SESSION 7A

EFFECTS OF PESTICIDES ON NON-TARGET ARTHROPODS

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Papers	7A-1 to 7A-4

The value of field studies with pesticides and non-target arthropods

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ABSTRACT

Field studies represent the only reliable tool available to investigate the response of populations and communities of invertebrates to pesticides. Current experimental practices are relatively unrefined and focus on mortality as the primary end point. Field effects may be evaluated both in terms of their magnitude and their duration. Both mortality and species diversity reduction can be assessed by comparison with untreated control plots and, in the case of insecticides, by reference to a selective soft standard. The duration of toxicity can be evaluated by conducting bioassays with crop material and by demonstrating species recovery within treated plots. More sophisticated field studies incorporate semi-field tests with species not found to be naturally occuring at the site.

INTRODUCTION

Field studies represent the final stage in a sequence of tests to evaluate the effects of pesticides on non-target arthropods. Laboratory tests on glass or sand and extended laboratory tests on plant substrates are designed to screen out the harmless products. Semi-field tests expose the individual indicator species outdoors but within cages or enclosures incorporating the crop, giving more realistic residue conditions. Whilst semi-field testing is currently an under-utilised step in the sequence which can accommodate powerful experimental designs, it typically generates data for individual species of laboratory reared animals. The field study is usually the last test in the sequence and is designed to investigate potential effects on naturally occurring populations of arthropods. Historically, field studies have investigated effects of simulated commercial applications of test products on pests, predators and parasites. A review of the literature of U.K. cereal studies will reveal an emphasis on cereal aphids and polyphagous predators, mostly carabid and staphylinid beetles and linyphiid and lycosid spiders. After the Escort Workshop in 1994 (Barrett et al, 1994) there was a shift in emphasis in the regulatory requirement for testing pesticides away from "beneficial" arthropods (predators and parasites of pest species) and towards non-target arthropods (everything other than the target species).

In a single seemingly small step the objective and rationale behind the terrestrial testing of pesticides was fundamentally altered. Despite this major change in direction the indicator species selected to represent the terrestrial environment (and therefore tested in laboratory tier 1 tests) have remained predators and parasites. The value of some of these species as indicators is questionable. The predatory mite *Typhlodromus pyri* is relatively immobile and is known to have regionally differing strains resistant to different products. Unless the resistance status of tested strains is reported then the results with test products cannot be relied upon when making regulatory decisions. The carabid beetle *Poecilus cupreus* is an extremely robust Pterostichine adapted to burrowing in soil. Because of its thick cuticle and burrowing habit *P. cupreus* is not killed in the laboratory by substances which may harm smaller or more surface active species.

At the field level it is possible to consider a wider range of naturally occurring species and functional taxa other than predators and parasites. Components of the terrestrial ecosystem which appear to have been overlooked in the switch to non-targets include Collembola and soil dwelling mites, both of which can be numerous in agricultural crops and which play an important part in decomposer trophic systems (Curry, 1994).

Whilst the laboratory test will always be a worst case scenario, with artificial exposure of laboratory bred caged organisms, the field study offers realism. The exposure of the organisms to the substance will be the same as would occur if a farmer was to spray the product in commercial use. Invertebrates may receive a topical dose from overspray, a contact dose from treated surfaces or an oral dose from consuming treated prey or a combination of all three. The pesticide residue will be adsorbed onto plant and soil surfaces and degrade at realistic rates according to the weather conditions. The behaviour of the organisms will be natural and not altered or compromised by the test itself.

The disadvantages of field studies are their high cost, the risk that the data will be difficult to interpret and concern that the results will be applicable only to the locality in which the study was conducted. The skillful field scientist works to maximise the advantages and to minimise these negative factors. Two of the three disadvantages are directly affected by the selection of the study site. If a site contains low numbers of non-target arthropods or they are patchily distributed then the best study design possible will be unable to make sensible conclusions. When data are presented, for example, with mean numbers of carabid beetles per pitfall sample of less than one then it is likely that the field site was unsuitable. A good site will contain high numbers of arthropods in a situation that can be considered to represent a realistic worst case for the invertebrates present. For epigeal arthropods, a fairly sandy soil with low organic matter is desirable so that the product will not be strongly bound to the soil. For any one field study, it is sometimes necessary to pre-sample five or six possible sites so as to select one with the most promising populations. Field scientists who accept the first available site to carry out an experiment should "spray and pray". They may be fortunate and have good numbers of many relevant species or they may not be. Investment in site selection will only become apparent when the in-life phase has been completed and samples are being analysed.

One of the major drawbacks of multi-application field studies, for example with fungicides which can be applied to crops 10 or 14 times per season, is that the spraying programme starts so early in the season that arthropod numbers may not have begun to increase. In this situation, it is not possible to choose a site based on species abundance and only the previous years data can be used to indicate that a site might be acceptable.

EXPERIMENTAL DESIGN

Study size

Field studies work best for small and comparatively immobile invertebrate taxa. The more active the species under investigation then the larger the experimental plots required. When a species is capable of flight then even larger plots are needed. For highly active alate orders, such as Diptera and Hymenoptera, open field designs are often inappropriate and field cages should be considered.

For "within season" field studies in arable crops a plot size of between 1 and 2 ha has been found to work well. Such a plot size in a study with four replicates of the test substance, water treated control and a positive reference would be expected to generate interpretable data for several carabid beetle species, the major sub-families of Staphylinidae and probably one or two staphylinid species, two or three species of linyphiid spider as well as Entomobryoidea and Sminthuridae.

In an orchard arthropod study a plot size of greater than 120 trees has been found to work well for many important predatory taxa (for example predatory Heteroptera such as *Anthocoris* spp., *Orius* spp., and *Heterotoma planicornis*, Coccinellidae, particularly larvae, Chrysopidae, as well as for spiders and earwigs).

Because they are relatively sessile, predatory mites can be studied on experimental plots with only a few adjacent trees. This means that mite studies can usually include sufficient replication and may include several reference substances.

Just as important as plot size is the experimental design and the homogeneity of the study site at the start of the experiment. The UK guideline for a cereal arthropod study (PSD and HSE. 1995) proposes a design involving four replicates of four treatments in a single field. With 1 ha plots this results in a field of at least 16 ha. Working in very large fields causes its own problems. There will be species which have relationships with the field margins and others which are found only in one part of the field. Although all other aspects of the U.K. guideline work wel, it is preferable to work in four adjacent fields, each of approximately 4 ha. This results in four plots per field, each with the same amount of field margin and interplot margin. Smaller fields are also more likely to be homogeneous. Any differences between the fields can be accommodated by using a blocked design with fields as blocks.

Replication

Large scale field studies are often a compromise between a sufficient number of replicates and large enough experimental plots (Brown, 1989). For each invertebrate type there is a plot size below which immigration could occur so rapidly that the data will be meaningless. It is rarely worthwhile compromising on the size of experimental plots used in a study. If plot size is not an issue then five replicates (as typically used for mite studies) is a safe approach, four replicates (as used in arable studies) is generally acceptable, three replicates makes the data vulnerable to anomolies. Less than three replicates is clearly compromising the study with respect to replication. Where replication is an issue it can be worthwhile to include more replicates of the test substance and the water control and to reduce the degree of replication of the positive reference. In the orchard study from which Figs. 1 and 2. are taken there were ten plots each of approximately 150 trees. The study design included 4 replicates of the water control and the test substance treatment but only two replicates of the positive reference. In most cases the positive reference is being used to say "is the study capable of detecting effects which we know should occur". Since there is an in-house data base describing the effects of fenpropathrin on orchard species over many years, there is a reduced need for replication with this reference.

Treatments

When there is concern about effects of spray drift it is not uncommon for studies to include the test substance at its maximum proposed field rate and at a lower rate considered representative of spray drift. It is also necessary to include a positive reference in the study design. For cereal studies, dimethoate or triazophos applied at their commercial field rates serve as good reference products. For predatory mite studies, there is debate about the choice of reference since the field population under test may be organophosphate resistant. Mancozeb is considered a suitable reference for multi-application studies and the pyrethroid fenpropathrin will certainly act as a toxic reference for single application studies. It is a good idea to test the field populations of mites for resistance to the major pesticide groups before conducting a study. In large scale non-target studies in orchards, the number of suitable reference products is diminishing although pyrethroid insecticides usually give reproducible results as a toxic reference.

Sampling methods and frequency

Of the numerous methods available for sampling non-target arthropods in terrestrial ecosystems, each has its own strengths and weaknesses. There is no right or wrong sampling method. Different field scientists become familiar with their own preferred method and become more competent at using it to collect samples. Pitfall traps have always been the mainstay of sampling in arable studies but can be complemented by night time sweep net sampling or by D-vac sampling. Washing leaves has become the accepted method for predatory mite studies but visual observation of leaves under a binocular microscope (particularly if carried out in situ when the leaves are collected) can provide a greater insight into the distribution of a species and its prey. The key to any successful field study is to be aware of the characteristics of each sampling method and to consider these when making conclusions about the data. Often this leads to different methods which complement each other being used in the same study. Whatever methods are used, appropriate steps should be taken to avoid edge effects.

The frequency with which sampling is undertaken will depend on the objectives of the study. It is usually worthwhile sampling at regular intervals throughout an experiment to be able to interpret the results with reference to the trends in abundance that occur naturally and are demonstrated in the results from the control plots. The German BBA guideline for field trials with predatory mites in vineyards involves repeated applications of the test substance but only involves post-treatment sampling on two occasions after the last treatment date. Although the end of season results will certainly give the greatest indication of the consequences of a whole seasons use it is a risky strategy to rely on these dates alone. Early decline in mite numbers or particularly noisy data at the end of the season render the whole study uninterpretable.

Weather

Field studies are obviously at risk from adverse weather conditions. Field scientists quickly become competent meteorologists and tend not to apply treatments in the path of approaching low pressure systems. Since many arthropods are active above threshold temperatures and are

favoured by high humidity, it is worthwhile installing a weather station at each field study site. This allows a comparison of the weather at the site with previous years and with different regions. Interpretation of pitfall trap data is made much easier with reference to daily maximum temperature. Pitfall trap catch sizes are a function of abundance and activity and for most arthropods warm weather results in greater activity. The "alpine skyline" plot of invertebrate numbers in control plots (as shown for example in Fig. 3 for July and August) very often mirrors the graph of daily maximum temperature.

INTERPRETATION

The first stage in interpreting results from multi-species studies is to plot the mean numbers of the more abundant taxa collected, by treatment, on each sampling occasion.

Comparisons of the trends in the test substance treatment with those in the control and toxic reference treatment will indicate whether any harmful effects have occurred. It is often relatively clear when a product is very harmful or completely harmless since numbers will follow closely the positive reference or the control treatment. Intermediate effects are much more difficult to determine. For species which are relatively abundant in the study, the next step is to conduct appropriate statistical analyses. Pre-treatment data from more than one sampling occasion are always useful to provide a measure of the inherent variability in the test system.

Fig.1 shows numbers of *Anthocoris nemoralis* (Hemiptera: Anthocoridae) nymphs collected in inventory samples from a pear orchard study in southern France during 1997. Pre-treatment numbers were similar (approximately 40 per tree) in all three treatments on two consecutive sampling occasions. After treatment, numbers of *A. nemoralis* nymphs fell sharply in the plots treated with fenpropathrin (applied as the reference product in this study) but remained at similar levels in the control and test substance treated plots. The decline in nymphal numbers between the first and second post-treatment sampling occasions coincided with increasing numbers of adult *A. nemoralis* in the control and test substance treated plots (Fig.2). and is likely to represent final instar nymphs undergoing their moult to adults. Since the increase in adult numbers is greater than the decline in nymphal numbers it is likely that immigration of adult *A. nemoralis* occurred between the third and fourth sampling dates. The relatively shallow increase in the number of adults in fenpropathrin treated plots followed by an increase on 1 July shows that his product remained toxic to these bugs for between 12 and 16 days, after which time there was a gradual recovery. The fact that the number of nymphs remained close to zero until the final sampling occasions demonstrates an important point when interpreting such data. The period of time that numbers in a treatment remain lower than the control doesn't necessarily correspond to the duration of a toxic effect. *A. nemoralis* nymphs cannot fly and were therefore unable to re-colonise the fenpropathrin treated plots as soon as they ceased to be toxic to them. The duration of harmfulness for fenpropathrin observed in this study, of about 14 days, is similar to that found in previous studies using the same reference.

In this instance, it is relatively clear that the test substance did not adversely affect immature or adult *A. nemoralis*. Statistical analysis of these data confirmed that significantly lower numbers ($P > 0.05$) were present in samples from fenpropathrin treated plots than control or test substance treated plots until 10 July for adults and 15 July for nymphs.

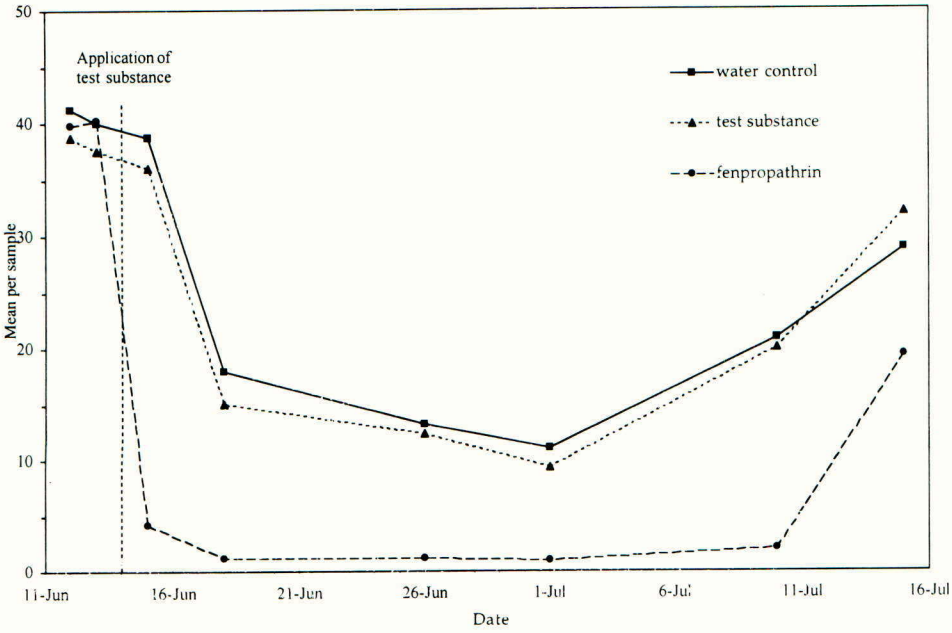

Figure 1. Mean number of *Anthocoris nemoralis* nymphs collected per inventory sample (pear orchard, France).

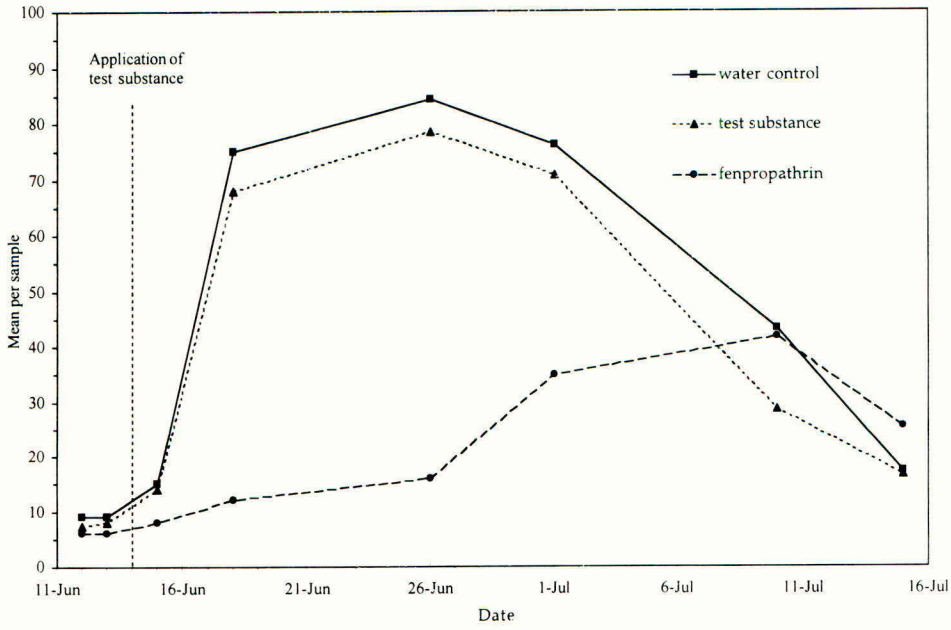

Figure 2. Mean number of *Anthocoris nemoralis* adults collected per inventory sample (pear orchard, France).

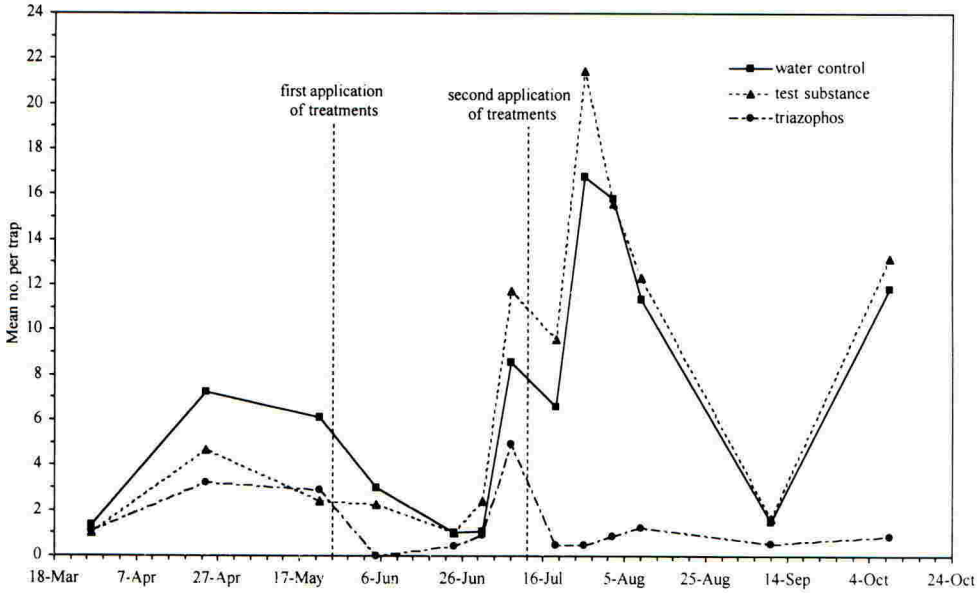

Figure 3. Mean number of *Oedothorax fuscus* per pitfall trap (grassland, Devon, England).

Field data are rarely this clear and it is true to say that these represent a particularly homogeneous population of anthocorids feeding on a large and uniform outbreak of pear psylla. Another factor which probably contributes to the clarity of these data is the sampling method used. Inventory sampling involves treating individual trees with a volatile insecticide whilst collecting sheets are in place beneath those trees. Virtually all the arthropods present in the tree fall onto the sheets and can be collected. The tree seems to represent an appropriate unit of biology to sample within a fruit orchard. Because the trees are of similar age and size they appear to contain similar numbers of the main taxa, particularly when these numbers are high. Other sampling methods which sample branches introduce a qualitative bias in that all branches are not equal and do not have the same number of arthropods on them.

Fig.3 shows more typical field data from a field study in grassland in South West England. The spring population of *Oedothorax fuscus* (Araneae: Linyphiidae) in 1996 was relatively small, with high variability between samples prior to the first treatment date. The summer population of *O. fuscus* was larger, with smaller variances about the mean values for each treatment. After the second treatment date, numbers of *O. fuscus* fell in the triazophos treated plots and never recovered. Numbers in the test substance treated plots mirrored those in the control plots.

It is sometimes tempting to draw more from a single study than can reasonably be concluded. This is particularly true in the contract industry when a sponsor may wish to tease every last drop of possible interpretation. In every multi-species field study there will be taxa sampled in very low numbers for which no conclusion can be made except to say that they were not eradicated by the treatment.

THE FUTURE

Many believe that modelling, Quantitative Structure Activity Relationships (QSARs) and more advanced single species laboratory tests will see an end to the need for field studies. The limitations of using single species tests to predict chemical effects at higher levels of organistion are clearly discussed by Forbes & Forbes (1994). Dynamic biological processes are so complex that any such model would quickly become unwieldy. Whilst it is possible that such an approach could predict major effects, it is unlikely that it would be able to detect refined effects (Mount, 1979).

The challenge facing those designing and conducting field studies is to ensure that their work doesn't also fall short of detecting the refined effects. Field studies have already begun to move away from the straightforward spray and sample approach to multi-faceted designs where a single large scale study is augmented by semi-field tests. Bioassays of treated crop material from the field site can be made in the laboratory with species such as *Aphidius rhopalosiphi* and *Chrysoperla carnea,* which might not be expected to occur in high numbers naturally in the field experiment. The interpretative power of such studies is high and they are often able to address a number of the regulatory concerns in one experiment.

Well designed and carefully conducted field studies will continue to be the final test of acceptability for new and existing pesticides. Tc make such studies easier to interpret and to reduce the quantity of misleading or poor quality data being generated there is a need for a series of comprehensive field guidelines. Each guideline should set out what can reasonably be achieved by employing a given study design and the steps to be taken to enhance the quality of the data. A few guidelines are already in existence. The UK cereal guideline was produced more than 10 years ago by a group of English experts specifically to address the effects of pyrethroids on predators and parasites in summer cereals. This could be upgraded relatively easily to make it applicable to non-target arthropods and to include reference to a wider range of taxa. A predatory mite working group is currently in the process of producing field guidelines for tests in orchards and vineyards.

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The complementary roles of laboratory and field testing in ecotoxicological risk assessment

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ABSTRACT

Despite widespread concern over possible adverse effects of pesticides on non-target arthropods, the scientific basis for evaluating and interpreting such risks is still hotly debated. Using examples drawn largely from work on carabid beetles and aphid parasitoids, this paper reviews some critical aspects of trials conducted at different levels in the testing hierarchy. For laboratory tests, multiple dose bioassays offer considerable advantages over ones at a single dose, providing a more precise comparison of effects between species and chemicals, and a more objective basis for decision making. The transition from simple laboratory bioassays to more realistic trials poses additional challenges that are best addressed in the short-term by focusing on 'semi-field' tests (conducted in the laboratory or field), allowing adequate replication and some insight into mechanisms underlying observed results. Although the primary objective is to assess mortality, such experiments can relatively easily be extended to investigate indirect and sub-lethal effects, providing an added benefit.

BACKGROUND

In the UK and elsewhere, evaluation of the risks posed by agrochemicals to non-target organisms is now an integral part of the pesticide registration process. Regulatory agencies charged with implementing and interpreting such risk assessment schemes face a number of difficulties. Firstly, it is necessary to ensure that the techniques adopted are scientifically sound and focus on appropriate indicator species. Secondly, the methodology should be sufficiently unambiguous and standardised to yield data that are at least broadly comparable between laboratories. Finally, there is the challenge of assimilating a wide range of possible laboratory bioassays and field trials into a sequential testing scheme that is as cost-effective, logical and informative as possible. Each stage in the scheme should be designed to address a complementary set of questions formulated specifically to determine the need to move from one level in the testing hierarchy to the next.

Although a number of different testing schemes have been suggested, there is general agreement that an effective scheme must encompass a succession of tests of increasing scale and complexity, with the requirement for each being based on objective decision-making criteria (Hassan, 1989; Oomen, 1998). A conditional and sequential series of tests has obvious logistical and financial advantages, and also recognises that the need for certain kinds of data depends on the results of earlier stages in a test programme; this cannot be predicted at the outset (Greig-Smith, 1991). For non-target arthropods other than honey bees, however, the exact nature and sequence of tests required, the criteria for moving between them, and the interpretation of data obtained at each stage, are still subject to much debate. The causes of this lack of agreement are undoubtedly complex, reflecting the

large number of species and interactions that could in principle be investigated, the piecemeal way in which various approaches to testing have evolved and been evaluated, conflicts over the use and definition of terminology, and the inevitable bureaucracy arising from the multitude of committees and organisations involved in this subject. Another potential problem has been the temptation towards 'over-standardisation' of the design and interpretation of test protocols for different organisms and biological systems. While it is important to ensure that tests adopted for a particular species are as standardised as possible, the optimal design and sequence of tests for different species may vary considerably, depending on factors such as generation time, mobility, and amenability to laboratory bioassays and/or field trials.

This paper reviews some of the key scientific and practical considerations for the design and interpretation of two different stages in risk assessment testing process for non-target arthropods. Large-scale field trials are not discussed, but receive emphasis in another paper in this volume (Brown 1998). Examples cited refer primarily to work conducted over the last five years on carabid beetles and aphid parasitoids. While emphasising the need for scientific rigour throughout, the paper also emphasises the importance of flexibility and pragmatism to facilitate a rapid and widespread implementation of testing protocols in accordance with relevant Governmental policy statements and EU Directives.

LABORATORY BIOASSAYS FOR INTRINSIC TOXICITY

Of all the types of test currently employed in risk assessment schemes, the 'first tier' laboratory bioassay is arguably the most critical from a regulatory standpoint since it is routinely applied to all chemicals, and is intended to identify compounds that require no follow-up testing under more realistic conditions. Its primary purpose is to quantify the intrinsic toxicity of a chemical, nearly always expressed in terms of direct mortality, although sub-lethal effects on behaviour or fecundity can often be accommodated within the same type of experiment. Based on recent developments, the three issues of greatest concern at this stage of testing are bioassay methodology, whether single or multiple doses are investigated, and how resulting data can be used to trigger (or not) a move to the next stage of the test sequence.

Design of bioassays

The availability of precise and repeatable bioassay methods has long been the cornerstone of research in arthropod toxicology. For obvious reasons, such methods have been best developed for pest species (e.g. Busvine, 1957). The most fruitful supply of methods for non-target species has been the 'Pesticides and Beneficial Organisms' Working Group of the International Organisation for Biological Control (IOBC), whose publications detail standardised bioassays for a diverse range of predatory and parasitic species, developed primarily to investigate the compatibility of chemicals with IPM programmes (Hassan, 1989; Hassan *et al.*, 1994). With suitable modification if necessary, many of these should be broadly applicable to ecotoxicological risk assessment as well. Despite its advantages, however, standardisation should not be allowed to inhibit improvement of existing methods or the introduction of new ones, especially where standard methods have been adopted without the benefit of supporting research (ffrench-Constant & Roush, 1990). Failure to tailor bioassays to the biological or physicochemical properties of pesticides (life-stage

specificity, systemicity, etc.) may also result in inappropriate routes of exposure and, potentially, grossly misleading impressions of toxicity. The most important consideration of all is to ensure that any methods adopted are carefully validated, clearly described, and incorporate appropriate controls (e.g. untreated subjects, recommended field rates and toxic standards) needed to facilitate a scientific interpretation of results.

Multiple vs. single doses

The most common practice with laboratory bioassays at present is to investigate the 'worst case scenario' of exposing individuals to the maximum envisaged field rate, and to use an arbitrary level of mortality (generally 30% or more) to trigger further tests. This has some operational advantages but is also flawed in important respects. Firstly, it is generally recognised that this approach leads to far too many high-risk classifications, and hence to too many higher tier data requirements (Oomen, 1998). Secondly, it provides no useful information on the distribution of tolerances within populations of test subjects that could be used, for example, to anticipate the consequences of changing application dosages to contend with different crops or pest species. Multiple dose bioassays offer the best prospect for overcoming these limitations, and are already incorporated into a provisional new risk assessment scheme for non-target arthropods under review by the European Plant Protection Organisation (EPPO) (Oomen 1998).

A prerequisite for implementing this EPPO recommendation is to establish the feasibility of obtaining accurate and repeatable dose-response data for a range of species likely to attain importance in risk assessment schemes. Given the plethora of published data for pest species, there has been surprisingly little work reported in this area for non-target organisms, and what has been done has generated somewhat pessimistic conclusions. Cilgi *et al.* (1996) tested several species of ground beetle (Coleoptera: Carabidae) over a range of insecticide concentrations and concluded that the high control mortality encountered was likely to obscure exact dose-response relationships and greatly impair repeatability. However, work at IACR-Rothamsted involving carabid beetles and wolf spiders (Araneae: Lycosidae) has generated more optimistic results. Dose-response bioassays exposing the carabids *Pterostichus melanarius*, *P. madidus*, *P. cupreus* and *Nebria brevicollis*, and the lycosids *Trochosa ruricola*, *T. terricola* and *Pardosa* spp. to dimethoate and lambda-cyhalothrin (through a combination of direct exposure to sprays and subsequent exposure to residues) yielded dose-response data that were readily amenable to probit analysis (Birnie *et al.*, 1998 and unpublished results). Control mortality was generally negligible during the seven day holding period. Furthermore, results for all three *Pterostichus* species were very similar, implying that these species could be used interchangeably as indicator species to contend with regional variation in species composition or differences in phenology. Lycosids responded similarly to carabids to dimethoate, but proved much more susceptible to lambda-cyhalothrin, showing the ability to extrapolate results between such taxonomically-distinct groups to be compound-dependent. Clearly, there is still much more work needed to validate multiple dose bioassays for a wider range of non-target species, and to compare results across and within taxonomic and ecologically functional groups.

Potential triggers - are probit lines essential?

One assumption that is usually implicit in the use of multiple dose bioassays for risk assessment is that results will be subjected to probit (or logit) regression analyses to yield single parameters (e.g. LD_{50} values) that summarise the underlying dose-response relationship, and which could form the basis of a trigger for further testing. The use of a single value has intuitive appeal, but also introduces some potential difficulties with regard to the conduct of bioassays and their interpretation. To be applied accurately, probit analysis not only assumes a particular distribution of tolerances between individuals (log-normal with respect to dose), but also requires considerable effort to optimise the number and range of doses tested (Robertson *et al.*, 1984). The latter in turn means that a substantial number of individuals must ideally be sacrificed for initial 'range-finding' bioassays to pinpoint these doses. An added problem is that most commercially available probit analysis software is relatively forgiving of poor or inadequate experimental designs. As long as mortality shows even an approximate tendency to increase with dose, a regression line can be fitted and LD-values (especially LD_{50}'s) can be interpolated from this line. The dilemma facing regulators is therefore to interpret the precision and likely reliability of an LD_{50} estimate, assuming that 'raw' bioassay data would not normally be submitted for inspection.

An alternative, less time-consuming and potentially equally informative approach is to abandon probit analysis in favour of testing organisms at a smaller, pre-defined number of doses (e.g. stated proportions of the recommended field rate), and to base decision-making on empirical estimates of mortality at these doses rather than a derived parameter such as an LD_{50}. Since trigger values are likely to be set in due course as a specified level of mortality at a specified proportion of the field rate, as is the case currently with honey bees (EPPO, 1994), this approach could serve the dual purpose of both encompassing the trigger dose and helping to pinpoint the position of a dose-response relationship. It would also avoid the need to waste time and resources on initial range-finder testing. It would certainly be worthwhile running 'probit line' and 'prescribed multiple dose' bioassays side-by-side to compare the logistics, precision and repeatability of each type of test.

TOWARDS GREATER REALISM: THE 'SEMI-FIELD' APPROACH

Laboratory bioassays conducted at the first tier of testing will, by definition, be highly artificial and unrepresentative of pesticide effects in the field. If further testing is required, the next step is therefore to conduct trials simulating more realistically conditions of crop growth and spraying encountered in farmland, and hence the way that non-target species are likely to be exposed to the compound in question (Sotherton, 1989). Large field trials offer the most realistic scenario of all, but are often difficult to interpret and provide little information on the mechanisms of any effects observed (e.g. Jepson, 1987; Poehling, 1989). One compromise is to adopt an intermediate tier of testing involving smaller scale, more controlled and manipulative trials in which organisms may be confined and/or deliberately released, which can be assessed with greater precision, and allow greater replication. This corresponds to the 'semi-field' approach advocated by many authors (e.g. Jepson, 1987; Hassan, 1989; Poehling, 1989), but which is still under-exploited and poorly validated for many groups of non-target arthropods.

Owing to differences in the size, life-history and behaviour of potential test species, there is no universal prescription for how semi-field tests should be designed, or what they should attempt to measure. For species such as parasitoids with short generation times, the objective should be to span at least one complete generation in order to encompass effects against all life-stages. For longer-lived species (e.g. carabid beetles) this may prove impossible, with measurements being restricted to the life-stage considered at greatest risk from pesticide exposure. In all cases, however, these trials should incorporate realistic spraying regimes, in order to simulate pesticide deposition patterns in the field. They should also be designed to exclude or differentiate between factors such as mortality, recruitment, dispersal and immigration that often compound the interpretation of open field experiments. Although most discussion of semi-field tests has centred on their use in the field, opportunities for simulating realistic exposure to pesticides in the laboratory should not be ignored, especially for organisms (e.g. parasitoids) that are difficult to census accurately under field conditions. Two examples of recent work at Rothamsted serve to demonstrate the versatility of and considerations with this intermediate stage in ecotoxicological risk assessment.

Polyphagous predators

Field experiments, such as those conducted between 1993 and 1996 at Rothamsted, demonstrated significant short-term reductions in pitfall trap catches of polyphagous predators following treatment with dimethoate. Treatment with pirimicarb had no significant effect. The underlying mechanisms for such effects could not be determined partly due to the catch of pitfall traps being a combined measure of arthropod activity and abundance. In one experiment (Kennedy *et al.*, submitted) with winter wheat, encompassing both large (0.89 ha) and small (0.01 ha) plots and performed over successive seasons, few species were caught in sufficient numbers in both pre-treatment and post-treatment samples to warrant individual statistical analyses. Nevertheless, more species were caught and catch variability was less in large open plots than in small barriered plots. This effect was most marked for species of Carabidae and least for Staphylinidae. Due primarily to the low numbers of individuals caught, it was concluded that if small barriered plots are to be recommended for use in risk assessment, careful consideration needs to be given to the optimum density and distribution of traps to ensure adequate numbers of arthropods are caught for statistical analyses.

Problems apparent from the above field trials were addressed in more detail by establishing discrete circular enclosures, 10 m in diameter, bounded by polythene barriers in a field of spring barley (Kennedy & Randall, 1997). This experiment focused on carabids, exploiting indigenous insects that were either treated with dimethoate or left unsprayed. One innovation with this study was to give all individuals of certain species caught within the enclosures a unique mark, following which they were re-released at the centre of the appropriate enclosure. Mark-release-recapture models were then used to estimate pre- and post-treatment population densities, and to distinguish between the effects of pesticides on survival, recruitment and locomotory activity. By this means it was shown that recruitment to populations of autumn-breeding *Pterostichus* species could substantially mask any mortality effects of pesticides on insects already present at the time of spraying. Similarly, changes in the number of certain carabid species caught in pitfall traps were shown to be due, in part at least, to effects of chemicals on insect activity rather than on absolute population size. Given the obvious advantages of incorporating such biological information

into the interpretation of semi-field trials, opportunities for exploiting mark-release-recapture techniques on a more routine basis should be investigated further.

A complication affecting this experiment was that total numbers of insects present varied markedly between enclosures as a consequence of natural, spatial heterogeneity in carabid abundance. One way to overcome this would be to augment numbers by releasing marked individuals into enclosures at the start of a trial. Despite the advantages of using semi-field plots prior to committing resources to open field experiments, there is clearly more work needed to determine optimum densities, plot sizes, levels of replication and sampling strategies to realise their full potential and tailor them to different non-target species.

Aphid parasitoids

Hymenopteran parasitoids pose a number of problems for higher-tier testing due to their small size, mobility, specialised life-style and frequent specificity to few or even one host species. Although small-scale field trials are still possible (e.g. Longley & Jepson, 1997), there are also compelling scientific and logistical arguments for basing semi-field trials in the laboratory, where densities of hosts and parasitoids can be easily manipulated, and all life-stages can be monitored accurately. One such approach that has proved successful has been to establish combinations of crop plants, aphid hosts (*Myzus persicae*) and parasitoids (*Diaretiella rapae*) in large cages (1.7 × 1.2 × 1.0 m) that are sprayed with recommended doses of insecticides, and then monitored for at least one complete parasitoid generation (Birnie *et al.*, 1996 and unpublished data). Chemicals can be applied before or after the release of adult parasitoids, depending on whether contact plus residual or just residual activity is of primary concern. These experiments can also, if required, be continued for a second parasitoid generation to investigate the likely field significance of indirect effects, e.g. on parasitoid sex-ratios (Umuru *et al.*, 1996), that seem to require two generations after treatment to become apparent.

CORRELATING LABORATORY AND FIELD DATA

If laboratory bioassays and semi-field trials (whether field- or laboratory-based) are to form the two initial stages of a sequential testing programme, it is clearly desirable to be able to relate data obtained from the two types of test. The problem of correlating laboratory and field effects of pesticides is not unique to ecotoxicology. It applies also to screening new pesticides and to research on insecticide resistance, e.g. when attempting to determine whether resistance disclosed by simplistic bioassays is likely to impair the performance of insecticides under field conditions (Denholm *et al.*, 1984). The development of dose-response relationships for a wider range of non-target arthropods in laboratory bioassays would be an important step in this direction, since these could then be compared directly with the range of doses needed to elicit a similar gradation of effects in semi-field trials. Disparities between the two sets of dose-response data, attributable primarily to factors altering exposure under more realistic conditions, would in turn assist with establishing more reliable trigger values for the first tier of a more cohesive and complementary risk assessment scheme.

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Risk assessment and risk management of pesticide effects on non-target arthropods in Europe

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ABSTRACT

This paper compares the current decision making schemes for risk assessment and risk management of honey bees and other terrestrial non-target arthropods, used in the registration of pesticides in the European Union. It describes stepwise how the schemes have been built up and refers to available associated literature. The schemes process laboratory, semi-field and field test results to produce a risk classification of a proposed pesticide application. The classification is used for decisions about the authorisation and risk mitigation of the pesticide. The described schemes have been developed by the EPPO/Council of Europe Panel on Environmental Risk Assessment but specific risk management policy aspects are discussed in a wider context. The aim of this paper is to identify common principles and conditions for success to be applied for reviewing terrestrial non-target arthropod risk management in Europe.

INTRODUCTION

Environmental risks of pesticide use cause considerable public concern. One of the first non-target organisms to receive attention was honey bees, some of the last were other non-target terrestrial arthropods. These risks should be limited to acceptable levels by regulation of pesticide use during the process of pesticide authorisation. This requires assessment and management of these risks within the procedure for pesticide authorisation and regulation.

In 1991 the European Union adopted the well known Directive 91/414/EEC (European Union, 1991) in order to bring the registration of plant protection products for use in Europe under one common regulation. This Directive has been extended with a number of annexes that specify data requirements and principles for evaluation and decision making. One of these (Annex VI) is the 'Uniform Principles for Evaluation and Authorizarion of Plant Protection Products' (European Union, 1994). In these Uniform Principles, in the sections on 'Influence on the Environment' and 'Impact on non-target species', requirements for risk assessment and management of honey bees and beneficial arthropods are given. These regulations and requirements have now been brought into force by all member states.

The EU regulations and requirements for non-target arthropods are based on two decision making schemes of EPPO/Council of Europe: the honey bee scheme (EPPO, 1993) which appears to be successful and more or less definitive (Lewis et al, 1996), and the non-target terrestrial arthropod scheme (EPPO, 1994) which seems to have had somewhat disappointing initial results and to need further development (Oomen, 1996). In this paper both schemes are compared in order to find common principles and perspectives for success.

METHODOLOGY FOR REGULATORY RISK ASSESSMENT

The successive steps for developing an effective and efficient risk assessment approach are given in the first column of Table 1. The other columns describe how these steps have been taken in the honey bee scheme (EPPO, 1993), in the existing non-target arthropod scheme (EPPO, 1994; Barrett et al., 1994), and finally in the scheme under review by the responsible working group of EPPO/CoE as it is proposed to be adopted in the near future (Van der Valk & Oomen, 1998).

The first steps (1-2), defining the objective and target, and defining acceptability of effects, of course are essential to be able to start development of risk assessment and risk management. They are the responsibility of policy makers.

The next steps (3-4) are selecting or developing suitable testing methods for first tier laboratory testing, collecting test results, and collecting information from field or practice about observed hazards. The laboratory testing should produce standardised toxicity data, e.g. LD_{50} data. For honey bees, a suitable method for assessing the contact LD_{50} was available from Smart & Stevenson (1982). For non-target arthropods, usable methods were available from IOBC (Hassan, 1994) though not yet suitable for LD_{50} determination. For the non-target arthropods scheme under review, these methods need to be elaborated further to include dose-response tests. It is doubtful whether the IOBC-tests, that include measuring sublethal effects, as they are now will be regarded as sufficiently robust statistically for these registration purposes.

The next step (5) of comparing and analysing these data is needed as part of a simple but elegant method to establish the first trigger value for decision making. A simple but adequate illustration of this for the honey bees is given in Fig. 1 where field information about harmlessness (o) and (unacceptable) harmfulness (+) is considered together with theoretical exposure data (highest recommended use rates) and laboratory toxicity data (LD_{50}).

Thereafter (6-8), observations on acceptable effects of pesticide uses are correlated to the exposure/toxicity data. A threshold level has been drawn as a straight line in the honey bee example of Fig. 1, above which all pesticide uses are safe without exceptions. A trigger value for the exposure/toxicity ratio (the "hazard ratio") is derived. In the honey bee example this trigger value is 50 at the units used. This hazard ratio of exposure and toxicity is now used as a trigger value in the first tier of the honey bee risk assessment scheme (EPPO, 1994; European Union, 1994).

The current non-target arthropod scheme (Table 1, column 3) lacks such a derivation of the first tier trigger value. The value (30% mortality in laboratory studies) as prescribed by the European Union (1994) and described by EPPO (1994) has been copied rather arbitrarily from IOBC where it was in use as a (never validated) threshold for classifying laboratory test results between the classes of harmless and slightly harmful pesticides (cf. Hassan, 1994). When used for registration purposes, this trigger value leads either to far too many high risk classifications, and hence to far too many higher tier data requirements (Oomen, 1996). In the revised non-target arthropod scheme under review, this trigger value will be newly derived, in a way comparable to the honey bee approach (Van der Valk & Oomen, 1998), to be used in the first tier of decision making.

Based on this first tier, a sequential decision-making scheme is constructed (step 9), based on the principle that all pesticide uses with a hazard ratio < trigger value are classified as harmless, unless there are considerations that make this approach unreliable (e.g. Insect Growth Regulating insecticides that affect juvenile stages only). But if the hazard ratio is exceeded at this stage, it does not mean necessarily that the considered pesticide use is harmful. Eventual harmlessness could still be demonstrated during a series of testing studies (extended laboratory, cage, field), each more representative of practical use conditions than the previous study (tier). Testing conditions in each of these studies should represent realistic worst case conditions in a semi-field or field situation, in order to enable extrapolation of the eventual conclusion about harmlessness to all normal conditions of practical use.

The assessment scheme is then tested, verified and improved (step 10) until it is found to be sufficiently reliable. Reliability should be demonstrated by an independent validation. For honey bees this was done by Aldridge & Hart (1993) with data from monitoring the effects of pesticide use in the UK.

METHODOLOGY FOR REGULATORY RISK MANAGEMENT

Schemes for risk assessment like these finally deliver risk classifications (11-13), for the proposed pesticide use, which should be suitable for registration decisions. Such decisions in particular will have the form of prescriptions for risk management measures. However, possibilities for effective risk management appear to be limited. Effects of pesticides on organisms, including non-target arthropods, depend upon the toxicity of the specific pesticide to the specific arthropod (this can be considered as an intrinsic characteristic of the pesticide-organism combination) and upon the exposure of the arthropod to the pesticide. Risk management should, in order to mitigate risks, influence these aspects. However, toxicity as a given characteristic cannot be influenced by the regulator. So his exclusive option is to influence exposure. Examples of possible measures which regulators can use to manage risks are to forbid:

- use during specific periods of the year or the day when the organism might be exposed;
- use in specific crops or areas where the organism might be exposed;
- use within a certain distance from relevant areas (buffer zones, no spray zones);
- use with application methods/equipment/formulations by which organism might be exposed;
- use over a maximum dose or maximum frequency

Risk mitigation is to be implemented mainly by regulation of the use, i.e. by statutary label requirements. Effective risk mitigation requires that conditions of use can be clearly and systematically prescribed, and also that they can be, and are, enforced. In the Netherlands, prescription of a maximum dose is considered to be a measure that cannot be enforced. For this reason setting an upper limit to the dose is not usable as a risk management measure.

Some examples of statutory label requirements (13) are:
- Do not apply to crops in flower
- Do not spray more often than twice in a season
- Do not spray [crop] within 6 m of the field boundary.

In common practice of pesticide registration and regulation, the registration applicant will submit proposals for pesticide uses, including risk mitigation measures, which as a whole are to be evaluated by the authority. This feedback between assessment and management makes these two inseparable. Possible measures for risk management, therefore, should be known before the definitive risk assessment approach is done, in order to allow optimisation of the risks and benefits of the pesticide.

DISCUSSION

Comparison of successes and problems of both the honey bee and the non-target terrestrial arthropod risk assessment schemes, leads me to the following conclusions:

- The availability of simple but well functioning examples such as the scheme for honey bees is a great help in developing a risk assessment scheme for other groups such as the non-target arthropods.
- A clear definition of objective, target and criteria for acceptability of effects is a conditio-sine-qua-non for developing a well functioning risk assessment scheme. These definitions are a responsibility of policy makers. However, policy making in Europe is problematical. This difficulty is solved by convening workshops of European experts and national policy makers and have these jointly address the questions. This was done firstly in the SETAC/ESCORT meeting which brought together research, commercial and policy/regulatory interests (Barrett *et al.*, 1994). A similar meeting will be held in 1999.
- Mixed objectives, such as in the original EU non-target arthropod approach, where protection of non-target arthropods both within and outside the crop, and protection of natural enemies for use in biological control were mixed together, need careful separation in order to enable effective functioning of the risk assessment process. In the revised non-target arthropod scheme under review, the last objective (natural enemies) has been abandoned. This objective requires information that is very detailed and very dependent on each specific situation. Results from the risk assessment done for the other objectives may nevertheless be useful for implementing integrated pest management.
- The proposed criterion of recovery of an affected population within one year (Van der Valk & Oomen, 1998) is much more relevant than the former criterion of 30% effect in laboratory studies, but much more difficult to implement in a risk assessment scheme.
- The described development of a trigger value for honey bees appeared to be a simple, pragmatic and effective way to find an optimal value for first tier decision making. In contrast, the trigger value for non-target arthropods (EPPO 1994; European Union 1994) has been arbitrary and has led to unrealistic risk classifications. For the review of the scheme under way, the pragmatic honey bee experience is indicating the way forward.
- The availability of suitable testing methods and test results is a very important advantage in developing a risk assessment scheme. And even more, after comparing the development of both schemes, I conclude that effective risk assessment and management requires a long history of achievements in practical observations, test development, laboratory and field studies, well defined objectives and criteria for protection, and experience with mitigation measures. Predecessors with solid shoulders to stand on are indispensable requirements in effectively managing environmental risks.

Table 1. A comparison of the step-by-step development of the risk assessment schemes for the honeybee, the non-target arthropods as operational in the EU, and as proposed by the EPPO-CoE Working Group.

Step	Development procedure published in:	Honey bees EPPO, 1993	Non-target arthropods - EU EPPO, 1994; Barrett et al., 1994	Non-target arthropods: being reviewed v/d Valk & Oomen, 1998
1	Define objective and target	Protection of bees from killing by pesticide use	Protection of terrestrial non-target arthropods from killing by pesticide use	Protection of terrestrial non-target arthropods from killing by pesticide use
2	Define acceptability of effects, i.e. define risk classes	Statistically insignificant mortality in exposed bee hives is acceptable	IPM: effective biological control maintained In-crop: no significant mortalities Off-crop: no ecological significant effects	IPM: is excluded from this scheme. In-crop: significant mortality acceptable but recovery within one year. Off-crop: no significant mortalities acceptable in relevant off-crop areas at given distance
3	Select or develop laboratory testing methods	Dose-response tests LD_{50} (Smart & Stevenson 1982)	Single dose tests on inert surface on 4 - 6 relevant species (IOBC-method)	Dose-response tests (LD_{50}) on inert substrate for 2 indicator spp. (limit test if toxicity low)
4	Collect laboratory test results, and collect field information	Publications, registration data, experiments, incidents	-	To be collected, including from field studies and experience of biocontrol companies
5	Compare and analyse laboratory and field data	Diagram (Fig. 1)	-	To be analysed as hazard ratio
6	Apply acceptability criterion to field information	Discern known harmless (o) and harmful (+) uses	-	Discern between known harmless and harmful uses
7	Correlate criterion to laboratory data	Find typical hazard ratio of safe cases	-	Find typical hazard ratio of safe cases
8	Set trigger value for laboratory data with safety margin	hazard ratio < 50	IOBC, EU: <30% effect at recommended concentration	Hazard ratio < x (x is maximum hazard ratio of indicator species in typical safe cases)
9	Develop sequential decision making scheme, using laboratory information in first tier	EPPO/CoE Scheme 10 (1993) lab - cage - field	EPPO/CoE Scheme 9 (1994) lab - semi-field - field	New EPPO/CoE Scheme 9 (by year 2000) laboratory (mortality in indicator species) - extended laboratory - semi-field – field
10	Test, improve and validate scheme	EPPO/CoE exercise; (Aldridge & Hart 1993)	EPPO/CoE Workshop (in Bilthoven in 1997) No validation done yet	
11	Use scheme and classify risks	EU countries widely used	EU countries in use	
12	Apply risk management by regulating uses with high risks	Low: no limitations High: use not allowed where exposure expected	Low: no limitations High in-crop: use not allowed where exposure High off-crop: use not allowed where exposure	Low: no limitations High in-crop: use not allowed where exposure High off-crop: use not allowed where exposure
13	Examples of risk management	No spraying during crop flowering	Buffer zones prescribed. Row application prescribed.	Buffer zone between relevant off-crop area. Restricted frequency of use.

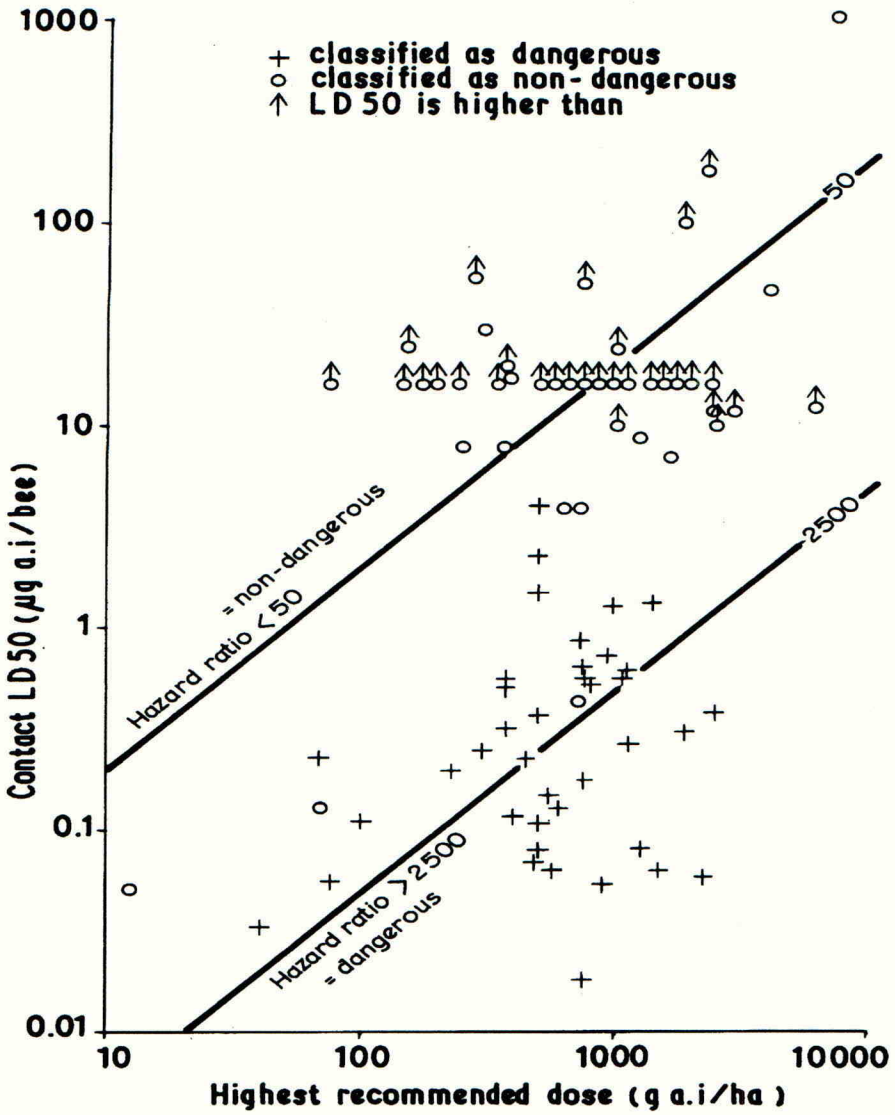

Figure 1. Toxicity (LD_{50} in µg a.i. per bee) and highest recommended dose rate (g a.i./ha) of 82 pesticides registered in the Netherlands in 1985 (cf. Oomen 1986). Pesticides designated with + are known from field observations and studies to be hazardous; those with **o** are known to be safe to honey bees. Pesticides designated with **o** with an upward arrow had an LD_{50} value higher than the indicated one. The two lines deliminate the area above which all pesticides are known to be safe, and below which all pesticides are known to be hazardous. The line with hazard ratio = 50 has become the trigger value for first tier decision making.

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Predicting susceptibility of non-target insect species to different insecticide applications in winter wheat

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ABSTRACT

The exposure of any insect species to a pesticide application depends on the intrinsic properties, behaviour and distribution of both insect species and pesticide. The short term pesticide exposure of several important cereal-dwelling natural enemy species was predicted and used to rank them in order of susceptibility to field applications of insecticide using different application systems. Individual susceptibility indices were attributed to individual beneficial insect species when subjected to an insecticide treatment applied by different nozzle types mounted on a boom sprayer. These indices were derived from a formula incorporating the distribution of insecticide and insect species within the crop, chemical toxicity, varying toxicity of insecticide at different crop strata and insect behaviour. From these indices, insect species were ranked in order of susceptibility to an insecticide application made under field conditions.

INTRODUCTION

The natural enemies of cereal aphids have been shown to have the potential to limit the population growth of these species and have played a role in the prevention of cereal aphid outbreaks (Chiverton, 1988). Assessment of the effects of pesticides on these natural enemies has received much attention in recent years with the agrochemical industry as a whole placing increasing importance on the safety of pesticide applications to beneficial insects.

The basic ecotoxicological approach to determine whether a pesticide constitutes a hazard to a natural enemy population is to establish the risk involved. This can be measured directly in terms of the percentage of the population affected (Brown, 1989), or predicted, from the pesticide's toxicity and the likely exposure of the insect to the pesticide.

Extensive work has been carried out establishing toxicities of different insecticides on natural enemies under laboratory, semi-field and field conditions (Hassan, 1989). There is much less information on predicting the risk posed when the insecticide is applied using a particular application system. The degree of exposure is affected by the pattern of pesticide deposit in the crop canopy after application and the behaviour patterns of the insect species. Different spray application systems produce spray structures having different physical characteristics (Legg &

Miller, 1989). Spinning discs produce droplets with a more horizontal droplet trajectory and with lower downward velocities compared with conventional hydraulic nozzles and can give greater deposits on the upper parts of the crop compared to the ground. This has implications for the level of exposure to pesticides by both plant and ground dwelling beneficial insect species.

The behaviour and distribution of non-target species within the crop is also an important factor in assessing the hazard posed by a pesticide application (Jepson, 1989). The risk that deltamethrin posed to an insect species is affected by the insects speed of movement, track width and area of contact with the leaf surface (Ford, 1992). A hazard index for several beneficial insect species has been developed using a function of these factors incorporating deltamethrin LD_{50} values, enabling a susceptibility ranking to be established (Wiles & Jepson, 1994). The index does not account for the distribution of the insect species within the crop canopy or the different pesticide deposit distributions given by different application systems. The toxicity of pesticide residues also varies depending on whether the deposit is on the plant or ground, with plant deposits having a higher toxicity (Unal & Jepson, 1991). This factor is not accounted for in the original hazard index.

This paper uses deposit distribution patterns from flat fan nozzle and spinning disc application systems, together with existing data on insect behaviour and toxicity to establish a simple susceptibility index for beneficial insect species in a winter wheat crop. Exposure and susceptibility are compared for several species in a crop treated with a deltamethrin spray, applied using spinning disc and flat fan application systems.

METHODS AND MATERIALS

A formula enabling the susceptibility of insect species to a winter wheat insecticide application to be assessed was developed. The formula incorporated variables existing both in the transfer of an insecticide from sprayer to insect and in the toxicity of the insecticide.

$$R = \frac{vwa}{LD_{50}} \sum_{n=1}^{N} p_n q_n k_n$$

where R is the Susceptibility Index: vwa is the exposure function (Ford, 1992), p_n is the probability of an insect being in strata n of the crop, q_n is the probability of insecticide being deposited in strata n of the crop and k_n is the toxicity constant assigned to strata n of the crop. Susceptibility indices were calculated for seven beneficial cereal dwelling insect species: *Agonum dorsale*, *Nebria brevicollis*, *Pterostichus melanarius*, *Demetrias atricapillus*, *Bembidion lampros*, *Tachyporus hypnorum* and *Coccinella septempunctata*. In each case the indices were calculated for two different spray application systems; a flat fan nozzle and a spinning disc.

Exposure function

This is a function where v = walking speed (cm/s), w = track width (cm) and a = contact area, the proportion of the insect area in contact with the substrate when in motion (Ford, 1992). Values for the seven species of cereal dwelling insects were taken from work by Wiles & Jepson (1994).

Distribution of insecticide

Spray deposits were measured using a tray-grown winter wheat crop sprayed from two different nozzles - a flat fan nozzle F110/1.6/3.0 (Lurmark Ltd) and a spinning disc at 5000 rev/min (Micron Sprayers Ltd). Winter wheat (cv. Riband) was drilled at a row spacing of 15 cm, on October 3rd 1997, in plastic trays 0.36 m long, 0.28 m wide and 0.14 m high. Spray treatments were applied at growth stage 59 (Zadoks *et al.*, 1974) when the crop had a tiller density of approximately 540/m^2 and a Leaf Area Index (LAI) of 5.2. Spray applications were applied to blocks of trays four wide by three deep. The spray solution was water with 0.5% wt/V Green-S dye (Merck Ltd) and 0.1% V/V non ionic surfactant (Agral, Zeneca Agrochemicals).

Spray was applied using a single nozzle travelling at 6 km/h, 350 mm above the canopy. A pressure of 3.0 bar was used for the flat fan nozzle and 0.6 bar for the spinning disc giving a flow rate of 1.60 l/min and 0.48 l/min respectively. The flat fan nozzle was directed downwards into the crop, the spinning disc was angled 12° down in the direction of travel.

After spray application, three tillers were removed at eight points across the spray swath. The ear, flag leaf, first, second and third leaves were cut from the tiller and dye removed by washing in test tubes containing 10.0 ml aliquots of water. Plant parts were then removed and dye deposits quantified by measuring absorbency using a spectrophotometer at a wavelength of 634 nm. A calibration curve was constructed from samples of the original dye solution and used to determine the total amount of spray solution on component parts of the canopy, area of each plant part was measured so as to give deposition in $\mu l/cm^2$. Ground deposits were measured by placing strips of chromatography paper between rows of the crop and dye deposits quantified similarly.

The target area was divided into three strata; level 1 was the ear, flag leaf and first leaf, level 2 the second and third leaves and level 3, the ground deposit. The sum of the deposit on each plant part at each target level was expressed as a fraction of the total deposit for each spray treatment. Toxicity constants were assigned to each of the three crop strata based on differences in residual toxicity of deposits as shown by Unal & Jepson (1991).

Distribution of insect species

The distribution and behaviour of the seven insect species was assessed, using a search of the literature. Values for the proportion of time spent by the species in each crop stratum were assigned for each species depending on behavioural patterns within the crop canopy, according to the literature on cereal dwelling insect movements and behaviour.

RESULTS

The spray deposition studies showed that a higher proportion of the spray was deposited on the plant in both strata 1 and 2 with the spinning disc treatment than with the flat fan nozzle, whilst the latter deposited a larger proportion on the ground (Table 1.).

Of the seven insect species investigated, four were predominantly ground active, rarely moving on to the crop and these had higher susceptibility indices with the flat fan treatment (Table 2.). Two of the species, *D. atricapillus* and *C. septempunctata*, forage on the crop, particularly at the

Table 1. Spray deposition patterns and toxicity constants at three crop levels.

Crop strata	Proportion of spray deposited: Spinning disc	Flat fan nozzle	Toxicity constant assigned
1	0.504	0.469	5
2	0.363	0.343	4
3	0.133	0.188	1

uppermost level. Both had a higher susceptibility index with the spinning disc treatment.

As more variable factors are introduced into the formula generating indices, the cumulative distributions of the datasets become more steeply inclined in the initial phase of the curve (Figure 1. I-iv). The susceptibility indices generated for species subjected to deltamethrin application with a spinning disc are generally lower than those for the flat fan treatment, resulting in a steeper cumulative distribution curve.

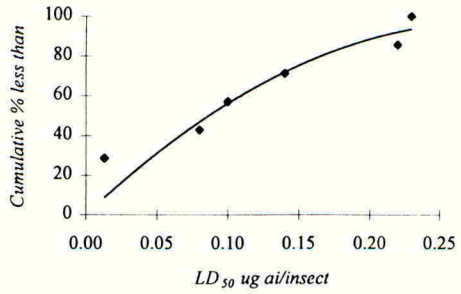
DISCUSSION

The overall order of susceptibility of the seven species tested generally agreed with previous work by Wiles & Jepson (1994), with *T. hypnorum* showing the greatest susceptibility to deltamethrin applied using either spraying system. The difference in susceptibility between species with the new index showed some differences between application systems as shown by the cumulative distribution curves. The model showed that the species at most risk, *T. hypnorum*, has an index approximately 20 times that of the species shown to be least at risk, *D. atricapillus*. This figure has 64 in the original model. The varying distribution of insect and pesticide, combined with varying toxicity of residues within the crop had the effect of reducing differences in predicted susceptibility between insect species in the most part. This could have consequences for the overall effect of field insecticide applications on crop dwelling beneficial insects in that fewer species may be at risk than previously thought. *Tachyporus hypnorum* was the exception, having an index of 4.4 times that of the second most susceptible species, *P. melanarius*, compared to 1.7 in the original index. It may be that this model identifies the extremes of species susceptibility under field conditions. To establish the model as a legitimate risk assessment tool further data sets for more insect species and spray application systems need to be included in the analysis. In addition field research is required to test the robustness of the model.

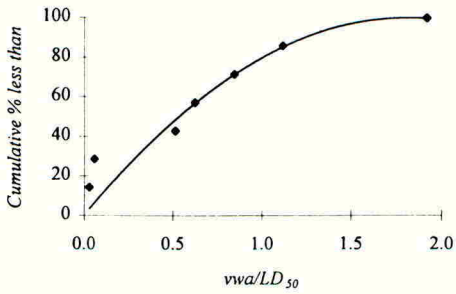
The lower susceptibility indices generated for five of the seven species by the spinning disc are due to the fact that the majority of the species are ground active. Plant active species such as *C. septempunctata* and *D. atricapillus* had higher indices for the spinning disc treatment, but in these cases there was less difference between the two treatments indicating that the spinning disc may have selective advantages when treating insect pests on the crop. This is supported by the fact that it showed increased plant deposits in this study and in other work (Holland *et al.*, 1997). The scheme does not account for risk associated with direct capture of pesticide spray by the insect. This is an important factor in estimating the effect of pesticide application on beneficial

Table 2. Distribution and susceptibility of beneficial insects within a winter wheat crop and their susceptibility to Deltamethrin residues applied using two different nozzles.

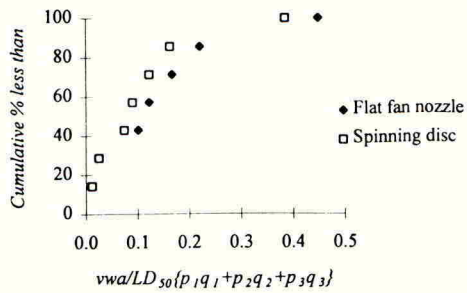
Insect species	Probability of insect being present at crop strata			LD50 Deltamethrin (μg ai/insect)	Exposure function	Susceptibility Index	
	1	2	3			Spinning disc	Flat fan nozzle
D. atricapillus	0.50	0.30	0.20	0.230	0.007	0.052	0.049
N. brevicollis	0.00	0.05	0.95	0.220	0.113	0.102	0.127
C. septempunctata	0.64	0.19	0.17	0.100	0.006	0.115	0.108
A. dorsale	0.00	0.05	0.95	0.080	0.050	0.124	0.155
B. lampros	0.00	0.05	0.95	0.013	0.011	0.168	0.209
P. melanarius	0.00	0.05	0.95	0.140	0.157	0.223	0.277
T. hypnorum	0.00	0.29	0.71	0.013	0.025	0.991	1.022



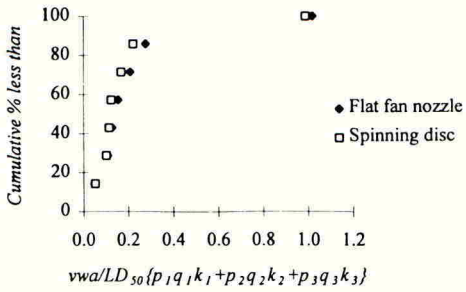
(i) LD_{50} values (from Wiles & Jepson, 1994)



(ii) Original susceptibility index (from Wiles & Jepson, 1994)



(iii) Incorporating distributions of insect and pesticide within the crop



(iv) New susceptibility values including distribution variables and toxicity constants

Figure 1. Cumulative distributions of data sets from component parts of the susceptibility index formula for beneficial insect species to deltamethrin applications.

insects and the model needs to be developed further to incorporate direct capture.

The risk assessment scheme acts as a simple testing framework for estimating susceptibility parameters from which possible toxic effects from pesticide applications in the field can be predicted. It has important properties because the comparative susceptibilities of both non-target and pest insect species to any winter wheat pesticide application can be determined once distribution parameters of spray, species and pesticide toxicity are known. This may allow selective dose reduction decisions to be made. The principles of the model could be applied to any cropping system where prediction of the susceptibility of insect species is desirable.

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