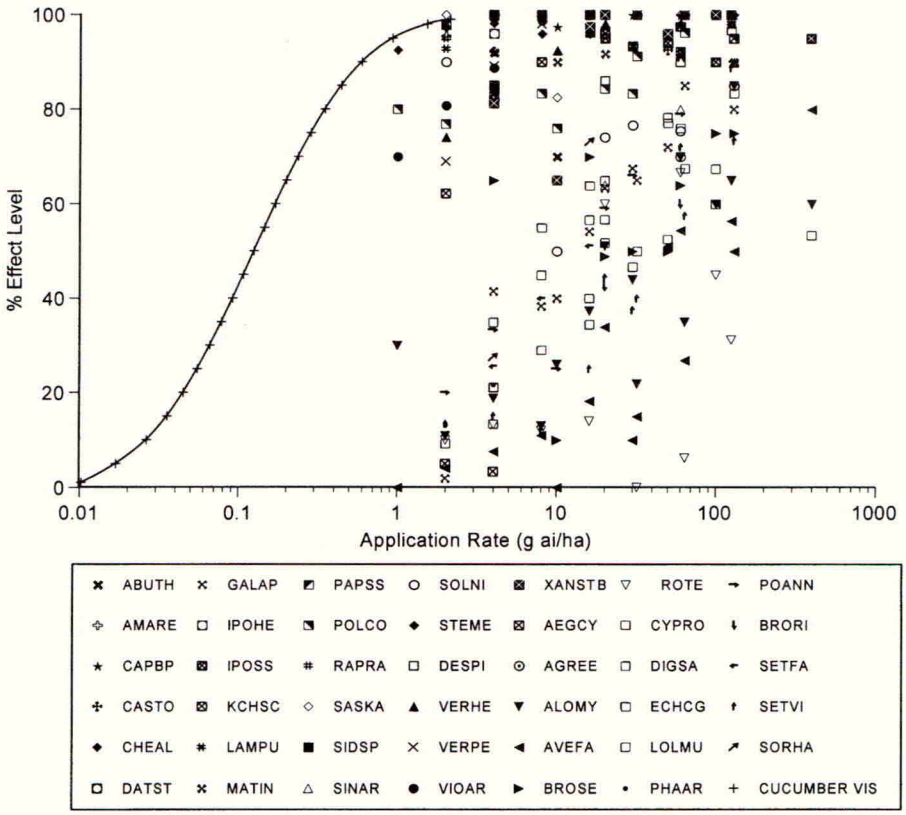

Figure 1. Post-emergence Data Comparison for a Sulfonylurea Herbicide between the Response for the Most Sensitive Regulatory Species (line) and Several Non-Domesticated Plant Species (Symbols)

OVERALL 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 180 to 6000 depending upon the product. Additionally, in the comparison of regulatory crop species to wild species for 11 herbicides, the most sensitive

regulatory species was shown to be as sensitive as any of the wild species tested. Based on this comparison plus the conservative test design and the assumptions used in the exposure assessment, the data suggest that an uncertainty factor of one should provide adequate protection to non-target plants.

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Emergence of wild species under OECD guideline conditions for terrestrial non-target plant testing

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ABSTRACT

For the assessment of the risk of pesticides to non-target terrestrial plants, proposals have been made to include wild species in studies alongside or in place of the crop plants currently tested. To examine the principle of testing wild plant species under strict GLP and guideline conditions, preliminary emergence studies with a range of proposed species were undertaken in a number of laboratories engaged in regulatory testing of terrestrial organisms. The studies indicated intra-species variation in germination and emergence both between laboratories and within seed batches. Emergence and growth rates for the majority of the wild species were inadequate to conduct biomass assessment under current guideline conditions. These findings were not unexpected and confirm the need for considerable further work to enable successful and meaningful inclusion of wild species in regulatory testing guidelines should this become necessary.

INTRODUCTION

Tests on terrestrial non-target plants (TNTP) are conditionally required for registration of plant protection products both in Europe, under Directive 91/414/EEC (Anon, 1991), and in the USA. These tests are currently conducted according to the Draft OECD guideline 208a+b (OECD, 2000). In these guidelines species are chosen from a variety of crop plants from different families, which serve to give a range of reliable responses representative of natural plant communities. Bot h guidelines have undergone recent revisions (OECD, 2003a+b), which have greatly broadened the list of plant species which could be selected for testing

with the inclusion of 150 wild plant species. These species were listed in Annex 3 of the draft guidelines, but with insufficient references on the conditions or treatment required to achieve germination. At an OECD ad-hoc committee of experts to review these guidelines in October 2004, the list was reduced to 52 species and some guidance was requested to assist germination and growth of these species.

Following a review of the revised Annex 3 in January 2005, concerns remained regarding the inclusion of many of these species. In particular, much of the guidance on treatment of seeds to achieve germination was unclear and contradictory, and for some species no information was cited. The OECD guidelines specify a minimum of 70% emergence in TNTP studies, and failure to meet such guidelines seriously compromises both the utility and validity of a test method for regulatory purposes. Whilst high and uniform levels of emergence are essential for the seedling emergence TNTP studies, it is also critical for the vegetative vigour studies where plants, prior to exposure to test substances, need to exhibit consistent and uniform plant vigour. Species showing erratic and poor emergence would not provide the necessary plant quality for the studies.

Industry therefore initiated a ring-test working group in order to investigate the suitability of the 52 species in meeting the emergence validity requirements for species in the new OECD guidelines.

MATERIALS AND METHODS

The emergence studies were carried out by four laboratories (3 in Europe and 1 in USA) that routinely carry out regulatory testing against terrestrial organisms according to OECD and US-EPA guidelines. A simple protocol was established which focused on determining germination and emergence under guideline conditions.

Forty seeds were sown for each species, with four replicate pots each containing 10 seeds sown in soil (<2% organic matter content) complying with OECD guidelines. The sowing depth for each species was as specified in references contained in the revised Annex 3. Where no information was available for a particular species, a standard depth of 2-5 mm was used. Watering was performed as described in the guideline with initial top watering followed by bottom watering. Mean glasshouse temperatures were 16-30°C and relative humidity was 60-90%. Assessment of emergence was recorded 14 and 21 days after sowing.

RESULTS

The data for emergence at 21 days was averaged across the four laboratories and is presented in Table 1 with the range of emergence (minimum – maximum) observed over the 4 facilities. These values are summarised in Table 2.

Of the 49 species tested, only one, purple morning glory (*Ipomoea hederacea*) met the OECD germination criteria of >70% emergence across all laboratories. A total of 35 species tested in two or more laboratories failed to achieve a mean emergence greater than 50% and 22 failed to achieve over 25% emergence. However, more than 50% emergence in two or more laboratories was observed for 11 species (see Table 2). Only two of these would introduce

new families to those already routinely tested in NTTP guideline studies, namely *Ipomoea hederacea* (Convolvulaceae) and *Rumex crispus* (Polygonaceae).

DISCUSSION

Based on the experiences gained from this initial ring test for emergence the following observations were made regarding the suitability of wild species for regulatory non-target plant testing.

Seed supplies

A range of wild plant species are available from commercial suppliers, however, cuckoo flower (*Cardamine pratensis)* and canada wild rye (*Elymus canadensis*) could not be accessed by any of the laboratories and consequently were not tested. canada goldenrod (*Solidago canadensis)*, purple nutsedge *(Cyperus rotundus)*, little barley (*Hordeum pusillum*), and spearmint (*Mentha spicata)* could only be sourced by one laboratory. A reliable supply of high quality seed is essential in order to run a non-target plant testing programme for regulatory purposes, and initial experience with these species indicates a potential issue with their global availability for testing.

Seed quality

Unlike seed for crop plants, none of the seed for wild plants was purchased with any guarantee of purity or viability. Canadian goldenrod (*Solidago canadensis*), little barley (*Hordeum pusillum*) and scarlet pimpernel (*Anagallis arvensis*) did not germinate, suggesting that they are unsuitable for inclusion in the guidelines without detailed guidance on germination. Other species such as common bentgrass (*Agrostis tenuis*), poppy (*Papaver rhoeas*), blackeyed susan (*Rudbeckia hirta*) did germinate, but subsequently grew very slowly. This could lead to issues determining biomass under current guideline procedures.

Considerable variation was observed in both the speed of germination and proportion of seeds which germinated. Considerable variation was observed both between laboratories and also within batches seed from the same supplier, which were supplied to more than one laboratory (*Inula helenium*, *Lychnis flos-cuculi*, *Phleum pratense*, *Geum urbanum* and *Galium* species). This result is not unexpected for wild plant species, but may cause problems for routine testing procedures.

Table 1. Mean emergence and range at 21 days after sowing for wild plant species in tests undertaken in four regulatory testing facilities

Family	Species name	% emergence	
		Mean	Range
Apiaceae	*Torilis japonica* (B)	17.5	2.5 - 45.0
Asteraceae	*Bellis perennis* (P)	30.0	20.0 - 40.0
	Centaurea cyanus (A)	51.9	37.5 - 67.5
	Centaurea nigra (P)	4.4	0.0 - 12.5
	Inula helenium (P)	25.6	5.0 - 62.5
	Leontoden hispidus (P)	28.1	2.5 - 52.5

Table 1 continued

Family	Species name	% emergence	
		Mean	Range
	Rudbeckia hirta (A)	31.3	15.0 - 47.5
	Solidago canadensis (P)	0.0	-
	Xanthium pensylvanicum (A)	53.8	50.0 - 57.5
	Xanthium spinosum (A)	10.0	2.5 - 17.5
	Xanthium strumarium (A)	37.5	0.0 - 82.5
Caryophyllaceae	*Lychnis flos-cuculi* (P)	36.3	0.0 - 92.5
Chenopodiaceae	*Chenopodium album* (A)	25.0	7.5 - 52.5
Clusiaceae	*Hypericum perforatum* (P)	0.6	0.0 - 2.5
Convolvulaceae	*Ipomoea hederacea* (A)	80.0	50.0 - 100
Cyperaceae	*Cyperus rotundus* (P)	2.5	-
Fabaceae	*Cassia* (*Senna*) *obtusifolia* (A)	15.0	0.0 - 30.0
	Lotus corniculatus (P)	45.0	7.5 - 92.5
	Sesbania exaltata (A)	61.7	17.5 - 85.0
	Trifolium pratense (P)	45.0	7.5 - 92.5
Lamiaceae	*Leonorus cardiaca* (P)	6.3	2.5 - 10.0
	Mentha spicata (P)	10.0	-
	Nepeta cataria (P)	2.5	0.0 - 5.0
	Prunella vulgaris (P)	5.6	0.0 - 20.0
	Stachys officinalis (P)	4.2	0.0 - 10.0
Malvaceae	*Abutilon theophrasti* (A)	42.5	22.5 - 70.0
	Sida spinosa (A)	41.7	37.5 - 47.5
Papaveraceae	*Papaver rhoeas* (A)	9.2	0.0 - 20.0
Poaceae	*Agrostis capillaris* (*Agrositus tenuis*) (P)	59.2	32.5 - 97.5
	Alopecurus myosuroides (A)	40.6	30.0 - 52.5
	Avena fatua (A)	62.5	27.5 - 87.5
	Anisantha tectorum (*Bromus tectorum*) (A)	58.1	35.0 - 80.0
	Cynosurus cristatus (P)	15.6	0.0 - 37.5
	Digitaria sanguinalis (A)	52.5	10.0 - 75.0
	Echinochola crus-galli (A)	60.0	30.0 - 75.0
	Festuca pratensis (P)	60.6	25.0 - 87.5
	Hordeum pusillum (A)	0.0	-
	Phleum pratense (P)	18.1	0.0 - 30.0
Polygonaceae	*Fallopia* (*Polygonum*) *convolvulus* (A)	4.2	0.0 - 7.5
	Persicaria (*Polygonum*) *lapathifolia* (A)	19.2	0.0 - 35.0
	Persicaria (*Polygonum*) *pensylvanicum* (A)	2.5	0.0 - 5.0
	Persicaria maculosa (*Polygonum persicaria*) (A)	6.7	0.0 - 12.5
	Rumex crispus (P)	61.9	20.0 - 100
Primulaceae	*Anagallis arvensis* (A)	0.0	-
Ranunculaceae	*Ranunculus acris* (P)	19.2	0.0 - 57.5
Rosaceae	*Geum urbanum* (P)	21.0	0.0 - 85.0
Rubiaceae	*Galium aparine* (A)	13.3	0.0 - 27.5
	Galium mollugo (P)	30.0	0.0 - 92.5
Scrophulariaceae	*Digitalis purpurea* (B)	30.6	0.0 - 100
	Veronica persica (A)	5.0	0.0 - 100

(A) Annual; (P) Perennial; (B) Biennial
(-) a range cannot be given (only one study, or no variation)

Table 2: Grouped mean emergence of wild plant species in tests undertaken in four regulatory testing facilities.

≥50% emergence	<50% and >25%	≤25%
Centaurea cyanus	*Bellis perennis*	*Torilis japonica*
Xanthium pensylvanicum	*Inula helenium*	*Centaurea nigra*
Ipomoea hederacea	*Leontoden hispidus*	*Xanthium spinosum*
Sesbania exaltata	*Rudbeckia hirta*	*Chenopodium album*
Festuca pratensis	*Xanthium strumarium*	*Hypericum perforatum*
Rumex crispus	*Lychnis flos-cuculi*	*Cassia (Senna) obtusifolia*
Agrostis tenuis	*Lotus corniculatus*	*Nepeta cataria*
Avena fatua	*Abutilon theophrasti*	*Prunella vulgaris*
Bromus tectorum	*Sida spinosa*	*Stachys officinalis*
Digitaria sanguinalis	*Alopecurus myosuroides*	*Papaver rhoeas*
Echinochola crus-galli	*Digitalis purpurea*	*Cynosurus cristatus*
	Trifolium pratense	*Fallopia convolvulus*
	Galium mollugo	*Persicaria lapathifolia*
		Persicaria penslvanicum
		Persicaria maculosa
		Anagallis arvensis
		Ranunculus acris
		Geum urbanum
		Galium aparine
		Veronica persica
		Leonorus cardiaca
		Phleum pratense

Note: Species tested in one laboratory only are excluded

Seed handling

A number of species were characterised by very small seeds, e.g. *Agrostis tenuis* (seed *wt* 0.07 mg), *Bellis perennis* (seed *wt* 0.09-0.17 mg), *Solidago canadensis* (seed *wt* 0.06-0.08 mg). This presented difficulties for exact counting into pots which is an important consideration for regulatory studies conducted under GLP. Difficulties were also encountered in retaining very small seeds on the soil surface during the test after top watering. This was necessary in order to meet the strict light requirement for germination for some species and may have accounted for some of the observed variation between laboratories.

CONCLUSION

Regulatory testing of any non-target species requires procedures which are reproducible, reliable and robust. These preliminary emergence studies, using the list of plant species provided in Annex 3 of the revised OECD guidelines for 208 and 227 for testing of plant protection products against TNTPs, suggest that none of these criteria would be met. Emergence was not predictable between laboratories or within batches of seed from the same supplier, and growth of a number of species was sufficiently slow to cause problems with biomass assessment. It is clear that the current guideline requirements for TNTP testing based on crop species cannot be easily applied to wild species. Considerable further work is required in order to more clearly define germination and growth characteristics of wild species in order to construct a reliable and pragmatic regulatory testing procedure for seedling emergence studies.

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