FIELD TRIALS FOR EVALUATING THE SIDE EFFECTS OF PESTICIDES

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Foreword to the English edition

The Dutch version of this report has been sold out in a very short time. Because of the international significance of the subject, the Dutch Ministry of Housing, Physical Planning and Environment commissioned us to prepare an English translation.

Thanks are due to the translater, Nigel Harle, who translated the report in a short time. Further, we like to thank Drs. Henk Bezemer for the final proof reading, and Mrs. Joke van der Peet-van Loon for typing.

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[1] A list of abbreviations is provided in the appendix.

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PART B: Guideline proposals for field trials for evaluating the side-effects of field use of chemical pesticides

1 Field trial proposal for terrestrial higher plants
2 Field trial proposal for earthworms
3 Field trial proposal for ground beetles
4 Field trial proposal for honeybees
5 Field trial proposal for birds
6 Field trial proposal for algae
7 Field trial proposal for midge larvae
8 Field trial proposal for water snails
9 Field trial proposal for water fleas
10 Field trial proposal for fish

Appendix part B: symbols used for characterization field trials

FIELD TRIALS FOR EVALUATING THE SIDE-EFFECTS OF PESTICIDES

This report consists of two parts. Part A, the main report, discusses the general findings of the study, while Part B consists of ten detailed proposals for field trials. Since Part B represents a detailed result of the main report, only Part A is summarized below.

INTRODUCTION (Chapter 1)

In 1989, the Centre of Environmental Science (CML) at Leiden University was commissioned by the Dutch Ministry of Housing, Physical Planning and Environment (Directorate-General for Environmental Protection: Directorate of Chemicals and Risk Management and Department of Watersupply, Water, Soil and Groundwater) to investigate the possibility of using field trials for pesticide approval. This study formed the third phase of the project on "Side-effects of Pesticides" (the so-called NB project) and was aimed specifically at drawing up proposals for such field trials.

For the study, three objectives were distinguished:

I. To indicate the possible role and status of field trials in pesticide approval procedures in the Netherlands (Chapter 2).
II. To develop a framework for selecting field trials for detecting pesticide side-effects (Chapter 3).
III. To develop field trial guidelines (Chapter 4).

In the course of the study, as much information as possible was collected on existing field trial guidelines as well as on field studies. Documents issued by a number of international organizations were also analyzed.

CURRENT STATUS AND USE OF FIELD TRIALS (Chapter 2)

In existing approval procedures, a decision to conduct field testing is usually based on predictions made using other information. Data obtained in laboratory testing is often first evaluated, after which a decision on additional field testing is made, using criteria that may or not be explicit.

Field trials in the Netherlands (Section 2.1)
In the Dutch pesticide approval procedure, a field study to ascertain the side-effects of a pesticide has not so far been a standard requirement. Neither have concrete criteria for carrying out such field trials been laid down, except for trials with honeybees. For a limited number of other fauna groups, an assessment of the toxic effects of a pesticide under field conditions may be necessary "in certain cases". To date, there has been one such case.

Field trials in an international context (Section 2.2)
The approval procedures and field trial guidelines of the OECD, IOBC, EPPO, FAO, EC and Council of Europe are discussed, in so far as they relate to side-effect studies. Only under the responsibility of the first three organizations are field guidelines actually issued, the other organizations referring to guidelines developed elsewhere. The approval procedures in force in the United Kingdom, the United States and Germany are also discussed.

As a rule, the criteria indicating when field testing is required are indicated only in broad outline. In most cases, they are based on uncertainty about the toxic effects of a pesticide, an uncertainty stemming from the results of the laboratory tests already performed. In isolated cases, a decision to conduct a field trial is motivated by the scale of use, the site of application (e.g. directly onto waterways), or the possibility of ecological food effects (only in the aquatic environment).

Survey of field studies (Section 2.3)
Although several guidelines for field trials exist, it is not clear whether they have actually been used in practice. At any rate, there are hardly any results available. A great deal of information is available from 'general' field studies, however, although these studies were not conducted in the context of pesticide approval.

An analysis of these general field studies shows that the aquatic environment has been less frequently studied than the terrestrial environment. Most aquatic studies have focussed on side-effects on fish and insects, with terrestrial studies concentrating mainly on beneficial organisms (insects, spiders and mites), birds and mammals. In most cases, direct toxic effects have been investigated, with ecological effects receiving far less attention.

Discussion (Section 2.4)
There is an ongoing debate about the extent to which the details of field trials should be laid down in strict procedures. It is argued, on the one hand, that variation in environmental factors and application methods excludes the possibility of standard field trial guidelines; on the other hand, a case-by-case approach (with the procedure determined in consultation between applicant and authorities) impedes standardization of both the methods and the interpretation and comparison of results.

In the present study, we have opted for a relatively precise specification of procedures. The guidelines can and should of course be adapted to cater for unusual circumstances; on no account should they be used to legitimize results by an applicant in case of extreme test circumstances.

PROPOSED FIELD TRIAL SELECTION METHOD (Chapter 3)

First of all, a proposal is made for assessing the general nature and magnitude of the toxic or ecological hazard (Figure 3.1). Serious or very serious ecotoxic hazards form grounds for advising against approval of a pesticide; if the hazard is minor, approval is recommended. Field testing is prescribed in the case of a moderate hazard or if some uncertainty remains.

For selecting the field trials most appropriate to a given situation, three aspects are discussed in further detail:
1 determination of the ecotoxic hazard
2 specification of the anticipated effect(s)
3 selection of one or more field trials.

These steps do not yet involve field testing itself, including evaluation of results, but are based solely on information obtained in laboratory testing and on any suspicions following from the intended use of the pesticide. In discussing the three aspects above, the following points are considered.

Nature and magnitude of potential side-effects (Section 3.1)
Two basic categories of side-effects are distinguished: toxic and ecological. Assessment of these is based on several aspects:

toxic side-effects	ecological side-effects
- existence of a toxic hazard	- spectrum of action
- uncertainty in evaluation	- widespread use
	- overlap with habitat
	- efficacy

As far as possible, criteria and norms are presented for each of these aspects, providing tools for deciding whether or not field testing is required.

Specification of anticipated effects (Section 3.2)
Effects may occur at the level of individuals, populations, communities and ecosystems. We take as a premise that field testing is justified if effects are suspected on at least the population level. An exception is made for protected or endangered species.

On the basis of compound properties, mode of application and 'receiving environment', the anticipated effects are narrowed down to one or more specific side-effects in specific environmental compartments, organisms, etc. Using an as accurate as possible categorization of types of effect, taxonomic groups, environmental compartments and ecosystem types, conclusions can then be drawn on the appropriate focus of field trial(s).

Selection of field trials (Section 3.3)
Based on the occurrence of taxonomic groups in each environmental compartment, an assessment is then made of the situations in which effects are likely to occur and thus of the situations for which trials should, in principle, be available (Table 3.11). Subsequent, more specific selection of experimental organisms is based on the following criteria:
- the organisms should play a major role in the ecosystem;
- in each environmental compartment, the principal biological processes should be considered;
- the organisms should be reasonably abundant in the agricultural area involved;
- there should be prospects of practical realization;
- the organisms should not be extremely insensitive to pesticides in general.

For each environmental compartment, an assessment is made of the most suitable species or group of species to use as experimental organism, and preferences are stated. The most appropriate type of trial for the different types of anticipated effect is also discussed, with a distinction being made between a field trial sensu stricto and a semi-field trial, which is taken to include enclosure, plot, ditch and cage trials.

For practical reasons, the stated preferences are finally narrowed down to ten species or groups of species, for which guidelines are proposed in Part B.

Method summary (Section 3.4)
The procedure (see Fig. 3.2) starts with laboratory testing and study of usage data. If this initial screening shows the hazard to be negligible or, conversely, (very) serious, no field trial is required. In the proposed

procedure, in all other cases the selection procedure is automatically followed, both for toxic and for ecological side-effects. The anticipated effect is specified and one or more appropriate field trials are selected. The procedure as described in this chapter has then been concluded; the next step is field testing (one or more trials) and evaluation of results, after which approval or rejection follows or, alternatively, reconsideration based on recommended alterations to the pesticide or to user guidelines.

Examples (Section 3.5)
Evaluation of the proposed screening procedure with reference to the specimen pesticides atrazine and pirimicarb shows that the field trial selection method can yield very different results. A fictitious application for use of atrazine results in far more field trials than a similar application for pirimicarb.

Discussion (Section 3.6)
The proposed procedure focusses on ecotoxic effects on non-target organisms. A decision on pesticide approval will always have to be based on other criteria as well.

Until now, it has not been customary to assess the ecological side-effects of a pesticide. The use of efficacy as a criterion for this purpose is debatable, for if it were to be used independently this would conflict with the very purpose of the pesticide, which is approved only after its efficacy has been proven. Nevertheless, eradication of all aphids on all plots in a polder, for instance, may be deemed undesirable in terms of the survival of aphid predators in the area. For this reason, efficacy is always viewed in conjunction with the scale of use and spectrum of action.

No information has been found in the literature that permits numerical values to be assigned to the criteria scale of use, spectrum of action and efficacy. The values used here are based on the idea that it should at least be possible to detect the effect at the population level. The values chosen are still open to debate and are in need of further substantiation.

DESIGN OF FIELD TRIAL GUIDELINES (Chapter 4)

For the species selected in Chapter 3, field trial guidelines have been developed for the following ten species groups: higher plants, earthworms, ground beetles, honeybees, birds, algae, midge larvae, water snails, water fleas and fish.

Approach adopted in guideline formulation (Section 4.2)
These field trial guidelines have been drawn up on the following premises:
1 there should be maximum conformity with existing guidelines;
2 wherever possible, methods should be adopted from field trials that have already been successfully carried out;
3 the field trials should be capable of demonstrating whether the NOEL of the non-target organisms examined is exceeded.

In addition, the guidelines must satisfy a number of general requirements: the trials should yield unambiguous results, and methods should allow effects to be observed with an acceptable degree of reliability, with other effects or combinations of effects being excluded. Furthermore, the field trials should allow conclusions to be drawn on practical usage situations.

In line with existing guidelines for efficacy evaluation, the proposed field guidelines have the following structure:
1 experimental conditions
2 pesticide application
3 observations
4 evaluation of results.

In each case, the guideline proper is preceded by a more general section explaining the methodological choices made. The guideline is followed by an estimate of the net costs of integral execution of the field trial. Subsequently, existing guidelines are discussed point by point and, finally, the results of available field studies are summarized and a list of references provided.

Cost (Section 4.3)
For each guideline proposal, a rough estimate is made of the cost of execution. This cost estimate comprises the following items:
1 material expenses (experimental organisms, plots, field equipment, etc.)
2 sampling of (a)biotic parameters (incl. experimental organisms)
3 measurement of parameters (abiotic and biotic)
4 data processing and interpretation
5 completion of test form.

To determine the scope of the work involved, scores are assigned to various trial characteristics and used as a basis for cost calculation. Table 4.1 gives a (very rough) estimate of the net cost of each field trial. Although there is considerable variation, the cost is always about Dfl. 100,000 to Dfl. 200,000 per field trial.

Discussion (Section 4.4)
In field testing, a conflict may arise between practicability and compliance with the basic premises. A test yielding a conclusion within the proposed statistical margins may prove be too comprehensive (= too costly), but a test of limited scope may lead to greater margins of uncertainty. To solve this dilemma, a trial can be focussed on a worst case situation. By applying a higher dose, the scope of the test can be limited. If effects are not then demonstrated, it may be assumed that the practical dose will not give rise to effects, either. If effects are found, however, there will have to be very careful translation to the practical dose.

The guideline proposals presented in Part B aim, provisionally, at testing the effects of a single new pesticide on a single (group of) organism(s) in the context of official approval procedures. On each of these three aspects, the guidelines could be extended. In principle, the same trials could also be used for evaluating the effects of a combination of pesticides. Effects on more than one group of organisms can be assessed by combining different tests or elements of tests. For post-registration monitoring of effects, however, the test method certainly needs to be adapted, although the same basic methods of observation could still be used.

Before the proposed guidelines can actually be implemented, practical validation is required. Since no guidelines exist for the aquatic environment, development of these field trials should be given highest priority. For the terrestrial environment, priority should be given to validating the guidelines for birds, ground beetles and higher plants.

CONCLUSIONS AND RECOMMENDATIONS (Chapter 5)

Conclusions (Section 5.1)
It is concluded that in the Netherlands field trials have always played a very minor role in pesticide approval procedures. Except for a honeybee trial, there are no standard guidelines available for use in pesticide approval in the Netherlands. In cases where this is deemed necessary, a guideline can be drawn up in consultation with experts from the Commission for the Registration of Pesticides (CTB). Guidelines do exist in the Netherlands for methods to be used in pesticide efficacy evaluation; these have been derived from the EPPO guidelines.

In an international context, at the level of both individual national governments and international organizations, there is a much greater emphasis on field trials, especially today. For various (groups of) organisms, there appears to be a reasonable number of field trials available, especially for honeybees, earthworms and beneficial insects and mites. However, field trial guidelines for the aquatic environment are lagging far behind in this respect.

In pesticide approval procedures, field trials are used in two different ways. Their first, and most widespread, use is for obtaining additional data on a pesticide before it is admitted to the market. In the second place, they can be used for post-registration monitoring of the practical side-effects of a pesticide. In the Netherlands, too, field trials could be integrated into the approval procedure in both these ways.

An analysis of field studies performed outside the scope of approval procedures shows that, compared with the terrestrial environment, very little work has been done on the aquatic environment. Terrestrial studies have concentrated mainly on beneficial arthropods (insects and arachnids), earthworms, birds and mammals. Most of these field studies have focussed on direct toxic effects, with ecological effects being studied far less frequently. Most studies in the aquatic environment have focussed on insects, molluscs, crustaceans and fish. Here, too, it is above all the direct toxic effects of a pesticide that receive the greatest attention.

A procedure (framework) has been designed for determining the need for field trials, and for selecting the most suitable field trial on the basis of available data. The type of field trial proposed focusses on ecotoxic effects on non-target organisms. Conclusions bearing on pesticide approval will always have to be drawn in conjunction with other data and criteria.

To assess the need for field testing, two keys are used, viz. toxic and ecological side-effects. To evaluate the toxic side-effects, use is made of commonly used criteria such as toxicity data, usage data and exposure data (PEC). This form of evaluation is very similar to the approach currently taken by CTB and also followed internationally.

Until now, it has not been customary to evaluate the ecological side-effects of a pesticide. As criteria for this purpose, we propose scale of use and spectrum of action, for widespread elimination of a food source for a variety of species may have a major impact on species at a higher trophic level.

In this respect, the efficacy of a pesticide is important, too. However, if efficacy were to be used as a criterion in its own right, this would conflict with the purpose for which approval is requested: a pesticide is approved only after its efficacy has been proven. Nonetheless, eradication of all aphids in all the plots in a polder, for instance, may be deemed undesirable in terms of the survival of aphid predators in the area. It is also of interest to investigate the 'General Environmental Quality'[1] in agricultural areas, which is jeopardized when the 'species survival ratio' is lower than 95%. For this reason, efficacy is always viewed in conjunction with scale of use or spectrum of action.

An evaluation of the proposed screening procedure using the specimen compounds atrazine and pirimicarb shows that markedly different results can be obtained. In the past, a request for approval of atrazine would have led to far more field trials than a request for pirimicarb.

In formulating the premises for the guidelines, a number of choices have been made, the aim being to design a test method offering the greatest chance of effects actually being detected.

Recommendations (Section 5.2)
The principal recommendation of this study is for policy makers and advisors to elaborate further - and preferably adopt - the proposals presented in Chapters 3 and 4. More specifically, the study leads to the following concrete recommendations with respect to the national situation and to international collaboration:

The Netherlands:
1 The Dutch pesticide approval procedure should be designed more as a tiered system, with field trials being given a clear status.
2 In such a procedure, post-registration monitoring should be explicitly mentioned and concrete instructions given for performing such monitoring.
3 Field trial guidelines should be integrated into the PCBB 'Fundamental Soil Research' programme, as is presently the case with laboratory trials.
4 The Netherlands should take the lead in developing field trial guidelines for the aquatic environment.

International:
5 All studies on side-effects should be coordinated by a single international organization.
6 The same organization should also be responsible for coordinating international harmonization of side-effect testing.
7 Efforts should be made to standardize field trial methods and improve their quality, which might be achieved through development of standard GFP (Good Field Practice).
8 There should be a more intensive exchange of field data and field trial data.

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[1] In Dutch environmental policy a distinction is drawn between 'General Environmental Quality' ('Algemene Milieukwaliteit') and 'Specific Environmental Quality' ('Bijzondere Milieukwaliteit'). The 'General Environmental Quality' lays quality standards for the Netherlands in its entirety, and the 'Specific Environmental Quality' lays standards for areas with a specific function, e.g. nature areas.

Follow-up (Section 5.3)
The proposed field trials still only have a paper status, together forming one of many possibilities for identifying side-effects by means of field testing. Before these guidelines can be considered for integration into approval procedures, validation in the field is absolutely essential and should be given high priority. It is conceivable that part of a field trial or a combination of (parts of) field trials will be tested in the field. The report therefore concludes with a proposal for a field validation programme.

1. INTRODUCTION

1.1 Background and motivation

In 1989, the Directorate-General for Environmental Protection of the Dutch Ministry of Housing, Physical Planning and the Environment (Directorate of Chemicals and Risk Management and Department of Watersupply, Water, Soil and Groundwater) commissioned a study into the potential for using field trials in pesticide approval procedures, to include development of a number of field trial guidelines. The study forms a sequel to previous research by CML, also commissioned by the Directorate-General for Environmental Protection.

This research, on the "Side-effects of Pesticides" (NB project), i.e. chemical pesticides in widespread agricultural use, was started in 1986. Phase 1 of the NB project focussed on side-effects on mammals, birds, amphibians and reptiles (De Snoo & Canters, 1990, English edition, 1988 Dutch edition). In a follow-up study, Phase 2 focussed on terrestrial invertebrates and the aquatic fauna (Canters et al., 1989).

A major finding of the research in both Phase 1 and Phase 2 of the NB project is that extrapolation of laboratory data to the field situation still involves major problems. In principle, two options are available for eliminating this bottleneck:
1) improvement of predictions based on existing laboratory data, or
2) collection of (supplementary) field data.

Great progress is currently being made in developing methods for extrapolating laboratory data to predict environmental hazards, and results are already being used as a basis for ecotoxicological standards (e.g. Stortelder et al., 1989). A survey of these extrapolation methods can be found in a report on ecotoxicological risk assessment published by the Netherlands Health Council (Gezondheidsraad, 1988).

In these laboratory-based predictions, data on toxicity and physico-chemical properties are combined with data on compound usage and possible species exposure. Predictions can be made using LC50 values (Slooff et al., 1983; Kooijman, 1987), NOEL or NOEC values (van Straalen, 1987; Stortelder et al., 1989), or alternatively by considering the structural properties of substances, e.g. QSARS. To determine the hazards posed to field organisms or ecosystems on the basis of laboratory data, use is made of extrapolation factors and safety factors. Van Straalen (1987) uses safety factors for sensitive species, extrapolation factors for the field situation, and 'habitat factors' for the relation with standard soil types. For extrapolating from acute toxicity to NOEL, from one species to several species, and from laboratory to field situation, the U.S. Environmental Protection Agency always uses a factor 10.

Although the use of extrapolation factors is becoming more common, experts agree that there is insufficient understanding of the scientific basis for extrapolation: '[The] Working Party members agreed that there is at present no scientific basis for the extrapolation of acute to chronic, species to species, and from acute/chronic data on individuals to communities and

ecosystems.' (OECD, 1988; p.18). However, it is questionable whether it is worthwhile to build up a precise understanding of the factors underlying extrapolation: due to the complexity of the environment and the large number of ambient chemicals, it would still remain necessary to incorporate uncertainty factors.

Besides the uncertainties involved in predicting the toxic effects of a compound in the field situation, there are also uncertainties in predicting the substance's concentration in the environment (Predicted Environmental Concentration, PEC) and the rate at which it disappears from the environment.

Despite current interest in risk assessment by means of extrapolation methods and modelling, many experts still recommend field study for assessing the risks under field conditions. Their arguments are summarized in De Snoo & Canters (1990) and Canters et al. (1989). Another argument in favour of field study is its value for practical model validation. In addition, field study is considered indispensable for setting up model ecosystems and environmental monitoring networks (Murk, 1987). Finally, field study is advised as providing an opportunity for assessing the side-effects of a pesticide after its approval (post-registration monitoring).

The use of field trials in pesticide approval procedures has pros as well as cons. Among the arguments in their favour are the following:

- Laboratory tests have insufficient predictive value for the field situation. Among the other factors involved, the differences between the laboratory and the field situations have their roots in differences in compound distribution and organism exposure.
- There are differences in sensitivity among the exposed species and, within one species, among exposed individuals (e.g. due to differences in population structure, background loading of the exposed organisms, environmental stress because of interactions with other substances and/or other activities).
- Field trials provide an opportunity to study ecological effects; these can hardly be determined in the laboratory.
- They provide an opportunity for field validation of predictions and models.
- They permit observation, under field conditions, of unexpected (unpredicted) side-effects which cannot be identified by laboratory research.

The limitations of field trials include:

- Field conditions fluctuate widely and results cannot be reproduced or compared (one reason being the absence of standard field trial guidelines).
- Since field trials are usually limited in scope (small area, short duration), in the field, too, it is difficult to measure effects at the population level.
- If the field trials are too insensitive, effects may be masked.
- Ethical arguments: exposing animals to potentially hazardous substances is generally considered undesirable.

Despite the above objections, the significance of field trials is generally recognized, also internationally, as witness the results of the workshop on terrestrial field testing of pesticides, held in Cambridge in September 1988 (Anon, 1988). Furthermore, in countries such as the United Kingdom and the United States, the initiative has been taken to develop field trial

guidelines and/or reference is made to previous field work meeting the standards of the relevant national authorities.

In the Netherlands, hardly any guidelines have been developed for field trials aimed at assessing side-effects or for use in approval procedures. Although field studies are performed by various Dutch institutes, this is rarely within the context of pesticide approval. These studies also differ in their scope. In addition, an extremely wide variety of methods and techniques is employed, without there being a synthesis of useful methods. In effect, there is a total absence of guidelines. Among other elements, field trial guidelines for use in the pesticide approval procedure should include:
- the conditions (criteria) for prescribing field testing
- the technical (experimental) design of the field trial
- the way the results are to be interpreted.

1.2 Objectives

Based on the above considerations, the present study aims to provide as concrete as possible building blocks for conducting field trials that can be used in the Dutch pesticide approval procedures. More specifically, the following three objectives are distinguished:

I. To indicate the possible role and status of field trials in pesticide approval procedures in the Netherlands.
II. To develop a framework for selecting field trials for detecting pesticide side-effects.
III. To develop field trial guidelines.

The study has proceeded from the following basic premises:
- There should be maximum conformity with existing approval procedures and with guidelines that are internationally recognized or already in use in other countries.
- The procedure used for determining the need for field trials and for selecting field trials should be as transparent as possible.
- The guidelines should be comprehensive, i.e. they should cover all environmental compartments and as many of the exposed functional groups within an ecosystem as possible. Agricultural functions should not be the only ones considered.
- The field trials should be laid down in the greatest possible detail.

On the last of these points, there is room for debate. While a well-defined and detailed guideline provides a firm reference point for both manufacturers and authorities, on the other hand care should be taken that the guideline is not so narrow and rigid as to offer scope for false legitimation on the part of the applicant, e.g. when the test conditions were extreme (pers. comm., Mineau, Canada).

1.3 Method

For the purposes of the study, as much information as possible has been collected on field trial guidelines and field trials conducted in the past. Use has been made of data from computerized literature databases and information obtained from specialists.

The literature search consisted of three sessions in the BIOSIS database. Two of these had already been carried out in the framework of Phases 1 and

2 of the NB project (for search profiles, see: De Snoo & Canters, 1990; Canters et al., 1989). These two searches were supplemented to cover the period from January 1987 to May 1989, using the following keywords: 'pesticide', 'herbicide', 'fungicide', 'insecticide', 'acaricide', 'nematicide', 'fumigant', 'granulate' or 'seed dressing'. These keywords had to occur in combination with: 'field', 'ecosystem', '(side) effect', 'surveillance', 'mapping', 'monitoring', 'population', 'ecological', 'forest', 'trap' or 'trapping', and in combination with 'effect', 'influence', 'plant', 'animal' or 'organism'. Studies with 'efficacy' and 'effectiveness' as keywords were excluded. This supplementary search profile yielded 53 publications.

The data on past aquatic field trials were supplemented using a literature database operated by RIVM (Netherlands Institute of Public Health and Environmental Protection). This yielded another 86 publications covering the period since 1970.

In addition to the literature search, representatives of ministries and institutes in various European countries were contacted to obtain field trials and relevant background information. Letters were sent out to the following bodies outside the Netherlands:

Austria:	-	Forschungsinstitut für Wildtierkunde, Vienna.
Belgium:	-	Ministry of Agriculture, Administration of Agriculture and Horticulture, Plant Protection Agency, Brussels.
Denmark:	-	National Agency of Environmental Protection, Copenhagen.
Finland:	-	Ministry of the Environment, Helsinki.
France:	-	Secretariat D'État Charge De L'Environnement, Neuilly-sur-Seine.
	-	l'Association Nationale pour la Protection des Plantes, Paris.
East Germany	-	Sektion Pflanzenproduktion der Martin-Luther-Universität, Halle.
West Germany	-	Umweltbundesambt, Berlin
		Der Bundesminister für Ernährung, Landwirtschaft und Forsten, Bonn.
	-	Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin und Braunschweig.
Norway:	-	Norwegian State Pollution Control Authority, Oslo.
	-	Pesticides Board of the Ministry of Agriculture, As.
Portugal:	-	Centro Nacional de Proteccao da Producao Agricola, Oeiras.
Sweden:	-	National Board on Environmental Protection, Research Department, Solna.
	-	Pesticide Approval Division, Chemicals Inspectorate, Solna.
Switzerland:	-	Federal Office for Environmental Protection, Bern.

Except for agencies in Denmark, Portugal and Switzerland, reactions were obtained from all those polled. In addition, material was obtained indirectly from the USA (EPA) and UK (MAFF) through personal contacts. Information on the procedure in Canada was obtained during a visit by Dr P. Mineau (Head, Pesticide Evaluation, National Wildlife Research Centre, Ottawa) to CML in August 1989.

Lastly, an analysis was made of the publications of several international organizations involved in pesticide approval, such as OECD, EPPO, IOBC,

FAO, EC and the Council of Europe. This analysis also included the results of a workshop on problems relating to field trials held in Cambridge in September 1988.

1.4 Report structure

The report consists broadly of three parts:

1. First, the current status and use of field trials in pesticide approval procedures is investigated; this includes a comparison of the approval procedures used in the Netherlands and in several other countries. These results are reported in Chapter 2.
2. In Chapter 3, the results obtained are used to formulate a proposal concerning the role of, and need for, field trials in the Dutch pesticide approval procedure. A screening procedure is formulated for selecting a suitable field trial in various situations, based both on the characteristics of the pesticide (e.g. fungicide, herbicide, etc., chemical group, physico-chemical properties and behaviour) and on usage data (mode of application, crop). This information is subsequently used to identify areas of suspicion and link potential effects to various groups of organisms. Specific side-effects can then be studied using a variety of field trials.
3. Finally, ten concrete proposals for field trials are presented. In preparing these guidelines, the following procedure was adopted: on the basis of the data collected, a draft field trial was first prepared. In each case, a summary was provided of existing guidelines and field studies on which the draft was based. The draft guideline was subsequently submitted to experts in the relevant fields and, where necessary, adapted to incorporate their comments. Our field trial guideline proposals were then finalized. The guidelines also include the criteria to be satisfied by results if they are to be used in the approval procedure. The general premises are formulated in Chapter 4. The proposed guidelines are presented in Part B of the report.

The present study restricts itself to the development of field trial guidelines. The proposed guidelines must of course be validated in the field, and the results obtained used to draw up definitive guidelines. Testing in the field and adaptation, where required, are outside the scope of Phase 3 of the NB project, but Section 5.3 makes recommendations for initiating and coordinating a validation programme.

1.5 Definition of terms

In this report a number of terms are used that require explanation:

- **Types of side-effects**

  Two types of side-effects are distinguished in the report: toxic and ecological side-effects (cf. De Snoo & Canters, 1990). A **toxic side-effect** is understood to be the influence of pesticide toxicity on an organism or population. This influence may be direct or indirect; in the latter case, it is due to secondary poisoning, with organisms at a lower trophic level acting as intermediaries. The consequences may be either lethal or sublethal (e.g. effects on reproduction or behaviour).

Ecological side-effects are always indirect. They occur as a result of factors other than intoxication and can be divided into effects brought about via food and via habitat. In the case of food effects, pesticide use affects the availability of food for non-target organisms; with habitat effects, the habitat of a species is altered (e.g. cover or shadow).

- **Types of field study**

In the literature, a variety of different - often implicit - criteria are used for characterizing field studies and semi-field studies. In this report a number of types of field study are distinguished:

Field study sensu stricto: study of the effects of pesticides on species occurring naturally in an existing ecosystem, which may be natural, semi-natural or cultivated. The ecosystem to be studied is integrated with its surroundings, with no form of artificial isolation. Use of nestboxes is also considered compatible with field study sensu stricto.

NB: A field study is not the same as an ecosystem study; the former may involve study of one species or a limited number of species or processes only.

Semi-field study: In this type of study, the initial situation is deliberately altered, i.e. there is manipulation. Three forms are distinguished:

- **enclosure trial:** an existing ecosystem is enclosed spatially. This may involve the use of nets to isolate part of a lake, or a grid laid over part of a field. This category also includes the use of beehives, for in practice the bees' movements are limited to the field in which the hives are placed.
- **ditch trial/plot trial:** a new ecosystem is created by digging experimental ditches or ponds or by laying out trial plots. In most cases, these test set-ups are spatially isolated for the species to be studied.
- **cage trial:** the abundance of one or more species enclosed in a highly restricted space is artificially increased. Examples include small underwater cages containing water fleas or fish, or beehives placed in cages. Cage trials can be carried out as part of an enclosure or plot trial, as well as in a field study sensu stricto.

2. CURRENT USE OF FIELD TRIALS IN THE APPROVAL PROCEDURE

In this chapter, the current status and use of field trials in assessing pesticide side-effects in approval procedures are discussed. Field trials can be incorporated into approval procedure in either of two ways (Anon, 1988)[1]:

a) as an essential and standard step in the approval procedure;
b) as a non-essential and non-standard step in the evaluation process, depending on the hazard level predicted from other information.

No concrete examples of the first approach (a) have been found, although proposals to this end have been made, for instance in the Netherlands (cf. Canters et al., 1989). In current approval procedures, the need for field testing is determined mainly by predictions based on other information (b). This usually involves a phased approach, i.e. a tiered system, in which data from initial laboratory testing are evaluated to establish whether field testing is required. This calls for criteria with which to determine the necessity of such testing. In this chapter, the field trials themselves and the criteria for their use are inventoried with reference to data from a number of international organizations, several national approval procedures and the report on a field study workshop held in Cambridge in September 1988.

First, the role of field testing and the criteria for its use in the Netherlands are discussed (Section 2.1), followed by practice in other countries (Section 2.2). The content of the trials is not considered in this chapter; this is the subject of Chapter 4. This is preceded by a proposal for a field trial selection method in Chapter 3.

2.1 Field trials in the Netherlands

In the Dutch pesticide approval procedure, field trials have never been a standard requirement, not for assessing side-effects at any rate. For fauna, additional data from cage and/or field trials are prescribed for assessing hazards to honeybees only, if the ratio between the LD50 and the highest recommended field dosage gives rise to suspicions (CTB, 1987: Application Form A, Section H.3.1). In this case, field trials are to be conducted according to the 'Guideline for evaluating the hazards of pesticides to honey bees, Apis mellifera' (EPPO, in prep.) or a comparable method. It should be noted that data on pesticide hazards to honeybees are required only if the pesticide is applied on flowering crops or plants visited by bees (CTB, 1987). In current approval procedure practice in the Netherlands, this is broadly interpreted, which means that hazards to bees are automatically studied if there is a perceived risk of exposure (pers. comm., Oomen).

For other faunal groups, assessment of the toxicity of a pesticide under field conditions may be required as 'supplementary data' and 'in certain cases' (CTB, 1987: Application Form A; Section H.7.2). Supplementary data may be requested if a need is indicated by replies to other questions, by

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[1] Field study of the (side-)effects of pesticides can also be conducted after the approval procedure, both in the framework of provisional approval and in the form of incident registration or area-based monitoring.

the nature of the application or by data on the behaviour of the pesticide in soil or water. To date, however, this has resulted in only one field study in the Netherlands, viz. on the possibility of secondary poisoning of insectivorous birds due to the use of diflubenzuron in orchards (de Reede, 1982). The study was conducted because, at the time of request for approval, diflubenzuron was a pesticide with a new mode of action: inhibition of the moulting hormone in insects.

Additional study under field conditions may also be requested in order to assess the influence of a pesticide on nitrification (soil microflora and related enzymatic processes), viz. when there is a risk of protracted influence. However, there is no reference to standard field trial guidelines.

From the above, it is evident that, except for honeybees Apis mellifera, at the moment there are no guidelines for conducting field tests to assess the side-effects of pesticides to be approved for use in the Netherlands. The application form states merely that 'it is most important that the experimental method and conditions are accurately described. Guidelines for this study can, if necessary, be drawn up in consultation with Commission [= CTB] experts' (CTB, 1987: Application form A, Section H.7.2). The form also refers to Working Document 7/1 of the British approval procedure (see Para. 2.2.2: UK)[1]. When a pesticide is claimed for integrated control, study of the hazards to beneficial insects and mites is also prescribed, however. The IOBC tests used for this purpose may in part consist of field trials (cf. Section 2.2: IOBC).

For evaluating the **efficacy** of a pesticide, guidelines for field testing do exist (PD: Netherlands Plant Protection Service, undated). These guidelines generally specify:

1 Experimental conditions: incl. choice of crop and variety, conditions relating to occurrence of attack, plot size, dimensions, number of objects, duplicates and plot arrangement.
2 Application: incl. compound(s) to be tested, application equipment, amount of spray liquid, number and time of treatments, safety period and data relating to treatments.
3 Observation: incl. methods, time and frequency of inspection for attack and further observations, such as phytotoxicity, plant development, visible residues and yield.

In total, 36 different Dutch field guidelines for efficacy testing have been drawn up. They differ in terms of the pest to be controlled and the crop groups to be protected and sometimes the spraying methods. Examples include field trial guidelines for chemical control of whitefly on vegetables and for red spider mites in fruit.

The Dutch field trial guidelines for efficacy evaluation are derived from the EPPO Guidelines for Efficacy Evaluation of Plant Protection Products. Since 1977, EPPO has drawn up 141 guidelines for efficacy evaluation, the majority for field study; for a full list, the reader is referred to EPPO (1989). In these efficacy trials, marginal attention is paid to possible side-effects: 'Any observed environmental effects should also be recorded, especially effects on wildlife and/or beneficial organisms' (EPPO, 1989).

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[1] It should be mentioned that the evaluation of side-effects of pesticides to be approved for use in the Netherlands often takes place abroad.

In addition, there are two EPPO guidelines for assessing side-effects in the field (cf. Section 2.2: EPPO).

Finally, the Dutch pesticide approval procedure also provides for the use of a pesticide for experimental purposes (so-called experimental exemption) (Van Rijn, 1989). Approval for such exemption might also include field testing but, again, standard guidelines are lacking.

NB: To date, only a limited number of species have been the subject of laboratory testing in Dutch pesticide approval procedures. As part of the Netherlands Integrated Soil Research Programme, a relatively large number of new test guidelines with other organisms are being drawn up (Eijsackers & Bosma, 1989). So far, these guidelines relate primarily to laboratory tests, and then only involving the soil fauna and the aquatic fauna. For the latter group, mesocosmos research is also being carried out by DBW/RIZA (National Institute of Inland Water Management/National Institute of Wastewater Treatment), TNO (Netherlands Organization for Applied Physical Research) and WL (Delft Hydrodynamic Laboratory) and other institutes; see also Eijsackers & Bosma (1989). For certain groups such as plants and birds, however, there are still no guidelines in preparation.

2.2 Field trials in an international context

In Para. 2.2.1, the activities of a number of international organizations are discussed, at least in so far as these relate to field trials for use in the context of pesticide approval. This subsection also reports on the Cambridge workshop. In Para. 2.2.2, the approval procedures of several national governments are discussed individually.

2.2.1. International organizations and the Cambridge workshop

Below, the activities of the following international organizations are discussed in turn: OECD, IOBC, EPPO, FAO, Council of Europe and EC. As a general remark it can be stated that, under the responsibility of the first three organizations guidelines are actually in preparation at the moment, while the other three refer to guidelines developed or being developed elsewhere.[1]

OECD

Under the responsibility of the Organization for Economic Cooperation and Development (OECD), many guidelines have been prepared for testing the effects of chemicals (including pesticides) on biotic systems (OECD, 1986). These guidelines are for laboratory tests only. In addition, the OECD has published a list of prpcedures used in the approval of new compounds (OECD, 1984).

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[1] This subsection provides no information on approval procedures outside the Netherlands; such information can be found in publications by the OECD (1984, 1988) and the Council of Europe (1989).

In June 1988, the OECD organized a workshop in Washington on the theme of 'Ecological effects assessments' (OECD, 1988). At this workshop, existing test guidelines were examined and focal points indicated for further research as well as for new guidelines. It was stated that, in general, three stages can be distinguished in approval procedures, characterized by an increase in complexity of study and a shift in emphasis from laboratory to field data:

- the initial, or screening stage (above all, acute toxicity tests)
- an intermediate stage (including chronic effects)
- a comprehensive stage (including broader ecosystem studies).

With regard to field trials, the following emerged during the workshop:

- Aquatic organisms: The experience gained in the aquatic environment renders practical micro- and mesocosmos trials feasible; in addition, the cost/result ratio has become more favourable. Still, it was stressed that more experience should be gained with the various systems and that the scope for using enclosures should be extended.
- Earthworms: Field trial aimed at observation of the effects of chemicals on (the activity of) earthworms (leaf decomposition) and their population structure; this test is being developed in West Germany (cf. Para. 2.2.2 and Chapter 4).
- Microflora and soil micro/mesofauna: Litterbag test: litter decomposition is measured in nylon bags buried 5 cm deep in the soil; decomposition is regarded as a variable, yielding information on the microflora and micro-/mesofauna.
- Beneficial arthropods: Cage, tent and field trials to assess mortality, behaviour and loss of (predatory) function.
- Honeybees: Cage, tent or field trials to evaluate the effect of chemicals applied to flowering crops; the parameters studied are mortality, foraging behaviour, brood development and larval toxicity; the need for a guideline was emphasized.
- Birds: Cage and field trials; cage trials for natural exposure. However, when cages are used, the number of uncontrollable variables is very large. An EPA draft protocol was referred to. Field trial: mortality, binomial analogous to the EPA test, and quantification of mortality. Radiometry can enhance the reliability and usefulness of field trials, but costs increase proportionately.

Despite these conclusions and the key role of field trials in the approval procedures presented, the workshop made no separate recommendations on the development of field trial guidelines. This is probably due to the participants having serious doubts about the scope and feasibility of standard field trials.

One of the recommendations workshop participants did make to the OECD was that this organization should encourage creation of a databank to facilitate comparison of data from field studies and mesocosmos research with the effects predicted on the basis of laboratory results.

IOBC

The International Organization for Biological Control of noxious animals and plants (IOBC, West Palearctic Regional Section, Working Group on Pesticides and Beneficial Organisms) monitors the side-effects of pesticides on beneficials (parasites and predators of pest organisms). A multitude of guidelines for laboratory tests have been developed (cf. Hassan et al.,

1985; IOBC, 1988), as well as a number of semi-field and field trial guidelines.

IOBC employs a tiered approach to evaluate side-effects on beneficials. First, an initial toxicity test is conducted in the laboratory; if the pesticide appears to be hazardous, testing is continued in a semi-field context, with initial toxicity and persistence being assessed. If the results confirm suspicions of a pesticide's hazard, field trials are then prescribed.

For assessing hazards, IOBC employs four categories (harmless, slightly harmful, moderately harmful, and harmful), based on the degree of damage (e.g. toxicity) to the non-target organisms examined and the persistence of the harmful action (cf. Hassan et al., 1985). These four evaluation categories are also used to interpret the results of field trials. A pesticide is considered harmless at < 25% mortality, slightly harmful at 25-50% mortality, moderately harmful at 51-75% mortality, and harmful at > 75% mortality (Hassan et al., 1985).

In all, seven IOBC field trials have been described to date (Hassan et al., 1985;, IOBC, 1988):

1 predatory mite Typhlodromus pyri in orchards
2 predatory mite Typhlodromus pyri in apple orchards
3 predatory mite Amblyseius finlandicus in apple, pear and cherry orchards
4 predatory mite Phytoseiulus persimilis in greenhouses
5 ichneumon fly Encarsia formosa in greenhouses
6 arthropods in field crops
7 arthropods in apple orchards.

It should be noted that in the first five trials the species referred to are actively used for biological control.

NB: The IOBC approach to hazard assessment, moving in steps from laboratory test to semi-field trial to field trial, with an attendant reduction in uncertainty margins, is also considered adequate by Dutch specialists, at least for pesticides (Eijsackers & Bosma, 1989).

EPPO

The European and Mediterranean Plant Protection Organization (EPPO) is engaged primarily in evaluation of the efficacy of pesticides (see Section 2.1). However, there are also two EPPO field trial guidelines for assessing side-effects, viz. for the ichneumon fly Encarsia formosa (EPPO, 1989) and the honeybee Apis mellifera (EPPO, 1986). The test for Encarsia formosa is in conformity with the IOBC standard. In testing the side-effects on these two species, a tiered approach is adopted, in which the last step is always a field trial.

In the case of the ichneumon fly, a beneficial organism, a field trial is conducted if the compound under review cannot be classified as hazardous or innocuous to this species on the basis of prior laboratory testing and usage data. Laboratory testing consists of a residual toxicity test, a direct contact toxicity test, and a persistence test. (EPPO proposes using 50% harmfulness or harmlessness as a hazard threshold). The aim of the field trial is to provide a definite conclusion as to whether or not a

pesticide is harmful to the ichneumon fly and to indicate the conditions under which a pesticide may be harmless.

The field trial for honeybees, too, is carried out only if the pesticide cannot be classified as harmful or harmless to bees on the basis of prior laboratory tests and usage data. In this case, laboratory testing comprises a test for oral toxicity, a test for contact toxicity and, optionally, a cage trial. The harmfulness is expressed in terms of a 'hazard ratio' defined as LD50 x dose/ha. A hazard ratio of $\geq$ 2500 is considered hazardous to bees, and a ratio of $<$ 50 innocuous. For honeybees, too, the aim of the field trial is to yield conclusive results on hazards in cases where previous tests have not provided absolute certainty.

FAO

The U.N. Food and Agriculture Organization (FAO) has published 'Guidelines on environmental criteria for the registration of pesticides' (FAO, 1985). This document deals with the environmental criteria for pesticide approval, in the context of both pre-registration and post-registration. The FAO adopts a four-tier approach to pesticide registration. The first tier focusses on both the usage data and the primary data of the active ingredient, such as physico-chemical properties and laboratory-assessed toxicity. In the second tier, supplementary laboratory data may be requested (e.g. on bioaccumulation). In the third tier, further research is performed, if so desired, which usually implies field study. The fourth tier, finally, comprises post-registration monitoring, focussing on both chemical and biological aspects. The last tier is carried out in cases where there still remain doubts on the validity of earlier predictions.

FAO has elaborated this general approach for six groups of species, specifying test guidelines for each group and each tier. It should be noted that for this purpose FAO uses existing guidelines, developed in a different context. The six species groups are: non-target soil micro-organisms, non-target soil macro-organisms, honeybees, beneficial arthropods, non-target aquatic organisms and birds. For each group, the criteria for testing, the test sequence, the test conditions and the appropriate experimental design are specified. There follows a brief description of the criteria and test guidelines for the various species groups (FAO, 1985):

1 Non-target soil micro-organisms: The effects considered are on functional soil processes, such as respiration and nitrogen conversion. Field trials are conducted if significant changes in these processes are observed during laboratory testing. The field trial then aims to assess the severity of these changes. For nitrogen-fixing plants (with root nodules), it is indicated that effects can only be evaluated in greenhouses or in small-scale field trials.
For suitable tests and supplementary information, the reader is referred to the literature and to a number of international workshops and symposia at which problems have been discussed (e.g.: Greaves et al., 1980).

2 Non-target soil macro-organisms: For this group of organisms, too, a functional approach is adopted. The effects considered are litter decomposition (especially by earthworms) and predation. The latter parameter is also studied in the test on beneficial arthropods (see 4, below). In the case of earthworms, the potential importance of bioaccumulation for effects on birds is mentioned. To monitor effects on earthworms, field trials are recommended in addition to laboratory testing. For studying

litter decomposition, field trials are even stated as being the only practicable approach, laboratory simulation being impossible.
For a field trial guideline with earthworms (population study), reference is made to the British approval procedure (MAFF, 1986). For earthworm collection and soil treatment, reference is made to previous studies. For a litter decomposition field trial, too, the British guideline is referred to.

3 Honeybees: Honeybees are deemed important because of their role as pollinators and for their honey production. Field study is called for if laboratory tests show the contact LD50 of a pesticide to be below 10 µg active ingredient per bee, or if bee mortality exceeds 10% after application of double the recommended dose in a direct spray test; in addition, the bees in the field must run a risk of exposure when the pesticide is used.
FAO specifies a cage trial or a field study, depending on the specific properties. For a (small-scale) cage trial, the method described by the 'Commission des Essais Biologiques' (CEB, 1982) or the guidelines of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft' (BBA, 1980) are considered suitable. If no reliable conclusion can be drawn on the basis of earlier tests, a larger-scale field trial may be conducted. For this trial, in addition to the methods already mentioned, the FAO refers to the British field trial guidelines for honeybees (MAFF, 1986: Working Document 7/4) and to the results of a workshop on the harmonization of test methods for honeybees (ICBB, 1985).

4 Beneficial arthropods (parasites and predators): These tests are required, above all, for pesticides claimed for use in integrated pest management. The results obtained in field trials with the pest-predator complex should weigh heavier than the results of laboratory testing.
For suitable field trial methods, the German guidelines (Para. 2.2.2) and the methods developed by IOBC (p.10) are referred to. In addition, reference is made to the British approval guidelines (MAFF, 1986).

5 Non-target aquatic organisms: If the hazard cannot be adequately assessed on the basis of laboratory testing, or if a pesticide is to be used intentionally in water courses, field testing (incl. enclosure studies) is required. Only by means of field trials can the recovery of an affected population be observed and the indirect (food) effects for the various species in the system be studied.
No existing field trial guideline or field study is referred to, but it is reported that methods are in preparation and that tests are to be carried out in waters where the population dynamics of the occurring species are well known.

6 Birds: Cage studies with birds are required if no realistic hazard evaluation can be made on the basis of laboratory tests alone, i.e. only if this is indicated by the oral toxicity of the pesticide in relation to the exposure pattern or residues in the diet of important species. If cage studies yield no reliable hazard assessment, field trials under practical conditions can be conducted. The data thus obtained should carry the greatest weight.
For suitable cage studies, reference is made to an EPA guideline (EPA, 1978). [N.B. This guideline has meanwhile been withdrawn because of methodological problems.] For a field trial method, reference is made to the British guideline (MAFF, 1986).

Council of Europe

Another organization involved in the evaluation of pesticides in the context of approval procedures is the Council of Europe (CoE, 1984). In terms of the side-effects of pesticides, the most important chapter of this book is 'Guidance on environmental phenomena and wildlife data'. This chapter was rediscussed in Paris in May 1989 at a hearing of the Council of Europe and EPPO (CoE, 1989).

The Council of Europe also favours a tiered screening procedure. Following laboratory tests, 'if any doubts remain regarding the validity of initial appreciations, studies should be carried out in the field' (CoE, 1984). The objective of the field trials is 'to confirm predictions based on toxicology tests and to determine effects which cannot be predicted from laboratory experiments'.

In the assessment of side-effects, several groups of organisms are distinguished (CoE, 1989): vertebrates (birds and mammals), soil flora and soil fauna, insects (beneficial insects and arachnids, pollinators and insects 'without significance') and aquatic organisms.

The main outcome of the hearing was that cooperation in this field between the Council of Europe and EPPO should be focussed on the following points (CoE, 1989):

- the need for an evaluation system for ecotoxic effects based on a set of basic data, but at the same time providing adequate flexibility and allowing incorporation of expert opinion;
- the need to know whether a test is aimed at the active ingredient and/or the various possible formulations;
- the question whether effects should first be studied at an ecosystem or a species level, and in what detail this should be done;
- the question whether the approval procedure should have a more rigid or a less specified character;
- the extent to which alternatives are needed alongside species toxicity tests;
- the premises underlying approval control and post-approval monitoring;
- use of degradation rates to determine the DT50 and the DT90 instead of the half-life;
- how to ensure adequate interpretation of data on exposure, behaviour and toxicity.

In addition, it was strongly recommended to continue the initiated cooperation between the Council of Europe and EPPO and also to involve other (international) organizations.

The European Community

In February 1989 the Commission of the European Communities issued a 'modified proposal for a directive of the Council [of Ministers] relating to market introduction of EC-approved crop protection agents' (EC, 1989). This included a specification of the data required for preparation of 'a communal, positive list of active ingredients, the use of which is deemed a priori safe for man, beast and the environment'. This positive list will specify those compounds that have been approved for use in the EC; approval of formulations will continue to take place at the level of individual member states. The EC also aims at mutual recognition of approvals issued

in member states. The active ingredients currently approved for use in the EC (about 440) are scheduled for re-evaluation in 10 years' time.

Among the data required before a substance can be considered for inclusion in the community's positive list are data on organisms collected under field conditions, in the framework of ecotoxicological study. Among the organisms to be monitored are non-target soil macro-organisms such as earthworms (EC, 1989: Annex II, Section 7.1.9). However, all that is presented is a list of required data, without any indication of the conditions under which field and other data are to be submitted.

In January 1990, the British Crop Protection Council (BCPC) organized a symposium in Reading (UK) to discuss the harmonization of pesticides registration as proposed by the EC ('Future changes in pesticides registration within the EC'). The participants, mainly from industry, were (very) critical of the proposal, one reason being that many essential details had not been provided - probably because no agreement could be reached on them. One of the problems mentioned was the protection of data. It was suggested the EC proposal should be introduced stepwise: harmonization of, first, the required data, then the tests and finally the registration. Representatives of the member states made it clear that the economic basis for harmonization should not be provided at the expense of environmental protection. The industry suggested an alternative in the form of provisional registration, with which experience has been gained in the UK, France and Ireland. The EC proposal for pesticides registration within Europe resembles the current situation in the United States, where approval for the active ingredient is issued centrally by the EPA, with individual states responsible for approval of formulations, which may be subject to supplementary requirements.

Cambridge workshop

In September 1988, a workshop was organized in Cambridge (UK) on the theme 'Terrestrial field testing of pesticides' (Anon, 1988). At this workshop, the need for field trials was evaluated. The following main elements were discussed: objectives, design, interpretation of results, and the potential of using field trials for post-registration monitoring. In particular, the side-effects of pesticides on vertebrates were discussed.

The participants concluded that there is a great deal of uncertainty involved in field evaluation of pesticides. For this reason, a field trial should have a flexible design, as expert know-how is essential for proper planning, execution and interpretation of results.

On the need for field testing it was concluded that:

> 'Field testing is an essential part of safety evaluation for some, but not all, products. The need should be judged on predictions of hazard based on toxicity, metabolism, use pattern, environmental fate and likely exposure of wildlife.'

The impossibility of employing well-defined procedures to decide on the need for field testing was also mentioned, there being so much variation among field situations as to automatically rule out the use of standard procedures. For deciding whether field testing is required, it was conclu-

ded, a case-by-case approach should be adopted, allowing for due flexibility as well as for expert opinion.

While there was general agreement on the need for criteria to determine whether or not to go ahead with field testing, it was considered impossible to formulate universal criteria that could serve as 'automatic triggers' for tests covering all forms of pesticide usage. Instead, a number of general recommendations were made on the type of information that might be used for this purpose:

- estimates of possible wildlife exposure to the pesticide, based on measurements of the product's concentration and distribution (in soil, water, vegetation, etc.; degradation and persistence) and the ecology and behaviour of the fauna coming into contact with the pesticide [comparable to the use of *agro-ecosystems* by De Snoo & Canters (1990)];
- toxicity, measured by standard laboratory procedures or extrapolated using data obtained on 'surrogate species';
- the assumed scale of use and methods of application;
- the comprehensiveness of post-registration observations and biomonitoring schemes.

In themselves, however, none of these recommendations suffice for indicating a potential risk, which should be assessed on the basis of expert judgment, possibly supported by existing risk assessment methods and development of a sophisticated predictive model employing a large number of chemical and biological parameters.

Under certain circumstances, the workshop participants considered field testing undesirable, viz.:

- if protected or endangered species would be exposed, or
- if the pesticide is to be used on a small scale, not justifying a (large-scale) field trial.

In terms of the scope of a field trial, a distinction was made between intensive trials (detailed study at a few locations) and extensive trials (less detailed study at a larger number of locations). Both methods have their pros and cons, but intensive studies usually require more manpower.

Field test methods may be of either a qualitative or a quantitative nature, but they must always be sensitive enough to detect adverse effects. It was noted that although a search for casualties will bring to light any substantial mortality, it will not enable it to be quantified.

With respect to the post-registration phase, a distinction was made between monitoring (planned, active sampling of populations at risk) and surveillance (study in response to reported mortality). The latter may provide information on certain aspects not studied in controlled experiments, e.g. unforeseen hazards, effects under unusual conditions, effects on rare species, pesticide abuse.

2.2.2 Approval procedures in other countries

In the pesticide approval procedures of most Northwest European and North American countries, use is made of decision procedures and/or trial guidelines such as those drawn up by the OECD, EC, EPPO, Council of Europe and FAO. A number of countries, however, use their own schemes and guidelines side by side with the international standards. Three countries that have

elaborated this approach, the United States, England and West Germany, will now be discussed.

United States

In the United States, one of the tasks of the Environmental Protection Agency (EPA) is ecological risk assessment. This has resulted in the development of Pesticide Assessment Guidelines, which provide clear guidance to applicants on how to conduct the tests. The required data are classified into four tiers. The first tier comprises basic toxicity data, such as LD50/LC50 values for birds. In additional tests (tier 2), reproductive and life cycle effects are studied; these tests are required when basic data and/or environmental conditions point to likely problems. Semi-field or field trials (tiers 3 and 4) are required when widespread impact is anticipated that cannot otherwise be observed (EPA, 1982a, 1986a).

The decision to conduct field trials is taken in ad hoc consultations between the EPA and the applicant (EPA, 1982b). Only for tests involving plants are well-defined criteria employed (see below).

For field trials, the EPA distinguishes the following species groups:

1 Terrestrial flora (non-target area testing): This test is to be conducted - on an ad hoc basis - if anticipated exposure exceeds the EC25 determined in the laboratory.

2 Terrestrial invertebrates:
- **Honeybees** Apis mellifera: Testing is required if the preceding (laboratory) tests have indicated a hazard: more specifically, a field trial is required if other than direct toxic effects are anticipated; if such effects are indeed likely, the hazard to bees is restricted by adapting the user guidelines.
- **Predators and parasites:** For this group of species, no guidelines are provided; the details of a tier system are still being discussed.

3 Birds: Field testing is required (ad hoc and after consultation with the EPA) if prior testing has indicated a likely hazard and if field trials can contribute useful data for assessment. For semi-field trials, a summary of a field trial guideline acceptable to the EPA is given (EPA, 1982a).

4 Mammals: Field trials involving mammals are subject to the same conditions as those for birds.

5 Aquatic flora: For aquatic plants, field testing is required - again on an ad hoc basis - if exposure is anticipated to exceed the laboratory EC50 value (EPA, 1982b).

6 Aquatic fauna: A decision on a field trial is again taken ad hoc and in consultation with the EPA. A short-term semi-field trial is required if adverse short-term effects are anticipated and if such a trial is expected to yield useful results. The decision to proceed with field testing takes into account all laboratory, usage and exposure data. Long-term field or semi-field trials are conducted (ad hoc and after consultation with EPA) if i) adverse long-term effects are expected, ii) there is a risk of cumulative effects, or iii) life-cycle tests indicate such a need. Again, there must be reasonable prior grounds for supposing that such testing will yield useful data for evaluation. There are no standard guidelines for such tests; instead, the EPA refers to the literature.

On closer inspection, it transpires that with the EPA, too, details of the tests remain largely unspecified. According to the EPA, field conditions may be so diverse as to render a standard field trial guideline pointless. For procedural details of field trials, EPA generally refers to the literature.

However, the EPA does wish to standardize evaluation, by means of so-called Standard Evaluation Procedures for the interpretation of trial results (EPA, 1986b). The risk criteria employed in this context indicate whether the risk involved in application of a pesticide is zero, acceptable or unacceptable risk (EPA, 1986a). Because of the strict requirements imposed, for instance in terms of experimental design, these Standard Evaluation Procedures in part determine the boundary conditions of the test.

United Kingdom

The British approval procedure is also based on a tiered system of hazard assessment. In the UK, there are no 'automatic triggers', decisions to proceed with further testing being taken on the basis of expert judgment. Application is first discussed by the Scientific Sub-Committee, which studies the applicant's data in conjunction with other available information. The application is then passed on, via the Advisory Committee on Pesticides, to the Registration Departments of the competent ministries.

In the British approval procedure, field testing focusses on indicator species, viz. **honeybees** Apis mellifera and **'birds most at risk'**. For these species, there are detailed field trial guidelines in the form of Working Documents (MAFF, 1986), which set out the trial background and method as well as evaluation of results.

Only some of the criteria for determining the need for field trials with these species are indicated. For honeybees, a field trial (with the formulation) is to be carried out if the crop is sprayed during the flowering season. For birds, field testing is required if there is a risk of direct ingestion of the pesticide, in the case of seed treatment for instance. In such cases, there is a need for data on the diet of the species anticipated to be at greatest risk as well as on the formulation used (MAFF, 1986).

The field trial guidelines for honeybees and birds are designed to assess short-term hazards, following mainly from the acute toxicity of a pesticide. Field testing - according to the British philosophy - is not suitable for assessing long-term hazards that may arise from cumulative or chronic toxicity, accumulation in the food chain, or disturbed ecological balance in the natural flora and fauna. Short-term hazards are considered especially important if the pesticide is planned for widespread use and if substantial mortality is caused by its use. Short-term hazard field testing is of limited scope, its duration being restricted to a single season.

Since long-term hazards do not normally lead to sudden manifest mortality, they may go unnoticed until after considerable damage has been done. There are no simple (field) experimental procedures for measuring such hazards. For this reason, and because of the limited suitability of field trials for evaluating short-term hazards to wildlife, it is sometimes considered desirable to monitor actual pesticide usage, particularly in the case of pesticides with physical, chemical or toxic properties suspected of having

a potential impact on wildlife. In these cases, temporary approval may be granted, so that effects can be monitored before considering definitive approval of the pesticide. A case in point is the study by Bunyan et al. (1981) on the side-effects of a new aldicarb formulation (granulate) used to control nematodes in beets and potatoes. After an intensive plot study, the side-effects of a commercial application were also monitored. On the basis of the data thus obtained, a less harmful application method was recommended.

In addition to the species already discussed (honeybees and birds), there is also a Working Document for evaluating the impact of pesticides on soil macro-fauna. This guideline consists of three parts, dealing with effects on: i) litter decomposition, ii) beneficial arthropods (carabid and staphylinid beetles) and iii) earthworms. The field test is elaborated in much less detail than those for birds and honeybees, but the experimental design and background are indicated and literature references provided. No clear criteria are given for undertaking field trials with these species, tests being conducted only when a need is 'indicated' by laboratory study.

Finally, field study with aquatic organisms may also be required to evaluate the 'overall effect' of the formulation on invertebrates (as the basic diet of fish). There is no Working Document for such study, nor are any decision-making criteria specified. The only 'indicators' for field trials are direct spraying of water or banks, or widescale use of the formulation in forests.

West Germany

The West German approval procedure, too, comprises essentially three tiers, the last of which is a field study. In general terms, the basis philosophy is that, in field testing, the less a standard test procedure is adhered to, the harder it becomes to achieve reproducibility and valid interpretation of results (as is the case in laboratory situations). For this reason, it is aimed to screen out the hazards of as many compounds as possible in the first and the second tiers of the approval procedure (laboratory and semi-field testing). Field testing is then prescribed only for those compounds whose hazards have not yet been adequately assessed.

For honeybees, this approval procedure has meanwhile been developed to a point where it is considered 'ideal'. The BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) intends to develop similar methods for other faunal groups as well as for the microflora. Because of the status of field trials in the approval procedure, there are still only a limited number of field trial guidelines. To date, they have been developed for the following species groups (pers. comm., Kohsiek):

- soil microflora: guideline available (since 1987[1]);
- soil mesofauna: guideline in preparation;
- earthworm test: guideline in preparation;
- honeybees: guideline available (BBA, 1980);

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[1] BBA Richtlinien für die amtliche Prüfung von Pflanzen-schutzmitteln, Teil VI, 1-1: Auswirkungen auf die Aktivität der Bodenmikroflora, März 1987 [BBA Guidelines for official testing of plant protection compounds, Part VI, 1-1: Effects on soil microflora activity; March 1987].

- beneficial arthropods: guideline available for two species , viz. beneficial arthropods in arboriculture (BBA, 1981) and predatory mites in viniculture (BBA, 1986).

For aquatic organisms or terrestrial vertebrates, a field trial can be set up in consultation with BBA in cases where this is deemed necessary. However, there are no guidelines available, nor have any criteria been formulated for assessing the need or desirability of such a trial.

N.B.: Since December 1989, the West German approval procedure requires submission of data on the effects on beneficial organisms. These data are obtained using the IOBC tests (pers. comm., Oomen).

2.3 Survey of field studies

A great deal of study has also been carried out outside the framework of pesticide approval. In this section we review these studies, to give an idea of the types of effects already studied, the type of field tests employed and the organisms involved. To this end, a semi-quantitative analysis has been made of the available literature. Many of the experimental aspects of the individual studies, such as design and method, have been used in drawing up the field trials guidelines proposed in Part B.

This review of practical field trials - i.e. those actually carried out- is based on the database of literature so far collected for the 'Side-effects of Pesticides' project (for search profile, see Chapter 1). From this database, all literature on field studies of pesticide side-effects has been singled out (with the exception of trials for incident registration and studies in the tropics).

In total, some 250 articles were found, about 170 of which relate to terrestrial organisms and about 80 to aquatic organisms. It can thus be concluded, at least on the basis of our results, that terrestrial field studies outnumber those carried out in the aquatic environment.

The selected literature has been categorized in terms of 1) type of field study, ii) type of effect, and iii) species group. The results are presented in Table 2.1 and Table 2.2, which show which types of study (see Section 1.5) have been used to examine the various types of effect on the principal species groups. On the basis of estimates and subcounts, the tables indicate to what extent studies have been conducted with each group of organisms. Below, the two tables are discussed in further detail.

Type of field study

Field studies have been classified as follows: field study sensu stricto, enclosures, experimental plots/ditches and cage studies (cf. Section 1.5). It can be seen from Table 2.1 and Table 2.2 that by far the majority of studies are field study sensu stricto or studies employing experimental plots and ditches. Cage studies and enclosure studies are rare, exceptions being cage studies with fish and, to a very limited extent, with other vertebrates, and enclosure studies with insects, most of which concern beehive trials.

AQUATIC	type of field study				type of effect			
	fld.ss	encl.	exp.d.	cage	tox.d.	tox.i.	eco.f.	eco.h.
plankton/algae	+	+	+		++	n.a.		
higher plants	+		+		+	n.a.		
unsegm. worms	+		+		+			
molluscs	++		++		++			
segm. worms	+		+		+			
crustaceans	++		++		+++			
spiders/mites	+		+		+			
insects	+++	+	+++	+	+++			
fish	++		++	++	+++		++	
amphibians	+				+			
reptiles								
birds								
mammals								

Table 2.1 Aquatic field studies, by species group, type of study and type of effect; + = rare, ++ = frequent, and +++ = very frequent; fld.ss = field study sensu stricto; encl. = enclosure; exp.d. = experimental ditch; tox.d. = toxic, direct; tox.i. = toxic, indirect; eco.f.= ecological, food, and eco.h. = ecological, habitat. (n.a. = not applicable)

TERRESTRIAL	type of field study				type of effect			
	fld.ss	encl.	ex.pl.	cage	tox.d.	tox.i.	eco.f.	eco.h.
higher plants	++		+		++	n.a.		
unsegm. worms	++		+		++			
molluscs					+			
segm. worms	+++		+++	+	+++		+	
crustaceans	+				+			
spiders/mites	+++		+	+	+++			
insects	+++	++	++	+	+++		+	+
amphibians								
reptiles								
birds	+++			+	+++	+	++	+
mammals	+++			+	+++	+	+	+

Table 2.2 Terrestrial field studies, by species group, type of study and type of effect; + = rare, ++ = frequent, and +++ = very frequent; ex.pl. = experimental plot; for other abbreviations, see Table 2.1.

Type of effect

The following types of side-effects have been distinguished: direct toxic effects, indirect toxic effects, food effects and habitat effects (cf. Section 1.5). Tables 2.1 and 2.2 show that by far the majority of field studies have focussed on direct toxic effects. In a small number of cases, indirect toxic effects have also been studied; these studies focussed almost exclusively on vertebrates. Only a few field studies have been found on the ecological food effects of pesticides and, again, these were concerned primarily with vertebrates. By far the least common were studies relating to habitat changes due to pesticide use.

Comparing the type of field study with the type of effects studied, it is interesting to note that toxic effects are examined in field studies sensu stricto, in trial plot/ditch studies and in cage studies. Ecological effects, however, are investigated mainly in field studies sensu stricto.

Species group

In terms of species groups, in the terrestrial environment invertebrate studies have focussed mainly on segmented worms, insects, spiders and mites; in most cases, earthworms, honeybees and 'beneficial organisms' are involved. There has also been frequent study of birds and mammals. By comparison, studies on molluscs, crustaceans, amphibians and reptiles are rare.

In the aquatic environment, studies have focussed largely on molluscs, crustaceans and insects. Spiders and mites have received little attention, certainly in comparison with terrestrial studies. Among aquatic vertebrates, it is mainly fish that have been studied.

2.4 Discussion

From the above sections, it can be concluded that field study plays only a minor role in the Netherlands. Except in the case of honeybees, field trials are not a standard requirement and there are hardly any guidelines for such trials. In the Netherlands, moreover, scarcely any experience has been gained with field studies conducted in the context of pesticide approval. The Dutch procedure is thus based almost exclusively on laboratory testing. A large proportion of current research is aimed at broadening the scope of such laboratory testing (more types of test) or at improving extrapolation from the laboratory to the field.

In an international context, i.e. in the procedures specified by national governments and by various international organizations, field trials feature more prominently. The approval procedures examined, viz. those used in the United Kingdom, West Germany and the United States, are characterized by a more explicitly tiered structure, as also favoured by the OECD and FAO, for example. In these procedures, the general principle is that maximum clarity about the substance under review must first be obtained by means of the simple laboratory tests. If these tests do not dispel all doubts, a semi-field or field study is conducted. In most of the approval procedures examined, the criteria for moving from one phase to the next are indicated only sketchily, and relate to toxic side-effects only.

Internationally, there are a reasonable number of field trials available. Trials with honeybees, beneficial insects and earthworms are often mentioned as an option, and for these species and species groups a number of guidelines have now been published. For birds, too, a number of guidelines are now available. However, for other groups of organisms such as plants, aquatic organisms and mammals, there is considerably less interest.

Although various field trial guidelines are available on paper, the extent to which such trials are actually in use is unclear. At any rate, there are very few results available. In contrast, there is a great deal of information on 'general' field studies, i.e. studies not conducted in the context of pesticide approval procedures. An analysis of these studies shows that, compared with the terrestrial environment, very little work has been done on the aquatic environment. Studies in the terrestrial environment have focussed mainly on beneficial arthropods (insects and arachnids), earthworms, birds and mammals. There have been only a few field studies on the side-effects of pesticide use on (terrestrial) molluscs (e.g. snails), crustaceans (e.g. woodlice), amphibians and reptiles. Most of these are field studies sensu stricto, focussing on direct toxic effects, with ecological effects being studied far less frequently.

Most studies in the aquatic environment have focussed on insects, molluscs, crustaceans and fish. Besides field studies sensu stricto, much work has also been carried out with experimental ditches. In the aquatic environment, too, it is above all the direct toxic effects of a pesticide that receive the greatest attention.

In comparing existing field trial guidelines and 'general' field studies, it is surprising to note that, even though many studies involve aquatic organisms, this has not resulted in a proportionate number of guidelines. On the basis of the data from 'general' studies, however, it should be possible to formulate proposals for such guidelines. The same also holds for (groups of) terrestrial organisms (see Chapter 4).

In various circles, there is currently a debate about the extent to which the details of field trials should be laid down in strict procedures. It is argued, on the one hand, that the variation existing in environmental factors and pesticide application methods excludes the possibility of standard field trial guidelines; on the other hand, a case-by-case approach (with the procedure determined in consultation between applicant and authorities) impedes standardization of both the methods and the interpretation and comparison of results. In drawing up our proposals for field trial guidelines we have opted, provisionally at any rate, for a relatively detailed specification of procedures. There are several reasons for this decision. Contrary to the situation in Canada or the United States, for instance, there is little need in the Netherlands to allow for wide variations in environmental conditions such as climate. At the same time, we have aimed as far as possible to eliminate arbitrary elements from the approval procedure. One of the advantages of this approach is that third-party control can be carried out in an objective manner. To cater for unusual circumstances, however, there should be scope for modifying the field trial guideline. On no account, though, may a standard field trial guideline be used to legitimize test results by an applicant confronted with extreme test circumstances. There will always be a need for consultations between the applicant and the authorities (with scope for a certain degree of public control). To a major extent, however, acceptance of the

proposed guidelines automatically implies establishment of these boundary conditions.

The proposed guidelines (see Part B) can therefore be characterized as 'basic guidelines', allowing for adaptation to specific circumstances dictated by the nature of the pesticide (formulation) or mode of application. A similar role for field trial guidelines was also advocated by Clegg (Health and Welfare, Tunney's Pasture, Ottawa, Canada) at the EC Symposium held at Reading in January 1990.

In view of the above considerations, for the time being at any rate, field testing will not be a routine operation, but will require a 'customized' approach. It should also be noted that the proposed procedure for initial screening also allows for a certain degree of flexibility.

In the Dutch approval procedure, the role of field trials should be more clearly defined, with field testing taking place only if prior laboratory tests indicate a need or if ecological hazards are suspected. The criteria used for deciding on a field trial should be underpinned as explicitly as possible. A proposal to this end is elaborated in Chapter 3.
It should be added that experience gained with field trials can also contribute substantially to validating extrapolation techniques currently under development, particularly in the Netherlands.

3. PROPOSED FIELD TRIAL SELECTION METHOD

Chapter 2 reviewed the role of field trials in national and international approval procedures, specifying when trials are to be performed. In this chapter, a proposal is presented for an approach towards improving the situation in the Netherlands. First, the proposed procedure is outlined and the need for field trials in the Dutch context explained (Section 3.1).

Once the desirability of a field trial has been established, the question arises which trial (which effect, which organism) can or should be conducted. In the following, a selection procedure is elaborated for this purpose, on the premise that use should be made of the data available at the time of the request for pesticide approval, i.e. laboratory and usage data. It is indicated how these data can be used to translate a suspicion of side-effects into a choice for one or several field trials. To this end, an assessment is first made of the anticipated effects (Section 3.2). Next, a decision is made on the preferred organisms for field trials and the type of field trial to be used (Section 3.3). In Section 3.4, the entire procedure is summarized in diagrammatic form. Section 3.5 illustrates the procedure with reference to two pesticides.

The proposed procedure leads to conclusions on the occurrence of ecotoxic side-effects[1]. Results should thus be viewed in this same perspective: it is on the basis of anticipated ecotoxic side-effects that a recommendation to approve or reject a pesticide is made. Weighing of other aspects such as human health, or comparison with other, approved, pesticides should be undertaken within a different framework; these aspects are outside the scope of the present study. Provisionally at least, the proposed procedure focusses on the side-effects of individual pesticides. The impact of a combination of pesticides may also be evaluated prior to approval if there is particular cause for suspicion. In other cases, viz. for pesticide combinations actually occurring in agricultural practice, due monitoring should be performed after approval.

The need for field testing arises because laboratory testing has been found inadequate for predicting side-effects occurring in the field. At the moment, considerable efforts are being devoted to improving extrapolating power (see Section 1.1), so as to minimize the need for field trials. If field testing is considered desirable, the procedure described in this chapter will chart a route leading to the most suitable field trial.

3.1 Nature and magnitude of side-effects

Broadly speaking, all the procedures considered in Chapter 2 have the same basic structure: an initial assessment is made on the basis of laboratory data; in certain cases - if there is an anticipated hazard - field testing

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[1] Besides effects on biota, there may also be abiotic effects, in particular pesticide concentrations in the various environmental compartments. In the context of the present study, these concentrations are relevant only in so far as they may be expected to give rise to ecotoxic effects. For this reason, field concentrations are determined only when there is an ecotoxic hazard.

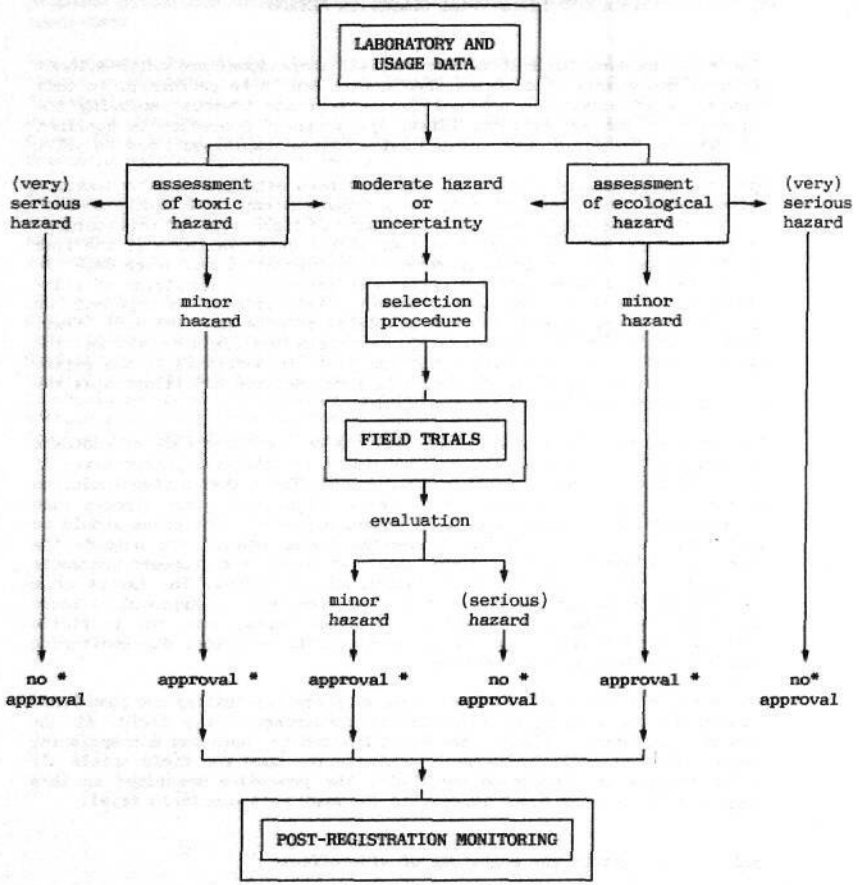


Figure 3.1 Proposed procedure for assessing the ecotoxic hazards of pesticides.
* This procedure leads to a recommendation for pesticide approval or rejection based solely on the risk of ecotoxicological effects; screening for other aspects or comparison with other pesticides is still required.

The pesticide is first subjected to laboratory testing and then its ecotoxic side-effects are assessed. If this does not lead to rejection - which means there is no (very) serious hazard - there may still be a moderate hazard or there may be too much uncertainty to permit evaluation. In that case, it is assessed whether field testing can provide conclusive evidence on the occurrence of side-effects. If the hazard is minor, a field trial is not considered necessary. We distinguish two types of effects: toxic side-effects and ecological effects, which will be discussed in detail in Paras. 3.1.1 and 3.1.2, respectively.

Upon completion of the field trials, the results must be interpreted and evaluated, leading to approval, rejection or proposals for application under modified conditions. After approval, finally, the procedure provides for a post-registration field trial. Among other things, such a trial may yield results on unexpected effects that could not have been detected prior to approval, or only through disproportionate efforts. This also permits investigation of effects caused by the action of the pesticide in combination with other pesticides or activities.

3.1.1 Toxic side-effects

The chances of toxic side-effects occurring is calculated on the basis of a pesticide's toxicity and the potential exposure of non-target organisms. To determine this potential exposure, in current Dutch procedure the Commission for the Registration of Pesticides (CTB) uses manufacturers' data to estimate the expected concentration in various environmental compartments (Predicted Environmental Concentration, PEC). The data supplied relate to the prescribed dose, the application method and the formulation. In addition, data on persistence and mobility are employed. The calculated concentration is then compared with the toxicity of the formulation to several groups of organisms, and - for calculation of actual exposure- the bioconcentration factor, lipophility and other factors are also considered. This comparison gives an impression of anticipated toxic side-effects. CTB then assesses these side-effects on the basis of the magnitude of the expected hazard.

This initial screening may lead to one of three conclusions (see Fig. 3.1):

1. There is a (very) serious toxic hazard to non-target organisms; because of the anticipated toxic side-effects, approval is not recommended; in any case, a field trial to demonstrate these side-effects is unnecessary and, for ethical reasons, even undesirable.
2. There is a moderate hazard; this may be indicated directly by initial assessment, but there may also be specific exposure hazards involved: granular formulations may, for instance, be picked up by birds, resulting in very high exposure. Other indications pointing towards side-effects, from the open literature, for instance, may also indicate a need for field testing.
   Furthermore, uncertainty may arise due to a lack of quantitative data or methods for evaluating laboratory tests, for instance in the case of a pesticide with a new mode of action. A pesticide may also increase an organism's risk of being preyed on, greatly increasing the hazard of secondary poisoning. In such cases, too, a field trial is required.
3. There is a minor hazard; in this case, no field trial is required to assess toxic side-effects.

We take this opportunity to point out that laboratory tests are required for a limited number of standard organisms only. For the aquatic environment, data on algae, crustaceans and fish are a (standard) requirement, and for the terrestrial environment this holds for mammals (rat), birds and earthworms and, in certain cases, honeybees. Data on effects on vegetation can be derived in part from the efficacy evaluation. However, it is quite conceivable that in some cases supplementary data will be needed for proper assessment. In the case of an acaricide, for instance, it makes sense to study effects on mites or spiders. To this end, the most desirable course would appear to be to increase the number of laboratory tests available so as to improve the scope for assessment. Only after all these laboratory tests have been conducted and a moderate hazard has been found should a field trial be prescribed.

3.1.2 Ecological side-effects

In international procedures, ecological side-effects are not explicitly assessed. We propose a procedure (see Fig. 3.1) analogous to that for toxic side-effects: an initial assessment is made on the basis of laboratory and usage data, and if there is a moderate hazard a field trial is considered as an option for providing more information on the occurrence of ecological side-effects.

A pesticide always has a toxic side-effect on a given group of organisms: that is what it is intended to do. Determination of ecological side-effects, on the other hand, involves an assessment of whether certain properties make it likely that non-target organisms will indirectly become victims of the pesticide. Ecological side-effects may be anticipated if a pesticide has a broad spectrum of action or if it is to be employed on a large scale. Suspicion may also be based on a high efficacy in combination with the spectrum of action or the scale of use. Below, these aspects are discussed in turn.

Spectrum of action

If a pesticide has a very specific mode of action (killing one or several species), its spectrum of action forms no reason to suspect ecological side-effects. If the spectrum is broader (for instance, a pesticide toxic to all arthropods or all dicotyledons), a larger group of organisms will be affected. If, in the latter case, an ecological function (such as decomposition or pollination) becomes impaired, at least on this point clarity should be obtained before approval is granted. If it is suspected that the impact on such a function exceeds 10%, a field trial is deemed necessary.

In the case of broad-spectrum pesticides, the group of organisms indirectly affected (predators, or species depending on a certain habitat) may also be larger. It is difficult to give general criteria for field testing, for much depends on the organisms present in the areas where the pesticide is to be used. It is the feeding and other habits of these species that determine the occurrence of ecological side-effects. Critical periods in the life cycle of a species include the rearing of young, for instance. Other factors of influence are natural fluctuations and the persistence of the effect. It is therefore of crucial importance that these aspects be taken into account in assessing potential hazards; field trials should

consequently be performed under conditions expected to demonstrate the greatest effects.

Subject to the above-mentioned conditions, a normative criterion can be drawn up for food and habitat effects: a field trial is called for if there is a risk of more than 10% of the food of a non-target species or more than 10% of its habitat being affected.

Scale of use

There are a number of questions relating to the scale of use. Is it possible for predators of the affected organisms to forage elsewhere? Does a given species lose its habitat over a relatively small area or is there substantial loss? In this context, two kinds of large-scale effects can be distinguished: pesticide treatment may extend over a large area, or there may be a high degree of overlap between the areas treated and the habitat of a specific organism, ecosystem or type of landscape.

Widespread use

We propose using the term 'widespread use' for cases where approval is requested for application on crops covering an area of at least 10,000 ha (approximately 0.5% of Dutch arable land), Table 3.1 presents a breakdown of arable land use in the Netherlands and the area covered by major individual crops. If a pesticide is claimed for use on one of the crops in Table 3.1, it is treated as a claim for 'widespread use'. If use is claimed for various minor crops, this also constitutes 'widespread use' if the total area covered by these crops exceeds 10,000 ha.

Overlap with habitat

If there is a large degree of overlap between the area in which a pesticide is to be used and a certain habitat (e.g. ditch banks), there is a potentially large hazard to this habitat. For this habitat, then, it is necessary for side-effects to be studied in the field. Likewise, if the area in which a pesticide is to be applied overlaps certain types of landscape (e.g. polders), there may be a risk of ecological side-effects for such areas. In these cases the 10,000 ha criterion does not apply. When connected areas are treated, the occurrence of ecological side-effects will have to be examined on a case-to-case basis; here again, field testing may still be required even if the area to be treated is less than 10,000 ha.

Particular attention should be drawn to hazards for rare, endangered and/or protected species and habitats. If there are suspicions that a claimed pesticide will come into contact with such habitats or species, extra caution should be applied. However, there are ethical objections to field trials involving endangered species or habitats, and a field trial in a comparable situation will have to be conducted to find out whether exposure or effects will occur. These findings can be used to formulate additional approval requirements relating to the endangered species or habitats.

Efficacy

In principle, every pesticide is designed for optimum efficacy. Use of a highly efficacious pesticide involves a serious hazard, however, since certain groups of organisms may, at least locally, be completely eradicated.

general	
grassland	1125
arable land	787
horticulture	88
greenhouse culture	9
agricultural land, total	2014

specific crops	
maize	198
sugar beet	128
wheat	111
eating potatoes	76
industrial potatoes	58
spring barley	42
vegetables*	39
green peas	36
seed potatoes	35
fruit*	22
grass seed	22
bulbs*	16
seed onions	11
beans	10

Table 3.1 Use of agricultural land in the Netherlands, in 1000 ha (1987 data); * denotes a number of similar crops grouped together. (Source: Anon, 1988.)

For this reason, field testing for ecological side-effects is considered necessary for pesticides so effective that 100% of target organisms are *eradicated. In all other cases, efficacy within the target area cannot be* used as a criterion in its own right. However, a very effective pesticide may still have ecological side-effects if it has a broader spectrum of action, or if it is used on a large scale. It is therefore proposed to take as a criterion the efficacy, in combination with the spectrum of action and scale of use. If there are still doubts after hazards have been estimated from spectrum of action or scale of use, field testing may be indicated by an efficacy of more than 90%.

Resumé

Table 3.2 summarizes the indicators for field testing.

toxic side-effects	ecological side-effects
presence of toxic hazard uncertainty in assessment	spectrum of action of pesticide widespread use overlap with habitat extremely efficacious pesticide

Table 3.2 Summary of criteria for field testing.

3.2 Specification of anticipated effects

The criteria given in Section 3.1 can be used to assess the need for field testing. In a broad sense, therefore, the anticipated effects have already been specified to a certain degree, viz. narrowed down to a toxic or ecological side-effect. In this section, the compound characteristics (3.2.1), usage data (3.2.2) and data on the 'receiving environment' (3.2.3), are used to narrow down the anticipated effects further, to one or more specific side-effects in particular environmental compartments, organisms, etc. An overview of these specific effects is given in Para. 3.2.4.

First, however, a number of general premises are discussed. Effects may occur at the level of individuals, populations, communities and ecosystems. To justify field testing, an effect should be expected at least at the population level. An exception is made for protected or endangered species; in this case, not even individuals should be put at risk. When populations are affected, this may have consequences for the composition of communities and for the ecosystem as a whole. Ecosystem effects can be anticipated on the basis of two types of suspicion. In the first place, a pesticide may have (toxic or ecological) side-effects on groups of organisms fulfilling specific ecosystem functions, and in the second place a pesticide may interfere with ecosystem processes, quite a conceivable possibility in the case of soil processes, for example.

3.2.1 Compound properties

The properties of the compound may already provide an indication of the effect or mechanism on which testing must eventually be focussed. Table 3.3 summarizes these relationships for the properties considered most important. Below, the properties in Table 3.3 are classified in terms of (inter)nationally accepted classes, which can be used to assign a relative weight to a potential effect.

Toxicity

Table 3.4 distinguishes a number of toxicity classes for non-target organisms, using limit values based on Van Gestel (1985), the Dutch Ministry of Housing, Physical Planning and Environment (VROM, 1985) and Hill et al. (1975). The classes 'toxic' and 'highly toxic' have been combined. Ideally, the aim should be to use 'no-effect levels', too; in view of data availability, however, we have provisionally taken the LC/LD50 values.

In the case of a Class I pesticide, its toxic properties alone may be sufficient grounds for rejection. However, if the PEC is so low that

approval is still considered, the compound's toxicity will anyway provide a clear indication of the group of organisms in which effects are likely to occur. Classes II and III may also form grounds for suspicion, but in this case the degree of exposure should be investigated. The toxicity of Class IV compounds is such that effects will not be suspected for a specific group of organisms.

Not all types of non-target organisms are included in the table. In some cases, comparison with specified organisms is possible: it might for instance be assumed that the same values hold for flower-feeding/pollinating insects such as butterflies and bees. For other organisms, comparison may not be as feasible. An alternative approach may then be to collect supplementary toxicity data or to use safety factors.

For the flora, a similar classification can probably also be made, involving effects on growth and flowering in addition to mortality. Data may perhaps be derived from the efficacy test, which in any case yields data on effects on the crop.

Persistence

In the Dutch government memorandum 'Milieucriteria' [Environmental criteria] (Dutch Parliament, 1988-1989, 21 012), the following limits are used:

- *half-life > 2 months: no approval* (NB: the government's environmental advisory body CRMH (Central Environmental Protection Council) advises 1 month rather than 2 months)
- half-life < ½ - 1 month: no problems expected.

In the intermediate range (1 - 2 months), there may be a suspicion of elevated concentrations in certain environmental compartments.

Lipophility

The lipophility of a compound can be expressed in terms of the partition coefficient between octanol and water (P_{ow}). In the case of compounds with log P_{ow} > 3, there is a (potential) risk of bioaccumulation. This value is taken from the same memorandum as the persistence parameters above; it is more stringent than the value currently used in the approval procedure. It implies that pesticides with log P_{ow} > 3 should no longer be approved in the future. At values between 2 and 3, the anticipated effect should be sought in the area of indirect toxic effects. If these norms are too strict, a value of 3 - 5 can be taken as grounds for suspicion, particularly for effects on organisms at the end of the food chain.

Mobility

The degree of mobility can be expressed in terms of the parameters adsorption (K-ads = adsorption constant), Rf value (for soil thin-layer chromatography), water solubility (S) and evaporation (P = vapour pressure). To assign numerical values to these parameters, use has been made of data provided by Van Gestel (1984, 1985). For mobile to very mobile compounds, potential effects are to be anticipated above all in surrounding ecosystems, notably in and around ditches.

property	mechanism	specific effects anticipated
toxicity	direct intoxication	direct toxic effect on related organisms/processes
pe rsistence	accumulation in environment	organisms in soil/aquatic sediment
lipophility	accumulation in food chain	indirect toxic effects on organisms at end of food chain
mobility	dispersal in environment	surrounding ecosystems, particularly ditches
type of action	secondary intoxication via increased availability or anomalous behaviour of target organism	indirect toxic effect or food effect on predators of target organisms
efficacy	complete eradication of target organism	food or habitat effect on predators, flower feeders/ pollinators, organisms dependent on target organism
spectrum of action	eradication of broad spectrum of food org.'s, habitat destruction	food or habitat effect on predators of target organisms or habitat-dependent organisms

Table 3.3 Specification of anticipated effect based on compound properties.

organisms / toxicity classes	aquatic fauna (LC50, mg/l)	earthworms (LC50, mg/kg soil)	bees (LC50, µg/bee)	birds & mammals (LD50, mg/kg body wt.)
I toxic/highly toxic	< 1	< 1 - 10	< 0.1 - 1	< 200
II moderately toxic	1 - 10	10 - 100	1 - 10	200 - 1000
III slightly toxic	10 - 100	100 - 1000	10 - 100	1000 - 5000
IV very slightly toxic	> 100	> 1000	> 100	> 5000

Table 3.4 Toxicity classes for non-target organisms.

Type of action

The intended action of a pesticide can also be regarded as a compound property, the target organisms providing an indication of likely side-effects. Table 3.6 categorizes the various types of pesticides on the basis of target organisms. If a toxic side-effect is anticipated, suspicions will be focussed primarily on non-target organisms from the same group and occurring in the same environmental compartment as the target organisms.

	K-ads (dm^3/kg)	Rf	S (mg/l)	P (Pa)
immobile	> 10	0.0 - 0.10	< 0.1	< 10^{-4}
slightly mobile	1.0 - 10	0.10 - 0.35	< 10	10^{-4} - 10^{-2}
moderately mobile	0.3 - 1.0	0.35 - 0.65	< 1000	10^{-2} - 1
mobile	0.1 - 0.3	0.65 - 0.90	> 1000	1 - 100
very mobile	< 0.1	0.90 - 1.0	>>1000	> 100

Table 3.5 Mobility classes on the basis of K-ads, Rf, S and P.

If an ecological side-effect is anticipated, this will involve the predators of the target organisms and/or habitat effects. In identifying the non-target organism to be investigated, exposure dynamics and the compound's mode of action should always be taken into account. Even when these organisms are unrelated to the target organism, the toxicity to the former should be investigated.

intended action	organisms at risk
bactericides	prokaryotes
virucides	prokaryotes
fungicides	fungi
algicides	algae
herbicides, incl. defoliants and growth regulators	plants
nematicides	unsegmented worms
molluscicides	molluscs
acaricides	mites & spiders
insecticides	insects
rodenticides	mammals

Table 3.6 Specification of effects based on target organisms.

Efficacy and spectrum of action

If the efficacy or spectrum of action point to the need for field testing for ecological side-effects (see Section 3.1), trials should be focussed on organisms dependent upon the target organisms for food or habitat. If the need for field testing is indicated by other compound properties, then efficacy and spectrum of action may form grounds for suspecting effects on non-target organisms related to the target organisms.

3.2.2 Usage data

Usage data, too, may be useful for further specification of anticipated effects. Table 3.7 summarizes the type of effects deducible from the type of pesticide formulation and its method of application. Usage data permit further specification of likely effects, in terms of the probable nature and extent of the compound's environmental distribution.

formulation	mechanism	species, ecosystem at risk
scatter-granules	direct ingestion roll-/run-off	soil birds, small mammals ditch ecosystem
wettable powder wettable granules spray liquid	direct loss run-off drift inhalation	vegetation, soil border ecosystems, esp. ditches ditch ecosystem nearby ecosystems fauna
poured liquid	direct	soil
aerial spraying	direct major loss drift inhalation*	vegetation, soil border ecosystems, esp. ditches nearby ecosystems fauna
injected gas/vapour	escape, drift	nearby ecosystems
can-sprayed gas**	drift inhalation	nearby ecosystems fauna

Table 3.7 Specification of anticipated effect based on pesticide formulation and application method.
* Very serious hazard due to large-scale application.
** Only seldom used outdoors.

3.2.3 Receiving environment

The nature of sites where the pesticide is to be applied allows for further specification of effects, in two respects, viz. in terms of risk to the specific environmental compartment in which the compound is to be used and the specific ecosystem or type of area in which it is to be employed. The environmental compartment is of course important for narrowing down anticipated effects to certain groups of organisms in a general sense (Table 3.8). The ecosystem or type of area (Table 3.9) may focus suspicions on certain communities, enabling a further specification of indications obtained from Table 3.8. An important aspect to be considered here is the rarity of the species or communities concerned.

compartment	treatment	species at risk
water (+ sediment)	ditch treatment ditch bed treatment	aquatic flora/fauna/ecosystem ditto
soil	soil fumigation ditch bank treatment	soil fauna and ecosystem riparian and aquatic flora aquatic fauna and ecosystem
crop	crop treatment row treatment seed pretreatment defoliation weed control	fauna bound to non-target vegetation ditto soil fauna vegetation-bound fauna non-target vegetation ditto; also species dependent on affected habitat
animals	pest control	birds and mammals predators of affected organisms
indoors	soil treatment greenhouse treatment seedling treatment seed treatment building treatment stock treatment	possibly via leaching ditto not relevant not relevant not relevant not relevant

Table 3.8 *Specification of anticipated effect based on environmental compartment of compound application.*

type of area/ecosystem	species, ecosystem at risk
ditch	ditch ecosystem, rare species
ditch bank	ditch bank ecosystem, rare species
cropped land, horticulture and bulb-growing	field flora and fauna, rare species, also possibly through leaching/run-off
grassland	grassland ecosystem, rare species, also possibly through leaching/run-off
forest	*forest ecosystem, rare species*
orchard	orchard flora and fauna, rare species
greenhouse	possibly through leaching

Table 3.9 Specification of anticipated effect based on area or ecosystem of compound application.

3.2.4 Resumé: Specification of effects

In the previous sections it is indicated how, using the data available at the time of application, anticipated effects can be narrowed down to suspicions regarding a specific effect on specific groups of species or ecosystems in specific types of environment. In this approach, a number of properties relating to the compound as well as its usage are used to particularize, as accurately as possible, the expected type of effect and the organism (or taxonomic group), environmental compartment and type of ecosystem at greatest risk. Table 3.10 summarizes the possibilities distinguished for each of these categories. These potential effects form the point of departure for designing field trials, as discussed in the next section.

Identification of the non-target organisms at greatest potential risk is based on affinity with the target organism and on toxicity data. This explains why the total number of taxonomic groups distinguished extends beyond those groups with a specific affinity with target organisms (Table 3.6).

type of effect	taxonomic group	environmental compartment	type of ecosystem
direct toxic effect	fungi	sediment	ditch
indirect toxic effect	plankton/algae	water	ditch bank
food effect	higher plants	soil	arable land
habitat effect	bacteria	vegetation	grassland
	unsegm. worms		forest
	molluscs		orchard
	segm. worms		
	crustaceans		
	spiders/mites		
	insects		
	fish		
	amphibians		
	reptiles		
	birds		
	mammals		

Table 3.10 Summary of specific effects identified.

3.3 *Selection of field trials*

In this section we discuss the trials to be used for detecting side-effects in the field. The basic premise is that the trials must be capable of demonstrating occurrence of the effects mentioned in the previous section, in so far as they actually occur in the field. Para. 3.3.1 identifies the groups of organisms that occur in the various environmental compartments *and where effects are to be expected.* Para. 3.3.2 discusses the specific species (groups) for which field trials should be developed, and in Para. 3.3.3 a number of field trials are selected for further elaboration.

3.3.1 Embodying specific effects in field trials

Para. 3.2.4 summarizes the range of specific side-effects distinguished, in terms of type of effect and groups of organisms at risk in specific environmental compartments and types of ecosystem. Table 3.11, a matrix of taxonomic groups and environmental compartments, provides a summary of the field trials that are in principle required.

Each cross in Table 3.11 represents, in principle, a field trial. The ecosystems distinguished in Table 3.9 are an important aid for selecting the species for individual field trials: preferably, the species selected should occur in the type of ecosystem at risk.

environmental compartment / taxonomic group	sediment	water	soil	'vegetation'
fungi			X	
plankton/algae		X		
higher plants		X	X	X
bacteria	X		X	
unsegmented worms	X		X	
molluscs	X	X	X	X
segmented worms	X		X	
crustaceans	X	X	X	
spiders/mites		X	X	X
insects	X	X	X	X
fish		X		
amphibians		X	X	
reptiles			X	
birds		X	X	X
mammals		(X)	X	X

Table 3.11 Occurrence of taxonomic groups in environmental compartments; X: effects anticipated and field trial therefore required, in principle; (X): may be required.

3.3.2 Choice of experimental organisms

Table 3.11 indicates the taxonomic groups and environmental compartments for which a field trial should, in principle, be available. The organism eventually chosen for a given field trial depends on a number of aspects, such as the role of the organism in the ecosystem or its exposure.

Below, an experimental organism is proposed for each of these field trials. For the compartment soil, a distinction is made between ground-dwelling organisms and soil organisms, on the one hand because exposure may differ, and on the other because entirely different groups of organisms are involved.

In selecting experimental organisms, the following criteria have been used:

- the organisms should play a major role in the functioning of the ecosystem or be representative of this function (of importance in evaluating results);
- due account should be taken of the major processes occurring in each environmental compartment (in the soil, decomposition, for instance, and on the ground, the complex formed by primary producers, herbivores and carnivores);
- the organisms should be fairly abundant in agricultural areas;
- trials should be practicable to perform, to be achieved, inter alia, by seeking conformity with existing guidelines;
- the organisms should not be extremely insensitive to pesticides in general.

Below, for each of the taxonomic groups in each environmental compartment a single organism is chosen on the basis of its ecological role and occurrence. Our preference is underlined, where applicable. In each case, existing trials or trial guidelines with which conformity can be sought are indicated.

Organisms living in and on aquatic sediments

Bacteria

Decomposition is the most important process occurring in aquatic sediments. The decomposition rate might be a suitable parameter to study, possibly by methods comparable to those used in terrestrial studies (litterbag test). However, such a test would have to be developed from scratch.

Unsegmented worms

In the benthic environment, unsegmented worms (e.g. Nematoda) play a less important role than segmented worms. Although taxonomically very different, segmented and unsegmented worms are so similar in habits and exposure dynamics that a separate test is considered superfluous.

Molluscs

The freshwater mussel is already being used as an indicator for the presence of pesticides in surface waters. In ditches, however, they are not abundant, and the same generally applies to most other bivalve molluscs. Consequently, development of a ditch test involving snails is to be preferred. Snails, moreover, are representative of herbivores. It is recommended to make use of the results of the test-pond work with snails being carried out by TNO-MT (Netherlands Organization for Applied Physical Research, Division of Technology for Society, Den Helder laboratories).

Segmented worms

Of the segmented worms (Annelida) living in aquatic sediments, the Oligochaetae (e.g. Tubifex sp.) form the most important group. They provide an abundant group of organisms exposed via the sediment pathway. Many of its representatives play an important role in the fragmentation of benthic litter, and a test with Tubifex sp. is therefore proposed. Coordination is possible with the laboratory test being developed by RIVM (Netherlands Institute of Public Health and Environmental Protection) and RIN (Netherlands Research Institute for Nature Management).

Crustaceans
A test involving water isopods (Asellus sp.) is recommended, possibly proceeding from the work being done at the Staring Centre (SC; Leeuwangh, cf. Beurskens, 1986; Romijn, 1988, and others). Our own research (Canters et al., 1989) also points to the suitability of these organisms for such a trial. Water lice play an important role in the decomposition of plant litter. As they also often frequent plants, however, they cannot be considered specifically benthic organisms, making them less indicative of exposure via sediments.

Insects
The most common insect inhabitants of the benthic environment are midge larvae, which graze on its surface. Midge larvae are also extremely abundant. In developing a test, use can be made of the studies at DBW/RIZA (National Institute of Inland Water Management/Institute of Wastewater Treatment), where sublethal effects (jaw deformities) have been found in midge larvae (Van Urk & Kerkum, 1986). There are other ongoing projects involving the use of midge larvae in laboratory and mesocosmos studies (Eijsackers & Bosma, 1989).

Organisms living in the water body

Algae
Algae are key primary producers, the first step in the food chain. A ditch trial should focus on both phytoplankton and epiphytic algae. An algae trial can be developed along the lines of existing laboratory tests (NEN 6506 and OECD Guideline 201) and experimental-ditch studies at the Staring Centre (pers. comm., Leeuwangh).

Higher plants
In the aquatic environment, higher plants constitute the other main group of primary producers. Because of their spatial configuration, they also provide a habitat for many species of aquatic fauna and epiphytes. In line with the EPA guidelines (EPA, 1982a), a test could be developed involving several species of higher plants.

Molluscs
On the vegetation, snails, as herbivores, play an important role. They are usually very abundant and are also representative of herbivores. Coordination with the TNO-MT experimental-pond studies is recommended.

Crustaceans
A test with water fleas should be developed, if only because of the high degree of correlation with available laboratory tests (NEN 6501 and OECD Guideline 202), offering potential for progress on the extrapolation problem. Moreover, as filter feeders (phytoplankton), water fleas represent an essential link in ditch food chains.

Spiders & mites
Arachnids should be studied because of the hazards posed by acaricides. A test with water mites makes more sense than one with water spiders, as mites are far more abundant. In addition, useful experience has already been gained with water mites (cf. Canters et al., 1989).

Insects
A test with insects is needed mainly because of the hazard of insecticides. Since the larvae of aquatic insects are generally more sensitive than adults, a test with the former should be developed. By choosing predatory insects, this aspect of the ecosystem can also be covered. For reasons of size, a test with the larvae of predatory beetles or nymphs of predatory bugs could be developed, for instance, though other organisms, such as (the larvae of) dragonflies and caddisflies, might also be used. With the latter group, there is already some laboratory experience (Heinis & Crommentuijn, 1988).

Fish
Test with sticklebacks. Sticklebacks are representative of ditch predators and they are also abundant, especially in smaller ditches. In developing a trial, the TNO-MT mesocosmos studies can be taken as a starting point.

Amphibians
Aside from any other considerations, the protected status of amphibians makes them a group for which side-effects are undesirable. This also implies that the greatest possible caution should be exercised in field testing. It may therefore be best to use frogspawn or tadpoles, which can be collected in the field. By taking frogspawn from sites where tadpoles cannot survive, for instance in ditches that are drying out, the risk of damage to frog populations is kept small. The work of Cooke can be further developed (1970, 1977, 1981). For a review of laboratory and field research on pesticide side-effects on amphibians, see Harfenist et al. (1989).

Organisms living in and on the soil

Fungi
Lower fungi and toadstools play an important role in the decomposition of organic matter in the soil. In addition, symbiotic fungi (mycorrhiza) play a major role in nitrogen fixation, and development of a field trial with this group therefore seems particularly desirable.

Bacteria
Bacteria, too, are important in litter decomposition. In several cases, tests are already prescribed, but no guidelines for field trials have yet been formulated. Such trials might be based on the Dutch laboratory guideline for nitrification tests (NEN 5795) and the West German soil microflora test (pers. comm., Kohsiek).

Higher plants
In the terrestrial environment, higher plants are undoubtedly the major primary producers, the basis of the food chain. They are also important as host plants for other species and in determining the spatial configuration of habitats. Due to the widespread use of herbicides, the wild flora in agricultural areas is under great pressure, as discussed in the Netherlands' National Environmental Policy Plan (NMP, 1989). Development of a field trial with higher plants is therefore urgently required. This can be based on existing trials with this group of organisms, viz. efficacy tests (EPPO, 1989) and the EPA guidelines.

Unsegmented worms
Nematodes form a relatively diverse group of organisms, fulfilling various functions in the ecosystem (Bongers, 1987). In the context of efficacy

testing as carried out by the Netherlands Plant Protection Service (PD, undated), the species composition of non-target nematodes can be readily determined at the same time as sampling of target nematodes. Use might also be made of the laboratory experience gained at RIVM (van Gestel et al., 1989).

Molluscs
Slugs constitute the major group of ground- and soil-dwelling snails, and there may in principle be a high degree of exposure to pesticides. In addition, slugs are an important element in the diet of other organisms. For this reason, a field trial seems desirable.

Segmented worms
Earthworms convert a great deal of soil matter and contribute substantially to the decomposition process. At the same time, exposure is high. They are also important as a source of secondary poisoning (e.g. as the staple diet of meadow birds, the little owl and the badger). A field trial with earthworms can be designed along the lines of the guideline currently in preparation in Germany (see Para. 2.2.2) and that already in force in the UK (MAFF, 1986), incorporating the developmental work of RIVM (cf. van Gestel & Ma, 1989).

Crustaceans
Woodlice play a role in decomposition. At the same time, much is already known about the effects of heavy metals on these organisms. Relevant information can be derived from the studies carried out at Amsterdam Free University (VU, e.g. van Wensem, 1989) and the work - of an older date- carried out by RIN (Eijsackers, 1978).

Spiders & mites
In the soil environment, a test with mites could be developed. A useful starting point might be the work of Van de Bund (1980), who demonstrated that certain types of predatory mites are particularly sensitive to pesticides.

On the soil surface, spiders play a role as predators. Use can be made of the studies carried out at LUW (Wageningen Agricultural University, Everts et al., 1986a, 1986b, 1989).

Insects
For soil-dwelling insects, a test with springtails seems a logical choice. Springtails are abundant and play an important role in the decomposition and mineralization of organic matter. Possible starting points for development of a trial include work at VU and LUW (cf. van Straalen & Everts, 1989), RIN and PD (van de Bund, 1980) and developments in Hungary (pers. comm., Oomen).

For ground-dwelling insects, it is proposed to develop a test with ground beetles. As major predators, ground beetles play an important role from both an ecological and an agricultural point of view. As a starting point, the British guideline can be taken (MAFF, 1986). Use can also be made of the work at RIN and PD (Eijsackers & van de Bund, 1980) and LUW (Everts et al., 1986a, 1986b, 1989) and the tests developed within the IOBC framework (IOBC, 1988).

Amphibians & reptiles

Since this group has a protected/endangered status (except for frogspawn), the negative impact of a field trial should be avoided. However, precisely because of this status it is necessary to know whether a pesticide can be expected to have adverse effects. It is proposed to assess the likelihood of effects on amphibians and/or reptiles on the basis of the specification procedure outlined above. If effects are anticipated, limitations can then be imposed on use in situations where exposure is likely, or user guidelines adapted.

Birds

Birds are important both because of their overall significance for ecosystems and because of the importance attached to them by the public at large (as also reflected in policy). Among the ground-living species, birds foraging on arable land and grassland are especially important (e.g. meadow species and gallinaceous birds). In developing trials, use can be made of the general bird inventorying techniques developed and used in the Netherlands (cf. Hustings et al., 1989). In addition, the UK guideline (MAFF, 1986) can be used as a basis.

Mammals

Small mammals are the obvious choice, and a test with mice is recommended. A number of common species with varying diets might be considered, e.g. a herbivorous species (field vole, Microtus agrestis), an insectivorous species (common shrew Sorex araneus) and an omnivorous species (wood mouse Apodemus sylvaticus) (cf. de Snoo & Canters, 1990). In addition, the mole Talpa europaea can be considered for a field trial. The research on the effects of heavy metals by RIN and IVM (Institute for Environmental Studies, Free University of Amsterdam) can be drawn on (e.g. Ma, 1989; Denneman et al., 1989). In any case, the British guideline (MAFF, 1986) can be taken as a starting point.

Organisms living on and/or consuming vegetation

Molluscs

Snails are representative of herbivores. Although snails may frequently form the target of the pesticide in question, a field trial may be worthwhile, especially if it focusses on ecological effects. As weed consumers, snails may become crop consumers, for instance. At the same time, they are a source of food for other species. A field trial guideline might be developed on the basis of efficacy testing.

Spiders & mites

A field trial with spiders or mites is recommended, linking up with the research work at PD on integrated pest control. In an international context, moreover, an enormous amount of research is being done on mites as 'beneficial organisms' (see Chapter 2).

Insects

For vegetation-bound insects, a field trial with honeybees would be an appropriate choice, given the key role of honeybees in flower pollination as well as their importance as producers of honey. A guideline can be based on the work of the Ambrosiushoeve and PD, for instance, and on existing international guidelines (EPPO, 1986; MAFF, 1986; pers. comm., Kohsiek, BBA).

Birds
See also under ground-living birds. For species living in and/or consuming vegetation, a field trial can be developed with insectivorous or herbivorous birds. In practice, it will be difficult to distinguish between species exposed via vegetation and via the soil, so that both groups should be studied in a single field trial.

Choice of field trial guidelines for elaboration

In the framework of this study, ten species (groups) have been selected for elaboration of field trial guidelines. In each environmental compartment, representatives of the major ecosystem processes have been chosen, distinguishing between producers, consumers and decomposers. In making a choice, the quantitative importance of the species for a given function was taken as the primary criterion. In addition, it was assessed whether there is sufficient data available for drawing up a guideline.

Producers
For producers, the division into environmental compartments is open to debate. In the context of trial selection, however, this question is unimportant. In the terrestrial environment, higher plants are the main primary producers and have therefore been selected for a field trial. As the main primary producers in the aquatic environment, algae have similarly been selected for guideline elaboration.

	terrestrial		aquatic	
	in/on soil	vegetation	in/on sedim.	water body
producers	**higher plant**	n.a.	n.a.	**algae** higher plant
consumers	nematode slug mite spider **ground beetle** **bird** mouse/mole	snail spider/mite **honeybee** **bird**	**snail**	**snail** **water flea** water mite beetle/bug ***stickleback*** tadpole
decomposers	mycorrhiza bacteria nematode **earthworm** woodlouse springtail	n.a.	bacteria tubifex waterisopod **midge larva**	

Table 3.12 Organisms selected for field trials; for the species in bold type, a guideline has been elaborated (see Part B).

Consumers
Birds have been taken as being representative of consumers in the terrestrial environment, as this enables exposure via the soil and vegetation to be studied in a single test. Moreover, a (British) guideline is already available, which can serve as a starting point. The choice for ground beetles and honeybees is also based on the fact that guidelines for these species already exist; these can be adapted to the Dutch situation. In addition, both species are important not only biologically: ground beetles are also agriculturally beneficial, while honeybees are important because of their function as pollinators and play an economic role in their own right as honey producers.

In the aquatic environment, water snails have been chosen because they allow exposure via the sediment and the water body to be studied in a single test. In addition, field trials for water fleas and fish have been developed because of the important role of these organisms in the aquatic ecosystem and because of the immediate links with laboratory tests.

Decomposers
For the soil environment, earthworms have been chosen; in terms of quantity, earthworms (may) play a very significant role in decomposition, making them important from an agricultural point of view, too. In aquatic sediments, midge larvae are generally rather more sensitive to pesticides than tubifex. Waterlice are not restricted to the sediment, and for bacteria hardly any field studies are available.

3.3.3 Choice of trial type

In Section 1.5, various types of trial were distinguished. The choice of trial depends in the first place on the type of organism to be studied. For organisms active over a large area, a semi-field trial is not that suitable. On the other hand, conditions can be better controlled in a semi-field trial, usually enabling the anticipated effect to be studied more accurately. Then again, precisely because of the smaller scale of a semi-field trial, exposure may be different from that under practical circumstances. The type of field trial chosen depends, further, on the parameters to be measured; mortality can probably be adequately assessed in a semi-field trial, but for migration, say, clearly no barriers should be present.

Whether cages or enclosures should be used for observing effects also depends on the organizational level to be studied and the effect anticipated. In principle, population effects can be investigated using any trial method, as long as organisms are employed that are representative or indicative of the non-target populations exposed in practice. For assessing effects at a community and ecosystem level, cage studies are not really appropriate.

For direct toxic effects, cage studies and enclosures will suffice, but for indirect toxic effects (secondary poisoning), a field study sensu stricto is required, to ensure realistic exposure dynamics, among other reasons. For ecological effects, too, a field study s.s. is required.

3.4 Summary of the proposed selection method

In the preceding paragraphs, the four steps involved in selecting the most suitable field trials for various situations were explained:
- initial assessment of the hazard or degree of uncertainty
- specification of the anticipated effects
- choice of an experimental organism
- selection of one or more types of field trial.

This procedure does not yet include actual field testing or evaluation of results. It is based solely on data from laboratory testing on the compound under review and on indications following from the use for which approval is requested.

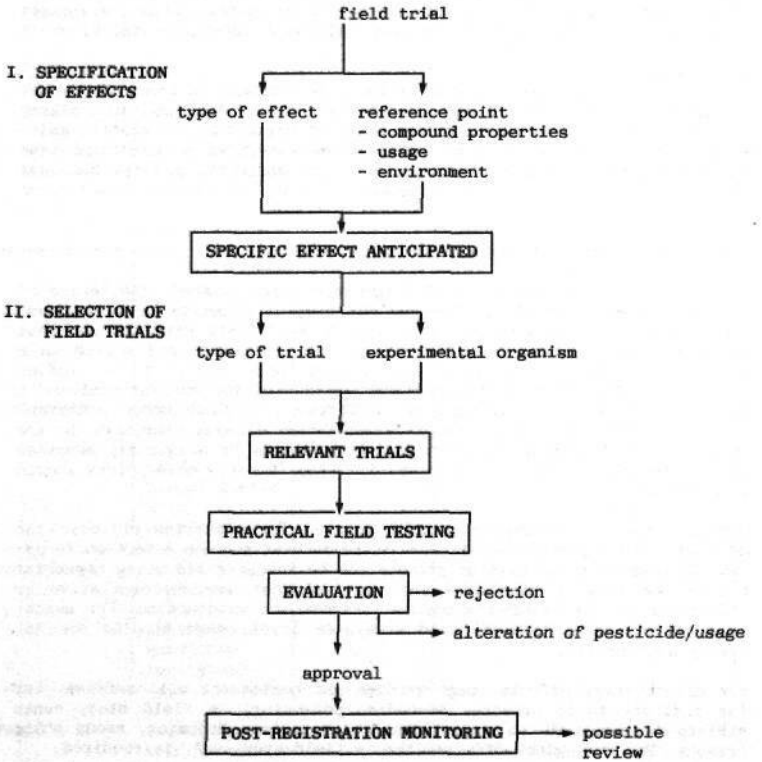


Figure 3.2 Proposed field trial selection method

Figure 3.1 (p. 26) outlines the basic steps of this procedure. From Sections 3.1 to 3.3, further details can now be filled in. The procedure starts with laboratory testing and usage data. If initial screening indicates a negligible hazard or, conversely, a (very) serious hazard, a field trial is not required. In all other cases, the proposed selection procedure is automatically followed. This procedure is shown schematically in Figure 3.2. It is to be followed both for toxic and for ecological side-effects. In Step I, the anticipated effect is specified; in Step II, one or more appropriate field trials are selected. This completes the procedure described in this chapter; the next step is actual field testing and evaluation of results, after which follow approval, rejection or reconsideration on the basis of recommended alterations to the pesticide itself or changes to user instructions. Approval may be followed by post-registration monitoring, which may give rise to later withdrawal of approval. Such monitoring is beyond the scope of the present study.

3.5 Examples

To demonstrate the practicability of the described approach, the proposed procedure is illustrated below with reference to the suspected field side-effects of two pesticides that have already been approved. The pesticides chosen for this purpose are atrazine, a widely used broad-spectrum herbicide, and pirimicarb, a selective insecticide. The examples are intended to illustrate the principles of the outlined procedure. On certain details, these worked examples may deviate from actual experience. This can be explained by the fact that we have not calculated the predicted environmental concentration (PEC), nor do we possess all toxicity data.

3.5.1 Atrazine

General

Herbicide: systemic action, acts on photosynthesis; recommended use: against annual weeds and couch grass; long-term action.
Application: granulate, wettable granules, wettable powder and liquid; recommended for asparagus, maize, field margins and uncultivated land.

Need for field testing

Toxic side-effects
The compound's long-term action may lead to such a high PEC that non-target vegetation is also affected, in which case there is a toxic hazard. The compound is in any case toxic to algae. Granular application may lead to high exposure of birds and small mammals; in view of this possibility, the toxicity should first be assessed in laboratory tests. If the compound is found to be toxic, a field trial may be required, depending on the toxicity class.

Ecological side-effects
The broad spectrum and widespread use (maize) implies a risk of ecological side-effects. In addition, application along field margins involves an overlap with a certain habitat, which might lead to severe restrictions being imposed on use along margins.

On the basis of the anticipated toxic and ecological side-effects described, the hazard must be deemed so great as to necessitate field testing.

Specification of effects

Compound properties
Toxicity: algae: toxic effect on algae
field margins: non-target plants
Persistence: organisms in soil and aquatic sediment
Spectrum: effect on habitat and food of vegetation-bound organisms

Target organism
Vegetation.

Form of application
Various formulations imply risks of the compound being picked up/eaten by non-target organisms as well as dispersion risks.
granulate: - potential hazard to birds and small mammals
- ditches
- soil
spraying: - ditches
- soil
- neighbouring ecosystem

Compartment
Systemic effect; compound is absorbed via the soil: soil organisms.
Ditch bank treatment: riparian and aquatic flora, fauna, ecosystem.

Type of area/ecosystem
Ditch bank ecosystem.
Non-target field flora.

Specific effects anticipated

Considering the above facts in juxtaposition, it is above all the following effects that are to be anticipated:

Toxic side-effects
1. Direct toxic effect on ditch vegetation: algae and higher water plants.
2. Direct toxic effect on field margin vegetation.
3. Possible direct toxic effect on birds and small mammals.

Ecological side-effects
1. Habitat effect on dwellers of herbaceous plants.
2. Food effect on vegetation-dependent fauna.

Field trials

Based on the above considerations, it is recommended to undertake the following trials:
- A trial with algae or higher aquatic plants: since a direct toxic effect is involved, a cage study in an experimental pond should in principle provide adequate information.
- A trial with field margin vegetation.

- For habitat effects, a trial might be conducted with ground beetles inhabiting field margins.
- For food effects, a test involving snails might be considered; for instance, it is interesting to see whether snails switch to the crop instead of their original food source.

3.5.2 *Pirimicarb*

General

Insecticide, cholinesterase inhibitor; permitted on many crops, selective action against aphids; application as wettable powder and wettable granules.

Need for field testing

Toxic side-effects
The compound is toxic to birds. Since it is not applied as a granulate, there is only a slight hazard of direct intoxication. The risk of secondary poisoning is also probably low, for the compound is not particularly persistent. It may, however, have a toxic effect on birds via consumption of plants. It is necessary to investigate when the pesticide is applied and then establish whether there is a consequent risk of birds being exposed. In the light of the evidence, it should be considered whether user guidelines can be adapted or whether a field trial should be carried out.

The compound is also moderately toxic to bees and predators. Since it is applied to the crop, a field trial is desirable (cf. Oomen, 1986). The compound is only slightly toxic to fish, and it is questionable whether the PEC would here give rise to field testing; this is probably not the case.

Ecological side-effects
The compound is in widespread use for aphid control; it is doubtful, however, whether its efficacy is so high as to affect aphid consumers. On the basis of the efficacy data, it should be considered whether the percentage of aphids killed is high enough to suggest an ecological side-effect.

Specification of effects

Compound properties

Toxicity:	toxic to birds; slight to moderate toxicity to bees and predators, slight toxicity to fish. No toxicity data are available for aquatic invertebrates or earthworms.
Mobility:	high solubility in water: hazards for ditches and groundwater.

Target organism
Insects.

Form of application
Wettable powder: risk of spray drift reaching ditches.
Applied to control aphids on standing crop: high risk for crop compartment, risk to soil probably minor.

Compartment
Crop: non-target vegetation-bound fauna.

Type of area/ecosystem
Fauna on field or in ditches.

Specific effects anticipated

The following effects can be anticipated:
Toxic effects on vegetation-bound non-target insects.
Depending on PEC, toxic effects on ditch insects or fish.

Field trials

Based on the above considerations, the following tests are recommended:
- A field trial with honeybees; although there is a direct toxic effect involved, it is recommended not to place the bees in a cage, for it is important that there is realistic exposure.
- Optionally, a field trial with aquatic insects or fish can be conducted, depending on the estimated degree of exposure.
- A field trial to study the ecological food effects can be carried out using predatory beetles or spiders; the need for such a trial depends on the percentage of aphids killed.

3.6 Discussion

In this chapter a procedure has been presented for determining the need for field trials and, subsequently, for selecting the most suitable field trial on the basis of available data. The type of field trial proposed focusses on ecotoxic effects on non-target organisms. Conclusions bearing on pesticide approval will always have to be drawn in conjunction with other data and criteria.

To assess the need for field testing, two keys are used, viz. toxic and ecological side-effects. To evaluate the toxic side-effects, use is made of commonly used criteria such as toxicity data, usage data and exposure data (PEC). This form of evaluation is very similar to the approach currently taken by the Dutch authorities (CTB) and also followed internationally.

Until now, it has not been customary to evaluate the ecological side-effects of a pesticide. As criteria for this purpose, we propose scale of use and spectrum of action, for widespread elimination of a food source for a variety of species may have a major impact on species at a higher trophic level, for instance. The efficacy of a pesticide is important, too. However, if efficacy were to be used as a criterion in its own right, this would conflict with the very purpose of the pesticide, which is approved only after its efficacy has been proven. Nevertheless, eradication of all aphids on all plots in a polder, for instance, may be deemed undesirable in terms of the survival of aphid predators in the area. It is also of interest to

investigate the degree to which the 'General Environmental Quality' in agricultural areas (which requires a 'species survival ratio' of 95%) is jeopardized. For this reason, efficacy is always viewed in conjunction with the scale of use and spectrum of action, rather than serving as an independent criterion.

No information has been found in the literature that permits numerical values to be assigned to the criteria scale of use, spectrum of action and efficacy. The values used here are based on the idea that it should at least be possible to detect the effect at the population level. For certain species (endangered and protected species) other choices may be made, however. The values chosen are still open to debate and are in need of further substantiation.

On the basis of the properties of the pesticide, its use and the 'receiving environment', suspicions are subsequently narrowed down to effects on specific groups of organisms, divided over four environmental compartments. In assigning groups of species to environmental compartments, we have striven for completeness. In principle, a field trial must be developed for each of these groups of organisms. We have made a provisional selection based on ecological relevance. However, other choices are obviously conceivable.

The examples illustrate that the proposed procedure leads to a limited number of field trials for a given pesticide. In the case of a selective pesticide, a field trial is recommended for directly exposed and related organisms and possibly for a species to which the compound is found to be toxic (despite its selectivity). For a far less selective pesticide, a greater number of field trials are necessary.

4. DESIGN OF FIELD TRIAL GUIDELINES

In Chapter 3 a method has been presented for describing the anticipated effects of a pesticide as accurately as possible and for selecting (groups of) organisms for use in field trials. This chapter explains the methods and premises employed in elaborating the trials, to produce trial guidelines that are as specific as possible. The actual guideline proposals are presented in Part B of the report. Section 4.1 identifies a number of general premises used in drawing up these guidelines. In Section 4.2, the design of the guidelines is discussed in greater detail and the underlying, more specific premises explained. Section 4.3 provides information on the costs of field testing. The chapter concludes with a discussion, in Section 4.4.

As indicated in Chapter 3, the findings of a field trial must enable a firm conclusion to be drawn on whether or not a pesticide represents a hazard. It is on the basis of these results that a recommendation for approval is based. In evaluating the findings, two aspects can be distinguished. In the first place, there are the technical aspects, including such matters as the experimental design of the trial and statistical processing of results. These points are discussed in Para. 4.2.4. In the second place, there will be a societal evaluation, involving, among other things, consideration of other properties of the pesticide and comparison with other pesticides; for this latter aspect, we refer to Canters et al. (1989).

4.1 General premises

In drawing up the guidelines, the following three premises have been taken:

1 There should be maximum conformity with existing guidelines. Especially if these guidelines are already in use in the Netherlands and have been found to work, there is no point in diverging from them to any major degree, particularly as these guidelines are the result of intensive (international) expert consultation.
2 Wherever possible, methods should be adopted from field trials that have already been successfully carried out (successful in the sense of yielding, within a statistically acceptable margin, a conclusion on the occurrence of side-effects); literature concerning studies in the tropics or on crops and organisms not found in the Netherlands has been used only if the methods described are also practicable for use in the Netherlands.
3 The field trials should be capable of demonstrating whether the NOEL of the non-target organisms examined is exceeded by use of the pesticide under review.

In addition, the guidelines to be developed must meet a number of general requirements which, though seemingly trivial, will be stated explicitly for the sake of completeness. In the first place, the trials should yield unambiguous results, i.e. it should be clear whether an observed effect is to be attributed to pesticide use. This has implications for experimental design: it necessitates use of a control (blank or positive), for example. The design (number of organisms, number of replicates) should also allow for observation of (differences in) effects with an acceptable degree of reliability. Furthermore, the influence of other factors or combined effects should be excluded as far as possible. The field trials should also allow conclusions to be drawn about the practical field situations in which the

pesticide is to be (or may be) applied. This means that the crop to be protected must be grown at the test site and that the dosage and method of application must be similar to those used under practical circumstances.

4.2 Specific premises

In their basic structure, the proposed field trials follow the guidelines in force for efficacy testing (PD, undated). Our guidelines are thus built up as follows: 1. Experimental conditions, 2. Application of compounds, and 3. Observation. In line with the NEN standards, an additional section has been added: 4. Validity.

In each of the guidelines presented in Part B, the actual method is preceded by the reasons for opting for the given method and its constituent elements discussed in a fixed sequence. In each case, this introduction is also preceded by a more general section. The guidelines proper are followed by a review of the cost of the trial and (in the present report) by summaries of existing guidelines (Appendices 1) and summaries of the most relevant literature (Appendices 2). Each guideline proposal concludes with references.

4.2.1 *Experimental conditions*

Crop
For the field trial, a crop is chosen for which the highest dose is prescribed and/or for which the highest exposure is to be expected. In practice, this means that the crop chosen is that for which the highest PEC has been calculated. This need not automatically be the same crop for the terrestrial and the aquatic environment.

Species
In a field trial, it is of great importance to determine effects on species actually found in the area of study. These should always include species that are common in the Netherlands. In addition, it is important that a comparison can be made with the results of the laboratory tests. Wherever possible, therefore, the specific test species employed should be the same as those used for laboratory testing; it is proposed to place these species in a cage in the trial plot.

For mammals, birds and fish, field trials should be carried out only if there remain no other options for obtaining relevant information. Such trials must be conducted on a larger scale than those for invertebrates; at the same time there is less social acceptance of tests involving vertebrates. On the other hand, in case of doubt, it is better to conduct a field trial prior to approval than to discover side-effects after approval.

Reference
In all cases, the experimental plot should be compared with a reference plot. This reference must have undergone the same mechanical operations and also, for instance, be treated with a substance resembling the formulation used, e.g. water or granules. If ecological effects are anticipated, it may be necessary to distinguish between toxic and ecological side-effects. In that case, a second reference plot should be treated with a compound having the same intended effect as the pesticide under review but

with a much lower toxicity to the experimental organism. In the case of a herbicide, a comparison of the two treated plots can provide an indication of whether or not the observed effects are due to the direct toxic action of the compound. Ecological effects can be studied by comparison with a reference that has not undergone chemical treatment.

Positive control
In addition to an untreated reference, a positive control is also often desirable. This is a test plot treated with a pesticide having the same intended action and which is known to be harmful to the experimental organism. This enables it to be established whether there are unusual conditions leading to the absence of effects. In the case of mammals, birds and fish, a positive control is less desirable, for two reasons: i) field trials with these organisms are on a larger scale, implying exposure of a larger area to a harmful substance, and ii) the deliberate killing of mammals, birds and fish is socially unacceptable, as reflected in opposition to hunting and (the side-effects of) pest control, for instance.

Trial duration
The duration of the trial depends on the anticipated effect. To draw maximum benefit from the potential offered by a field trial, however, it is desirable to continue a field trial for at least one (field) season. This also means that the pesticide can be applied several times, in accordance with practical use. In addition, any medium- to long-term effects can also thus be traced.

4.2.2 Pesticide application

Dosage
In all trials, it is proposed to apply the highest recommended dose, for this will, in principle, constitute the greatest hazard occurring in normal use. In practice, however, there are several circumstances that may lead to (locally) higher loads, for instance when spraying zones overlap. For this reason, it is also proposed to treat a trial plot with four times the maximum dose prescribed, thus incorporating a worst case situation into the field trial. This also allows for use - deliberate or not - of a dose in excess of the highest prescribed dose. At the same time, the chances of effects not being observed because of unforeseen circumstances are thus reduced. A problem may arise if the maximum dose produces no observable effect, while the fourfold dose does. In this case, there is evidently a potential hazard, which is not apparently encountered during normal use. In this case, a solution may perhaps be found in higher safety margins, to be achieved by prescribing lower user doses, for instance. A study can also be conducted using graded doses of the pesticide under review, enabling a dose-effect relationship to be established. For this type of study, a different, more comprehensive test method is required, however. A field trial employed in the framework of pesticide approval should lead to a firm conclusion on the occurrence or non-occurrence of side-effects. For this reason, the maximum dose and four times this dose are used in the standard trials. Once a hazard is found to exist, more accurate tests can always be carried out if so desired.

To detect any effects on the non-target flora in field margins outside the field sprayed, the same doses are applied. If these doses result in an observable impact, effects can also be expected at a greater distance, due to spray drift, for instance. In this case, a plot should also be treated

with 1% of the maximum dose in order to simulate exposure at 100 m distance.

Formulation and method of application
The selection procedure (Chapter 3) may indicate that use of a certain formulation or method of application is hazardous. The same formulation and/or method of application should therefore be employed in the field trial. If other formulations and/or methods of application likewise involve a hazard, the field trial should initially focus on the situation in which the greatest hazard is anticipated. If necessary, a separate trial should be performed for the other situations. As regards exposure of organisms outside the target area, attention should be focussed on those situations in which exposure will be highest. Under conditions of normal use, greatest drift takes place in fruit-growing applications, and if pesticide approval is requested for such applications, a field trial should be conducted in an orchard setting.

In addition, there are some applications in which the load on ditches and field margins may be as high as 100%, for instance when ditch banks and ditches themselves are treated. In such cases, however, the ditches and ditch banks constitute the target area, and the effects can no longer be termed side-effects. The desirability of such treatment should be debated in another context. A loading of 100% may also occur if aerial spraying is employed. In this case, there will often already be a serious hazard, obviating the need for a field trial. If a moderate hazard is anticipated, a field trial can be conducted; for the time being, however, we have opted for field trials focussing on more standard pesticide use.

4.2.3 Observation

The methods of observation depend primarily on the effect anticipated. As stated in Chapter 3, the issue under study is impact at the population level. Apart from any other parameters, therefore, changes in numbers of organisms must be monitored. In addition, it is useful to observe mortality, if possible, as this enables short-term effects to be established. In the case of ecological effects, too, the aim is to observe changes in populations, so that in principle the same observations can be made. Although in this case direct mortality is not anticipated, effects on migration, say, may now be involved.

In a field trial, it is always essential to know whether the experimental organisms are actually exposed. Although a positive control yields important information on this point, measurement of pesticide concentrations in the exposed environmental compartments and in the organisms tested should be part of standard procedure, as these data can provide support in establishing causal relationships.

4.2.4 Validity

In the United States, the EPA has drawn up Standard Evaluation Procedures (SEP) for test results. In principle, there is a separate SEP for each test. These procedures ask detailed questions about the way the test is carried out. If a detailed guideline is available, this means that the SEP strongly resembles this guideline, the difference being that it is in

the interrogative form. In view of the level of detail of the guidelines proposed here, there would appear to be less need for such an evaluation.

In our opinion, a number of points can be distinguished that are of importance in evaluating test results. These can be summarized as follows:

Statistical significance
Effects must be demonstrated with 90% (one-sided) certainty. This condition places high demands on the test method. In a number of cases, it will be necessary to determine, say, the abundance of a certain organism in the field prior to the trial. Using these results, the number of random tests required for obtaining a statistically verifiable result can be determined. In this context, it is also important what differences are to be demonstrated. The premise here is that it should be possible to establish when the NOEL is exceeded by 10%.

Effects in the blank control
In laboratory tests, mortality in the blank may not exceed 10%. In the field, this figure will depend on the natural mortality rate. One way to assess the natural mortality rate is to transfer the laboratory organisms to an untreated field. The mortality rate of these organisms can then serve as a standard for blank mortality in the field. In several places in the literature, a field mortality rate of 15% in the blank is quoted as being acceptable (e.g. EPPO, 1986).

Effects in the positive control
In the positive control, a 80-100% effect should be found, in conformity with the laboratory tests. This effect may be mortality or another anticipated effect.

4.3 Cost

For each proposed guideline, a very rough estimate has been made of the costs of field testing. In preparing these cost estimates it has been assumed that the proposed guideline is carried out in its entirety. This means that no account has been taken of the possibility of combining certain trials or of carrying out only part of a trial, for instance using only individuals occurring naturally in the environment or performing only cage studies.

To establish the scope of the work involved in performing the integral field trial, the following aspects have been rated:

A species used:
- e = explicitly studied species
- l = locally occurring wild flora/fauna
- n = non-indigenous flora/fauna, but resistant to (a)biotic conditions in the Netherlands
- w = wild flora/fauna

B aspect of organism on which test is focussed:
- a = numbers
- b = biomass or production/growth
- c = condition, incl. any deformities
- h = behaviour
- p = population dynamics

B (continued)
r = reproduction
s = species composition
x = post-mortem examination

C type of test:
f = field study sensu stricto
c = employing cage(s) and/or enclosure(s)

D dosage (of formulation):
hp = highest practical dosage
4hp= four times highest practical dosage

E blank / reference material:
b = experiment including blank/placebo situation
t = experiment including well-known toxic compound

F replicates
1 = single experiment
2 = in duplicate
4 = in fourfold

G/H number of samples per sampling operation(s) during entire field trial

The cost estimate is based on the following items:
1 material expenses (experimental organisms, plots, field equipment, etc.)
2 sampling of (a)biotic parameters (incl. experimental organisms)
3 measurement of parameters (abiotic and biotic)
4 data processing and interpretation
5 completion of test form.

For each of these five elements, the net number of working days is indicated, including the associated material costs. To form an initial impression of overall costs, the average cost of one net working day has been assumed to be Dfl. 750. This is an average figure, for some of the work may be done by an analyst (medium or higher vocational training) and other work by a (junior) graduate. VAT and overheads have not been budgeted, since i) it is not clear which elements are VAT-taxable (and at which rate), and ii) overheads are not considered relevant for actual field testing.

The required laboratory equipment, especially that used for measuring residue concentrations, has been included as a cost item yet to be quantified. It is assumed that use can be made of equipment that is already available, but a certain figure must still be included for depreciation. As this figure includes a highly arbitrary element, it has been left open provisionally.

Table 4.1 presents a very rough estimate of the net cost of each field trial. As can be seen, there is considerable variation in cost; however, all trials fall in the approximate range of Dfl. 100,000-200,000.

	net cost of field trial
higher plants	Dfl. 80,000
earthworms	175,000
ground beetles	90,000
honeybees	100,000
birds	100,000
algae	195,000
midge larvae	120,000
water snails	220,000
water fleas	220,000
fish	85,000

Table 4.1 Cost of proposed field trials, based on a very rough estimate and rounded off to the nearest Dfl. 5,000.

4.4 Discussion

In formulating the premises for the guidelines, a number of choices have been made, the aim being to design a test method offering the greatest chance of effects actually being detected. This means that a number of parameters have been left out of consideration. Further elaboration will depend on the one hand on the statistical margins chosen, and on the other on such factors as the field abundance of the organisms.

In field testing, a conflict may arise between practicability and compliance with the basic premises. A test yielding a conclusion within the proposed statistical margins may prove to be too comprehensive (= too costly), but a test of limited scope may lead to greater margins of uncertainty. To solve this dilemma, a trial can be focussed on a worst case situation. By applying a higher dose, the scope of the test can be limited. If effects are not then demonstrated, it may be assumed that the practical dose will not give rise to effects, either. If effects are found, however, there will have to be very careful translation to the practical dose.

The guideline proposals presented in Part B aim, provisionally, at testing the effects of a single new pesticide on a single (group of) organism(s) in the context of official approval procedures. On each of these three aspects, the scope of the guidelines could be extended. In principle, the same trials could also be used for evaluating the effects of a combination of pesticides. There may be various reasons for combined testing. In an existing practical application involving multiple treatment, an old pestici-

cide may be replaced by a new compound. Alternatively, there may be suspicions of combined toxicity or synergistic effects. In such cases, the pesticides involved can be tested simultaneously.

Effects on more than one group of organisms can be assessed by combining different tests or elements of tests. For post-registration monitoring, however, the test method certainly needs to be adapted, although the same basic methods of observation could still be used.

Before the proposed guidelines can actually be implemented, practical validation is required. Since no guidelines exist for the aquatic environment, development of these field trials should be given the highest priority, particularly those involving algae, water fleas and fish, because of similarities with existing laboratory tests. These organisms should preferably be tested in combination. There is a paucity of data on midge larvae, and especially on snails; however, it seems logical to include these groups, too, in an overall guideline validation test for the aquatic environment.

For the terrestrial environment, a number of guidelines are already available. A guideline for earthworms is currently in preparation in Germany. Although the guideline proposed here deviates in some respects, the German validation procedure will probably yield so much data as to render specific validation of our guideline superfluous. For honeybees, too, a guideline is already effective, and here, too, extra validation of the proposed guideline does not appear strictly necessary. In the case of ground beetles and (especially) birds, the extent to which existing guidelines have actually been field-tested is far less clear. For these trials, therefore, more extensive validation is required. For higher plants - outside the target area - there are no guidelines at all. Priority might therefore be given to validation of the guidelines for ground beetles, birds and higher plants. Alternatively, it might be opted to finalize the guidelines for honeybees and earthworms, as this would involve relatively little extra effort. Validation of the earthworm trial could be combined with validation of the ground beetle trial, since both cases involve exposure via the soil.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In Chapter 1 the following objectives were formulated for this study:

I. To indicate the possible role and status of field trials in pesticide approval procedures in the Netherlands.
II. To develop a framework for selecting field trials for detecting pesticide side-effects.
III. To develop field trial guidelines.

For each of these objectives, the main conclusions of the study are summarized below. For more detailed information, the reader is referred to relevant discussions in individual chapter sections.

I. Possible role and status of field trials in the Netherlands (Chapter 2)

1 The Dutch approval procedure
The current Dutch approval procedure is based almost entirely on laboratory testing. Field testing plays only a minor role and, except for honeybees, is not a standard requirement. Guidelines for such trials are almost entirely lacking, moreover. Most of the work currently in progress is aimed at broadening the scope of laboratory testing (more types of test organisms) or at improving extrapolation from the laboratory to the field.

2 Field study in an international context
In an international context, there is a greater emphasis on field studies than in the Netherlands. This holds both at the national level as well as on the part of various international organizations.

3 Field trials in approval procedures outside the Netherlands
In approval procedures outside the Netherlands, field trials are used mainly to obtain supplementary data before a pesticide is admitted to the market. At that stage, laboratory testing has already taken place. If these tests have not dispelled all doubts, semi-field or field testing is prescribed. In this case, the field trial constitutes an explicit step in a tiered approval procedure. Field trials may also be used, after the compound has come onto the market, for post-registration monitoring of the side-effects of practical use - this is, at least, often prescribed on paper. If serious or hitherto unknown side-effects are detected, appropriate measures may be taken.

4 Field trials in the Dutch procedure
The status of the field trial within the Dutch approval procedure should be defined more clearly. Field testing should take place only if prior laboratory tests have indicated such a need, or if ecological hazards are anticipated. The criteria governing the progression to field testing should be made as explicit as possible (see Chapter 3). In addition, in the Netherlands, too, field trials can be used for post-registration monitoring and for assessing overall environmental quality.

5 Criteria for field trials

The criteria for moving from one phase of the approval procedure to the next are indicated only sketchily in most of the approval procedures studied, and are concerned with toxic side-effects only.

6 Existing guidelines

Internationally, there appear to be a reasonably large number of field tests available. Tests with honeybees, other beneficial insects and earthworms, particularly, receive frequent mention. For these organisms, a number of guidelines are now available. For birds, too, field trial guidelines are available. However, for other groups of organisms such as plants, aquatic organisms and mammals, there is considerably less interest.

7 Availability of field trial results

Although various guidelines for field trials exist, it is not clear to what extent these are actually in use. At any rate, there are very few results available.

8 Analysis of terrestrial field studies

An analysis of field work performed outside the scope of approval procedures shows that, compared with the terrestrial environment, very little work has been done on the aquatic environment. Terrestrial studies have focussed mainly on beneficial arthropods (insects and arachnids), earthworms, birds and mammals. There have been only a few field studies into the side-effects of pesticides on (terrestrial) molluscs (e.g. snails), crustaceans (e.g. woodlice), amphibians and reptiles. Most available studies are field studies sensu stricto, focussing on direct toxic effects, with ecological effects being studied far less frequently.

9 Analysis of aquatic field studies

Most studies in the aquatic environment have focussed on insects, molluscs, crustaceans and fish. Besides field studies sensu stricto, much work has also been carried out with experimental ditches. In the aquatic environment, too, it is above all the direct toxic effects of a pesticide that receive the greatest attention.

10 Development of guidelines

In comparing existing field trial guidelines and 'general' field studies, it is surprising to note that, even though many studies involve aquatic organisms, this has not resulted in a proportionate number of guidelines. On the basis of the data from 'general' studies, however, it should be possible to formulate proposals for such guidelines. The same also holds for (groups of) terrestrial organisms (see Chapter 4).

11 Standard guidelines

In various circles, there is currently a debate about the extent to which the details of field trials should be laid down in strict procedures. It is argued, on the one hand, that the variation existing in environmental factors and pesticide application methods excludes the possibility of standard field trial guidelines; on the other hand, a case-by-case approach (with the procedure determined in consultation between applicant and authorities) impedes standardization of both the methods and the interpretation and comparison of results. In drawing up our proposals for field trial guidelines we have opted, provisionally at any rate, for relatively detailed specification of procedures. There are several reasons for this decision. Contrary to the situation

in Canada or the United States, for instance, there is little need in the Netherlands to allow for wide variations in environmental conditions such as climate. At the same time, we have aimed as far as possible to eliminate arbitrary elements from the approval procedure. One of the advantages of this approach is that third-party control can be carried out in an objective manner. To cater for unusual circumstances, however, there should be scope for modifying the field trial guideline. On no account may a standard field trial guideline be used to legitimize test results by an applicant confronted with extreme field circumstances. There will always be a need for consultations between the applicant and the authorities (with scope for a certain degree of public control). To a major extent, however, acceptance of the proposed guidelines automatically implies establishment of these boundary conditions.

12 Proposed guidelines

The proposed guidelines (see Part B) can be characterized as 'basic guidelines', allowing for adaptation to specific circumstances dictated by the nature of the pesticide (formulation) or mode of application. In view of the above, for the time being at any rate field testing will not be a routine operation, but will require a 'customized' approach.

13 *Validation of extrapolation from laboratory to field*

The experience gained with field trials can also contribute substantially to validating extrapolation techniques currently under development, particularly in the Netherlands.

II Development of a framework for selection of a suitable field trial (Chapter 3)

14 Proposed selection procedure

A procedure (framework) has been presented for determining the need for field trials and for selecting the most suitable field trial on the basis of available data. The type of field trial proposed focusses on ecotoxic effects on non-target organisms. Conclusions bearing on pesticide approval will always have to be drawn in conjunction with other data and criteria.

15 Assessment of the need for field testing

To assess the need for field testing, two keys are used, viz. toxic and ecological side-effects. To evaluate the toxic side-effects, use is made of commonly used criteria such as toxicity data, usage data and exposure data (PEC). This form of evaluation is very similar to the approach currently taken by the Dutch authorities (CTB) and also followed internationally.

16 *Ecological side-effects*

Until now, it has not been customary to evaluate the ecological side-effects of a pesticide. As criteria for this purpose, we propose scale of use and spectrum of action, for widespread elimination of a food source for a variety of species may have a major impact on species at a higher trophic level.

17 Efficacy as a criterion

The efficacy of a pesticide is important, too. However, if efficacy were to be used as a criterion in its own right, this would conflict with the very purpose of the pesticide, which is approved only after

its efficacy has been proven. Nevertheless, eradication of all aphids on all plots in a polder, for instance, may be deemed undesirable in terms of the survival of aphid predators in the area. It is also of interest to investigate the degree to which the 'General Environmental Quality' in agricultural areas (which requires a 'species survival ratio' of 95%) is jeopardized. For this reason, efficacy is always viewed in conjunction with the scale of use and spectrum of action, rather than serving as an independent criterion.

18 Criteria for ecological field trials

No information has been found in the literature that permits numerical values to be assigned to the criteria scale of use, spectrum of action and efficacy. The values used here are based on the idea that it should at least be possible to detect the effect at the population level. For certain species (endangered and protected species) different choices may be made, however. The values chosen are still open to debate and are in need of further substantiation.

19 Choice of experimental organisms

On the basis of the properties of the pesticide, its use and the 'receiving environment', suspicions are subsequently narrowed down to effects on specific groups of organisms, divided over four environmental compartments. In assigning groups of species to environmental compartments, we have striven for completeness. In principle, a field trial must be developed for each of these groups of organisms. We have made a provisional selection based on ecological relevance. However, other choices are obviously conceivable.

20 Specimen compounds

An evaluation of the proposed screening procedure using the specimen compounds atrazine and pirimicarb shows that markedly different results are obtained. In the past, a request for approval of atrazine would have led to far more field trials than a request for pirimicarb.

III Development of field trial guidelines (Chapter 4; see also Part B)

21 Choices made in guideline design

In formulating the premises for the guidelines, a number of choices have been made, the aim being to design a test method offering the greatest chance of effects actually being detected. This means that a number of parameters have been left out of consideration. Further elaboration will depend on the one hand on the statistical margins chosen, and on the other on such factors as the field abundance of organisms.

22 Application of field trial guidelines

The guideline proposals presented in Part B aim, provisionally, at testing the effects of a single new pesticide on a single (group of) organism(s) in the context of official approval procedures. In principle, the same trials could also be used for evaluating the effects of a combination of pesticides. There may be various reasons for combined testing. In an existing practical application involving multiple treatment, an old pesticide may be replaced by a new compound. Alternatively, there may be suspicions of combined toxicity or synergistic effects. In such cases, the pesticides involved can be tested simultaneously.

23 Effects on more than one group of organisms

Effects on more than one group of organisms can be assessed by combining different tests or elements of different tests. For post-registration monitoring, however, the test method certainly needs to be adapted, although the same basic methods of observation could still be used.

24 Validation of aquatic guidelines

Before the proposed guidelines can actually be implemented, practical *validation is required. Since no guidelines exist for the aquatic environment,* development of these field trials should be given the highest priority, particularly those involving algae, water fleas and fish, because of similarities with existing laboratory tests. These organisms should preferably be tested in combination. There is a paucity of data on midge larvae, and especially on snails; however, it seems logical to include these groups, too, in an overall guideline validation test for the aquatic environment.

25 Validation of terrestrial guidelines

For the terrestrial environment, a number of guidelines are already available. In the case of earthworms and honeybees, the guidelines proposed here do not deviate sufficiently from existing guidelines to render field validation strictly necessary. For ground beetles and (especially) for birds, the extent to which existing guidelines have actually been field-tested is far less clear. For these trials, therefore, *more extensive validation is required. For higher plants - outside the* target area - there are no guidelines at all. Priority might therefore be given to validation of the guidelines for ground beetles, birds and higher plants.

5.2 Recommendations

In Chapters 3 and 4 of Part A of this study, we propose a decision-making scheme for conducting field trials. In Part B, we make proposals for guidelines for ten field trials. *Field trials to assess toxic side-effects* can be readily incorporated into existing procedures. Current laboratory testing may indicate a minor, moderate or (very) serious hazard: whenever there is a moderate hazard, field testing is required.

For ecological side-effects, the current procedure provides insufficient scope. To assess the need for a field trial focussing on these side-effects, the scale of use, spectrum of action and efficacy of the pesticide must also be considered. Using these parameters, the magnitude of the ecological hazard can be estimated. In the case of a moderate hazard, again, a field trial focussing on ecological side-effects is required.

The principal recommendation of this study is for policy makers and advisors to elaborate further - and preferably adopt - the proposals presented in Chapters 3 and 4. More specifically, the present study leads to the following concrete recommendations:

The Netherlands:

1 In line with the situation in a number of other countries, the Dutch pesticide approval procedure should be designed more as a tiered system, with field trials being given a clear status. Among other things, this implies that Form H will need to be adapted.

2 In such a procedure, post-registration monitoring should be explicitly mentioned and concrete instructions given for performing such monitoring.

3 At the moment, development of field trial guidelines for the Netherlands can probably be readily integrated into the PCBB 'Fundamental Soil Research' programme. A number of laboratory tests are already being developed within this same framework. It is desirable that these field trial guidelines be prepared by specialists working on the respective species groups; this approach also ensures a better assessment of the relevance of conducting the respective trials under (semi-)field conditions.

4 For the aquatic environment, the Netherlands might be able to play a pioneering role in the development of field trial guidelines. Not only is the aquatic environment of major importance to the Netherlands. The country also has excellent research facilities, including experimental-ditch and mesocosmos environments, and has built up a great deal of know-how and expertise.

International:

5 In an international context, several different organizations are involved with (development and use of) field trials. EPPO focusses on efficacy, but has also published several field trial guidelines on side-effects. IOBC is interested above all in beneficial organisms, and consequently field trials are not covered entirely by this organization, either. To date, the OECD has only been concerned with laboratory testing for side-effects, though the organization did recently recommend development of field trial guidelines. We recommend charging a single organization, for instance the OECD, with coordination of all side-effects studies, i.e. including all field studies and all the work performed until now by EPPO and IOBC.

6 The OECD could also coordinate efforts towards international harmonization of work on side-effect testing. In this process of harmonization, progress is still slow, and appears to be taking place in a relatively random fashion. This is illustrated by the organization of work around groups of organisms, vertebrates having been discussed at a one-off workshop in Cambridge, honeybees being the subject of regular meetings (again in 1990) and work on beneficial organisms being handled by IOBC. In this situation, aquatic organisms threaten to be forgotten.

7 At the moment, the EC, the Council of Europe and the FAO each have their own proposals for approval procedures. There should be a greater degree of coordination than appears to be the case at present.

8 Efforts should be made to standardize field trial methods and improve their quality. Greater efforts should be devoted to development of

standard GFP (Good Field Practice), analogous to laboratory GLP (Good Laboratory Practice).

9 There should be a more intensive exchange of field data (including population dynamics) and field trial data, as has also been recommended by the OECD (1988).

5.3 Follow-up: validation of field trial guidelines

The ten proposed field trial guidelines (see Part B) are among the most important results of this study. Before these guidelines can be integrated into the approval procedure, however, they must be validated in the field. Without such validation, the proposed guidelines cannot be used in the approval procedure. A follow-up to the present developmental study is therefore desirable.

In validating the designed guidelines, maximum use should be made of the facilities and potential of a number of Dutch institutes with relevant expertise, viz. RIVM, PD, DBW/RIZA, SC, RIN and various testing stations.

We conclude Part A of the report with a proposal for field validation of the proposed guidelines.

Validation procedure

Before field validation can take place, a certain amount of preliminary preparation is necessary, relating, on the one hand, to the organizational aspects of validation and, on the other, to the actual content of the validation procedure. Organizational aspects include the financial scope and limitations of the validation exercise, including its integration into existing or planned research at the various institutes. Aspects relating to content include the choice and use of trial areas and the pesticides that are to be studied. It is anticipated that a well-designed programme can lead to combined experiments yielding substantial savings in costs.

It is proposed to carry out a number of preparatory activities in 1991 to arrive at a coherent research programme and arrange project financing and execution. It is expected that actual field validation can then be started in 1992. The duration of the validation exercise is not yet clear, but it has already been established that the programme, or parts of it, will most probably take at least two field seasons. The eventual duration will depend on the organisms being studied, the available know-how and the progress of validation itself.

Primary objective of validation and preparatory sub-objectives

The primary objective of the follow-up study on the potential of field trial guidelines for evaluating the side-effects of pesticides is:

To investigate the extent to which the proposed field trials can be implemented in practice and how these field trials can be integrated into the approval procedure.

This primary objective is to be achieved in three phases:
a. preparation (1991)
b. field study (1992-93)
c. integration into procedure (1994)

Here, only the preparatory phase (a) is further elaborated. In this phase, the following sub-objectives can be distinguished:
1. To investigate the financial scope for conducting validation studies, including the possibilities of coordination with ongoing research efforts.
2. To investigate the willingness of various research institutes to contribute to validation studies, as well as their (in)capacity, requirements and conditions for doing so.
3. To prepare a scenario for project execution, including coordination of tasks and setting of priorities.

Planning of preparatory phase

Financial scope (Objective 1):
- Discussion of the project with representatives of ministries and coordinators of research programmes, to establish the potential for (joint) financing of the field validation exercise.
- Investigation of the scope for coordinating the project with similar research being conducted elsewhere or currently in preparation, including the form collaboration might take.

Result: insight into the financial feasibility and scope for execution of the proposed validation studies, possibly directly coordinated with other studies of a similar nature.

Establishing interest (Objective 2)
- Discussion of the project with representatives of research institutes, to assess the scope for conducting the validation programme in 1992 and 1993 (expertise, know-how, etc.), with or without the cooperation of, or support from, CML.
- Investigation of the scope for collaboration in given experimental areas and/or ditches; wherever possible, preference should be given to testing under identical conditions.

Result: report(s), tentative conclusions and preparation of draft 'Validation Research Programme' (experimental areas, materials and other requirements, locations, manpower, costs, etc.).

Scenario, coordination and priorities (Objective 3):
- Establishment of a project committee.
- Presentation of draft 'Validation Research Programme' to representatives of ministries and research institutes, possibly in the form of a workshop (emphasis on policy).
- Preparation of a scenario; specification of the technical details of the validation programme, in consultation with and at the level of those who will be executing the work; establishment of further details for 1992, again possibly in the form of a workshop (emphasis on programme execution).

Result: presentation of scenario for field validation in 1992-93 to project committee, and approval.

Reporting

The results are to be reported in the form of a document that can serve as a scenario for the work to be performed in the years ahead. It should include a description of the tasks and competences of the project committee as well as a definition of the responsibilities of the parties participating in the project.

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APPENDIX

Abbreviations

CABO	Centre for Agro-Biological Research (NL)
CML	Centre of Environmental Science, University of Leiden
CRNH	Central Environmental Protection Council (NL)
CTB	Commission for the Registration of Pesticides (NL)
DBW/RIZA	National Institute of Inland Water Management/National Institute of Wastewater Treatment (NL)
EPA	Environmental Protection Agency (US)
EPPO	European and Mediterranean Plant Protection Organization
FAO	U.N. Food and Agriculture Organization
IOBC	International Organization for Biological Control (of noxious animals and plants)
IVM	Institute for Environmental Studies, Amsterdam Free University (NL)
LUW	Wageningen Agricultural University (NL)
MAFF	Ministry of Agriculture, Fisheries and Food (UK)
NEN	Netherlands Standardization Institute (NL)
OECD	Organisation for Economic Cooperation and Development
PCBB	Programme Committee on Fundamental Soil Research (NL)
PD	Plant Protection Service (NL)
RIN	Research Institute for Nature Management (NL)
RIVM	Institute of Public Health and Environmental Protection (NL)
TNO	Organization for Applied Physical Research (NL)
TNO-MT TNO,	Division of Technology for Society (NL)
VROM	Ministry of Housing, Physical Planning and Environment (NL)
VU	Amsterdam Free University (NL)
WL	Delft Hydrodynamic Laboratory (NL)

(NL) indicates a Dutch institute or authority.

Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

TERRESTRIAL HIGHER PLANTS

FIELD TRIAL FOR TERRESTRIAL HIGHER PLANTS

General

In the United States (see: Appendix 4.1.1), there exist separate field trial guidelines for assessing the side-effects of pesticides on plants growing inside and outside the target area. In the context of U.S. admission procedures, this distinction is useful because efficacy tests and tests on crop side-effects are very separate, on paper at any rate. Within the cropped area, these side-effect studies are thus focussed purely on the crop itself. In the Netherlands, efficacy testing embraces *effectiveness of control as well as potential toxic side-effects on the* crop.

The aim of a field trial for higher plants (= Cormophyta) is to establish any effects on non-target plants. From an agricultural point of view, it may be debated whether a field contains any 'non-target plants', apart from the crop itself. Although selective herbicides do exist, any plant that is a potential competitor of the crop is in principle deemed undesirable. The side-effects on the crop itself are already investigated within the framework of efficacy testing, as indicated above. For the purposes of the present protocol, therefore, the plants in the field *itself are not taken into consideration. The most directly exposed non-*target plants are thus to be found in the field margins, and it is with this category of plants that the present field trial guideline is concerned. In scope, therefore, the present guideline differs from other existing protocols, although the experimental approach is based as far as possible on EPA and EPPO methods (see: Appendix 4.1.1).

The substantial impact of pesticides on plant life in arable farming regions is borne out by studies in which field margins were left unsprayed (see: Appendix 4.1.2: Boatman, 1988; Dean, 1989). In these untreated margins, the number of wild flowers increased dramatically.

Even away from the fields and their margins, a certain impact is to be anticipated. Because of spray drift and evaporation from the treated crops, considerable amounts of pesticides find their way into the atmosphere and may lead to additional impact further afield. This protocol also covers these more remote effects.

Experimental conditions

When testing compounds other than herbicides, in addition to the unsprayed control field, a field must also be treated with a compound targeted at the same pest but known to be non-phytotoxic, in order to distinguish toxic from ecological side-effects. In the case of an insecticide, for example, the ensuing depression of insect attack on the plants may be so great as to overrule any toxic side-effects (Fox, 1958; Brown et al., 1987). This obviously also depends on the type of effect studied. In the case of impact on biomass, for instance, the effects of insect attack are difficult to distinguish from those of the chemical itself and a control sample, as outlined above, is therefore essential. Toxic effects (e.g.

necrosis), on the other hand, can readily be distinguished without requiring a control sample.

In the Netherlands, field boundaries/margins will in practice consist of ditches, roadside verges, woodland or hedges. When considering woodland, a field trial must include two species of deciduous tree, in addition to the species specified by the EPA. In the case of ditches, the impact on riparian species must also be assessed; here, the EPA guideline reflects the major groups and thus appears satisfactory.

Application

Spraying is carried out on the field on which the test plots border, applying both the maximum recommended dose of the compound as well as four times this dose. The latter simulates a worst-case situation, representing a situation in which fields on both sides of a margin are treated as well as the fluctuations that are likely to occur in actual agricultural practice. If measurements reveal lack of exposure in the test plots, the field trial can provide for direct spraying onto these margins, using 10% of the maximum recommended dose. It should be noted, however, that in the latter case extrapolation to actual spraying practice will involve a greater degree of uncertainty.

If, after the standard spraying procedure, the plants in the field margins are observed to be affected, there is a probability that plants growing further away from the field will also have been affected (by spray drift deposits, etc.). In such a case, therefore, the protocol prescribes additional trials on separate plots, using a lower dose (1% of the maximum recommended dose), to simulate exposure originating from further afield.

Observation

Higher plants and (a)biotic environmental factors
The impact on species composition and on the abundance of individual species is assessed, along with any phytotoxic symptoms. In addition to the weather conditions, any other treatment of the field should be accurately recorded. Finally, soil characteristics should also be recorded, being relevant for general plant condition and growth.

Compound
In field-spraying exercises, it is essential to make a good assessment of the degree of test plot exposure. For this purpose, soil and crop residue levels are regularly determined; a dye or other marker may also be added to the compound to aid assessment of coverage. In directly sprayed experimental fields, too, soil and crop residues must be measured in order to determine the degree of exposure.

Proposal: **FIELD TRIAL GUIDELINE FOR TERRESTRIAL HIGHER PLANTS**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

The experimental plots must contain the following species of higher plants (= Cormophyta): three dicotyledon species from three families; three monocotyledon species from three families; ferns from two families; one species of moss or liverwort; one species of coniferous tree and a species of deciduous tree growing in a moist habitat (e.g. willow, Salix sp.) or a deciduous tree growing in a dry habitat (e.g. hawthorn, Crataegus sp.). The plants may either be growing naturally on the plot or, alternatively, they may be sown or transplanted there. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage.

1.2 Experimental conditions

Define test plots in the margins of the fields to be treated. At a single session, treat the respective fields with the compound being assessed, with a markedly phytotoxic agent and with water, or with another innocuous carrier substance similar to the compound under review. If the compound under review is not a herbicide, also spray a field with a markedly non-phytotoxic agent with the same intended spectrum of action, to distinguish toxic from ecological effects.

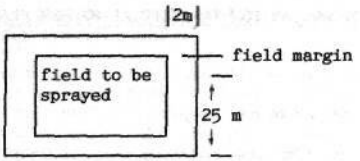
1.3 Other requirements

Spray during weather conditions favouring drift to the field margins, i.e. preferably dry weather with a moderate wind towards the experimental plots. Avoid extremes such as prolonged drought or rain. If effects are observed after following the described test procedure, repeat using a lower dosage (1%) to assess any impact from drift over greater distances. For this purpose, prepare special new plots, and treat them (directly on the plots), respectively, with the lower dosage, with a phytotoxic agent and with controls.

1.4 Experimental design

Plot size

The experimental fields should measure at least 2500 m². Along the edges of these plots, select trial plots (strips) 2-3 m wide and at least 25 m long. These strips are not sprayed. (See figure below.) If new fields are required for additional spraying using a lower dosage (see: 1.3), these should measure at least 50 m².



Test plot

Plot arrangement

Perform the tests in duplo. To exclude the possibility of mutual spraying impact, the test fields should be located at least 100 metres apart.

2. APPLICATION

2.1 Compounds

Apply the compounds being studied according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. If measurements indicate that the plots have not been exposed, immediately spray 10% of the maximum recommended dose and four times that amount directly onto these plots. If effects are already observed after spraying of the field itself, also apply 1% of the maximum dose directly to special new plots.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sampling consists of determining, within each plot, the species composition and the abundance of individual species. In addition, four times 25x25 cm of vegetation should be harvested and, for each species, the ground-level cover and the fresh and dry weight determined.

For each sample, record phytotoxic symptoms such as chlorosis, necrosis, wilting, leaf or stem damage, anomalous growth or development and any other observable damage.

Sample each plot one week prior to treatment and two weeks after treatment, and subsequently each month until the end of the growing season.

Record the meteorological conditions on the day of treatment: precipitation, temperature, wind, cloud cover, sunshine and atmospheric humidity (incl. leaf moisture). Ten days before and after treatment, record precipitation, temperature, cloud cover and sunshine. On the day of treatment, these data must be recorded at the experimental site. On other days, an on-site record is preferable, but data from a nearby meteostation are also acceptable. Subsequently, only extreme weather conditions need be recorded. Record data on spraying and fertilizer regime as well as the following soil characteristics: pH, organic matter, soil type and moisture.

Measure pesticide residues in the trial plots, taking both plant and soil samples, daily during the first week and subsequently concurrently with the vegetation sampling schedule. When the compound can no longer be detected, sampling frequency can again be reduced.

4. VALIDITY

Test results are not valid if results are unclear, or if there is high observable impact in the blanks or little impact in the positive controls. Results are also invalid if plots already showed significant differences in the measured parameters prior to treatment.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
n w	a b c s	f c	hp	b t')	2	4	6 - 8

') For compounds other than herbicides, also test with a markedly non-phytotoxic agent.

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials - test organisms - test plots - field equipment	5	 500 1,000 ( )
2 sampling (4x2x4x8 plots) - test organisms - abiotic parameters	 32 6	
3 parameter measurement - test organisms - residue measurements	 32 20	 500 2,000
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 100	n.a.
cost estimate (subtotal)	Dfl. 75,000	Dfl. 4,000
overall cost (very rough estimate):		Dfl. 80,000

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.1.1 Review of existing field trial guidelines for higher plants

United States (EPA, 1982, 1986a, 1986b)
General testing requirements:
- All methods must be scientifically accepted.
- Trial to be performed by trained personnel, with individual accountability for each element of the trial.
- Trial to be performed using active ingredient or formulation of known composition.
- Application method itself must have zero impact on the compound or organism under investigation.
- Healthy plants to be used.
- No protected or threatened plant species to be used.
- For each treatment or replicate, population size must be such as to provide a 90-95% confidence level for 25% or 50% damage to plants.
- Control treatments to be performed identically, but without the compound. If a solvent or formulation other than water is employed, apply this formulation.
- Wherever possible, employ the commercial method of application.
- In non-target area trials, use the following species: three dicotyledon species from three families; three monocotyledon species from three families; two species of cryptogams from two families; one species of moss or liverwort; one species of coniferous tree.
- Target-area trials are concerned with toxic effects on the crop.
- Trial duration: as long as permitted by repeated application according to manufacturer's instructions. Measurements: twice weekly and subsequently until at least 2 weeks and max. 4 weeks after final application.

Observation:
- Any variations between control plants and trial plants, e.g. phytotoxic symptoms (chlorosis, necrosis, wilting etc.), leaf and stem damage, growth and development.

EPPO (1989)
Guidelines for efficacy evaluation of pesticides:
- Evaluation of efficacy of non-herbicides is based solely on effects (positive and negative) on the crop.
- Evaluation of efficacy of herbicides is obviously also based on effects on field weeds. In these studies, the following procedure is followed:
- Plots are selected with a varied but homogeneous weed vegetation that corresponds with the compound's spectrum of action.
- Duration of trial may be as long as 2 years or more in the case of persistent compounds.
- Plot size for each crop varies from about 10 to 100 m^2.
- Use the compound being assessed, a reference compound and a blank. Perform four replicates.
- Spray according to manufacturer's instructions and at the stage of crop/weed growth recommended by the manufacturer.

Observations:
- Record the meteorological conditions on the day of treatment: precipitation, temperature, wind, cloud cover, sunshine and atmospheric

humidity (incl. leaf moisture). Ten days before and after treatment, record precipitation, temperature, cloud cover and sunshine. An on-site record is preferable, but data form a nearby meteo-station are also acceptable. Subsequently, only extreme weather conditions need to be recorded. Data on spraying should always be recorded.

- Record soil characteristics: pH, organic matter, soil type, moisture, seedbed condition and fertilizer regime.
- Vegetation sampling: record numbers, ground-level cover or weight (measured or estimated). Describe any observable damage to the vegetation.
- Sampling schedule of weeds.
  Treatment by pre-emergent herbicide: before the treatment, in the middle of the growing period, and just before the harvest.
  Treatment by compound sprayed during the growing period: before the treatment, 2-3 weeks after the treatment, and just before the harvest.

Appendix 4.1.2 *Review of field studies involving higher plants*

Fox (1958) Effects of insecticides (aldrin, dieldrin and heptachlor) on grassland wireworms and vegetation. Plots measuring 7.6x9.2 m were used, leaving half of each untreated as controls. The impact on vegetation was assessed by taking two 20x20 cm samples from each sub-plot, measuring the ground-level cover of each species. Observed effects were mostly indirect, caused by checked attack by wireworms.

Henderson & Clements (1977) Four locations were selected in meadows of different ages (3-6 years). At each location, two pairs of 3x7 m plots were defined. One plot per pair was treated with aldrin and phorate (granules). The yield was assessed 4-5 times a year by harvesting a 0.9x6 m strip. Species composition was determined from the year's first harvest by hand-sorting approx. 200 g (fresh-weight) of vegetation. In all cases, insecticide-treated meadows yielded a greater harvest; fluctuations in species composition were observed (in the two dominant sown grasses), but there were no lasting effects.

Heijbroek & Van de Bund (1982) Effects of crop rotation and pesticides in sugar beet. Duplo testing: two fields, each comprising two blocks 9 m apart with different crop rotation schedules. These blocks contained six plots with twelve 18 m rows of sugar beet (50 cm apart). In one field, sugar beet was cultivated every year. In the other, winter wheat was grown, alternated every third year with sugar beet. Concurrent with sowing, the following treatments were applied to the respective fields: no insecticides, lindane, aldicarb, chloradizone, no pre-emergent herbicide. Weeds were counted between the rows, over a total area of 9 m^2; when plant cover was high, abundance/distribution were estimated using pre-defined classes. The major impact of herbicides was confirmed. With continuous beet cultivation, perennial/biennial weeds were not adequately controlled and constituted a problem. When a pre-emergent herbicide was applied, annual weeds served as an alternative source of nutrition for the springtail Onychiurus armatus, leading to less damage to seedlings.

Brown et al. (1987) In ecological studies, attack by herbivore pests is often experimentally manipulated. In such cases, the effect on vegetation of removing the herbivore load is often so great as to overrule any impact of the compound itself. In this study, it was investigated whether malathion also has a direct effect on the vegetation. An assessment was made of the impact on the early stages of succession in terrestrial vegetations, measured in terms of biomass one year post-ploughing. Four 3x3 m sub-plots were defined in a 30x20 m field. Two plots were treated with malathion and two with an equal volume of water. Spraying took place in the morning or in the evening, using a ULV (ultra-low-volume) spraying unit. In principle, spraying was either performed every 10 days or when surveillance on alternate days yielded more than five living invertebrates within 10 minutes. The control field was kept free of invertebrates by means of a 'vacuum cleaner' unit. Vegetation was sampled on three occasions (25x25 cm), in mid-August, October and the end of October. At the end of October, the root mass was also determined. A distinction was made between annual and perennial plants and grasses. Four dominant species were found: Spergula arvensis, Trifolium pratense, Rumex acetosella and Holcus lanatus. In each sample, the biomass of each of these

species was determined (t-test and F-test). In addition, parallel experiments were performed on species grown from seed under controlled conditions (in the lab). Results: no significant differences, due in part to the natural spread in species occurrence. In the lab experiments, too, no differences were found. The lab tests were reported to be more time-consuming and expensive (space requirements!) than field tests.

Ibrahim et al. (1987) Study of the impact of various herbicides on oilseed rape and weeds (in Egypt). Untreated fields and hand-weeded fields were used as controls. Seven different compounds were applied in two concentrations + two blank controls, all in four replicate experiments during two seasons. Each 10.5 m^2 plot consisted of six 3.5 m rows, 50 cm apart. Weed samples were hand-picked from 1 m^2 (60 days after sowing and at harvest) and sorted into three categories: grass-like, broad-leaved and perennial species. The individual species were also identified. Fresh and dry weight were measured and reductions relative to the untreated control recorded. The herbicides employed were not effective against perennials. The method was judged suitable for demonstrating differences in compound effectiveness.

Lund-Hoie & Gronvold (1987) Effects of forest spraying with glyphosate. Plots consisted of sprayed, unsprayed and hand-cleared areas measuring 25 m^2, located in different regions. The impact on succession patterns was investigated. Taller trees were found to be reasonably tolerant, with the exception of conifers. Spraying as well as hand-clearing led to an increase in species diversity; in the sprayed plots, this increase was even greater for taller-growing species, because spruce also ceased to form a competitive factor.

Boatman (1988) In the 'Cereals and Gamebirds Research Project (UK)', field margins are left unsprayed to allow flora and fauna to develop more naturally. Using a well-designed herbicide programme, undesirable weeds can be controlled while still retaining relatively rich field margins.

Dean (1989) Margins of meadows and arable land are managed for nature conservation and recreation (incl. hunting). Non-treatment of field margins with insecticides and herbicides leads to a high increase in floristic diversity as well as in the variety of insect, bird and mammal life.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

EARTHWORMS

FIELD TRIAL FOR EARTHWORMS

General

A field trial guideline for earthworms (of the class Oligochaeta) is available in the United Kingdom (see: Appendix 4.2.1), while a draft guideline presented by the West German BBA at a Guideline Meeting in April 1989 (see: Appendix 4.2.1: pers. comm., Van Gestel). The present guideline proposal is based principally on the latter, for the main reason that Dutch researchers have up to now followed the BBA approach. In several respects, however, this proposal has been supplemented and modified, with reference to the British guideline.

Experimental conditions

In the BBA draft guideline, preference is expressed for a trial in meadow grassland, where worms are abundant. In arable soils, there would have to be at least 100 worms of the species Lumbricus terrestris and Apporectodea caliginosa per m^2. Ma (pers. comm.) also states that Lumbricus rubellus should be present. Van de Bund (1980) reports an average of 200 earthworms per m^2 in arable land, Van Rhee (1970) 50 to 300. A possible approach to ensuring observable effects at low densities is to bury boxes already containing earthworms (Ebing et al., 1984). Although sufficient numbers are thus ensured, the sensitivity of the worms in the field is not taken into due account. Another option is to bury 'dung bags', containing farmyard manure to attract earthworms (Satchell, 1971). The test method can, alternatively, be adapted to increase the frequency or size of sub-samples. In view of the numbers of worms encountered in the Netherlands, we opt for trials employing the crop to which the formulation is normally applied instead of in grassland: after all, the very objective of a field trial is to make predictions about the field situation. For the same reason, we are hesitant to use boxes with captive worms, preferring, if necessary, to trap the local worms using dung bags.

In order to distinguish ecological from toxic effects, when testing herbicides a blank should be run with a herbicide that is non-toxic to earthworms. If necessary, weeding can be done by hand.

Application

Fourfold replicates, as proposed in the BBA guideline, is a minimum requirement for validity of the various statistical procedures employed (e.g. variance analysis).

Observation

Earthworms and (a)biotic environmental factors

The BBA guideline does not require prior sampling preparatory to the field trial. In our opinion, however, this is essential for identifying any 'natural' clustering or other variations in abundance among trial plots, e.g. through inhomogeneous distribution of organic matter in the

soil. Initial sampling should be carried out one month post-treatment; any early effects can thus also be observed (pers. comm., Van Gestel).

In principle, the proposed 'formalin method' is carried out according to Raw (1959), but adapted according to Satchell (1971). Because a plot size of 0.25 m^2 is opted for in the BBA protocol, as well as in many other studies (see: Appendix 4.2.2), the quantity of formalin prescribed is half that used by Satchell for 0.5 m^2.

There are various reports in the literature (e.g. Satchell, 1971) of correction methods for such parameters as temperature, soil moisture, collection method and dry/fresh weight correlation. In the present guideline, however, all comparisons are between different treatments. As long as the plots chosen exhibit no differences in the above parameters, therefore, there is no need for correction. However, when comparing various different test series, correction may be advisable. For this reason, it is recommended to measure soil temperature and moisture.

Compound

Soil exposure and, consequently, earthworm exposure may vary greatly with crop coverage. Coverage should therefore be determined regularly, as should residues in and on the soil and in the earthworms.

Proposal: **FIELD TRIAL GUIDELINE FOR TERRESTRIAL EARTHWORMS**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

The experimental plots must always contain earthworms (of the class Oligochaeta) of the following species: Lumbricus terrestris, Apporectodea caliginosa and Lumbricus rubellus. Select an experimental site having a soil type on which the formulation will be used in practice - to model the worst-case situation, preferably on the lightest soil type. Select a crop requiring the highest recommended dosage.

1.2 Experimental conditions

At a single session, treat the respective experimental plots with the compound being assessed, with a markedly earthworm-toxic agent (e.g. benomyl) and with water, or with another innocuous carrier substance similar to the compound under review. If it is a herbicide being assessed, in addition to the blank, also spray a plot with a herbicide that is innocuous to earthworms. Conduct the trial when earthworm diapause is unlikely, i.e. not during dry and/or hot weather.

1.3 Other requirements

Perform the trial at a site with at least 100 earthworms per m^2 and with individuals of the species indicated in 1.1. The trial duration is one year.

1.4 Experimental design

Plot size

The experimental plots should measure at least 10x10 m^2.

Plot arrangement

Perform the tests in fourfold or as often as is required for valid statistical analysis (Healy, 1961; Neuhauser, 1989). To exclude the possibility of mutual spraying impact, the test plots should be located at least 100 m apart.

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. Spray a buffer zone at least 2.5 m wide on each side of the plots.

2.4 Spraying schedule

Carry out spraying at a time at which the agent is normally applied. If possible, spray the various plots simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions. Perform further treatment series according to the terms of the trial.

3. OBSERVATION

Methods and frequency

Sampling consists of taking ten 0.25 m^2 samples from each plot, employing the formalin method. It is recommended to use three times 5 litres of a 0.275% formalin solution at 10-minute intervals. If earthworm abundance is low, more samples may be needed. To establish the requisite number of samples, see Healy (1962).

If abundance is extremely low, capture with the aid of 'dung bags', containing farmyard manure, may provide an indication of differences in activity dynamics among the plots; 600-ml bags are recommended. These are placed in clay flowerpots, which are buried in the field and recovered after 14 days for laboratory analysis (Satchell, 1971).

Sample each plot one week prior to treatment and four weeks, four months and one year after treatment. In the case of recurrent treatment, always sample four weeks post-treatment. During the first week post-treatment, visually inspect the plots daily for anomalies.

Record abundance and biomass of both individual worm species and overall population. Record dominance and adult/juvenile ratio. For true comparison, it is essential that biomass measurements on the various samples are made at the same time post-sampling.

In order to determine the contribution of earthworms to leaf litter decomposition, bury litter-bags with two different mesh sizes (0.5 and 7 mm) according to Heath et al. (1966). This allows decomposition rates with and without earthworms to be assessed. In each plot, bury 4 bags of each mesh size, each containing 50 discs cut from leaves (2.5 cm diameter). Depending on the hardness of the leaves, check decomposition rates every week or less frequently (e.g. every month). For background information, cf. Swift et al. (1979).

To support causal relationships, pesticide residues must also be measured. Determine residues in and on the soil one day and one week post-treatment. Each time worms are sampled, determine residues in some of the specimens. Also record crop coverage data at the time of treatment as well as during sampling.

Measure temperature and soil moisture at fixed times each day.

4. VALIDITY

Test results are not valid if results are unclear, or if mortality is high in the blanks or low in the positive controls. Cf. data on earthworm population dynamics (e.g. Boström, 1988).

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
1	a b p	f	hp 4hp	b') t	4	10	4

') When testing a herbicide, in addition to the blank also test with a compound innocuous to earthworms.

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		500
- test plots		1,000
- field equipment		500
2 sampling (5x 160 samples)		1,000
- test organisms	10	
- abiotic parameters	5	
3 parameter measurement		
- test organisms	160	-
- residue measurements	20	1,000
- abiotic parameters	10	1,000
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 225	n.a.
cost estimate (subtotal)	Dfl.168,750	Dfl. 5,000
overall cost (very rough estimate):		Dfl. 175,500

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.2.1 Review of existing field trial guidelines for earthworms

United Kingdom (MAFF, 1986: Working Document 7/6)
- Experimental plots measuring at least 10 m^2 in a cropped field.
- Application of two dosages of the trial compound and a toxic standard, e.g. benomyl.
- Application according to instructions.
- Sampling between August and November for springtime treatment.
- Sampling according to Raw (1959), i.e. using 0.5 m^2 frames into which a formalin solution (50 ml of 40% formalin in 9 l water) is poured from a watering can onto the soil, without creating pools; most worms surface within 15 min. and can be collected in 10% formalin.
- In each plot, 3 or 4 such samples are taken.
- For the purpose of residue measurements, worms can be collected using formalin or an electric-shock method or by digging. With formalin, it is important to rinse the worms directly in clean water. With the electric-shock method, two electrodes are inserted 10-20 cm into the soil and a 220 Volt (A.C.) shock applied; when using the mains, a single shock is sufficient, but if a portable generator is used, two shocks may be necessary, depending on the strength of supply. In all cases, the worms must be placed on damp filter paper to ensure gut evacuation.

West Germany (BBA, 1989. Protocol under development: status report based on Guideline Meeting, as reported by Van Gestel (pers. comm.)
- Experimental plots preferably in meadows, but alternatively on arable land.
- Highest permitted dosage and 4 or 10 times this amount.
- Plot area: 10x10 m; four replicates.
- Include toxic standard, e.g. benomyl.
- Both Lumbricus terrestris and Apporectodea caliginosa should be present in sufficient numbers (> 100/m^2).
- Sampling after 4 weeks, 4-6 months and 1 year.
- 8-10 samples of 0.25 m^2.
- Sampling using formalin method, optionally in combination with electric-shock method.

Observation:
- Parameters: abundance, biomass, dominance, adult/juvenile ratio.

Evaluation:
- According to Heimbach (Bayer), 43, 10 and 8 samples are required to demonstrate significant differences of 10, 20 and 25% between plots.
- According to Bauchenss (BLBP), surveillance must be continued for 5 years to assess recovery.

FAO (1985)
For field trial guidelines, FAO refers to Wright (1977), Raw (1959) and the British guidelines.

Appendix 4.2.2 Review of field studies involving earthworms

Raw (1959) Description and comparison of earthworm sampling methods. In the formalin method, 25 ml 40% formalin is dissolved in 3.785 l water and the solution applied to a 0.37 m^2 plot; if necessary, the treatment is repeated after 20 minutes. The same procedure can also be followed using a solution containing 7 g potassium permanganate. In the hand-sorting method, 2-3 hours are spent counting worms in a soil sample 21.6 cm in diameter and 20.3 cm deep. The methods were compared by taking 10 samples according to each of the first two methods and 8 samples using the latter method; all samples were collected in orchards. Many more worms were collected with the formalin method than with the permanganate method. This holds for both deep-living and for surface-living species. In the hand-sorting method, the sampling depth was judged too shallow for deeper-living species such as Lumbricus terrestris. For shallow-living species, however, this method was found to give the best results.

Zicsi (1962) Hand-sorting trials employing differing plot areas. Sixteen 1/16 m^2 samples provide a representative indication of populations, at least for the Hungarian soils tested.

Heath et al. (1966) Study of the contribution of lumbricid earthworms to leaf litter decomposition by burying litterbags with different mesh sizes (0.5 and 7 mm, the latter accessible to worms). Bags contained 50 discs (diameter: 2.5 cm) cut from leaves of 11 different plant species, in fourfold for each species. Every 7 weeks the bags were dug up and decomposition examined. Hard leaves were decomposed in max. one year; for soft leaves, 7 weeks was found to be too long. During the first 5 weeks, it is therefore better to sample once a week.

Long et al. (1967) Study of effects of chlordane in sugarcane cultivation. Six plots, containing three 43 m rows, were sprayed; six were not. In each plot, 36 samples were collected (11.4 dm^2 and 30 cm deep). One third of these samples was hand-sorted during the first 3 months post-treatment (the remainder being used for tests involving other organisms). This method was found adequate for detecting effects on worm abundance.

Van Rhee (1970) One hectare of arable land contains 0.5-3 million earthworms. The recommended sampling procedure is to dig up 50x50x50 cm of soil and hand-sort the material.

Satchell (1971) Description of various sampling methods: driving the worms from the soil electrically or with chemical irritants, collection plus hand-sorting, and trap collection. For formalin, 3 times 9 litres (2 gallons) of a 0.275% solution was recommended, applied at 10-minute intervals on a 0.5 m^2 plot. A capture ratio of 95% was thus achieved. To achieve greater accuracy, more plots must be sampled. For Lumbricus terrestris, a correction factor is given for temperature and soil moisture: corrected population = observed population x $\exp\{0.0075\ (T - 10.6)^2\}$ x $\exp\{-0.0214\ (M - 40)\}$, where T = soil temperature at 10 cm depth (°C) and M = percentage soil moisture.

Hand-sorting is only practical for larger earthworms (> 0.2 g). Additional data on smaller worms can be obtained by transferring the soil to a

formalin solution or MgSO. The latter also causes the egg capsules to float to the surface, providing a complete picture of population structure. Flushing through a sieve with water was found to be a less practical method. An alternative is thermal extraction: an example is mentioned in which a 20x20x10 cm soil sample was placed on a screen 5 cm from the bottom of a plastic baby's bath (55x45 cm). There were several centimetres of water in the bath and fourteen 60W lamps suspended above. The water was heated for 3 hours. The results appear to be generally better than those obtained with either the formalin or hand-sorting methods.

When earthworm populations are very low, particularly, collection using dung bags (with farmyard manure) can provide an indication of the relative activity in test plots. The worm population is found to correlate with the size of the bags. Bags of 600, 300 and 150 ml are discussed. To facilitate work in the lab, it is recommended to place the bag in a clay flowerpot, which is buried in the field and recovered after 14 days.

With hand-sorting, larger samples were found to be less efficient; 25x25 cm samples taken to a depth of 40 cm were found to suffice. The number of samples required is very much dependent on the sample size and the population density of assessed species. To achieve an overall error of <5% for each species, it was experimentally determined that 32 to 358 samples should be collected.

As worm biomass decreases during conservation, it is necessary either to introduce a correction or to measure the biomass of all samples at the same time post-conservation. Allowance must also be made for gut content and, when weighing conserved animals, the dry/fresh weight ratio must also be measured or a fixed ratio employed. More subtle parameters are also reported, such as size category distribution, growth curves, survival and relative productivity, in addition to metabolic data (feeding, excretion and respiration).

Henderson & Clements (1977) Ten grassland test sites were selected, each with 6 pairs of 2x6 m plots. One plot in each pair was treated with aldrin and forat (granules). The plots had been sown between 5 and 48 months previously with ryegrass. The invertebrate fauna was sampled for a period of 2 years. Earthworms were sampled in November by treating two 0.37 m^2 sections of each plot with a dilute formalin solution (Raw, 1959). Effects on worm populations are reported, but it is not indicated whether or not this is due to the pesticides.

Wright (1977) Study of earthworms in plots in experimental orchards one year after spraying with various fungicides, including benomyl. Sampling was according to Raw (1959). The control plots were left unsprayed in the year of testing, but had been sprayed in the previous years. Population and biomass were measured. Effects were identified: with benomyl, the population of all species declined, while Lumbricus terrestris and Allobophora chlorotica totally disappeared.

Eijsackers & Van de Bund (1980) For 'larger soil fauna', plots measuring 10x10 m are recommended, with 0.25 m^2 samples being hand-sorted. Earthworms are suitable indicators of disturbed soil dynamics. Due to the

relatively small number of species, however, earthworms are less suitable for assessing effects on diversity.

Bunyan et al. (1981) Study of environmental pathways using aldicarb (granules). Two beet fields were sampled, one treated and the other with an untreated margin. Samples were taken from 10 plots in the selected field, prior to treatment and 14, 28 and 56 days post-treatment (for method, see: Raw, 1959). Few worms were found, probably due to the light soil and agricultural influences. The largest group was that found dead or dying on the surface, 6 days post-treatment, probably due to dissolved aldicarb. Residues could not be detected.

Edwards & Brown (1982) Long-term studies (2-5 years) on the impact of pesticides on earthworm populations in grassland on three different soils. Grassland was selected because it is more suitable for this kind of study than arable land, which is being continually disturbed. For earthworms, moreover, populations as well as species diversity are higher in this habitat. Plots measured 6 m^2, with 1.5 m margins; all tests were carried out in triplo. In each experiment, plots were tested with a standard dose of the compound being assessed as well as with 10 times this dose. In addition, there were two untreated control plots and one plot treated with a toxic standard (benomyl). When the compound being assessed was a herbicide, a plot was also treated with paraquat: this is non-toxic to worms, enabling the toxic impact of the compound to be distinguished from its environmental impact. In general, compounds were applied in spring. Sampling took place shortly before treatment, 1, 6 and 12 months post-treatment and subsequently in the spring and autumn, when worms are active. Two 60x60 cm areas were sampled, with a standard configuration in the field. With formalin, the collecting method was to cut 60x60 cm of the crop very short and apply 9 l of 0.22% formalin (4.5 l with wet or non-porous soils). The worms were identified, counted and weighed within 20 minutes, and sorted into adults and juveniles whenever possible. Numbers were transformed logarithmically and variance analysis applied. When differences were significant (F-test, p=5%), the t-test (p=5% or 1%) was applied to identify which particular plots were affected. Exposure to paraquat also caused a decline in population, in this case indirectly, through 'burning' of the grass cover.

Ma (1982) Especially in orchards and grassland, earthworms are very abundant and fulfil a major role in soil processes. Through soil tillage, etc., populations in arable land are severely depressed. Earthworms are important because they can transfer toxins through food webs. A field trial method is described to investigate the impact of fertilizer application, in sandy meadowland. Various quantities and types of fertilizer were applied to 2x2 m plots, in duplo. At the centre of each plot, a 50x50x50 cm sample was dug up and the soil hand-sorted for earthworms. The biomass was measured after the worms had been stored for two days at 15°C on damp filter paper in a Petri dish (empty gut). Statistical analysis was by linear regression and the chi-square test. Parameters measured: numbers, (changes in) species composition and age structure. In a second experiment using 5x5 m plots (all tests in duplo), the influence of high copper concentrations on fecundity, growth and development was investigated. Earthworms were collected by applying vibration to the upper 30 cm of the soil for 15 min. and sampling within a 1x2 m area,

permitting detection of any differences between populations. Sampling took place 3 years post-treatment. Parameters measured: numbers and biomass for each annual class and per m^2, both per species and for the whole population. In a parallel test series investigating the impact of sludge from wastewater treatment plants, 3 m^2 was sampled in the same way 60 cm from the edge of the plots.

Forsyth (1983) Study on DDT (granule) levels in (a)biotic ecosystem compartments in orchards. One focus of the study was on earthworms, which were sampled according to Raw (1959). Sampling took place 6 days post-treatment, weekly during the first month and then monthly. Residues in earthworms were found to be relatively high, decreasing after several years.

Ebing et al. (1984) Earthworms are useful bio-indicators because they are exposed to chemicals both on and below the surface; they moreover process large quantities of soil. On the other hand, earthworms are generally less sensitive to xenobiotics. In one experiment, an asbestos-cement box was buried measuring 120x60 cm by 65 cm deep and covered underneath by wire mesh. Sixty Lumbricus terrestris adults were introduced into each box. After a one-week habituation period, various boxes were sprayed with various pesticides (using practical doses and formulations). Residues in worms and soil were determined 21 days post-treatment. Differences were detected. In a second experiment, 100-200 g of worms were collected from various crops and residues analyzed.

Conrady (1986) Effects of atrazine and pentachlorophenol on soil invertebrates in grassland. Plots of 15 m^2 were sprayed with two concentrations of atrazine and pentachlorophenol; one plot was left unsprayed. The experiment was repeated with 9 m^2 exclosures. On each plot, 5 samples were taken with a diameter of 20 cm and a depth of 5 cm; deeper-living worms were sampled by pouring 1 litre of formalin (0.55%) into the holes, three times, at 10 min. intervals. Sampling began 3 days post-treatment and was repeated fortnightly, until 3 months post-treatment. Parameters measured: numbers of each species, per age class, and biomass. Pentachlorophenol was found to have a significant effect on the juveniles of one species. With earthworms, the use of exclosures made no difference. Additionally, the distribution of worms over the plots was sampled taking 15 subsamples from each plot. Analysis was feasible for two species only (sufficiently large population density). With these species, aggregation was found after treatment, probably due to differences in vegetation causing differences in pesticide seepage rates into the soil.

Boström (1988) Comparison of the formalin method with hand-sorting. The formalin method gives a 7% higher abundance, but a 17% lower biomass. One drawback of this method is that it only works when worms are active, i.e. at certain temperatures and moisture levels. Formalin method: frames measuring 0.5 m^2 were let 5 cm into the soil, four in each plot. Within the frames, the crop was removed and twice 10 l of 0.2% formalin poured in (15 min. interval). All worms appearing within 1.5 h were collected, cleaned and stored at 5°C. Within 24 h the worms were sorted into adults and juveniles, counted, rolled in filter paper and weighed, including gut content. By comparing these data with an identical area in which the

worms were hand- and screen-sorted, a correction factor could be defined (for this specific situation).

Ma (1988) Effects of copper-enriched manure on earthworms. Various categories of copper-enriched manure were mixed annually with the soil. After three or four years, worms were sampled by hand-sorting two to four replicate plots and then applying formalin. The manure was found to have an impact on earthworms.

Neuhauser et al. (1980) Study on the impact of oily waste on earthworms, using twenty 4x4 m plots separated by 4 m margins. To exclude corner effects, the 4 corner plots were not sampled. All tests were in fourfold, with two types of blank: one completely untreated and one only managed. Sampling was several days pre-treatment and each month post-treatment during the growing season, using a 20x20x15 cm metal sampling frame. In each plot, 3 samples were collected and hand-sorted to determine numbers and biomass. Data was analyzed by one-way variance analysis and by the Student Newman-Keuls multiple comparison test.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

GROUND BEETLES

FIELD TRIAL FOR GROUND BEETLES

General

British pesticide approval procedures (see: Appendix 4.3.1) include a guideline for a field trial with ground beetles (= Carabidae). The IOBC also gives instructions for this group. As far as possible, instructions from both have been incorporated into the present guideline. The available literature (see: Appendix 4.3.2) indicates that significant effects are not usually found in small, open plots (Bunyan et al., 1980; Stinner et al., 1986). As reported by the workers themselves, this does not necessarily mean that effects were indeed absent: the plots employed were probably too small, allowing outside beetles to very rapidly replace affected animals. In tests with larger plots, observable effects have been reported (Berger, 1985; Heimbach, 1988). For practical reasons, it is recommended to employ small enclosure-type plots to establish toxic side-effects. A study of field trial methods (Basedow, 1987) also points in this direction. However, for ecological side-effects not immediately lethal to the organisms but causing emigration, for example, larger trial plots are more appropriate.

Edwards & Thompson (1975) conclude that a field trial yields more information than a laboratory test, noting, however, that the former is extremely laborious. British approval procedures indicate that there is now special equipment for setting up the test enclosures.

Experimental conditions

British guidelines recommend an area of 10 m^2 for enclosures. In many studies, a larger area has been employed, however. The present proposal is based on the recommendation of Eijsackers & Van de Bund (1980) for an enclosure of 10x10 m. To achieve uniformity with laboratory testing procedures currently under development, it is desirable that each enclosure contain at least one Bembidion species. For non-enclosed plots, the British guideline specifies a minimum size of 0.5 ha. The IOBC specifies 3 ha. For practical reasons, we prescribe a minimum area of 0.5 ha. We also prescribe trials in duplo rather than triplo. To obtain results with a certain statistical significance, however, it may be necessary to perform several replicates.

Application

The compound is applied using both the maximum recommended dose as well as four times this dose, the latter simulating a worst-case situation. This anyway gives greater certainty in predicting the likely effects of recommended use.

Observation

Ground beetles and (a)biotic environmental factors

Pitfall traps are used to assess the number of organisms as well as their activity (Eijsackers & Van de Bund, 1980). Activity is affected not only by the pesticide, but also by the weather, food supply, soil structure and so on. These parameters must also therefore be included in the field trial. The organisms should preferably be taken alive from the pitfalls and returned in the same condition to the enclosure. This implies a high rate of sampling, for otherwise the beetles will prey on one another in the traps. To arrive at an absolute estimate, organisms may alternatively be tagged and retrapped, or a given area be exhaustively sampled.

Compound

To establish actual exposure levels, it is important that soil and crop residues also be measured. Crop coverage should also be estimated. Residues in the beetles themselves may also be determined, to provide support for any effects observed.

Proposal: **FIELD TRIAL GUIDELINE FOR GROUND BEETLES**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial for ground beetles (= Carabidae): experimental plots must contain at least one Bembidion species. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage.

1.2 Experimental conditions

At a single session, treat individual plots with the compound being assessed, with an agent markedly toxic to carabids (e.g. dimethoate) and with water, or with another innocuous carrier substance similar to the compound under review. If it is a herbicide being assessed, perform an extra blank using a herbicide that is non-toxic to carabids, or a blank in which weeding is done mechanically. Carry out trial on cultivated land. Undertake spraying during weather conducive to beetles activity, i.e. during warm and (fairly) dry weather.

1.3 Other requirements

Prior to the trial, determine carabid abundance on the site, to calculate the number of replicates required for statistically significant results.

1.4 Experimental design

Open plots

Open plots must have an area of at least 0.5 ha. Perform trials in duplo, at least.

Enclosures

Enclosures must have an area of at least 100 m^2. They can be made using polythene film, supported by small poles. The film should be buried 10-15 cm deep in the ground and be more than 35 cm high. Cover the top of the enclosures with wire mesh to prevent birds preying on beetles trapped in pitfalls. Prepare enclosures after sowing, but before compound spraying. Carry out tests in fourfold.

Enclosure arrangement

To exclude the possibility of mutual spraying impact, the enclosures (or open plots) should be located at least 100 m apart.

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various plots simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sample each enclosure or plot by means of 8-10 pitfall traps 10 cm in diameter and 10-15 cm deep, four of which should be placed at enclosure corners. If the traps are left open for a short period of time (24-48 h), the animals can be taken alive for identification and then released; this procedure is particularly recommended for trials involving enclosures. In this case, pitfalls should be emptied at least once a day. Place a layer of litter in each trap, so that beetles can hide. If sampling is less frequent, beetles must be killed in the traps. If larger plots are employed, the animals can be conserved directly in the pitfalls using a layer of 4% formalin and soap. In this case, the traps can be left for longer (e.g. 5 days). To prevent flooding after rain, pitfalls should be shielded using a slightly larger cover positioned about 10 cm above the trap.

Establish species composition and the abundance of individual species. If possible, count the number of juveniles. Reproduction can also be estimated by counting the number of active ovaries.

Sample one week prior to treatment, then once a week during the first month post-treatment and subsequently once a fortnight or month. When using open plots, also sample soon after treatment.

Prior to testing, as well as once a month subsequently, estimate the absolute number of carabids by counting all the beetles found within a

square frame on the ground. The size and number of frames must be such that the estimate has a 95% confidence level.

Together with sampling, record the temperature and soil moisture content and measure residues in soil and crop. Also estimate crop coverage. Analyse residues in beetles; this can be done a week after treatment.

4. VALIDITY

Test results are not valid if results are unclear, or if there is high observable impact in the blanks or little impact in the positive controls.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
1	a s	f or c	hp	b') t	f: 2 c:4	8-10")	8

') When testing a herbicide, in addition to the blank also test with a compound innocuous to carabids.
") May be combined into one or several mixed samples.

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		-
- plot preparation		2,000
- 16 enclosures/traps		5,000
2 sampling (4x4x1[0]x8)		500
- test organisms	20	
- abiotic parameters	5	
3 parameter measurement		
- test org.'s (count+ident.)	40	-
- residue measurements	20	1,000
- abiotic parameters	5	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 110	n.a.
cost estimate (subtotal)	Dfl. 82,500	Dfl. 9,000

overall cost (very rough estimate): Dfl. 91,500

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.3.1 Review of existing field trial guidelines for ground beetles

U.K. (MAFF, 1986: Working Document 7/6)
- Trial between June and September, on cultivated land.
- Either very large open plots (min. 0.5 ha) or use of enclosures (Edwards & Thompson); in latter case, 10 m^2 for example is sufficient. Enclosures can be made using polythene film supported by small poles; film buried 10-15 cm in soil and standing 37-45 cm high. Barriers to be placed after sowing but before treatment. There is even a machine available for setting up enclosures.

Observation:
- Sampling by means of plastic pitfall traps (7-10 cm diameter; 10-15 cm deep). 8-10 traps are generally enough per plot. Traps are generally closed, being opened for a 24-48 h period prior to treatment and then 1, 2 or 4 weeks post-treatment. Trapped beetles are counted and identified and then returned to the plot. If traps are left open longer, a small volume of 50% alcohol can be added to kill the beetles and discourage predators.
- Trials with local application or other procedures can yield additional data.

IOBC (Stevenson et al., 1985) General guidelines for field trials with arable crops.
- In addition to test compound, trial also performed with an untreated (water) control and preferably with a positive control (dimethoate).
- Procedure specified for winter wheat: 3 large fields with the same crop variety, agricultural history and tillage regime, preferably in the same vicinity. These are divided into 2 or 3 (in the case of a positive control) plots of min. 3 ha.
- Carabids are sampled using pitfall traps: 5-10 per plot, sampling period 5 days. Small carabids additionally sampled by visual search.
- Sampling 10 and 5 days pre-treatment and 2, 5, 10, 20 and 50 days post-treatment.

Although there exist several other guidelines for beneficials, e.g. FAO Guidelines and West German 'Richtlinien', these do not include concrete proposals for carabids.

Appendix 4.3.2 Review of field studies involving ground beetles

Edwards & Thompson (1975) Carabid and staphylinid beetles may be important predators of various pests, motivating study of the impact of insecticides on beetles. Because these insects are extremely active, field trials require either very large plots or 8x5.5 m enclosures made of polythene film, supported by small wooden poles; film was dug in 15 cm and stood 40 cm high. The enclosures were spaced 4.5 m apart. The barriers were erected when beetles become active, i.e. May/June. Each enclosure contained 8 pitfall traps (10 cm diameter), marked by a 1 m stick. Beetles were sampled every two days and then released again in the enclosure. Plots were treated one week after the census procedure was started. Each year during the four-year trial one plot was sprayed with chlorfenvinphos, with one blank; the other two plots were treated with other test compounds and formulations. With chlorfenvinphos, more beetles were trapped than in the blank, probably because this non-toxic compound stimulates activity, e.g. by reducing food supply. Although this field study was laborious (enclosure preparation and census), useful and reproducible results were obtained. Laboratory trials were not always effective in extrapolating to the field situation.

Eijsackers & Van de Bund (1980) Design experiment such that pesticide exposure is sole difference. For carabids, use 10x10 m enclosures. Pitfall capture establishes animals' activity above all. Probability of capture varies with species and is influenced by soil surface structure and other factors.

Bunyan et al. (1981) Pitfalls were placed in a sugar beet field, with 12 on an untreated strip. Traps were surveilled for 16 weeks, being transferred weekly. Although results were difficult to interpret due to low abundance, authors report no indication of differences, possibly because of rapid recolonization.

Kreutzweiler (1982) Effects of forest spraying with permethrin. In one control and one treated plot, carabids were trapped in pitfalls (12x12x10 cm) during three 5-day periods. 20 traps were set in each plot, spaced 5 m apart, and emptied daily. No significant differences were found.

Berger (1985) Field study of the impact of pirimicarb and other insecticides on soil fauna, with special reference to species consumed by pheasants and partridges. It was investigated whether populations of these species were depressed after control of aphids in beet fields using pirimicarb and oxydemeton methyl. Two 30x100 m plots were sprayed with pirimicarb (50% active ingredient, 300 g/ha) and two with oxydemeton methyl (600 ml/ha, 250 g/l); there were 4 control plots. Soil arthropods were counted weekly from 7 June to 2 August using 12 pitfall traps per plot. Spraying was on 28 June; sampling was more intensive during the initial period post-treatment (every 2 days). Pirimicarb was found to depress the carabid population. Population recovery set in after 7 days and after 14 days there was no longer any difference between treated and untreated plots. Spraying with oxydemeton methyl decimated the carabid population for a 6-day period, with recovery setting in after 14 days. After 27 days there was no difference between experimental and control

plots. It was concluded that pirimicarb spraying leads to faster recovery of non-target insect populations than oxydemeton methyl.

Kelly & Curry (1985) Effects of methiocarb on winter wheat. Five treatments carried out in fourfold in 20x20 m plots spaced 3 m apart. Carabids were trapped using 5 (glass) pitfalls per plot; traps contained 150 ml picric acid and were left for one week. Limited effects were observed, although there was sometimes a sharp increase in the number of animals trapped following treatment.

Everts et al. (1986 & 1989) Study of animal indicators for side-effects in fields of oilseed rape. In a single field, 60 pitfall traps were placed in 20 groups of 3, in 4 rows 100 m apart. As a control, 20 traps were placed in a field of winter wheat, a very suitable crop because natural fluctuations are the same as with oilseed rape. In addition, 3 groups of 8 pitfalls were placed in another rape field, each of which received a different treatment. The three fields each measured 60x125 m. Traps were emptied weekly throughout the trial period (September-December). With spiders, differences were always observed; for beetles, this was not always the case.

Stinner et al. (1986)
The influence of a carbamate and an organophosphate compound were compared with a blank using three fields with different tillage regimes: ploughed and unploughed with corn, and fallow. For each regime, the three treatments were applied to four replicate plots. Soil fauna was sampled by means of pitfall traps (1 l) containing 50 ml ethylene glycol and opened for 24 h every 3 weeks. After each treatment, 12 samples were taken. Tillage regimes were found to have a greater impact than insecticides. Only limited effects were observed on carabid beetles, possibly due to high mobility.

Basedow (1987) Study of pros and cons of pitfall trapping method. Mainly cons: e.g. hyperactivity of beetles, plot size (open plots), laboriousness and expense. Results on the broad action spectrum of dimethoate were confirmed, although the absence of data on beetle larvae introduced a measure of uncertainty. Field trials continue to form a necessary complement to laboratory testing, however. Improved methods are suggested, involving enclosure-type plots with pre-determined numbers of beetles (often readily bred or trapped).

Booij & Noorlander (1988) Carabids were trapped for six years in succession in a variety of crops cultivated on conventional, integrated and alternative farms. The short-term effects of pesticides could not be traced, because of differences in cultivation methods and the complexity of pesticide regimes. Only parathion and diazinon had a distinctly negative impact. For the longer term, too, unambiguous conclusions could not be drawn, partly because of major variations between individual years. It was however concluded that weed coverage played a major role, thus confirming the indirect impact of herbicides.

Von Heimbach (1988) Study on the effects of pyrazophos as a fungicide for winter wheat. Four 1.3 ha plots were defined in a 10 ha field, one serving as a control, one treated with pyrazophos and the other two with

another fungicide. In each plot live beetles were trapped in pitfalls, 3-7 days pre- and post-treatment. In the pyrazophos-treated plot, a relatively distinct depression of activity/density of carabid beetles was observed - confirming laboratory studies. With staphylinids, no significant differences were found. Following treatment, dead insects were surveyed on 20 m^2 of tractor tracks: many were found in the pyrazophos-treated plot (88 dead carabids), but not in the others (2). Extraction of soil samples confirmed this picture, although the numbers involved were too small for statistical significance. A clear increase in aphid abundance in the pyrazophos-treated plot could not simply be attributed to the disappearance of predators; pyrazophos also affects aphid disease dynamics and wheat condition.

Von Heimbach & Giri (1988) Field and laboratory tests on the impact of parathion and herbicides on carabid and staphylinid beetles. Four plots were defined (see: Von Heimbach, 1988): one untreated control, one treated with parathion and two with other herbicides. In the lab, parathion was found to be highly toxic to both carabids and staphylinids. This effect was not confirmed in the field, possibly because it was overshadowed by the number of beetles emerging from the soil at the time of application (early in the year). Although soil samples appeared to indicate an impact, populations were too small for statistical significance.

Jarvis (1988) The side-effects of pesticides were studied in extensive trials at an agricultural testing centre. A major reduction in the abundance of beetles was observed (in particular, Bembidion obtusum). This species hibernates near the soil surface rather than in the field margins. With a species that does hibernate in the field margins (Agonum dorsale), effects were observed much later, in the second and third years of the trial.

Canters et al. (1989) Pilot study in which epigeal invertebrates were pitfall-sampled (single sample) in various crops with various pesticide regimes. In each plot, 6 pitfalls containing formalin and soap were left for one week. Significant differences were found for a number of groups of epigeal invertebrates, but not for carabid beetles.

Maaskamp et al. (1989) Bio-monitoring of the impact of grassland management on sandy soils using invertebrates. Carabids were trapped in pitfalls (11 cm diameter, 14 cm deep). Each plot contained 5 traps, 3-5 m apart and 50 m from field perimeters. In some cases, pots were covered, e.g. using hardboard with three long nails. Traps were set during favourable weather (warm and fairly dry) and emptied after 3 days.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

HONEYBEES

FIELD TRIAL FOR HONEYBEES

General

Field trials for assessing the side-effects of pesticides on honeybees Apis mellifera have been carried out successfully for several decades (see: Appendix 4.4.2). They are practicable and provide useful results. Today, experimental design has been so standardized that results are reproducible, which also permits comparison of results obtained by different procedures. Guidelines for honeybee field trials are included in the pesticide registration procedures of various countries (see: Appendix 4.4.1).

The present guideline proposal is based on the EPPO protocol (see: Appendix 4.4.2), already internationally accepted and also used in the Netherlands.

Experimental conditions

In principle, the cage studies focus on the short-term toxic effects of a single application of a compound. In the cage setting, food availability is restricted and would constitute a major limiting factor in the longer term. Field trials s.s. also allow medium-term effects to be studied and permit repeated application of the compound under review, if so desired. Effects on foraging can also be observed, as diet can be readily established by means of pollen analysis. The present guideline proposal comprises a cage trial as well as a field trial. A third type of trial is the tunnel trial (see: Appendix 4.4.1), designed for situations in which a field trial, although more appropriate, would be impracticable for the type of observation concerned, for example exposure via honeydew. Because the principles involved are similar to those of the cage trial, this type of trial has not been included in the present proposal.

Most studies employ a blank as well as a positive control. Use of both provides the best guarantee of interpretable results. All existing guidelines use both forms of control, except for the West German protocol, in which only the compound under review is tested, although at twice the maximum recommended dosage. Contrary to other protocols, the German procedure also calls for repeated treatment. Since normal, single doses are generally satisfactory, and because the German procedure is the only exception in this respect, preference is given to the former type of trial. Our proposal involves application of the maximum recommended dosage and four times this dose, the latter covering a worst-case situation. This also makes it easier to predict whether effects will occur following everyday application at slightly above the maximum dosage, for instance. In other respects, current guidelines differ mainly in the detail with which certain aspects are elaborated, and in a few technical aspects.

In principle, a blank can be run with water or another innocuous carrier substance similar to the compound under review. If the compound is anticipated to have a major ecological impact (e.g. in the case of a herbicide), an extra control should be run alongside the blank (if

possible), using a compound innocuous to honeybees and with the same intended spectrum of action. Only in this way can toxic effects be distinguished from ecological effects. In the case of cage trials, duplicates are recommended (and feasible).

The EPPO protocol specifies a minimum plot size of 1500 m². Virtually all other studies use a minimum plot size of 3 ha, however. Since EPPO does not argue its choice, we have opted for 3 ha.

Application

The compound is applied using both the maximum recommended dose as well as four times this dose, the latter simulating a worst-case situation. This anyway gives greater certainty in predicting the likely effects of recommended use.

Weather conditions are very important for honeybee activity. It is vital that bees are actually in flight during application. There should be no differences among the plots; to this end, ensure that the various treatments are carried out simultaneously or within two hours at the most.

Observation

Honeybees and (a)biotic environmental factors

The major additions to the EPPO guideline relate to observation. In a number of guidelines, the frequency of observation is higher than that employed by EPPO. In view of the basic expenses already incurred in setting up and performing the trials, a higher frequency would seem to be called for. Although expenses may be slightly higher, there is a far greater chance of unambiguous results. Especially when testing compounds with a growth-regulating action on insects, additional observation of the brood is also recommended.

Temperature and relative humidity are also measured, to establish differences between plots.

Compound

To assess the relative importance of different exposure routes and to support causal relationships between the compound and effects, residues are measured in bees, pollen and honey.

Proposal: **FIELD TRIAL GUIDELINE FOR HONEYBEES**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Perform the trial on a flowering crop or on plants visited by honey bees Apis mellifera. Select a crop requiring the highest recommended dose of the compound. The cage trial permits assessment of the short-term toxic effects of a single application, while the field trial is directed towards long-term toxic effects and ecological impact. If, for the latter case, repeated application is recommended, this must still be included in the trial guideline.

1.2 Experimental conditions

Perform the trial using healthy honeybee colonies, as assessed by an experienced bee-keeper. In the field, treat at a single session: one plot with the compound under review, one with a compound recognized as being toxic to bees (e.g. parathion or dimethoate) and one with a compound non-toxic to bees or with e.g. water. Cooperate with an experienced bee-keeper during the trials, which should be performed during a period of weather conducive to foraging. Perform the cage trials with at least one duplo, or as many more as are required for adequate statistical analysis.

1.3 Other requirements

Cage trials

Use one small colony in each cage. Each colony should be queen-right, have at least three full frames and be large enough to ensure about 10-12 bees/m^2 during foraging. The colonies should at any rate be balanced, with brood at all stages of development. The colonies should all be taken from the same location. Continue the trial for 1 week.

Field trials

Use colonies that are queen-right and comprising at least 10,000-15,000 individuals. The hives should contain at least 10-12 frames, including at least 5-6 brood frames. Perform the trial at least 5 km from where the colonies originate. The various colonies should have foraged on the same crop. Use at least 4 hives in each trial plot. Continue the trial for at least 2 weeks, or longer if this is required by the compound's mode of action or method of application, e.g. with systemic pesticides or if multiple spraying is called for.

1.4 Experimental design

Plot size

Cages should measure at least 2x2 m by 3 m high, with a maximum mesh of 3 mm. If desired, a strip can be left open on the inside of the cages (by clearing the vegetation), to facilitate collection of dead bees. Do not omit to place drinking-water receptacles in the cages.

Trial plots must extend at least 200 m from the hives and have an area of at least 3 ha. Smaller areas may also be used, but duplicates must then be run.

Hive placement

In cage trials, place the hives in position two days prior to treatment. If necessary, attach UV-absorbent film to the sunny side to stop bees flying towards the sun (and into the mesh).

In field trials, place at least four hives at one of the corners of each plot two days prior to treatment. During these two days make sure that the bees are doing most of their foraging on the trial plot (pollen analysis, flight observation).

Plot arrangement

To exclude the possibility of mutual spraying impact, cages should be located at least 100 metres apart.

In field trials, a minimum distance of 500 m apart is recommended, to ensure that bees do not forage in nearby plots; plots should be interspersed with crops that are unattractive to bees.

2. APPLICATION

2.1 Compound

Apply the compound according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compound using standard equipment, according to standard practice. If the hives are also exposed during application, shield them briefly with film during spraying.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount.

2.4 Spraying schedule

Carry out spraying two days after the hives have been put in position at a time when the bees are foraging on the crop, preferably in the morning, after the dew has disappeared, but certainly between 10.00 and 16.00 h. If possible, spray the various plots simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Mortality is assessed with the aid of dead-bee traps placed at hive entrances (see, for example: Free et al., 1967). Dead bees are rejected from the hive by the colony. Place the traps so as not to obstruct flight activity. During the two days prior to treatment, empty the traps once daily; just prior to treatment and on the day of treatment, every two hours; on the next day, three times; and subsequently at least once daily. Make sure that dead bees are not carried off by other animals (e.g. ants); in the event of signs of removal, take appropriate measures (see: Robinson & Johansen, 1978). In cage trials, dead bees must also be collected from the cages (especially along the sides). To this end, clear a strip of vegetation.

The **population** is estimated on the frames on the evening before treatment and at the end of the trial, using Jeffree's method (Jeffree, 1951).

Foraging activity and behaviour on the crop and at the hive are assessed once daily the first two days before treatment, just before and just after treatment, then every two hours on the day after treatment, and subsequently once daily. In field trials, estimate foraging populations by walking a fixed route in a fixed time, e.g. 200 m long and 2 m wide (depending on the crop) in 15 minutes; this can be combined with a census estimate at the hive. If so desired, flight intensity can be measured electronically using a photoelectric cell (Kingsbury et al., 1981). By means of a cell differentiating between incoming and outgoing bees, bee mortality can also be calculated.

General colony condition, e.g. juvenile development, growth and mortality are assessed in each trial by an experienced bee-keeper one day pre-treatment and six days post-treatment. In the case of field trials, this may be done 14 days and one month post-treatment.
If **effects on the brood** are anticipated, brood condition should also be assessed, both prior to treatment and 14 days post-treatment.
Temperature and relative humidity should be recorded throughout the trials.
In the case of field trials, pollen traps can be used to establish **pollen-collecting activity**; it is also useful to monitor pollen composition, to establish whether the bees are indeed foraging on the sprayed crop and whether diet composition changes through/after treatment. If pollen-collecting activity is monitored, it is recommended to do so only in some of the hives, as traps influence flight dynamics.

Dead bees, pollen and honey can be chemically analyzed to provide additional data and insight into exposure dynamics.

4. VALIDITY

Test results are not valid if results are unclear, if there is more than 15% mortality in the blanks or if there is little mortality in the positive controls. The raw data as well as the statistical methods employed should be included with the trial results.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
e	a c h r	f or c	hp 4hp	b t	f:1 c: 2	4/col.[1]	20

[1] col. = colony

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test org. (6x4 colonies)		12,000
- test plot preparation		2,000
- field app. about 8 hives)		4,000
2 sampling (4x2x4x20 plots)		1,000
- test organisms	30	
- abiotic parameters	5	
3 parameter measurement		( )
- test org. (behav. etc.)	40	
- residue measurements	10	1,000
- abiotic parameters	5	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 110	n.a.
cost estimate (subtotal)	Dfl. 82,500	Dfl. 20,500
overall cost (very rough estimate):		Dfl. 100,000

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.4.1 Review of existing field trial guidelines for honeybees

Germany (BBA, 1980; pers. comm. Petzold; pers. comm. Kohsiek)
Cage and field trial using twice the maximum recommended dose, in principle, except with aerial and LV (low volume) spraying, when the highest dose is used.

Cage trial
- Use blank (water or talc), markedly bee-toxic compound and compound under review; two replicates at different times.
- Cage at least 2x3x2 m high; metal or plastic netting, mesh < 3.5mm.
- Place drinking-water receptacle in cage; place dead-bee trap at hive entrance and clear a strip on three (non-hive) sides to retrieve dead bees. Discourage bees from flying too often towards sun-side by use of *UV-absorbent film*.
- Recommended crops: phacelia Phacelia tanacetifolia, oilseed rape Brassica napus, borage Borago sp. and mustard Sinapis sp.
- Number of bees required depends on cage area; foraging density at least 10-12 bees/m^2.
- Remove old, moribund bees by moving hive.
- Place hive about 2 days pre-treatment.
- Apply compound when bees are in flight.

Observation:
- Pre-treatment: once daily, count dead bees and assess flight density (in afternoon).
- Post-treatment: during first hour, assess behaviour and flight density/activity continuously. Subsequently, count dead bees in trap and assess behaviour every 2 hours. At end of day, count dead bees at edge of cage; depending on results, at least 2 or 3 assessments on 2nd and 3rd days. If no anomalies have been recorded after 72 h, discontinue the trial; *in the case of granules or systemic compounds, wait one* week.

Evaluation:
- Compare 3 treatments in terms of dead bees, behaviour and juvenile anomalies.

Special formulations and mode of action:
- Granules: applied according to instructions.
- 'Insect sticks': interspersed between flowering crops.
- *Systemic compounds: trial duration depending on mode of action.*

Presentation:
- Results presented on a standard form.

Field trial
- Use phacelia or oilseed rape in full bloom. As a rule, no blank or positive control, but comparison with hives remaining at point of origin.
- Plot area: *at least 0.25 ha.*
- Establish foraging in trial plot using pollen traps in hive and by observation of flight; if honeydew consumption is seen to be high, the trial may be postponed.
- Perform trial during weather conducive to foraging and between May and mid-September, repeating on two occasions.

- Place at least 4 colonies 3-4 days pre-treatment; same number as control.
- Apply compound in morning after plants have dew-dried and with a good weather forecast; spray 50 cm from crop; shield hive and flight board while spraying unit is passing.

Observation:

- Count dead bees on 1.5 m wide linen strip placed at hive entrance, at same time each day: just before spraying, 2-hourly after spraying, twice the next day and subsequently once daily.
- Foraging: estimate on five 1 m^2 sub-plots spread around the plot; once daily before spraying, just before and just after spraying; first hour, every 15 min., then 2-hourly; subsequent days, at least once daily.
- Observe at same time as for foraging: behaviour on flight board: anomalies and direction of flight.
- Estimate population (number of bees on frames) on evening before treatment and evening of 3rd day.
- Assess condition of brood and collected pollen on 5th and 6th evening; final assessment after 4 weeks, for any long-term effects.
- Record temperature on linen strip at hive entrance and on one of 5 sub-plots where foraging is assessed at same time as census and estimate; measure rainfall at edge of trial plot.
- Enlist the involvement of local bee-keepers.
- Additional test: diet test with juveniles: feed bees with pollen collected in trial plot and with control pollen.

Evaluation:

- Dead bees, changes in colony size, brood condition, foraging and flight-board behaviour and diet test.

N.B. Compare control with trial results; increased mortality usually occurs after transferral of hive and after frame assessment. Results are filled out on standard forms.

FAO (1985)

For methods, FAO refers to British, German and French protocols.

United Kingdom (MAFF, 1986: Working Document 7/4)

Protocol for field trial with one positive control, one blank (optional) and trial compound. Replicates not usually feasible, for reasons of cost.

Experimental conditions:

- Cooperate with experienced bee-keepers during trial.
- Colonies must be in good health.
- Perform test at least 3 km from original foraging area.
- Hives should be placed at the edge of the crop, providing an unimpeded flight path and not earlier than 2 days pre-treatment. At least four colonies are needed at each plot.
- Crop extending at least 200 m from hives; each plot e.g. 3-5 ha; at least 300 m between plots.
- Application when crop is in full bloom, with foraging between 10.00 and 16.00 h.
- Neighbouring fields not to be sprayed.
- Duration of trial: 2-3 weeks.

Observation:
Type of observation depends on mode of application and experience of personnel. As a minimum, toxicity must be assessed by counting dead bees in hive-entrance traps. Preferably, the following parameters should also be measured:
- population census: estimation of number of bees on frame (compared with photographs; Jeffree 1951)
- condition pre- and post-application (egg-laying, development)
- activity at hive: number of landings
- identification of foraging plant using pollen traps (according to Free, 1959)
- foraging activity: observers walk fixed route at fixed time and count bees, e.g. 200 m, 2 m wide, in 15 minutes, 3 or 4 per field
- honey production (hive weight)
- analysis of dead bees, beeswax and pollen (see: Needham et al., 1966)
- observation of other beneficials, e.g. foraging behaviour or mortality (using traps)

United States (EPA, 1986)
Atkins et al. (1976) and Robinson & Johansen (1978) are referred to as acceptable protocols. Compound application according to label instructions. There is a Standard Evaluation Procedure (SEP) for bees and a standard form for filling out results.

EPPO (1986)
The EPPO protocol is still under development, but is already used in the Netherlands (Oomen, pers. comm.). This protocol is based largely on results of 3rd Symposium on Harmonization of Methods for Testing the Toxicity of Pesticides to Bees (see Felton et al., 1986). Cage, field and tunnel trials are distinguished.

Cage trial
Use one blank (water or no treatment) and one positive control (e.g. parathion, dimethoate), with as many duplos as required for statistical analysis.
Experimental conditions:
- Use a single healthy colony, preferably queen-right, but at least with three frames; avoid trapping wild bees in cage; additional feeding may be required.
- Small colonies in cages measuring at least 2x2x3 m; max. mesh: 3 mm; plastic roof may be used if desired.
- Suitable crops: borage, phacelia, mustard or other crops that attract bees and are eligible for spraying with compound under review.
- Formulations only, using highest recommended dose on flowering crop.
- Apply on a day when bees are foraging; avoid spraying on cage sides.

Observation:
- Observe effects just prior to treatment and 0, 1, 2, 4 and 7 days post-treatment.
- Assess foraging activity and bee behaviour on crop and in cage.
- Count bees in traps and in rest of cage.
- Record temperature and relative humidity.
- Also record other observations, e.g. impact on brood, if compound properties give cause for suspicion.

Evaluation:
- Repeat trial if mortality with blank is > 15% or if mortality with toxic control is too low.
- Process results using appropriate statistical test; always report raw data and statistical test used.

Field trial
Perform field trial using a flowering crop visited by bees and eligible for spraying with compound under review. Use review compound, innocuous compound and positive control (e.g. parathion or dimethoate).
Experimental conditions:
- Set up at least 3 colonies at one corner of field several days prior to trial; make certain bees are foraging only on trial plot.
- Use healthy colonies with at least 10,000-15,000 bees and at least 10-12 frames (at least 5-6 brood frames); with different-sized colonies, distribute over fields.
- Plots at least 1500 m^2, larger for larger colonies; plots separated by at least 500-1000 m and at adequate distance from other flowering crops.
- Replicates desirable, but often not feasible for reasons of cost or space.
- Use formulation only.
- Use reference compounds approved for similar use.
- Apply compound according to manufacturer's instructions when bees are actively foraging, preferably treating all fields simultaneously, but at least within 2 hours.

Observation:
- Record temperature and relative humidity throughout trial duration.
- Record number of foraging bees, behaviour on crop and at hive, and dead bees in traps.
- Preferably also record: pollen collection (pollen traps), pollen composition in collected honey, number of bees on frames, brood condition and residues in dead bees, pollen and honey.
- Measure parameters just before or one day pre-treatment and preferably 0, 1, 2, 4, 7 and 14 days post-treatment. Continue until 3 months post-treatment, at greater intervals if so desired.

Evaluation:
- Repeat trial if mortality with blank is > 15% or if mortality with toxic control is too low.
- Process results using appropriate statistical test; always report raw data and statistical test used.

Tunnel trial
Perform if observations are required that cannot be made in a field trial, e.g. foraging on aphid-secreted honeydew.

OECD (1988)
A workshop on ecological effects indicated that bees are representative of social pollinators. It was noted that a colony is equivalent to a population and that it should be easy to design a life-cycle test. Development of a protocol is desirable.

Appendix 4.4.2 Review of field studies involving honeybees

Jeffree (1951) Describes a method for estimation the bee populations of a hive, comparing hive frames with photos of frames with known numbers.

Free (1959) Study of the impact of bee-hive transfer time (before or during flowering) and the resultant effects on foraging behaviour and preferences. With premature transfer, there is a risk of conditioning on other flowering plants. In each of 3 trial plots, 4 hives were placed. Pollen composition was sampled using pollen traps. Results showed that transfer time is of only limited significance. However, prior history constitutes an important factor; bees prefer to continue foraging on the same crop.

Free et al. (1967) Comparison of the effect on honeybees of aphid control using granules or sprays on flowering field beans. Assessment of the impact on aphids was unsuccessful because too few aphids were introduced to the fields. To investigate the (side-)effects of granules versus sprays, different compounds were employed for the two formulations, on the argument that there is no point in using a formulation that does not exist in practice. Four or five hives were placed at one corner of each plot. *The number of bees on the frames was assessed (for method, see:* Jeffree, 1951) and a method for retrieving dead bees was tested. To collect dead bees, an inverted hive was placed immediately outside the hive, covered with wire mesh. Since dead bees are ejected from the hive, these are found in the inverted hive. 74% of dead bees were thus retrieved. When dead bees were also recovered from pollen traps, 89% were retrieved. Correction to 100% was thus feasible. To correlate intoxication as cause of death, cholinesterase activity was determined. Activity > 66% of the control value was deemed normal, with activity < 33% *indicating intoxication. There were several inconsistencies in the study:* treatments were carried out at different times, under different weather conditions, etc. Spraying was found to have a direct toxic impact, particularly on workers foraging in a sprayed field. After spraying, the contribution of pollen from the sprayed field declined appreciably. With regard to the effect of granules, further study was recommended. An additional cage trial was conducted: nylon cages measuring 2.4x4.9 m were placed in sprayed and unsprayed sub-plots (the latter covered during application). In these covered cages no mortality was recorded, from which it was concluded that the pollen and nectar are non-toxic and that mortality was therefore apparently due to direct contact.

Robinson & Johansen (1978) Study of the impact on pollination of spraying in Douglas fir forest to control the moth Orgyia pseudotsuga. Two bee-hives were placed centrally in each plot. The impact was studied by daily counting of numbers found in dead-bee traps (Atkins et al., 1970). When numbers were large, an estimate was made by transferring insects through a funnel into a cylinder with a known ratio between volume and number of bees (Anderson et al., 1966). The condition of the colony was also assessed on the basis of the number of occupied frames, numbers per frame and the judgment of an experienced bee-keeper. Additionally, pollen traps (constructed to function also as dead bee traps inside the hive) were set up according to Johansen (1960). Pollen was chemically analyzed.

The method was found to be satisfactory for observing short- and long-term effects.

Kingsbury & McLeod (1979) Study of the effects of two aerial forest spraying runs with permethrin. In sprayed and unsprayed forest, bee hives were placed in a clearing and in coniferous and deciduous woodland. The hives contained dead-bee and pollen traps, which were sampled daily. The weight of the colony was measured regularly. At the end of the season, production was measured in each hive. In one treated and one untreated plot, pollen residues were determined. The weather during spraying was so poor that the bees did not forage the first two hours. No differences were found between the treated and untreated plots. The absence of observed short-term effects was possibly due to weather conditions.

Kingsbury et al. (1981) Study of the effects of forest spraying with aminocarb and nonyl phenol. Hives were placed, with separate controls for each treatment. The impact was assessed by means of dead-bee and pollen traps and by photocell activity measurements. Hive weight was measured by placing scales under one of the corners of the hive. General colony condition was assessed by regularly estimating brood size and honey production. At the end of the experiment, the hive population were counted. Compounds were applied in the evening; the only observable effect was slight mortality on the day after application. In one of the hives the queen was replaced, affecting the quantity of pollen collected.

Johansen et al. (1983) Study of the effects of insecticides on various bee species. At harvest of alfalfa and maize, part of the crop was left standing in the middle of the field (0.4 ha and 52.7 ha, respectively), creating an isolated test plot. In the two fields, 2 and 8-10 hives were placed, respectively, with Todd traps (Atkins et al., 1970). For wild bees, smaller enclosures were used. Results: honeybees are generally more vulnerable to insecticides than wild bees; residues are greater at lower temperatures. Wild bees react differently from honeybees: the latter often become immobilized, while wild bees become very active. Both residual exposure in the laboratory and numbers in dead-bee traps appear to be useful parameters for forecasting field mortality as well as general impact on the colony.

Stoner et al. (1983) Study of long-term food effects using artificial food, employing 'standard-size field colonies': 9 frames in a 10-frame Langstroth unit. With this size colony, normal mortality is 100 adult bees a day.

Shires et al. (1984a) Study of the effects of a new pyrethroid on honeybees foraging on oilseed rape, in four fields of 5.3-13 ha, each at least 3 km from other rape fields. In each field, 8 hives with 10 frames were placed one week prior to treatment. At the time of treatment, hives contained 40-80,000 bees. Of each 8 hives, 6 were fitted with dead-bee traps and 2 with pollen traps. Treatment: between 11.45 and 13.30 h, during warm, dry weather. From a helicopter, three fields were sprayed with the compound under review, with methyl parathion (toxic to bees) and with phosalone (innocuous to bees); the fourth field was tractor-sprayed with the new compound. Hives were not shielded off. Precipitation, sunshine and temperature were regularly measured; comparative data were

also obtained from a weather station. Dead bees were counted daily. The pollen trap consisted of a perforated piece of plastic. Three of the eight pollen traps did not function properly; the remainder were regularly sampled. Activity was assessed by walking 50 m and counting all bees flying within 1 m (measured with a rod); per field, 300 m^2 were thus assessed. To assess activity at the hive, 6 categories were defined, based on number of flights and behaviour. General colony condition was assessed 5 days before and 24 hours and 10 weeks after treatment by making colour photos of the frames and measuring the brood on these photos. At the end of the season, colony condition was assessed by a professional bee-keeper. Compound deposition on the crop was determined using pieces of aluminium foil (250x250 cm) placed in the field at crop height. In addition to dead-bee and pollen measurements, residues were determined in honey and wax, before and three days after treatment. In small cages (10x5x5 cm), a bio-assay was carried out on adult bees fed with pollen and nectar from the trial fields. Result: no extra mortality with the new compound or with phosalone, but increased mortality with parathion. With the new compound, the share of rape pollen was also reduced. Conclusion: effects with methyl parathion and the absence of effects with phosalon and the new compound point to inadequate experimental design. At a higher dosage, the new pyrethroid compound did have a repellant effect on foraging activity on the first day. This was probably the reason why mortality and residues were low, despite the high toxicity in laboratory trials.

Shires et al. (1984b) Similar to rape study (Shires et al., 1984a), but using mustard. In a 3 ha field, a 1.2 ha section was marked off and four 3000 m^2 sub-sections defined and sown with mustard at 2-4 week intervals. Each of these sub-sections contained three 20x50 m plots in which the mustard was sown in 1 m wide strips, 0.5-1 m apart. Each plot consisted of 10 strips, 2 of which were unsprayed. The three plots were treated with phosalone, methyl parathion and the compound under review. Four hives containing 20-30,000 bees were set in place 2-3 days prior to treatment. As an addition to the previous experiment, in this case dead bees were also sought in the open strips in the field; very few were found, however. Results were very similar to those of the previous experiment.

Wilkinson & Gough (1984) Two field trials based largely on the British guidelines, but with a few alterations. Using a helicopter, PF321 and a toxic standard were sprayed. Preference was given to the size of field common in practice: 20-50 ha, with individual plots separated by about 12 km. One drawback was that some plots were less comparable because of differences in local weather conditions. There were 5 hives on each plot, 3 with dead-bee traps and 2 with pollen traps. In a second experiment, spraying had to be postponed due to poor weather (after the hives were in place). This led to better results in terms of foraging in the plots; the time of placement appeared to be of minor importance. Logistical problems prevented simultaneous spraying; because of the unsettled weather, this again led to weather differences between the various spray runs. The number of dead bees gave the clearest result: no dead bees due to the new compound, in contrast to the toxic standard. Foraging activity was assessed in each plot on 6 strips 1 m wide and 50 m long. Results were not unambiguous, however, due partly to the fluctuations in weather:

sometimes foraging was concentrated, sometimes bees were spread around the field. Hourly observation gave a better picture than once every 2 hours. There was also great variation in at-hive activity because of weather conditions. Preference was give to electronic counting. Brood condition was assessed pre- and post-treatment by means of a polythene print, later photographically. Egg counts from photos proved difficult and time-consuming, however. The pollen traps did not function well and were only in fact suitable for determining the share of the experimental crop in the pollen. There were too few dead bees for analysis. Researchers' conclusion: satisfactory results, with room for improvement through increased observation. However, an alternative conclusion is that changes led to greater variation and thus to poorly interpretable results.

Arnold & Davies (1985) Effects of prochloraz in oilseed rape. Three plots at least 3 ha large and about 1 km apart were treated, with one blank and one positive control (dimethoate). Several days prior to treatment, 4 hives were set in place on each plot. Activity was assessed before, during and after treatment. Dead bees were collected daily. Residues were measured in bees, pollen and honey. The flowering crop was treated with a spraying unit. No negative impact was found. Residues were found in bees and pollen, but almost absent in honey.

Celli et al. (1985) Use of bees for monitoring pesticides and heavy metals. At each of 24 stations, 2 hives were placed with dead-bee traps. These were emptied weekly and when more than 500-700 dead bees were found, they were chemically analyzed. Of 19 samples with high mortality, 74% contained fungicides, 58% insecticides and 5% acaricides.

Czarnecki et al. (1985) Study of collection by bees of micro-encapsulated products in beet fields measuring at least 3 ha and spaced sufficiently far apart. Six 10-frame hives were used, 2 with dead-bee traps, 2 with pollen traps and 2 without traps. The formulation was sprayed in the evening, as well as 2 formulations of a product toxic to bees (methylparathion), a compound that is never used on beet (so that residues were necessarily from the experiment) and one blank. Flowers, pollen from traps and hives (collected during a 8-day period), living workers and dead bees were sampled and residues measured. Three to six samples were taken in the period from 1 day before to 8 days after treatment. A visual method was elaborated to retrieve the microcapsules (after colouration) from the hive.

Gerig (1985) Study of the effects of phenoxycarb treatment on the pollen-collecting behaviour of bees, in 3 orchards. No impact was found on population density, brood development or mortality. Over 3 years of observation, no effects were found, probably partly due to major fluctuations in pollen-collecting activity. The methods employed were apparently too insensitive for detecting effects on pollen collection. No deleterious medium-term effects were observed.

Oomen (1985) Study of the impact of pyrethroids used in oilseed rape cultivation to control beetles. Bee-toxic and bee-innocuous compounds were compared with the compound under review. Eight 24 ha plots were defined at least 500 m apart, with 4 bee colonies per plot. Parameters measured: dead bees (in traps and in the field) and foraging intensity

(before and 1 week after treatment). General colony condition was assessed before and several weeks after treatment. The experimental design was judged satisfactory. Results corresponded to those of Shires et al. (1984a,b).

Van der Steen & Van der Eijnden (1985) Field trial for assessing the impact of deltamethrin in oilseed rape in May. This is the month of most intense foraging on this crop, although tests are usually performed in the summer. Eight 100x24 m plots were marked, 500 m apart. There were 4 queen-right hives per plot. Average number of occupied frames: 14. Hives were placed after the start of flowering and prior to spraying. Each hive had a dead-bee trap and bees were collected the day before treatment, several hours after treatment and then daily until the sixth day post-treatment. In the middle of each plot was a cleared strip where wire mesh was laid to collect bees dying in the field. Foraging activity was also assessed on 100 m^2. The compound was tested in 3 concentrations (the lowest in duplo), in two test series, with additional treatment with phosalone and parathion. Data was analyzed using variance analysis, performing the t-test in the case of differences. In the lowest concentration, the compound under review was found to be no more harmful than phosalone.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

BIRDS

FIELD TRIAL FOR BIRDS

General

Vertebrates generally have a far greater home range than invertebrates. To yield results, a field study of the effects of pesticides on various species of bird (= Aves) must be conducted on a large scale. Mineau & Collins (1988) provide an indication of the considerable difficulties involved in field trials for birds. Trials must not only be conducted on a large-scale but meticulously, too. In field practice, it will be necessary to assess many different impact parameters. As a matter of principle, side-effects on birds are always to be considered undesirable and the suspicion of a moderate risk therefore warrants extensive study.

There are a number of objections to cage trials (see for example: Heijbroek & Koster, 1973; Messick et al., 1974) and a proposed EPA guideline employing caged birds was recently withdrawn. We therefore omit a cage trial in our proposal, describing only a field trial.

One of the greatest problems involved in field trials with birds is their low population density. In specific cases, it might be possible to increase this density, e.g. by placing nesting boxes (Mattes et al., 1980).

Appendix 4.5.2 gives an overall review of studies into the side-effects of pesticides on birds (partially reprinted from De Snoo & Canters, 1988). Studies have been performed in various environments (woodland, fields, orchards). Effects have been studied employing different methods (searches for corpses, population censuses, behavioural observation), focussed on different parameters (mortality, reproduction, behaviour). In a large-scale context, ecological as well as toxic side-effects have been demonstrated (e.g. Hunter, 1984; Hunter & Witham, 1985; McEwen et al., 1986). There has been a relative focus on rodenticides, because of the large risk of ingestion or secondary poisoning, and on granules, again because of the ingestion hazard. In the case of granules, the predictive value of various trial methods is the subject of debate (Mineau,1988). A survey of the literature shows that studies have also been carried out in the Netherlands (e.g. Heijbroek & Koster, 1973; Brugge, 1977; De Reede, 1982).

To satisfactorily assess the whole range of effects, it is essential to combine various different methods of observation. Our proposal is based mainly on two sources: the existing British protocol (see Appendix 4.5.1) and the methods described by Hustings et al. (1989), a review of methods frequently employed in avian studies.

Experimental conditions

Field trials with invertebrates generally recommend running a positive control, i.e. a control with a compound known to have a certain effect. More so than with invertebrates, however, it is hardly acceptable to cause deliberate harm to birds by using a positive control, and such a control is therefore lacking in our proposal. For greater confidence

about the occurrence of effects, an extra experimental field is treated with four times the maximum recommended dosage, creating a worst-case situation from which to assess whether effects will occur when 'normal' doses are exceeded. In addition, close observation is required to make sure that the birds are indeed exposed to the compound.

A minimum of 20 bird species at the trial site has been adopted from the British protocol. In the Netherlands, this number is only realistic if the trial field borders on a non-agricultural area. For a 'normal' agricultural area, this number may have to be reviewed.

Application

We have opted for application according to manufacturer's instructions. It is important that the reference field receives exactly the same treatment as the trial field, apart from spraying with the active chemical of course. The reference field may either be sprayed with an innocuous spray base (preferably water) or a granulate. In this way, any disturbance from equipment etc. will be the same in both fields.

Observation

Birds and (a)biotic environmental factors
In the proposal, we have opted for two large trial fields and one reference field (i.e. no duplos). In order to keep observational error to a minimum, therefore, observation in these fields must be extremely intensive. We have therefore adopted observational methods that are as accurate as possible, with territories and nests being recorded on maps (Hustings et al., 1989).

Compound
If there is a risk of secondary poisoning, residues in birds must also be measured. In the case of persistent pesticides, effects may occur in migrating birds. At the merest suspicion of such effects, residues must be measured. In order to gain a good idea of exposure, it may also be necessary to measure residues in the birds' food.

Proposal: **FIELD TRIAL GUIDELINE FOR BIRDS**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Perform the trial with birds (= Aves) on a soil type on which the compound is normally used, on a crop requiring the highest recommended dosage.

1.2 Experimental conditions

The experimental plot is compared with a reference plot; the latter undergoes the same treatment and spraying schedule, but employing water or another innocuous carrier substance similar to the compound under review. The trial lasts at least one full breeding season, so that any reproductive effects can be detected and in order to permit several applications of the compound (according to manufacturer's instructions).

1.3 Other requirements

When selecting a site, make sure there are a sufficient number of bird species present: 20 species is generally adequate. A definite choice of location depends on the presence of species running the greatest potential risk. The area should also include several hedgerows or thickets and preferably border on woodland or copses. The three trial plots should be as similar as possible, with the same species of bird present. In the surroundings, birdlife should not be too prolific, for otherwise any affected birds would be replaced too quickly by others; in the case of a territory count by means of birdsong, for instance, no effect would be found.

1.4 Experimental design

Plot size

Perform the trial on an plot measuring about 10 ha.

Plot arrangement

Select the plots in an area in which the compound will normally be applied. The experimental and reference plots must be in the same area, at a sufficient distance to avoid mutual spraying impact. The plots should also be located such that there is little chance of the birds feeding in the other trial plot.

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied.

3. OBSERVATION

Methods and frequency

In general, surveillance should be carried out by trained and experienced observers; for a number of types of observation, such as song and behaviour, surveillance by experienced ornithologists is in fact essential.

Corpse counts
Survey the plot on foot, investigating strips at most three metres apart. Also survey the area bordering on the trial plot as well as local roosting sites. Survey for, and remove, any corpses 24-48 hours before the start of the trial. Survey again for casualties on the day of spraying and subsequently at intervals determined by anticipated effects. Accurately record time, place, duration of survey, etc. Pack corpses individually in aluminium foil, subjecting them as quickly as possible to study and residue analysis.

N.B. The behaviour of predators should also be observed.

Population counts
Method: walk a fixed route in the morning and evening and record the presence of birds (singing males). Also carry out a census on the adjacent land several days before and after treatment. Wherever possible, identify territories and note these on maps (see: Hustings et al., 1989). Also note other, non-territorial species. These counts may be performed parallel with the search for casualties. Before spraying takes place, territories and nests should have been accurately mapped. Surveillance should be carried out directly after treatment and then daily during the first two weeks. Subsequently, the frequency can be reduced to once every two days during the first month post-treatment and then twice weekly. The census should be based on very close observation and should cover the plot as well as its margins. The neighbouring land can be covered less intensively. It is important to note which birds come to feed in the trial plot from the surrounding land. It is useful to have a good idea of all territorial birds within 100 m of the trial plot. Effects can then be assessed by observing these birds. Surveillance here may be less intensive than in the trial plot itself, for instance with weekly observation.

Nest monitoring and breeding success
When counting birds, if possible record the number of nests and establish clutch size, percentage leaving the nest and number of juveniles reaching maturity. Breeding success can be most readily established in nesting boxes, which can be hung up in the trial plot.

Behavioural observation
First study sub-lethal laboratory effects, and then try to establish changes in behavioural patterns in the field, including, for instance, changes in feeding behaviour and feeding site.

Residues
To follow up specific suspicions, additional observations may also be carried out. It may be desirable to establish compound dispersion and exposure by measuring residues in organisms. To this end, birds and eggs may be trapped/collected according to a prior routine and analyzed for residues and/or anomalies. In the case of a cholinesterase inhibitor, the cholinesterase activity of the organisms may also be determined. Residues in food can also be measured.

If food or habitat effects are suspected, food and habitat may be directly investigated; for instance, effects on insects may be assessed by means of a ground beetle field trial and effects on vegetation by means of a higher plant field trial. Any food effects will be most pronounced during the breeding season.

4. VALIDITY

Replicates in time are only required in the case of unclear results or if there is high mortality in the blanks. An extensive test report is absolutely essential for proper evaluation of the results. Every detail, however trivial, should be recorded. The raw data as well as the statistical methods employed should be included with the trial results.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
1	a h r x	f	hp 4hp	b	1	various	10-30

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test plots		5,000
- field equipment		( )
2 sampling (3x10 ha)		( )
- transect counts	20	
- corpse count	40	
- reprod. behaviour	30	
- abiotic parameters	5	
3 parameter measurement		
- residue measurements	5	500
- abiotic parameters	5	2,000
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 125	n.a.
cost estimate (subtotal)	Dfl. 93,750	Dfl. 7,500
overall cost (very rough estimate):	Dfl. 100,000	

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.5.1 Review of existing field trial guidelines for birds

United States (EPA, 1982)
Mentions field trials as an option; however, the only concrete example is a cage trial employing quails:
- 8 pairs in 3.1x15.2x2 m cages. At least 3 cages for each treatment and control. Place 2 weeks before first treatment until 3 weeks post-treatment.

United Kingdom (MAFF, 1986; Working Document 7/1)
Gives a number of general requirements for field trials and reviews available field trial options.
Experimental requirements:
- Surveillence of vertebrate populations must be carried out meticulously, based on the knowledge of experienced biologists.
- A site should be selected offering an adequate number of bird species present: 20 species is generally adequate. A definite choice should be based on the presence of species running the greatest potential risk. The area treated should have an area of at least 10 ha, with wide hedgerows, woodland or copses on at least one side; in the surroundings, birdlife should not be too prolific, for otherwise any affected birds would be replaced too quickly by others; in the case of a territory count by means of birdsong, for instance, no effect would be found.
- *Before embarking on a field trial, it may be necessary to first* develop laboratory methods, e.g. for analyzing residues.
- Apply the compound according to manufacturer's instructions, using the highest recommended dosage. Sample compound during application. Record details of treatment accurately.
- A detailed protocol should be drawn up beforehand, preferably with the approval of the authorities concerned. The field team should, however, have the scope to adapt to unforeseen circumstances if necessary.

Observation:
- Corpse count (to be performed during each trial):
  Survey the field along strips at most 3 m apart; also survey outside the plot and at local roosting areas; survey for, and remove, corpses 24-48 h pre-treatment; survey again for casualties on the day of treatment and later at intervals governed by anticipated effects; always accurately record the time, place and duration of search; pack corpses individually in aluminium foil, subjecting them as quickly as possible to study and residue analysis.
- Population counts (especially suitable for breeding and/or territorial birds): walk fixed route, in morning and evening, and record presence (singing males). Also make counts on adjacent land, several days before and after treatment.
- Monitor nests and breeding success.

Additional observations during population counts:
- Bird behaviour (by experienced ornithologists only): first study sublethal laboratory effects; then try to observe changes in behavioural patterns in the field.

- Residues in organisms:
  It may be desirable to determine compound dispersion by analyzing residual levels in organisms. To this end, birds and eggs may be trapped/collected according to a prior routine and analyzed for residues and/or anomalies.
- Cage trials: are the subject of debate, but may yield useful information on actual exposure, etc. Moreover, smaller plots may then be acceptable. Care must be taken that birds are not subjected to stress and are allowed to acclimatize to cage life. Adequate references are essential. Nevertheless, cage trials should be performed only after specific questions have arisen that can be answered with this type of trial.
- Food residues can be measured and compared with the laboratory LC50. It is mentioned, however, that for birds the LC50 has little bearing on the field situation.

Presentation:

- An extensive report on the field trial is absolutely essential for proper evaluation of the results. Every detail, however trivial, should be recorded.

Netherlands (CTB, 1987; Part H)
With respect to birds, it is stated that a field trial may sometimes be needed; for methods, reference is made to the British approval procedure.

Appendix 4.5.2 Review of field studies involving birds

McEwen et al. (1972) Field study of the impact of diazinon on birds and mammals. Diazinon was applied on 24 trial plots in Wyoming to control grasshoppers (5.0-8.0 oz./acre). Before spraying, an average of 26.6 birds were observed per ¼ mile, dropping to 11.1 6-8 days after spraying. Eight dead birds of the 4 common species in the area were also found. Four birds were subjected to further analysis and contained 0.06-0.57 mg diazinon/kg body weight. It was concluded that the use of diazinon depressed the avian population in the area.

Tait (1972) Field study of the potential impact of aldicarb (Temik, 10% granules) on vertebrates in sugarbeet cultivation. In April, 20 acres were drilled with sugarbeet and granules and 10 acres with sugarbeet alone. The plots were systematically surveyed for the presence of corpses: 4 were found, one of which (a linnet Carduelis cannabina) contained residual aldicarb. No differences in diversity or numbers were found after aldicarb treatment, nor did fewer birds reach maturity.

Buckner et al. (1973) Field study of the impact of carbaryl on birds and small mammals. A 20 acre woodland plot was sprayed with carbaryl (Sevin oil; dosage unknown). The number of singing males was taken as a measure of population density. Observations were made before spraying and 1, 2 and 3 weeks afterwards. There was a reference plot. No significant difference was found in the numbers of species or individuals or in egg hatching before and after spraying. Validity of results was limited by the small plot area and the lack of replicates.

Heijbroek & Koster (1973) Birds and small mammals may suffer from aldicarb intoxication, either directly - by ingesting granulate - or indirectly - by eating contaminated plants. In this semi-field study, performed by IRS (Netherlands Institute of Rational Sugar Production), RIN (Netherlands National Institute of Nature Management) and the Toxicology department of Wageningen Agricultural University, the possibility of indirect intoxication of pheasants was studied. On 4 plots (6x30 m) various concentrations of aldicarb were applied to the soil (10-50 kg Temik 10 G [= 10% aldicarb granules] per ha). 12 cages with a total of 24 pheasants were placed in the plots for 2 weeks. Food consumption was determined. It was found that aldicarb residues on the sugarbeets increased with time. No lethal effects were found in the pheasants, however. It was concluded that there was no indirect intoxication of pheasants (via the vegetation). Direct intoxication did occur, however, ingestion of even a few granules proving lethal.

Jennings & Brown (1973) Field study in which soil and wildlife were analyzed for the presence of aldicarb residues. In the soil, the aldicarb concentration decreased from 0.134 mg/kg on the day of application to 0.043 mg/kg after 97 days. Animals studied (found and shot): mice, shrews, hares, rabbits, blackbirds, skylarks, wood pigeons, pheasants, red-legged partridges and eggs of various bird species. On the basis of residues in the birds, the risk for the various species was evaluated as: red-legged partridge > skylark > pheasant > blackbird. Surprisingly, the highest aldicarb concentrations in the birds were often found only a long time after application of the compound: skylarks max. 3 mg aldicarb per

kg body weight in the liver after 64 days, wood pigeons max. 1 mg/kg after 36 days. The highest aldicarb levels were found in the crop and gut of a red-legged partridge (found dead): 380 and 79 mg/kg body weight, respectively. With some trapped animals, despite the fact that no aldicarb residues were detected, behavioural anomalies due to intoxication were observed.

Rivera (1973) Several days after an adjacent field had been treated with paraquat (20%), 72% of a goose population (totalling 84 birds) died. The birds could not have fed on the field; following heavy rain, there was probably downhill run-off into puddles where the geese were exposed.

Kurtz & Studholme (1974) Field study of the impact of carbaryl (1 lb/A) on birds (finches) after spraying a 150 acre woodland plot. Birds were shot 3 days after spraying. Birds from sprayed and control fields were analyzed for carbaryl; in both groups, only minor traces of carbaryl were detected. Low exposure was probably due to little carbaryl reaching the soil. No effect of carbaryl was observed on ground-feeding woodland birds.

N.B. Study is only moderately reliable because of the small number of birds, which were probably only slightly exposed, since only living animals were analyzed for exposure. Moreover, samples were stored for a fairly long time, with storage time also unevenly spread over the trial groups.

Messick et al. (1974) Field study of the impact of pesticides on pheasants. In 1970, 24 ha were sprayed with parathion (47%, 0.9 kg/ha) and other compounds. Parameters measured: exposure, survival, breeding success, migration, insect consumption and cholinesterase inhibition, in free-living pheasants Phasianus colchicus as well as in juvenile pheasants held in cages on the trial plots. No mortality was observed. The juveniles (5-15 days) in the cages suffered from a loss of balance several minutes after spraying. Six-week-old pheasants exhibited no abnormal symptoms. In comparison with control animals, neither group of pheasants showed any difference in body weight or cholinesterase activity.

N.B. The caged birds were fed with uncontaminated food.

With the free pheasants, no difference in the number of successful nests was found between sprayed and unsprayed areas. Several pheasants tracked radiometrically were not observed to avoid the sprayed area. The impact of the pesticides on the food situation of the free-living pheasants was also studied. The number of insects in the sprayed fields was much lower than in the unsprayed fields. The crops of pheasants shot in the sprayed areas also contained far fewer insects than those of pheasants from unsprayed areas. It was concluded that the toxic side-effects of the pesticides could not be the main cause of the reduced pheasant population. In this study, the consequences of the ecological side-effects (food shortage) were not further investigated.

Moulding (1976) Field study of the impact of carbaryl on woodland birds in coniferous forest. Aerial spraying of two 5000 acre plots with 500 g

Sevin oil per gallon and per acre. Two control plots of the same size were unsprayed. Birds were observed by sight and song. After spraying, the number of individual birds, number of species and species diversity index decreased for 8 weeks to 55% of control plot levels. Until the next summer, the bird population remained lower than in the control plots (45%). It appears that the decrease was more marked for species feeding among the foliage than for ground-feeding species. The reduction was explained in terms of birds feeding outside the sprayed areas and a reduction in reproductive success. No dead birds were found. Song is unaffected after spraying. The number of woodland birds and species decreases after carbaryl treatment due to (probably) indirect food effects or effects on reproduction.

Bridges & Andrews (1977) Fifty-five wild turkeys Meleagris gallapavo shot in the USA (South Illinois) during the 1974 shooting season were analyzed for the presence of pesticides. Twelve different pesticides were identified. Only four were present in all samples: DDT, heptachlor epoxide, toxaphene and linuron. Linuron was found to be persistent and readily detectable in turkeys.

Brugge (1977) In 1975, chlorophacinone was used (experimentally) to control fieldmice in the Netherlands (Alblasserwaard). The effects and side-effects of control were studied. Chlorophacinone was applied to wheat (1.54/20 kg wheat = 1.5 g/ha). The wheat was broadcast on 60 ha grassland, 55 ha road verges, 39 ha orchards and 1 ha arable land. At the time of application, the fieldmouse population was no longer high. It was found that birds rather than mice ate most of the wheat. On the treated plots, more wood pigeons, sparrows, mallards and carrion crows were observed. Chlorophacinone was found in the crop/gut of homing pigeons, wood pigeons and mallards. The compound caused mortality in 6 homing pigeons and 1 wood pigeon and was probable responsible for mortality in 3 mallards 1 wood pigeon and 1 stoat.

Lauenstein (1978 & 1980) As part of the West German approval procedure for chlorophacinone, two large-scale experiments were carried out in Oldenburg. Chlorophacinone-treated wheat (0.0075%) was broadcast (10 and 20 kg/ha). A search for corpses was carried out in the treated fields and up to 5 km beyond. There were more birds in the treated than in the untreated fields. A total of 2000 birds (37 species) were counted. In the first experiment, in 1977, 12 dead birds were found. In the case of a black-headed gull, a herring gull and a moorhen, there were strong indications of intoxication. In the case of another black-headed gull and a starling, intoxication was suspected. In 1978, a number of birds were also shot and analyzed for chlorophacinone residues; the compound was detected in one crow, one jackdaw and one magpie. No chlorophacinone was found in pheasants, herons or buzzards. In the second series of experiments, in 1979-1980, no bird mortality due to chlorophacinone was observed.

Zinkl et al. (1978) Description of an incident in which 14 Canada geese died on a golf course months after diazinon treatment.

Bart (1979) Eight woodland plots, one of which was 100 ha, were treated with 1.1 kg Sevin oil/ha. Woodland birdsong was employed as a measure of

population density. After spraying, no impact was found on species occurrence, total number of individuals or birdsong duration. One species was found less in the sprayed area. Bird weight did not differ after spraying. It was assumed that there was no difference in feeding behaviour. Conclusion: very little or no effect of carbaryl on birdlife.

De Weese et al. (1979) Three fields (350-550 ha) were sprayed with 500 g carbaryl/0.5 gallon/acre (Sevin oil). The impact on the woodland bird population was studied, observing by sight and by sound. Parameters: breeding density, diet (gut content), breeding success, feeding height, cholinesterase inhibition and mortality of 20 species. Observation continued until 14 days post-treatment. No significant differences in the measured parameters were found between the sprayed and unsprayed plots. However, measured in terms of gut content, birds feeding in the vegetation suffered higher exposure than ground-feeding birds.

Kingsbury & McLeod (1979) Field study of the impact of permethrin on birds, mammals and terrestrial invertebrates in three different ecological habitats (total area: about 930 ha). An open plantation and deciduous and coniferous woodland plots were sprayed twice with 17.5 g permethrin/ha (6-day interval). In each habitat, a control plot was surveyed in addition to a 4 ha sprayed plot. A population census of the woodland bird population (visual + birdsong) was carried out from 5 days before spraying to 6 days after final treatment. On the day of treatment, the habitats were surveyed for dead or sick birds. A total of 44 to 56 bird species were recorded in the various habitats. In none of the three habitats was the bird population found to be affected by permethrin spraying. Only in the coniferous woodland plot did birdsong and feeding activity decrease on the second day after the first spraying run. Two days later, both birdsong and feeding activity had returned to the control level. Intensive surveillance yielded no dead or sick birds in any of the habitats. After spraying, bird territories also remained occupied.

Richmond et al. (1979) Field study of the impact of diflubenzuron and other pesticides on woodland birds. Two plots (each 129.5 ha) were sprayed with 0.14 and 0.28 kg diflubenzuron/ha. Number of birds, species diversity, breeding success and the presence of dead or sick birds were observed in the sprayed and control fields. The study lasted 2 years. No dead or sick birds were found in the dibenzuron-treated plots. Species diversity and the number of breeding pairs were unaffected. With 0.28 kg diflubenzuron, in the first year breeding success was 16% less than in the control plot. In the second year, breeding success was 11% greater than in the control plot. The authors' conclusion: diflubenzuron had no effect on woodland birds. Comparable results were found in the literature quoted by the authors.

Stanley & Bunyan (1979) Chlorfenvinphos is highly toxic to pigeons (LD50: 16 mg/kg body weight). This is reflected in the field, too, where most incidents involve pigeons. A total of 9 incidents were reported between 1974 and 1976 in which more than 150 pigeons (of various species) were killed due to consumption of chlorfenvinphos-treated winter wheat. In most dead birds, brain cholinesterase inhibition was > 90%.

Mattes et al. (1980) Field study of the effect of insecticides on the vitality and reproduction of great tits Parus major. Nest boxes were hung up in 5 German orchards to increase or stabilize the great tit population. In the period 1972-1975, the nest boxes were checked daily during the breeding season. The orchards were sprayed with pesticides 15-18 times, including 8 treatments with insecticides such as diazinon, dimethoate, parathion, azinofos, omethate, phosalone and endosulphan. Parameters measured: effect on survival of female parents, number and weight of eggs and hatchlings, eggshell thickness, age distribution of population and food situation (food supply and feeding activity). Residues in eggs and juveniles were also measured. The most striking effect of spraying was the impact of the food situation on the birds' vitality. Differences in population density, clutch size, mortality and juvenile growth rate could be related to the occurring food shortage. One direct effect of the insecticides was a thinning of the eggshells. With juveniles, deformities and increased mortality were found in the areas most heavily sprayed. Several female parents also exhibited abnormal behaviour.

Bunyan et al. (1981) Field study in UK (Suffolk) on the effect of aldicarb on soil, invertebrates and vertebrates. Aldicarb was sprayed on 31 ha of sugarbeet in granular form (10% active compound, 1.12 kg aldicarb/ha). Two ha at the centre of the sprayed plot served as an unsprayed control. The vertebrates were compared before and after spraying. There was no difference between the sprayed and unsprayed plots. The number of birds flying over was counted. Hatchling survival and activity were also recorded. A risk index was calculated on the basis of the number of individuals observed and the frequency with which these individuals were sighted. Red-legged partridges, followed by sky larks, were found to run the greatest risk. Aldicarb was not found to affect reproduction. The compound could not be detected in the eggs of the various species. Two dead red-legged partridges were found in the treated plots; cause of death: aldicarb intoxication. Of the 30 birds shot, 73% were found to contain aldicarb residues. Aldicarb was even found in birds shot 91 days after treatment. Of the birds shot, 30% exhibited one or more signs of aldicarb intoxication. Apart from the trial plots, 8 other fields where aldicarb was used in farming practice were surveyed for corpses. Two birds were found that had possibly been killed by aldicarb application. The authors identify the hazard of aldicarb as arising from ingestion of granules and from consumption of residue-containing earthworms.

De Reede (1982) Field study on the possible secondary poisoning of birds in the Netherlands following control of insect larvae with diflubenzuron. In 1976, three apple orchards were sprayed with 3.3 kg diflubenzuron/ha (Dimilin-25%). In 1977, an ash coppice was sprayed with 1.2 kg diflubenzuron/ha. Three unsprayed apple orchards and three unsprayed ash coppices served as controls. Diflubenzuron levels were measured in leaves and in leaf-eating insects. The effect of treatment was assessed in terms of the breeding success of tree sparrows, great tits and blue tits. Treatment was found to have no effect on juvenile weight or mortality. There was no difference in breeding success between birds on sprayed and unsprayed plots. It should be noted that in 1976 the birds from the sprayed orchards gathered 50-75% of their food outside the sprayed area. Although in 1977 the birds did gather all their food in the sprayed ash coppice, hardly any leaf-eating insects were consumed after spraying.

Smith (1982) Rabbits and Virginian quail, exposed three times under field conditions to 3 lb oxamyl/acre exhibited no clinical or major pathological anomalies.

Balcomb (1983) A 5 ha cereal field was surveyed for victims of carbofuran use (Furadan-10 granules, 1.12 kg active ingredient/ha). One dying and one sick buzzard Buteo lineatus were found. The secondary poisoning of these birds was probably due to consumption of small mammals.

Balcomb et al. (1984) In Maryland (USA), 15 cereal fields (total area: 195 ha) were treated with carbofuran (Furadan-10 granules, 1.12 active compound/ha) to control corn rootworm. The study comprised a systematic survey of some of the fields 1 and 3-4 days after treatment. A 2-3 m wide strip was surveyed on foot by observers for casualties. A total of 25-38 ha was surveyed: 6 dead birds (5 species) were found, 5 of which were most probably killed by carbofuran. On the basis of the number of carbofuran-induced mortalities (1 bird per 5-7.6 ha), the total number of birds killed annually by carbofuran in the USA was estimated at more than 1 million!

Blus et al. (1984) Study of the effects of heptachlor and lindane applied as seed disinfectants on Canada geese. In the period 1974-1979, the number of geese in a population in Umatilla decreased, most probably due to the use of heptachlor. In the period 1979-1983, heptachlor was replaced by lindane and the goose population recovered, with mortality decreasing and the number of breeding pairs increasing. There was no indication that lindane has adverse effects or that it accumulates in geese or goose eggs.

Hunter et al. (1984) Semi-field study of the effects on juvenile ducks of carbaryl pond treatment. Populations of aquatic invertebrates were found to be substantially depressed. From 2 days post-treatment onwards, growth of the ducks in the sprayed ponds was depressed. The birds spent more time feeding. Despite the extremely cold weather during the study, the ducks survived. There is therefore a carbaryl-induced effect in the short term; long-term effects were not studied, however. The decline of common scoter populations in recent years is ascribed to food shortage. Carbaryl may lead to juveniles obtaining too little food, resulting in depressed growth.

King et al. (1984) Field study in Texas on the effect of parathion on nest defence behaviour and reproduction rate in laughing gulls Larus atricilla. In a breeding colony, 14 birds were given an oral dose of 5 mg parathion (in oil) per kg body weight. Ten control birds were given oil only. The effect on nest defence behaviour 6, 24 and 30 hours after treatment was studied. Parameters: flight height above intruders and time before return to nest. The effect on reproduction was determined by counting the number of eggs and juveniles in the nests during a 3-week period. The study showed that a single dose of parathion has no effect on nest defence behaviour or reproductive success.

Blus et al. (1985) Study in Columbia Basin, Oregon and Washington on the effects of heptachlor and lindane applied as seed disinfectants on a

number of species of birds including ducks, pheasants and magpies. In the period 1978-1981, the eggs of 6 species were analyzed for residues of both compounds. Lindane could not be detected in the eggs, in contrast to heptachlor, neither could lindane be detected in the brains of several birds subjected to further analysis. Lindane has no adverse effects on the birds.

Hunter & Witham (1985) Two field experiments: in Expt. 1, 30% of a woodland plot (1577 ha) was sprayed with carbaryl (840 g/ha); in Expt. 2, an entire woodland plot (1487 ha) was sprayed. Parameters measured: feeding height in trees, ratio between numbers of each species, sex, tree species for feeding and number of feeding birds (chiffchaff). In Expt. 1, the number of feeding birds was depressed up to 7 days post-treatment. Feeding height decreased and different trees were visited. The number of leaf arthropods decreased after treatment. In Expt. 2, no effects on birds were observed, although there was a sharper drop in the number of arthropods. The differences between the two experiments was explained in terms of there being an alternative source of food in Expt. 1 but not in Expt. 2.

Stone & Gradoni (1985) Description of several incidents involving carbofuran-induced bird mortality.

McEwen et al. (1986) Field study of the effect of chlorpyrifos on shore larks Eremophila alpestris and McCown's buntings Calcarius mccownin. 16 ha of winter wheat and two 4-ha strips were sprayed with 0.56 kg and 1.0 kg pyrifos (1.8 g/l/ha), respectively. Larks and buntings were shot at the corners of the treated plots and in control plots. Brain cholinesterase activity and gut content were analyzed. It was found that 3 and 9 days post-treatment the cholinesterase activity of larks was lower than that of the control birds. On the 3rd day, 50% of the birds suffered $\geq$ 20% cholinesterase inhibition (max. 52.1%). On the 9th day, 44% of the birds suffered $\geq$ 20% inhibition (max. 44.2%). On the 16th day, there was no longer any difference between the birds from the treated and untreated plots; only one lark still exhibited $\geq$ 20% inhibition. Small numbers of buntings were analyzed on the 3rd and 9th days post-treatment. Cholinesterase inhibition was not detected (possibly due to the small sample). No larks or buntings were found dead or sick; however, no systematic survey was performed. Gut content consisted of both vegetable and animal matter; both species are omnivorous. On the 3rd day post-treatment, consumption of animal matter was greatest and greater than in the control birds. This was probably due to consumption of dead (controlled) insects (especially Agrotis orthogonia). On the 9th and 16th days post-treatment, the exposed birds consumed more vegetable matter than the control birds, possibly due to a shortage of insects following spraying. This shift in food supply may be especially important for the food supply of juveniles, which are normally 86-89% carnivorous.

N.B. It is stated that $\geq$ 20% cholinesterase inhibition indicates exposure to a cholinesterase inhibitor and that $\geq$ 50% inhibition can be a potential cause of mortality in birds, as well as affecting behaviour.

Brae et al. (1988) Comparison of bird populations in organically and conventionally farmed areas. Birds were counted, visually and audially,

from fixed locations. Where possible, breeding birds and territories were also mapped. 31 organic farms were selected and matched as closely as possible to 31 conventional farms. During a 4-year period, 8 censuses were held between 15 April and 15 June. Corrections were made for differences in habitat. On average, more individuals of each species were found at the organic farms. These differences appeared to be due more to pesticide use than to differences in fertilizer regimes.

Mineau (1988); Mineau & Collins (1988) Voluntary incident registration yields a lop-sided picture of avian mortality. Although the method may be satisfactory for large, conspicuous birds, it underestimates mortality in smaller species because these are less frequently recovered. Even when better-structured, however, casualty surveys still involve a number of difficulties: the time elapsing between exposure and death may vary widely, the population density and therefore the chance of corpse retrieval is generally low, the birds may die outside the experimental area, etc. Due caution is recommended in interpreting the results of field studies; there are examples of field trials indicating no effects, while practical use later indicates the contrary.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

ALGAE

FIELD TRIAL FOR ALGAE

General

In principle, side-effects on algae (= Phycophyta) can be studied with reference to two groups of algae, each with their own requirements as to experimental conditions (see: Appendix 4.6.2). Studies on phytoplanktonic algae are undertaken in enclosed sections of surface waters. Studies on algae which, under natural conditions, grow on plants (epiphytic algae) or on the stream or lake bed (benthic algae) are usually performed with the aid of an artificial substrate. With these groups of algae, too, compartments are generally created to prevent the test compound from spreading. Side-effect studies are carried out both in naturally occurring waters and in specially created experimental ponds and ditches.

Two approaches to (side-)effect studies can be distinguished. In the first, species are cultivated in cages and on artificial substrates, using the same species employed in laboratory trials, e.g. for phytoplankton, Scenedesmus sp. (see: NEN 6506 or OECD Guideline 201) and for epiphytic plankton, Stigeoclonium sp. (cf. De Vries, 1986). These algae can then be transferred to (experimental) ditches for subsequent evaluation of pesticide impact. In the second approach, the impact is evaluated with reference to the algae occurring naturally in the ditch.

In the Dutch context, the major focus of attention is on the most exposed surface waters, i.e. ditches in agricultural regions. In the EPA protocol (see: Appendix 4.6.1) the impact is mainly evaluated in artificial ponds. In this respect, therefore, our proposal deviates from the EPA protocol.

Quantitatively, epiphytic algae constitute an important group in ditches. Although phytoplanktonic algae are included in the present proposal, the major focus is on the epiphytic algae.

Experimental conditions

Ditches are taken to include the smaller drainage ditches between fields as well as the larger streams into which they flow. In line with the literature, this proposal is based on enclosing part of a ditch by means of impermeable barriers, to prevent the compound from spreading.

It is recommended to perform the trial using crops giving the highest risk of exposure. In general, greatest pesticide drift occurs in fruit growing and sylviculture. When an algae field trial is called for, it will therefore usually be in this setting.

Application

Because the aim of a field trial is to assess the impact of practical spraying operations, the compound should be applied to the field bordering on the ditch. The maximum recommended dosage is applied, as well as four times this amount, the latter simulating a worst case situation in which the fields on both sides of the ditch are sprayed as well as

reflecting variations that are likely to occur during practical spraying operations.

With the described method of application, the ensuing surface-water loading may vary. Weather conditions play a major role; in addition, the exact application procedure is especially important in this trial. Variations in distance to the ditch, e.g. in wedge-shaped fields, may result in relatively large differences in exposure. When performing the trial, therefore, due allowance should be made for weather conditions and application itself should be performed with great accuracy.

The concentration of the compound in the water must be established, either by direct measurement or by adding a marker (e.g. a dye) to the compound. Determination of water concentration is very important for establishing a causal relationship between the presence of the compound and any changes in the algae. If, through unforeseen circumstances, there is no exposure, the compound may be added directly to the water. In this case, a quantity can be added that leads to a calculated PEC (predicted environmental concentration) under practical conditions as well as four times this amount. However, extrapolation of the results to practical spraying conditions will involve a degree of uncertainty.

Observation

Algae and (a)biotic environmental factors

In addition to algal growth and reproduction, a number of physical and chemical parameters are measured. On the one hand, differences in e.g. food availability will lead to differences in algal growth; on the other, pesticide-induced effects on algae can lead to a lower oxygen content. The presence of invertebrates may also have a major impact on the algae. A compound may affect zooplankton or, alternatively, zooplankton predators. In this case, therefore, there may be a major impact on algal grazers. For this reason, the proposed guideline prescribes sampling of invertebrates, at least groupwise and semi-quantitatively.

Compound

The pesticide concentration must be monitored regularly. In the first few days following treatment, frequent measurements should be made (several times daily). This frequency can subsequently be reduced to twice weekly, and further still once the compound is no longer detected.

Proposal: **FIELD TRIAL GUIDELINE FOR ALGAE**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial using algae (= Phycophyta), covering species occurring in the field (i.e. occurring naturally in the ditches selected for study) as well as species employed in laboratory trials. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage and/or a crop in which drift percentages are highest. Several weeks prior to the trial, cultivate the algae occurring in the ditch or the laboratory algae on the substrate.

1.2 Experimental conditions

Perform the trial in ditches bordering on the fields to be sprayed. Field plots are treated with the compound under review and with a compound known to be toxic to algae. An additional plot is treated with water or with another innocuous carrier substance similar to the compound under review. Effects are evaluated in the adjacent ditches.

1.3 Other requirements

The experimental ditches should be representative, at least on a regional scale, i.e. of average dimension and with conventional use and management regimes. Perform the trial under weather conditions favouring exposure of the water body (moderate wind), avoiding extremes of weather (rain, severe drought, hard wind or no wind).

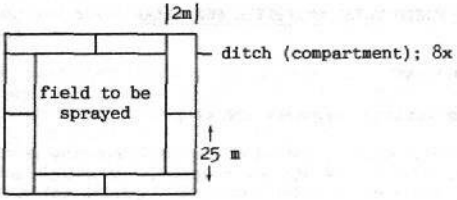
1.4 Experimental design

Plot size

In the experimental ditches, define 25-m compartments, treating the adjacent field with the compound under review.

Plot arrangement

For the plot arrangement, see the figure. The arrangement comprises eight ditch compartments, for a single application. Each treatment requires a single plot. The whole trial is performed in duplo. The proposed plot arrangement is such that, under all weather conditions, there is always maximum exposure in one of the ditches. Other arrangements satisfying the same conditions are also permitted.



Test plot

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 *Dosage*

Apply the maximum recommended dose of the formulation and four times this amount. If measurements show that the water is not exposed, add directly to the ditch the quantity calculated to give the PEC (predicted environmental concentration) ensuing from one and four times the maximum recommended dose.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various plots and duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sample the epiphytes by collecting artificial substrates from each compartment. These substrates can be made of nylon-reinforced PVC, glued to microscope slides. These are held in stainless-steel holders or affixed to a base. Allow them to grow over for several weeks prior to the trial: some with species occurring naturally in the ditch, others with laboratory species.

Sample the naturally occurring phytoplankton species using a test tube (20 cm long, 3 cm diameter); two tubes per sample and three samples per compartment. Sample the epiphytic algae by removing one slide from each compartment. At the same time, sample the naturally occurring fauna (zooplankton and macrofauna). Macrofauna are sampled using a standard net (Beltman, 1983). The quantity of zooplankton can be determined using the samples taken for phytoplankton. Identification down to the species level is not necessary, but must be accurate enough to reveal any changes in the dynamics of algal grazers and their predators.

In each compartment, also place ten cages with phytoplanktonic algae species used in laboratory testing. The cages consist of a tube of gauze (mesh width depending on the species chosen) capped at each end by a lid (diameter 15 cm). The tubes can be drawn out of the water by gripping the top lid, while still leaving the algae submerged in a layer of water. On each sampling date, remove one cage in order to monitor algal growth. During the trial, keep the cages as free as possible from epiphytic algae. If cages threaten to clog up, transfer some of the algae to a new cage.

Record species composition, the abundance of the various species and the chlorophyll a content.

Sample one week prior to treatment, weekly during the first month post-treatment and subsequently two-weekly or monthly. To enable field results to be compared directly with the laboratory results, it is also recommended to sample 48 h post-treatment.

Also sample daily, at fixed times: temperature, dissolved oxygen, total CO_2 content, organic carbon, phosphate, nitrogen, pH, dissolvable CO_2 and conductivity. Measure the pesticide concentration three times daily during the first week post-treatment; subsequently, make these measurements at the same time as biological monitoring is performed. When the compound is no longer detectable, reduce the sampling frequency.

4. VALIDITY

Test results are not valid if results are unclear, or if there is a high observable impact in the blanks or little impact in the positive control. The trial is also invalid in cases where there were already significant differences in the measured parameters between the plots before the trial was started.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
n l	a c') s	f c	hp 4hp")	b t	2	3 x 8	8 - 10

') i.e. chlorophyll content
") if ditch is not exposed: PEC of hp and 4hp

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test org.'s (cult. algae)		1,000
- test plots		1,000
- field equipment		500
2 sampling (4x2x3x8x10)		
- test organisms	40	
- other biotic parameters	10	
- abiotic parameters	10	
3 parameter measurement		
- test org. ident. (1920x)	120	2,000
- other biot. parameters	25	( )
- residue measurements	20	4,000
- abiotic parameters	10	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 245	n.a.
cost estimate (subtotal)	Dfl. 183,750	Dfl. 9,000
overall cost (very rough estimate):		Dfl. 192,500

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.6.1 Review of existing field trial guidelines for algae

United States (EPA, 1982, 1986)
General testing requirements:

- All methods must be scientifically accepted.
- Trial to be performed by trained personnel, with individual accountability for each element of the trial.
- Trial to be performed using active ingredient or formulation of known composition.
- Application method itself must have zero impact on the compound or organism under investigation.
- Healthy organisms to be used.
- No protected or threatened organisms to be used.
- For each treatment or replicate, population size must be such as to provide a 90-95% confidence level for 25% or 50% damage to organisms.
- Control treatments to be performed identically, but without the compound. If a formulation other than water is employed, apply this formulation.
- Wherever possible, employ the commercial method of application.
- Apply the compound in 5 concentrations at evenly spaced, but not more than doubling, intervals. The highest concentration must be in relation to application of the maximum recommended dose to a body of water 15 cm deep.
- Species: one species from each division of algae (incl. Cyanophyta). Algae to be present in approx. the following densities: Chlorophyta: 3000 cells/ml; Chrysophyta (marine): 1000 cells/ml; Cyanobacteria: 1000 cells/ml; Chrysophyta: 3000 cells/ml.
- Cultivate test organisms on a natural substrate and in natural water or on a similar substrate. Artificial light reduction may be necessary to simulate a natural situation. Other natural conditions also to be approximated as closely as possible.
- Perform trial in enclosed or controlled sections of water body or in aquariums, not in open water bodies where the compound can disperse.
- Trial duration: as long as permitted by repeated application according to manufacturer's instructions. Measurements: twice weekly and subsequently until at least 2 weeks after final application.
- Results to be reported: phytotoxicity data, weight, size or other growth parameters, measured environmental concentrations and statistical methods.

Appendix 4.6.2 Review of field studies involving algae

Butcher et al. (1977) Effects of chlorpyrifos in experimental ponds (2.5x1.8x0.6 m deep). Ponds were lined with plastic and contained leaf debris (48 kg dry weight) from woodland pools. After one year, in spring, 3 ponds were treated with increasing concentrations of chlorpyrifos, with 2 ponds serving as controls. For 80 days, a large number of biotic and abiotic parameters were measured daily at 14.00 h. The extent and duration of algal growth were also monitored. An impact was observed: both growth (algal bloom) and species composition of the algal populations were affected. This was an indirect effect: the herbivorous macrofauna were killed. In addition, phosphorus added to the compound was released during detritus decomposition.

Papst & Boyer (1980) In the pools described in Butcher et al. (1977), the effects of temephos and chlorpyrifos were studied. Plankton were collected using a test tube (3 cm diameter, 16.5 cm long). Chlorophyll and pheopigment were monitored. The phytoplankton population increased sharply after treatment, most probably because of the marked depression in the zooplankton population.

Scorgie (1980) A drainage ditch was divided into two sections, one of which was treated with cyanatryn. Algal growth was measured on 12 glass slides in a metal holder. Each week, one slide was removed and the algae counted and identified. Unfortunately, the control section was also contaminated with cyanatryn. No difference in species composition was found; however, in the treated section, the number of organisms was much higher. This was probably due to the higher plants being adversely affected and releasing nutrients as they decomposed. The cyanatryn peak was observed much later than expected.

Caspers & Heckman (1981, 1982); Heckman (1982) A comparison was made of the species composition in orchard drainage ditches, as it was in 1958 and in 1980. The flora was found to have been unaffected by pesticides. With fauna, however, the species composition was very different. The 1958 ditches were very similar to 1980 ditches that had been unexposed to pesticides.

Denoyelles et al. (1982) Six 0.045 ha ponds were refilled with water from a natural spring-fed pool. To enhance the ecological community, fish were introduced. Atrazine was added in a single dose, in 2 different concentrations; 2 ponds served as blanks. Between one week pre-treatment and 63 days post-treatment, daily surveys were performed; subsequently, surveys were monthly until day 136. A variety of physico-chemical parameters was measured. Phytoplankton was sampled with a cylindrical sampler, 2 grabs forming a single sample. From each pond, 3 samples were taken and species composition determined. Algae were concentrated in a sedimentation chamber and counted in one of the 3 samples per pond. Biomass was measured by counting algae in the samples used for chemical analysis. Counts were performed twice, with 7 intervals from 8 to 64 µm in each of the 3 samples per pond. CO_2 production was also measured. Effects on photosynthesis as well as on succession were monitored. Resistance set in after several days.

Goldsborough & Robinson (1983) Effects of simazine and terbutryn on epiphytic algae in the control of aquatic plants. Enclosures made of 1.5 mm thick PVC (240x120 cm) were dug 45 cm into the bottom. Epiphytic algae were grown on acrylic rods (1 m long and 0.62 cm in diameter, notched to obtain good subsamples). Submerged macrophytes were removed. The unformulated active ingredient was added in various concentrations. There were 7 enclosures: 1 blank and 3 different concentrations of each compound. Sampling was carried out 9 days after treatment and then weekly for 5 weeks. At each sampling session, 3 rods were removed and analyzed for chlorophyll a. Three others were used for monitoring CO_2 assimilation. Silicon, ammonium and dissolved oxygen were analyzed daily at 10 cm depth. Oxygen profile, light intensity and pesticide concentration were measured weekly. Serious effects were found. Factor analysis indicated that these were caused by water chemistry, light availability, time and treatment method. The effect is thus not only direct but more complex. However, recovery sets in after one week; the risk of long-term effects occurring after a single application was considered minimal.

Stephenson & Kane (1984) Effects of parathion and linuron on ecosystems in enclosures in a pond 10x5x1 m deep in the UK. Enclosures (polythene, metal frame) were sunk 5 cm into the pool bottom and protruded above water. The diameter of the enclosures was 115 cm, so that they contained about 1 m^3 of water. Three treatments (in duplo): blank, methyl parathion and linuron. On days 0 (treatment) and 28, macrophytic cover was estimated; on day 49, macrophytes were harvested. Zooplankton were sampled before (22, 12, 7, 4 and 2 days) and after (0, 1, 2, 4, 7, 12, 23 and 33 days) treatment at 3 locations in the pond and in the enclosures. Each sample consisted of 2 subsamples taken with 80 cm long tubes (3.5 cm diameter). Samples were sieved and organisms conserved and identified. The water was used for chemical analyses and filtered. The algae left behind on the filter were analyzed for chlorophyll a as a measure of biomass. Algal and plant growth occurred in enclosures that had not been treated with linuron. Water fleas survived only in the untreated controls. Combined with measurements and bio-assays, this is an extremely practicable method.

Herman et al. (1986) Effects of atrazine on lake epiphytic algae in so-called 'limnocorrals': water tight enclosures (5x5x5 m) prepared from nylon-reinforced PVC and also comprising the lake bottom. The top edges were buoyed up by floats, with the lower edges sunk into the bottom. Three enclosures were treated and three served as blanks. They remained in place for one year. Two treatments took place on day 1 (1 June) and day 35. Samples were taken on days 1, 5, 14, 24, 34, 37, 42, 54, 68, 96, 137 and 329. Substrates were inoculated with epiphytic algae 3 weeks prior to treatment; nutrients were added weekly. The substrate consisted of 6.5x4.0 cm PVC strips, glued in pairs to a holder consisting of 3 microscope slides. Four of these holders were tied together and suspended at depths of 0.5, 1.5, 2.5 and 3.5 m. Twelve of these sets were placed in each 'limnocorral', and one harvested on each sampling date. One of the strips was then immediately conserved for identification purposes, the second being used for measuring organic matter and chlorophyll a levels. Assimilation was measured using labelled carbon. The method was adequate for detecting differences in the measured parameters.

Yount & Richter (1986) Effects of pentachlorophenol in artificial streams. To 4 experimental channels 520 m long, with alternating 30.5 m stretches of fast-flowing water and pools, 3 concentrations of pentachlorophenol were added. One channel served as a blank. Using an artificial substrate (ceramic tiles), short-, medium- and long-term effects of various concentrations of pentachlorophenol were investigated. Effects were demonstrated in all experiments.

Fairchild et al. (1987) In three experimental streams, the effects of contaminated sediment were studied. The standing epiphytic algae crop was monitored by measuring chlorophyll a on glass slides. To this end, a perspex holder containing a number of slides was placed for 7 days on the stream bed, parallel to the current. Each week, 3 slides from each holder were replaced and extracted with 90% acetone. Phytoplanktonic algae were collected in brass-mesh nets (0.14 m^2, 364 µm mesh). Effects were detected, especially with the latter method.

Hamilton et al. (1987) Study of the effects of atrazine in enclosures (5x5x5 m) incorporating the lake bottom. Two concentrations were added to 2 different enclosures, with 2 others serving as controls. Algal growth was monitored as follows: nylon-reinforced PVC strips (7.5x2.5 cm) were glued to microscope slides, which were affixed 2 cm apart to bricks (8 per brick). These bricks were left in the lake for 53 days to grow over. At the start of the experiment, the bricks were suspended in the enclosures and sampling consisted of random collection of slides. Three subsamples were taken from each slide: for chlorophyll analysis, radiotracking and counting and identification. One year later, a comparable experiment was performed, in which the slides were affixed to a petri dish. Both methods were found to be satisfactory for detecting effects.

Yasuno et al. (1988) Effects of permethrin on phyto- and zooplankton in enclosures in a (small) lake. Enclosures (1 m diameter and 3.8 m deep) were made from 0.06 mm polythene film and a stainless steel frame. These were driven into the lake bottom to isolate water and sediment. To 2 enclosures, permethrin was added twice; one enclosure served as a blank. On every 3rd or 4th day, water samples were taken over the entire depth using a plastic tube (3 cm diameter). A 100 ml subsample was fixed and analyzed for phytoplankton. Four integral samples were sieved through a 55 µm brass net and conserved in formalin for zooplankton analysis. Other parameters measured: chlorophyll a, photosynthesis activity, assimilation, chlorophyll a sedimentation (in glass jars at 3 m depth), temperature, pH, dissolved oxygen and transparency. Residues in water and sediment were also measured. In the first application, the compound was fed to the bottom through a tube; the second application was to the water surface. No effects on photosynthesis were found. Of the species monitored, only Ceratium hirundinella was affected by permethrin. Water fleas were severely affected, especially after application directly to the water surface, but appeared able to recover well because their predators also virtually disappear after this kind of treatment.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

MIDGE LARVAE

FIELD TRIAL FOR MIDGE LARVAE

General

For trials in the benthic environment, i.e. stream and lake beds, trials with such commonly occurring organisms as tubifex (= Tubificidae) or midge larvae (= Chironomidae) would appear to be the best choice. Laboratory trials are currently being developed for both groups; for chironomids, for example, breeding guidelines are already available (pers. comm. Van Urk). Tubificidae have the advantage of being bottom-living and-feeding organisms; exposure via the skin may moreover be more direct and therefore have a greater impact than in the case of chironomid larvae. With the latter organisms, however, sublethal effects have been observed in the field (Van Urk & Kerkum, 1986). It is still debatable whether field experiments can yield additional information; DBW/RIZA hope to be able to provide a provisional answer to this question by the end of 1990 (pers. comm. Van Urk).

Effects generally occur sooner in insect larvae than in adults. In bioassays, too, midge larvae were found to react to pesticides (Heinis & Crommentuijn, 1988). In addition, a number of studies have demonstrated effects in midge larvae but not in tubificidae (see: Appendix 4.7.2: Grzenda et al., 1962; Crossland, 1984). We have therefore opted to develop a trial using midge larvae. In view of the differences in exposure and diet, however, it still remains desirable to develop a guideline for Tubificidae, too. Apart from the efforts of DBW/RIZA, very little field work has been carried out to date with midge larvae. The following guideline proposal should therefore be considered provisional.

In order to achieve a minimum of ambiguity in the results, two approaches have been taken: i) the midge larvae occurring naturally in the ditch are sampled, and ii) the midge larvae are introduced into the ditch in containers. The latter approach allows for comparison with the laboratory situation.

From Van Urk (pers. comm.) we received comments on the draft version of the proposed midge larvae trial guideline. From these comments, we reproduce in its entirety the section on "pitfalls in working with midge larvae", which gives a good indication of the type of problems likely to occur in the field:

- Chironomid development is often highly synchronized. This means that the majority of a population fly out in the same period, resulting in an enormous reduction in the number of larvae (e.g. by a factor 10 in one month). Chironomids also have a great capacity for colonization, in turn allowing large numbers of larvae to develop in a very short period of time. Consequently, fluctuations in numbers can only be properly interpreted if there is due knowledge of the life cycle of the species in question.
- Laboratory testing has shown that the youngest larval stages (1st and 2nd stage) are most sensitive to toxic agents. It is these stages that should therefore be studied in the field. However, they are only short-lived and consequently not always available for study. Moreover, the initial stages live as plankton and may pass through fine-mesh netting. This means that experiments are only practicable in sealed

aquariums; in cages of netting, the larvae emerging from the deposited eggs can readily escape, while other 1st stage larvae can readily enter. At DBW/RIZA, specially designed units are being developed for use in a project focussing on larger bodies of fresh water.

- Finally, Van Urk notes that the proposed experimental design does not consider any effects of pesticide application on the adult stages of the life cycle of aquatic insects. With chironomids, this may be justifiable, because populations have an over-capacity for colonization: no habitats suitable for larvae remain unoccupied, because there are no adults. For other insect groups, though, the situation may be completely different (e.g. dragonflies).

Our overall reaction to these comments on the proposed midge larvae trial is once again to emphasize its provisional character and to state that a trial of this kind may, in practice, still prove unfeasible. More in detail, our reaction is that for further development of a midge larvae trial: i) knowledge of the life cycles of the most relevant species must be improved in relation to population dynamics, and ii) for the time being, any study of initial larval stages should focus on 'naturally' occurring insects.

Experimental conditions

In the Dutch context, the prime concern is for those bodies of fresh water suffering greatest exposure, i.e. ditches in agricultural regions. These are taken to include the smaller drainage ditches between fields as well as the larger streams into which they flow. This proposal is based on enclosing part of a ditch by means of impermeable barriers, to prevent the compound from spreading.

It is recommended to perform the trial using crops giving the highest risk of exposure. In general, greatest pesticide drift occurs in fruit growing and sylviculture. When an midge larvae field trial is called for, it will therefore usually be in this setting.

Application

Because the aim of a field trial is to assess the impact of practical spraying operations, the compound should be applied to the field bordering on the ditch. The maximum recommended dosage is applied, as well as four times this amount, the latter simulating a worst case situation in which the fields on both sides of the ditch are sprayed as well as reflecting variations that are likely to occur during practical spraying operations.

With the described method of application, the ensuing surface-water loading may vary. Weather conditions play a major role; in addition, the exact application procedure is especially important in this trial. Variations in distance to the ditch, e.g. in wedge-shaped fields, may result in relatively large differences in exposure. When performing the trial, therefore, due allowance should be made for weather conditions and application itself should be performed with great accuracy.

The concentration of the compound in the water must be established, either by direct measurement or by adding a marker (e.g. a dye) to the compound. Determination of water concentration is very important for establishing a causal relationship between the presence of the compound and any changes in the midge larvae. If, through unforeseen circumstances, there is no exposure, the compound may be added directly to the water. In this case, a quantity can be added that leads to a calculated PEC (predicted environmental concentration) under practical conditions as well as four times this amount. However, extrapolation of the results to practical spraying conditions will involve a degree of uncertainty.

Observation

Midge larvae and (a)biotic environmental factors

The abundance, species composition and development of the midge larvae on the ditch bottom must be monitored. When they fly out, the adult midges may also be trapped for study. By placing containers in the ditch, the percentage of emerging larvae can be determined and development closely monitored. Besides midge larvae growth and development, a number of physical, chemical and biotic parameters are also monitored. On the one hand, differences in e.g. food availability will lead to differences in larval growth, while on the other, differences in midge larvae predators may also occur. The proposed guideline therefore prescribes sampling of invertebrates, at least groupwise and semi-quantitatively. Fish, too, should be observed.

As more becomes known about the species met with in practice, sampling of the natural populations requires further refinement. One factor that must be taken into due account is the time at which the compound is applied (pers. comm. Van Urk). The complexity of the chironomid life cycle is a given fact, multiplying the number of variants in terms of relevant environmental factors as well as sampling protocols. DBW/RIZA, for instance, is still developing an ideal sampling programme for ditches and other small freshwater streams; for Chironomus plumosus in larger bodies of water, there is already reasonable knowledge of how a sampling programme should be implemented (see: Kerkum & Van Urk, 1989).

With respect to sampling frequency, Van Urk advised us (pers. comm.) as follows: experience has shown that 10-20 samples are required to establish an interval of + or - 50% with 95% confidence; with a density of 10 individuals per grab sample, for example, the 95% confidence level is: lower limit, 5 individuals and upper limit, 17 individuals. In practice, this means that there is a significant reduction in density if the number of individuals per grab sample falls below five. The total of 10-20 grabs is based on experience in fairly homogeneous environments. However, ditches are often extremely heterogeneous, and even with 20 samples an interval of + or - 50% may not even be achieved. Anything up to 40-50 grabs may then be required. Because the sampling operation itself entails habitat disturbance, when repeated, such a lengthy sampling series also sets demands on minimum compartment size in the experimental ditch, which in this case should be at least 50 m.

Compound

The pesticide concentration must be monitored regularly. In the first few days following treatment, frequent measurements should be made (several times daily). This frequency can subsequently be reduced to twice weekly, and further still once the compound is no longer detected.

Proposal: **FIELD TRIAL GUIDELINE FOR MIDGE LARVAE**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial with midge larvae (= Chironomidae) occurring in the ditch bottoms under investigation and used in the laboratory. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage and resulting in the highest percentage drift.

1.2 Experimental conditions

Perform the trial in ditches bordering on the fields to be sprayed. Field plots are treated with the compound under review and with a compound known to be toxic to midge larvae. An additional plot is treated with water or with another innocuous carrier substance similar to the compound under review. Effects are evaluated in the adjacent ditches.

1.3 Other requirements

The experimental ditches should be representative, at least on a regional scale, i.e. of average dimension and with conventional use and management regimes. Perform the trial under weather conditions favouring exposure of the water body (moderate wind), avoiding extremes of weather (rain, severe drought, hard wind or no wind).

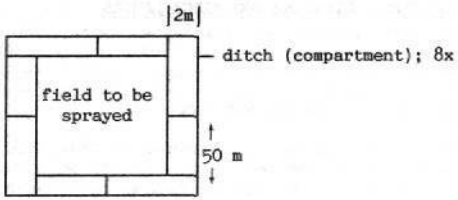
1.4 Experimental design

Plot size

In the experimental ditches, define 50-m compartments (the relatively large number of samples implies a larger minimum compartment size), treating the adjacent field with the compound under review.

Plot arrangement

For the plot arrangement, see the figure. The arrangement comprises eight ditch compartments, for a single application. Each treatment requires a single plot. The whole trial is performed in duplo. The proposed plot arrangement is such that, under all weather conditions, there is always maximum exposure in one of the ditches. Other arrangements satisfying the same conditions are also permitted.



Test plot

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. If measurements show that the water is not exposed, add directly to the ditch the quantity calculated to give the PEC (predicted environmental concentration) ensuing from one and four times the maximum recommended dose.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sample the midge larvae occurring naturally in the ditch bottom using a mud sampler. Take at least five samples from each compartment, or as many are required to estimate midge larvae abundance with 95% confidence. In this respect, seek a balance between (maximum) sampling frequency and (minimum) disturbance of the ditch bottom. Carry out sampling several days prior to treatment and once weekly after treatment. Record species composition and the abundance of the various species and any deformities.

In each compartment, place five containers with eggs and five with midge larvae. The containers should measure 50x50 cm and contain bottom mud, preferably from the ditches under investigation. To the larvae container attach a cylinder of netting/gauze protruding above the surface of the water. To the netting, affix a midge trap to trap midges flying out. The containers should be partitioned into 10x10 cm sections. At each sampling date, harvest one section to assess development. In their initial stages, larvae live as plankton and pass readily through netting. Therefore, when emergence is anticipated, seal the container with eggs using a glass cylinder; this can be removed when the larvae have grown larger and re-established themselves in the bottom.

Monitor abundance, biomass, any (jaw) deformities and development of the midge larvae; development is taken to include the percentage of successful eggs and the percentage of adult midges flying out.

If ecological effects are suspected, the algae or macrofauna may also be sampled. For the former, the reader is referred to the proposed algae trial. The macrofauna and fish population can be sampled using a standard net (Beltman, 1983). Identification down to the species level is not necessary, but must be accurate enough to reveal any major changes in the dynamics of midge larvae predators.

Also sample daily, at fixed times: temperature, dissolved oxygen, total CO_2 content, organic carbon, phosphate, nitrogen, pH, dissolvable CO_2 and conductivity. Measure the pesticide concentration three times daily during the first week post-treatment; subsequently, make these measurements at the same time as biological monitoring is performed. When the compound is no longer detectable, reduce the sampling frequency.

4. VALIDITY

Test results are not valid if results are unclear, or if there is high mortality in the blanks or little mortality in the positive control. The trial is also invalid in cases where there were already significant differences in the measured parameters between the plots before the trial was started.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
l n	a b c r	f c	hp 4hp')	b t	2	8	5 10

') if ditch is not exposed: PEC of hp and 4hp

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		1,000
- test plots		1,000
- field equipment		500
2 sampling (4x2x8x5x5)ples)		
- test organisms	20	
- other biotic parameters	5	
- abiotic parameters	10	
3 parameter measurement		( )
- test organisms (1600x)	50	2,000
- other biotic parameters	10	( )
- residue measurements	20	4,000
- abiotic parameters	10	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 145	n.a.
cost estimate (subtotal)	Dfl. 108,750	Dfl. 9,000
overall cost (very rough estimate):		Dfl. 117,500

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.7.1 **Review of existing field trial guidelines for midge larvae**

Concrete guidelines for field trials with aquatic organisms were not found. Field trials were mentioned in several procedures, however:

United States (EPA, 1982) A field study is desirable if the existence of a hazard is indicated by other tests. For procedures, reference is made to several handbooks and specific studies that 'can provide useful background information for conducting a simulated or actual field study ...'.

United Kingdom (MAFF, 1986) Working Document 7.1 merely mentions that field trials with aquatic invertebrates may be necessary under certain conditions.

OECD (1987) Indicates that field studies may have an important role to play. Possibilities mentioned include the introduction of artificial substrates and caged organisms. At the same time, the limitations of field studies are recognized.

Appendix 4.7.2 Review of field studies involving midge larvae

Grzenda et al. (1962) Study of the effects on pond fauna in a peach orchard; parathion, particularly, is used in large quantities in peach growing. There was no untreated control. In addition to a number of physico-chemical parameters, pesticide residues were extensively monitored. The bottom fauna was collected using an Ekman dredge: 15 grabs per sampling session. Zooplankton was sampled with a 3-l flask; a total of 15-46 l was sampled and filtered in the field using a plankton net. Significant differences pre- and post-treatment were found for insects and midge larvae; this was in contrast to results for oligochaeta. In terms of numbers and species composition, no effects were found on zooplankton. The impact on fish was considered debatable: one species, a large-mouth black bass Micropterus salmoides (introduced to the pond), disappeared, while a sunfish Lepomis macrochirus survived. On the basis of laboratory tests, no unambiguous conclusion could be drawn about the role of parathion.

Crossland (1984) Three experimental ponds were exposed to methyl parathion, with three serving as controls. For methods, see Crossland & Hillaby (1985). The macrofauna was sampled by drawing a net 5 metres through the water, 3 times for each sample. The bottom fauna was sampled by 'vacuuming' an area of 20x20 cm, 5 samples per pond. Rainbow trout Salmo gairdneri were bred prior to the experiment. 25 fish (4.4-4.9 g) were introduced into each pond. After 11 weeks, an electric shock was administered to sample and weigh the fish. As was anticipated from laboratory tests, water fleas and copepods (Cyclops) were severely affected by parathion, in contrast to fish. Other invertebrates were also affected. Recovery set in, however, and after 10 weeks there was little difference from the controls. With regard to the bottom fauna, effects on midge larvae were found, but snails and oligochaetes were unaffected. Fish grew better in the untreated ponds. Because no toxic effect was found in the laboratory, this was ascribed to a dietary cause.

Zischke et al. (1985) In experimental channels, it was investigated whether a toxic limit concentration calculated on the basis of laboratory data adequately protects the aquatic biocoenose. One channel was exposed to the calculated level, one to 3 times and one to 9 times this level; one served as a blank. The channels were 520 m long and made up of 30.5 m mud-bottomed pools alternating with 30.5 m gravel-bottomed, faster-flowing runs. To these channels, river water was added that was continually contaminated with pentachlorophenol. Grids were placed between the compartments to keep the fish in place. The water was sampled twice weekly and analyzed for pesticide. The following parameters were also monitored: dissolved oxygen, pH, temperature, alkalinity, hardness, ortho- and total phosphate, ammonia, total nitrogen and BOD. Traps were used to sample micro-invertebrates; these were set in the evening and removed in the morning, the organisms being trapped during nocturnal migration. Benthic invertebrates were sampled with the aid of steel containers (0.02 m^2) filled with channel substrate and let into the channel bottom. Invertebrate drift (dead or week organisms carried by the stream) was also sampled. Reproduction of the snail Physa gyrina was studied by counting the egg capsules deposited on an artificial substrate. Differences were assessed by means of variance analysis (significan-

ce: < 0.05). About 450 rock-basses Pimephales promelas and about 55 sunfish Lepomis macrochirus were introduced into the channels, some of which had been anaesthetized beforehand and weighed. Fifteen of the latter species were placed in a cage (1.3x0.9x0.9 m). Three times weekly, the number of dead fish was counted. The growth of 50 of the former species was monitored weekly. Egg deposition was monitored 3 times weekly by sampling 25% of 112 artificial substrates. Larvae were also sampled by monitoring the drift. At the end of the season, all fish were removed, counted and measured; 10% were weighed. Micro-invertebrates (including chironomids and water fleas) were found to be affected by the middle concentration, with greatest abundance found in the control. Snails were severely affected by the highest concentration; under these conditions, there was also fish mortality; however, effects were also found at other concentrations. It was thus concluded that the calculated limit concentration did not provide adequate protection of the aquatic biocoenose.

Stephenson & Mackie (1986) In 6 experimental ponds (10x20x2.5 m), the effect of macrophyte controlling with 2,4-D on benthic invertebrates was studied. Four pools were treated with 2,4-D (two forms), with 2 serving as controls. The study focussed on ecological effects, viz. those resulting from decomposition of macrophytes. The invertebrate fauna was sampled by divers using mud samplers (diameter 7.6 cm), 9 per pond. Samples were taken 1 day before treatment and 29, 63, 91, 126 and 338 days post-treatment. The samples were sieved (0.26 mm mesh) and then sorted in a white bowl. Effects were monitored by three means: i) using Ecological Community Analysis, the species were expressed as ecological entities (feeding habits, environmental preference, etc.); variance analysis was then employed to assess whether the populations subjected to different treatments showed any differences in these ecological characteristics; ii) cluster analysis was used to assess any differences in species disappearance characteristics; iii) for each subsample, a diversity index was prepared and variance analysis employed to detect differences between treatments. No direct toxic effects were found. In the longer term (338 days), the diversity of the treated ponds was significantly lower than in the untreated ponds: in the former, Tubificidae predominated, in the latter, Chironomidae, Gastropoda and Tubificidae.

Van Urk & Kerkum (1986) Study of jaw deformities in midge larvae in 19 medium-sized Dutch surface waters. Deformities were found to be positively correlated with contaminant levels in the benthic habitat.

Ali et al. (1988) Control of citrus rust mite in citrus orchards with diflubenzuron and side-effects on water organisms. Effects were studied in two lakes: one 2 ha lake with a shallow (1 m) and a deep (2-3 m) end, bordering directly on the orchard and one shallow (1 m) 1 ha lake that was not exposed. Motivation: diflubenzuron is also used to control midge larvae and is extremely toxic to a number of aquatic organisms. The lakes were sampled 4 and 1 days pre-treatment and 1, 3, 7, 14, 28 and 56 days post-treatment. In the exposed lake, 8 bottom samples were taken at the shallow end and 6 at the deep end; 7 and 4 zooplankton samples were taken, respectively. In the control lake, 6 bottom samples and 4 zooplankton samples were taken. The bottom samples (along 40 cm) were taken with a nylon net (20x20 cm, 55 cm long, 0.5 mm mesh). At the deep end, an Ekman dredge (15x15x15 cm) was used; the bottom samples were filtered

through a 0.5 mm filter, sorted in a white bowl, conserved and identified. The zooplankton was sampled in a shallow area using a 50 cm long conical net (20 cm opening, 125 µm mesh) over a length of 6 m. Samples from the deep end were taken with a plankton net drawn behind a boat. No significant differences were found, probably because of the extremely low levels in the water.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

WATER SNAILS

FIELD TRIAL FOR WATER SNAILS

General

Water snails (belonging to the Gastropoda) have been included in this series of field trials because of their role as herbivores in the aquatic ecosystem (see: Chapter 3 of the main report). The field trial focusses on water snails that live on aquatic plants. As very little field research has been performed on water snails, however, this field trial proposal is therefore of a very provisional character.

In order to achieve a minimum of ambiguity in the results, two approaches have been taken: i) the water snails occurring naturally in the ditch are sampled, and ii) caged water snails are introduced into the ditch, e.g. the Great Pond Snail Lymnea stagnalis. The latter approach can build on the experience of VU (Free University of Amsterdam) and TNO-MT (Netherlands Institute of Applied Scientific Research, Division of Technology for Society)(pers. comm. Scholten), involving use of an (artificial) substrate on which eggs can be laid, either in the field or in the laboratory, for transfer to the field.

In the Dutch context, the major focus of attention is on the most exposed surface waters, i.e. ditches in agricultural regions.

Experimental conditions

Ditches are taken to include the smaller drainage ditches between fields as well as the larger streams into which they flow. In line with the literature, this proposal is based on enclosing part of a ditch by means of impermeable barriers, to prevent the compound from spreading.

It is recommended to perform the trial using crops giving the highest risk of exposure. In general, greatest pesticide drift occurs in fruit growing and sylviculture. When a water snail field trial is called for, it will therefore usually be in this setting.

Application

Because the aim of a field trial is to assess the impact of practical spraying operations, the compound should be applied to the field bordering on the ditch. The maximum recommended dosage is applied, as well as four times this amount, the latter simulating a worst case situation in which the fields on both sides of the ditch are sprayed as well as reflecting variations that are likely to occur during practical spraying operations.

With the described method of application, the ensuing surface-water loading may vary. Weather conditions play a major role; in addition, the exact application procedure is especially important in this trial. Variations in distance to the ditch, e.g. in wedge-shaped fields, may result in relatively large differences in exposure. When performing the

trial, therefore, due allowance should be made for weather conditions and application itself should be performed with great accuracy.

The concentration of the compound in the water must be established, either by direct measurement or by adding a marker (e.g. a dye) to the compound. Determination of water concentration is very important for establishing a causal relationship between the presence of the compound and any changes in the water snails. If, through unforeseen circumstances, there is no exposure, the compound may be added directly to the water. In this case, a quantity can be added that leads to a calculated PEC (predicted environmental concentration) under practical conditions as well as four times this amount. However, extrapolation of the results to practical spraying conditions will involve a degree of uncertainty.

Observation

Water snails and (a)biotic environmental factors

The abundance and the species composition of the water snails must be monitored. It should be noted that a sampling procedure can often only be optimized for a single species. In the process of species-specific optimization, information on other species may be lost, due to differences in size, depth and life cycle (pers. comm. Van Urk).

Artificial substrates may also be placed in the ditch, enabling egg laying and development to be observed. Alternatively, caged water snails may be placed in the ditch. These cages should contain some of the ditch vegetation as a food supply for the snails. Egg laying and development can also be observed in these cages. If so desired, egg capsules can also be introduced into the cages.

Besides water snail growth and development, a number of physical and chemical parameters are also monitored. On the one hand, differences in e.g. food availability will lead to differences in snail growth, while on the other, differences in snail predation may also occur. The proposed guideline therefore prescribes sampling of invertebrates, at least groupwise and semi-quantitatively and a quatitative sampling of algae and macrophytes.

Compound

The pesticide concentration must be monitored regularly. In the first few days following treatment, frequent measurements should be made (several times daily). This frequency can subsequently be reduced to twice weekly, and further still once the compound is no longer detected.

Proposal: **FIELD TRIAL GUIDELINE FOR WATER SNAILS**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial with water snails (belonging to the Gastropoda) occurring naturally on the ditch bottoms under investigation as well as introduced there. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage and resulting in the highest percentage drift.

1.2 Experimental conditions

Perform the trial in ditches bordering on the fields to be sprayed. Field plots are treated with the compound under review and with a compound known to be toxic to water snails. An additional plot is treated with water or with another innocuous carrier substance similar to the compound under review. Effects are evaluated in the adjacent ditches.

1.3 Other requirements

The experimental ditches should be representative, at least on a regional scale, i.e. of average dimension and with conventional use and management regimes. Perform the trial under weather conditions favouring exposure of the water body (moderate wind), avoiding extremes of weather (rain, severe drought, hard wind or no wind).

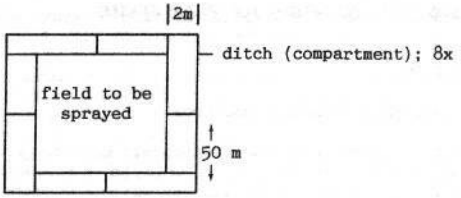
1.4 Experimental design

Plot size

In the experimental ditches, define 50-m compartments (25 m being considered too small; pers. comm. Van Urk), treating the adjacent field with the compound under review.

Plot arrangement

For the plot arrangement, see the figure. The arrangement comprises eight ditch compartments, for a single application. Each treatment requires a single plot. The whole trial is performed in duplo. The proposed plot arrangement is such that, under all weather conditions, there is always maximum exposure in one of the ditches. Other arrangements satisfying the same conditions are also permitted.



Test plot

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. If measurements show that the water is not exposed, add directly to the ditch the quantity calculated to give the PEC (predicted environmental concentration) ensuing from one and four times the maximum recommended dose.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sample the water snails occurring naturally in each compartment of the ditch with a net. Also harvest a fixed quantity of vegetation; use this to determine the numbers and species composition of the snail population. From each compartment take as many samples as are required to estimate water snail abundance with 95% confidence. In this respect, seek a balance between (maximum) sampling frequency and (minimum) disturbance of the ditch. If necessary, snail abundance in the ditch can be artificially increased by introducing laboratory-bred snails (which should be tagged to distinguish them from the naturally occurring snails and to enable the

distance moved to be assessed, as a measure of snail activity). Growth can also be monitored by measuring/weighing snail shells.

Carry out sampling several days prior to treatment and once weekly after treatment. Record species composition and the abundance of the various species and any anomalies in behaviour or development.

In each compartment, place five cages with 20 egg capsules and five cages with 100 adult water snails (e.g. L. stagnalis). The cages should measure 50x50 cm and be as deep as permitted by ditch depth. Seal the bottom of the cages with very fine gauze and place the cages on the ditch bottom, after introducing water plants (without snails) from the ditch as well as the artificial substrates. Sample the snails by counting the number of snails on the substrate; also monitor egg laying. In the cages with egg capsules, also monitor development of eggs and juveniles on the substrates.

If ecological effects are suspected, the epiphytic algae or macrofauna may also be sampled. For the former, the reader is referred to the proposed algae trial. The macrofauna can be sampled using a standard net (Beltman, 1983). Identification down to the species level is not necessary, but must be accurate enough to reveal any major changes in the dynamics of water snail predators and parasites.

Also sample daily, at fixed times: temperature, dissolved oxygen, total CO_2 content, organic carbon, phosphate, nitrogen, pH, dissolvable CO_2 and conductivity. Measure the pesticide concentration three times daily during the first week post-treatment; subsequently, make these measurements at the same time as biological monitoring is performed. When the compound is no longer detectable, reduce the sampling frequency.

4. VALIDITY

The trial need only be repeated if the results are unclear, or if there is high mortality in the blanks or little mortality in the positive control. The trial is invalid in cases where there were already significant differences in the measured parameters between the plots before the trial was started.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
l n	a h r s	f c	hp 4hp')	b t	2	8	5 x 5

') if ditch is not exposed: PEC of hp and 4hp

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		1,000
- test plots		1,000
- field equipment		500
2 sampling (4x2x8x5x15)		
- test organisms	25	
- other biotic parameters	10	
- abiotic parameters	10	
3 parameter measurement		( )
- test organisms (4800x)	150	4,000
- other biotic parameters	30	( )
- residue measurements	20	4,000
- abiotic parameters	10	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 275	n.a.
cost estimate (subtotal)	Dfl. 206,250	Dfl. 11,000
overall cost (very rough estimate):		Dfl. 217,500

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.8.1 **Review of existing field trial guidelines for water snails**

Concrete guidelines for field trials with aquatic organisms were not found. Field trials were mentioned in several procedures, however:

United States (EPA, 1982) A field study is desirable if the existence of a hazard is indicated by other tests. For procedures, reference is made to several handbooks and specific studies that 'can provide useful background information for conducting a simulated or actual field study ...'.

United Kingdom (MAFF, 1986) Working Document 7.1 merely mentions that field trials with aquatic invertebrates may be necessary under certain conditions.

OECD (1987) Indicates that field studies may have an important role to play. Possibilities mentioned include the introduction of artificial substrates and caged organisms. At the same time, the limitations of field studies are recognized.

Appendix 4.8.2 Review of field studies involving water snails

Harman (1976) The shallow parts of a lake (94 ha, max. 13 m deep) were treated with simazine to control algal bloom. Residues of other chemicals ($CuSO_4$ and diquat) were probably also present. An enclosed section served as a control. Side-effects on the benthic fauna were studied. Samples were taken with a mud samplers; water snails were sampled by divers. To this end, a 0.5 m^2 frame was placed on the lake bottom from which all snails were collected. There was high mortality in the snail Goniobasis livescens. The population recovered only in the spring of the following year. The benthic snail Viviparus georgianus was virtually eliminated. Foetuses were aborted and virtually all snails died within 2 days. Because this species does not usually continue to reproduce later in the year, recovery only set in the following year. Laboratory experiments had not indicated that the water snails were sensitive to the anticipated concentrations of simazine. The effects were possibly due to combined action, viz. the toxic effects on water snails and algae.

Zischke et al. (1985) In experimental channels, it was investigated whether a toxic limit concentration calculated on the basis of laboratory data adequately protects the aquatic biocoenose. One channel was exposed to the calculated level, one to 3 times and one to 9 times this level; one served as a blank. The channels were 520 m long and made up of 30.5 m mud-bottomed pools alternating with 30.5 m gravel-bottomed, faster-flowing runs. To these channels, river water was added that was continually contaminated with pentachlorophenol. Grids were placed between the compartments to keep the fish in place. The water was sampled twice weekly and analyzed for pesticide. The following parameters were also monitored: dissolved oxygen, pH, temperature, alkalinity, hardness, ortho- and total phosphate, ammonia, total nitrogen and BOD. Traps were used to sample micro-invertebrates; these were set in the evening and removed in the morning, the organisms being trapped during nocturnal migration. Benthic invertebrates were sampled with the aid of steel containers (0.02 m^2) filled with channel substrate and let into the channel bottom. Invertebrate drift was also sampled. Reproduction of the snail Physa gyrina was studied by counting the egg capsules deposited on an artificial substrate. Differences were assessed by means of variance analysis (significance: < 0.05). Two species of fish were introduced into the channels, some of which had been anaesthetized beforehand and weighed. Some of the fish were placed in a cage (1.3x0.9x0.9 m). Three times weekly, the number of dead fish was counted. The growth of 50 fish was monitored weekly. Egg deposition was monitored 3 times weekly by sampling 25% of 112 artificial substrates. Larvae were also sampled by monitoring the drift. At the end of the season, all fish were removed, counted and measured; 10% were weighed. Micro-invertebrates (including chironomids and water fleas) were found to be affected by the middle concentration, with greatest abundance found in the control. Snails were severely affected by the highest concentration; under these conditions, there was also fish mortality; however, effects were also found at other concentrations. It was thus concluded that the calculated limit concentration did not provide adequate protection of the aquatic biocoenose.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

WATER FLEAS

FIELD TRIAL FOR WATER FLEAS

General

Various publications (e.g. Zischke et al., 1985; Von Peichl et al., 1984) have indicated that the field effects of pesticides cannot always be predicted by laboratory trials. In part, this is because any impact other than direct toxic effects cannot be detected during short-term toxicity tests and not all species can be studied in the laboratory setting. In the field, moreover, secondary (ecological) effects occur that are unpredictable (Crossland, 1983). The desirability of field studies under certain conditions is also confirmed in existing procedures (see: Appendix 4.9.1). With regard to water fleas, however, no concrete field trial proposals have yet been suggested.

Side-effects on water fleas (= Cladocera) can be studied in two ways (see: Appendix 4.9.2): either the water fleas occurring naturally in the water are sampled, or caged water fleas are introduced. In the Dutch context, both approaches should be followed: i) water fleas are introduced into the water in cages of netting (for comparison with laboratory tests, it has been opted to use the same species as for the laboratory tests) and ii) effects on the naturally occurring species are monitored outside the cages.

Studies have been performed in natural waters as well as in specially designed experimental ponds and so on. In the Dutch context, we are most concerned with the most exposed surface waters, i.e. ditches in agricultural regions.

Experimental conditions

The main category of ditch we are concerned with are the smaller drainage ditches between fields. This proposal is based on enclosing part of a ditch by means of impermeable barriers, to prevent the compound from spreading. This implies some disturbance of the natural situation: the circulation pattern is discontinued. However, since no dilution or removal of the compound can occur, a worst-case situation is thus created.

It is recommended to perform the trial using crops giving the highest risk of exposure. In general, greatest pesticide drift occurs in fruit growing and sylviculture. When a water flea field trial is called for, it will therefore usually be in this setting.

Application

Because the aim of a field trial is to assess the impact of practical spraying operations, the compound should be applied to the field bordering on the ditch. The maximum recommended dosage is applied, as well as four times this amount, the latter simulating a worst case situation in which the fields on both sides of the ditch are sprayed as well as

reflecting variations that are likely to occur during practical spraying operations.

With the described method of application, the ensuing surface-water loading may vary. Weather conditions play a major role; in addition, the exact application procedure is especially important in this trial. Variations in distance to the ditch, e.g. in wedge-shaped fields, may result in relatively large differences in exposure. When performing the trial, therefore, due allowance should be made for weather conditions and application itself should be performed with great accuracy.

The concentration of the compound in the water must be established, either by direct measurement or by adding a marker (e.g. a dye) to the compound. Determination of water concentration is very important for establishing a causal relationship between the presence of the compound and any changes in the water fleas. If, through unforeseen circumstances, there is no exposure, the compound may be added directly to the water. In this case, a quantity can be added that leads to a calculated PEC (predicted environmental concentration) under practical conditions as well as four times this amount. However, extrapolation of the results to practical spraying conditions will involve a degree of uncertainty.

Observation

Water fleas and (a)biotic environmental factors

Besides water flea growth and development, a number of physical and chemical parameters are also monitored. On the one hand, differences in e.g. food availability will lead to differences in water flea growth; on the other hand, changes in the water flea population may lead to algal bloom, for instance. The invertebrate and fish population may have a major influence on the water fleas. A compound may influence predation dynamics. The proposed guideline therefore prescribes sampling of invertebrates, at least groupwise and semi-quantitatively. Depending on the nature of the fish population living in the ditches being studied, a decision will have to be taken as to whether the fish must be removed from or allowed to remain in the experimental compartments.

Compound

The pesticide concentration must be monitored regularly. In the first few days following treatment, frequent measurements should be made (several times daily). This frequency can subsequently be reduced to twice weekly, and further still once the compound is no longer detected.

Proposal: **FIELD TRIAL GUIDELINE FOR WATER FLEAS**

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial with water fleas (= Cladocera) occurring naturally in the experimental ditches as well as a species used in the laboratory, preferably Daphnia magna. The selected ditches must always contain a number of species other than D. magna. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage and resulting in the highest percentage drift.

1.2 Experimental conditions

Perform the trial in ditches bordering on the fields to be sprayed. Field plots are treated with the compound under review and with a compound known to be toxic to water fleas. An additional plot is treated with water or with another innocuous carrier substance similar to the compound under review. Effects are evaluated in the adjacent ditches.

1.3 Other requirements

The experimental ditches should be representative, at least on a regional scale, i.e. of average dimension and with conventional use and management regimes. Perform the trial under weather conditions favouring exposure of the water body (moderate wind), avoiding extremes of weather (rain, severe drought, hard wind or no wind).

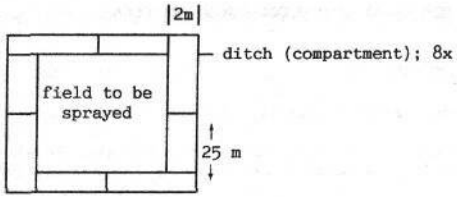
1.4 Experimental design

Plot size

In the experimental ditches, define 25-m compartments, treating the adjacent field with the compound under review.

Plot arrangement

For the plot arrangement, see the figure. The arrangement comprises eight ditch compartments, for a single application. Each treatment requires a single plot. The whole trial is performed in duplo. The proposed plot arrangement is such that, under all weather conditions, there is always maximum exposure in one of the ditches. Other arrangements satisfying the same conditions are also permitted.



Test plot

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. If measurements show that the water is not exposed, add directly to the ditch the quantity calculated to give the PEC (predicted environmental concentration) ensuing from one and four times the maximum recommended dose.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

Sample the naturally occurring water fleas in each ditch compartment using a perspex cylinder with a diameter of 30 cm and a length of 30 cm, or as long as is required to take samples just above the ditch bottom. Compensate for any differences in volume thus occurring. From each compartment, take at least 5 samples or as many as are required to estimate water flea abundance with 95% confidence. Record species composition and the abundance of the several species. Also monitor population dynamics: differences in various size categories, percentages of egg-bearing individuals and brood size.

In each compartment, place 10 cages each containing 10 water fleas, preferably including individuals of different ages. The cages should consist of a tube of gauze (mesh depending on species chosen) capped at each end by a lid (diameter 15 cm). The tubes can be drawn out of the water by gripping the top lid, while still leaving the water fleas submerged in a layer of water. Survival and development can thus be monitored.

Carry out sampling twice during the week prior to treatment, one day after treatment, twice weekly in the first month post-treatment and then once weekly. If ecological effects are suspected, algae and macrofauna may also be sampled. For the former, the reader is referred to the proposed algae trial. As a minimum, measure the chlorophyll a level daily, as a measure of the food supply of the water fleas. Assess the phytoplankton population once weekly. The macrofauna can be sampled using a standard net (Beltman, 1983). Identification down to the species level is not necessary, but must be accurate enough to reveal any major changes in the dynamics of water flea predators. Prior to the trial, make a rough assessment of the fish population; for methods, see Cazemier & Uijtendael (1985). If there are major differences between the various compartments, remove as many fish as possible.

Also sample daily, at fixed times: temperature, dissolved oxygen, total CO_2 content, organic carbon, phosphate, nitrogen, pH, dissolvable CO_2 and conductivity. Measure the pesticide concentration three times daily during the first week post-treatment; subsequently, make these measurements at the same time as biological monitoring is performed. When the compound is no longer detectable, reduce the sampling frequency.

4. VALIDITY

Test results are not valid if results are unclear, or if there is high mortality in the blanks or little mortality in the positive control. The trial is also invalid in cases where there were already significant differences in the measured parameters between the plots before the trial was started.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
e l n	a s p	f c	hp 4hp')	b t	2	8	5 x 15

') if ditch is not exposed: PEC of hp and 4hp

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		1,000
- test plots		1,000
- field equipment		500
2 sampling (4x2x8x5x15)		
- test organisms	25	
- other biot. parameters	10	
- abiotic parameters	10	
3 parameter measurement		( )
- test organisms (4800x)	150	4,000
- other biotic parameters	30	( )
- residue measurements	20	4,000
- abiotic parameters	10	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 275	n.a.
cost estimate (subtotal)	Dfl. 206,250	Dfl. 11,000
overall cost (very rough estimate):		Dfl. 217,500

( ) depreciation cost materials still to be included
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Appendix 4.9.1 Review of existing field trial guidelines for water fleas

Concrete guidelines for field trials with aquatic organisms were not found. Field trials were mentioned in several procedures, however:

United States (EPA, 1982) A field study is desirable if the existence of a hazard is indicated by other tests. For procedures, reference is made to several handbooks and specific studies that 'can provide useful background information for conducting a simulated or actual field study ...'.

United Kingdom (MAFF, 1986) Working Document 7.1 merely mentions that field trials with aquatic invertebrates may be necessary under certain conditions.

FAO (1985) Field studies (incl. enclosure studies) with Daphnia sp. and fish is only necessary if prior laboratory testing gives rise to doubts. Field trials may allow conclusions to be drawn about recovery and interactions between species. Methods are under development.

OECD (1987) Indicates that field studies may have an important role to play. Possibilities mentioned include the introduction of artificial substrates and caged organisms. At the same time, the limitations of field studies are recognized.

Appendix 4.9.2 Review of field studies involving water fleas

Grzenda et al. (1962) Study of the effects on pond fauna in a peach orchard; parathion, particularly, is used in large quantities in peach growing. There was no untreated control. In addition to a number of physico-chemical parameters, pesticide residues were extensively monitored. The bottom fauna was collected using an Ekman dredge: 15 grabs per sampling session. Zooplankton was sampled with a 3-l flask; a total of 15-46 l was sampled and filtered in the field using a plankton net. Significant differences pre- and post-treatment were found for insects and midge larvae; this was in contrast to results for oligochaeta. In terms of numbers and species composition, no effects were found on zooplankton. The impact on fish was considered debatable: one species, a large-mouth black bass Micropterus salmoides (introduced to the pond), disappeared, while a sunfish Lepomis macrochirus survived. On the basis of laboratory tests, no unambiguous conclusion could be drawn about the role of parathion.

Apperson et al. (1978) Study of the side-effects of Chaoborus astictopus control with diflubenzuron, various quantities of which were added to rectangular breeding ponds (0.06-0.2 ha, 3-5 m deep). A larger lake (18.6 ha, 5 m deep) was also treated. The larvae of C. astictopus were sampled using mud samplers, the adults being collected with traps at the water surface as they flew out. Zooplankton and algae were sampled with a net. Fish were also netted at certain intervals; they were weighed and measured and their gut content was conserved and analyzed for diet. The dry weight of a 10% subsample was measured. Water fleas were found to be affected, although the effect varied widely from species to species. Fish were found to switch to other food sources, but did not appear to be affected by this change. One surprising result was that the fish no longer consumed copepods after treatment, not even when these had recovered.

Rettich (1980) The side-effects of controlling mosquito larvae and pupae with permethrin and decamethrin were studied in flooded woods and orchards. The size of the resultant pools varied from 10-20 x 3-10 m, with a depth of 30-40 cm. Side-effects were assessed visually and only qualitatively (living and dead organisms). Despite the rough nature of the method, major differences were found: water fleas were killed, for instance, while midge larvae survived, depending on the concentration. In contrast, water snails survived even at the highest concentrations.

Crossland et al. (1982) In England, sugarbeet and potato fields were sprayed with cypermethrin; in France, the same treatment was applied in vineyards. In England, the effects were studied in adjacent ponds and in France in two streams and in a drainage ditch. In the laboratory, cypermethrin is highly toxic to aquatic organisms. Compound deposition was measured on flat discs. Residues in fish were measured and zooplankton were sampled with a perspex cylinder (diameter 10 cm, depth 50 cm); 4 of these samples were mixed to form a single sample and filtered in the field. Macrofauna were sampled with a net drawn through 10 m of water. No effects were found. Because worst-case situations were chosen, no effects are to be anticipated in other surface waters.

N.B. In the Dutch context, the situation might differ: ditches are generally narrower and shallower than the waters investigated in this study, so that i) the whole surface is contaminated and ii) the compound is diluted in a smaller volume of water. Moreover, ditches are usually exposed over their entire length (and sometimes on both sides).

Crossland (1984) Three experimental ponds were exposed to methyl parathion, with three serving as controls. For methods, see Crossland & Hillaby (1985). The macrofauna was sampled by drawing a net 5 metres through the water, 3 times for each sample. The bottom fauna was sampled by 'vacuuming' an area of 20x20 cm. Rainbow trout Salmo gairdneri were bred prior to the experiment. 25 fish (4.4-4.9 g) were introduced into each pond. After 11 weeks, an electric shock was administered to sample and weigh the fish. As was anticipated from laboratory tests, water fleas and copepods (Cyclops) were severely affected by parathion, in contrast to fish. Other invertebrates were also affected. Recovery set in, however, and after 10 weeks there was little difference from the controls. With regard to the bottom fauna, effects on midge larvae were found, but snails and oligochaetes were unaffected. Fish grew better in the untreated ponds. Because no toxic effect was found in the laboratory, this was ascribed to a dietary cause.

Von Peichl et al. (1984) In a pond measuring 4x2.5x0.8 m deep, PVC pipes (diameter 0.5 m) were set 30 cm into the sediment and protruding 20 cm above the surface. Before addition of the compound, a 4-1 water sample was taken every 2-3 days for 3 weeks. In a subsample, phytoplankton were monitored, with the whole sample being used to monitor zooplankton; the water was then returned to the compartment. After 3 weeks, atrazine and dichlobenil were added to 3 of the 6 compartments. For 19 weeks subsequently, zooplankton were monitored, as were the concentrations of the compounds in the water. Effects were observed: in the case of water fleas, after only 5 days; in the authors' view, this indicates toxic effects on reproduction or intake of the compound with diet. Consequently, there was no correlation with the results of short-term laboratory research.

Crossland & Hillaby (1985) In 6 experimental ponds treated with 3,4-dichloroaniline (DCA) and 3 serving as controls, it was assessed whether laboratory data retained their validity in the field. In the treated ponds (10x5x1 m deep), a given concentration was maintained for 28 days: in 3 ponds a high and in 3 a low concentration. Zooplankton (Cladocera and Copepoda) were sampled twice weekly in 2.5x5 m subcompartments. Each pond contained 3 subcompartments. In each compartment, 5 subsamples were taken using a perspex tube (10 cm diameter, 70 cm long); together these constituted a single sample. Species composition, diversity and population composition were determined. In the ponds a bio-assay was also performed with Daphnia longispina held in a cylinder (10 cm diameter, 1.25 m long). The authors report large differences in sensitivity among species; in this case, calculation of a maximum permissible concentration was based on one of the most sensitive organisms and thus proved to be a 'conservative' predictor of effects.

Kaushik et al. (1985) In 'limnocorrals', 5x5x5 m compartments including the bottom sediment, the effect of permethrin on zooplankton was studied. In the controls, acetone (the solvent used with permethrin) was added. Zooplankton was sampled weekly at different depths. A toxic effect was found at all concentrations, although this occurred much later with the micro-zooplankton than with the macro-zooplankton. Recovery was observed; the smaller species, confronted with reduced competition, are particularly quick to recover. Species diversity decreased at all concentrations, however. The authors conclude that this method is very practicable for assessing effects and recovery potential.

Shires & Bennet (1985) Effects of aerial spraying of winter wheat with cypermethrin on aqueous fauna in ditches. Sampling sites were chosen adjacent to the field and upstream and downstream in the same ditch; during the study, however, no current was observed. The ditch had a high fish population. In a different ditch, measurements were also made adjacent to the field and at a distance of several hundred metres; here, the only fish were sticklebacks Gasterosteus aculeatus and eels Anguilla anguilla. Temperature, dissolved oxygen, pH, conductivity, chlorophyll content and dissolved particles were measured in the water. Pesticide deposition was measured on series of 12 sheets of aluminium foil (250x250 mm). For residue analysis, at each location water samples were taken consisting of four 200-250 ml sub-samples, mixed to one litre, to which 50 ml of hexane was added. Residues in fish were also measured: 10 days before treatment, 2 galvanized steel cages (0.5x0.5x1 m high, 10 mm mesh) containing 10 carp weighing about 10 g were placed in the ditch. On each sampling day, one fish from each cage was sampled. Bio-assays were performed on Gammarus pulex in ditch water. Zooplankton were sampled by collecting 8 columns (9 cm diameter and 50 or 30 cm deep) of water from each location and extracting the zooplankton by means of a sieve. Macrofauna were observed visually and sampled using a net (0.95 mm mesh) drawn through 10 m of the ditch. The behaviour of the fish (both caged and free-swimming) was also observed visually. A slight toxic effect was found in air-breathing boat bugs (Corixdae); effects were more pronounced in water mites. In the bio-assays with Gammarus pulex, mortality was observed during the first 2 days post-treatment. No effects on fish were observed, nor were residues found. Zooplankton populations fluctuated so widely that any effects were masked. Deposition measurements showed that a substantial pesticide fraction was deposited on the leeward and windward ditch banks: 29% and 9%, respectively. In the ditch itself, these figures were 3% and 6%, respectively.

Zischke et al. (1985) In experimental channels, it was investigated whether a toxic limit concentration calculated on the basis of laboratory data adequately protects the aquatic biocoenose. One channel was exposed to the calculated level, one to 3 times and one to 9 times this level; one served as a blank. The channels were 520 m long and made up of 30.5 m mud-bottomed pools alternating with 30.5 m gravel-bottomed, faster-flowing runs. To these channels, river water was added that was continually contaminated with pentachlorophenol. Grids were placed between the compartments to keep the fish in place. The water was sampled twice weekly and analyzed for pesticide. The following parameters were also monitored: dissolved oxygen, pH, temperature, alkalinity, hardness, ortho- and total phosphate, ammonia, total nitrogen and BOD. Traps were

used to sample micro-invertebrates; these were set in the evening and removed in the morning, the organisms being trapped during nocturnal migration. Benthic invertebrates were sampled with the aid of steel containers (0.02 m^2) filled with channel substrate and let into the channel bottom. Invertebrate drift was also sampled. Reproduction of the snail Physa gyrina was studied by counting the egg capsules deposited on an artificial substrate. Differences were assessed by means of variance analysis (significance: < 0.05). About 450 rock-basses Pimephales promelas and about 55 sunfish Lepomis macrochirus were introduced into the channels, some of which had been anaesthetized beforehand and weighed. Fifteen of the latter species were placed in a cage (1.3x0.9x0.9 m). Three times weekly, the number of dead fish was counted. The growth of 50 of the former species was monitored weekly. Egg deposition was monitored 3 times weekly by sampling 25% of 112 artificial substrates. Larvae were also sampled by monitoring the drift. At the end of the season, all fish were removed, counted and measured; 10% were weighed. Micro-invertebrates (including chironomids and water fleas) were found to be affected by the middle concentration, with greatest abundance found in the control. Snails were severely affected by the highest concentration; under these conditions, there was also fish mortality; however, effects were also found at other concentrations. It was thus concluded that the calculated limit concentration did not provide adequate protection of the aquatic biocoenose.

Goodman & Cripe (1987) Describes cages for studying effects on aquatic organisms. Cages of various size and nets of varying mesh are used, depending on the kind of organism involved. The cages are made of halved polypropylene pots, with nylon netting stretched between these 'lids'.

Ali et al. (1988) Control of citrus rust mite in citrus orchards with diflubenzuron and side-effects on water organisms. Effects were studied in two lakes: one 2 ha lake with a shallow (1 m) and a deep (2 m) end, bordering directly on the orchard and one shallow (1 m) 1 ha lake that was not exposed. Motivation: diflubenzuron is also used to control midge larvae and is extremely toxic to a number of aquatic organisms. The lakes were sampled 4 and 1 days pre-treatment and 1, 3, 7, 14, 28 and 56 days post-treatment. In the exposed lake, 8 bottom samples were taken at the shallow end and 6 at the deep end; 7 and 4 zooplankton samples were taken, respectively. In the control lake, 6 bottom samples and 4 zooplankton samples were taken. The bottom samples (40 cm^2) were taken with a nylon net (20x20 cm, 55 cm long, 0.5 mm mesh). At the deep end, an Ekman dredge (15x15x15 cm) was used; the bottom samples were filtered through a 0.5 mm filter, sorted in a white bowl, conserved and identified. The zooplankton was sampled in a shallow area using a 50 cm long conical net (20 cm opening, 125 µm mesh) over a length of 6 m. Samples from the deep end were taken with a plankton net drawn behind a boat. No significant differences were found, probably because of the extremely low levels in the water.

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Proposal for

GUIDELINE FOR A FIELD TRIAL FOR EVALUATING THE SIDE-EFFECTS OF FIELD USE OF CHEMICAL PESTICIDES ON

FISH

FIELD TRIAL FOR FISH

General

Various publications (Crossland, 1984; Zischke et al., 1985) have indicated that the field effects of pesticides cannot always be predicted by laboratory trials. In part, this is because any impact other than direct toxic effects cannot be detected during short-term toxicity tests; in the field, moreover, effects on invertebrates can lead to secondary dietary effects. The desirability of field studies under certain conditions is also confirmed in existing procedures (see: Appendix 4.10.1 and Chapter 2 of the main report). No concrete field trial proposals are suggested, however.

The side-effects on fish (= Pisces) can be studied in two ways (see: Appendix 4.10.2): i) the fish occurring naturally in the water are sampled, or ii) caged fish are introduced. In the Dutch context, the first approach is recommended, using sticklebacks, which are profuse in most ditches and reproduce well. According to Van Urk (pers. comm.), a field trial of this nature should focus largely on sub-lethal effects (e.g. development of eggs and juveniles) rather than acute mortality. The species most suitable for this purpose is still the subject of conjecture, but it must anyway be a species with fairly large eggs and hatchlings.

Sticklebacks are employed to satisfaction in the 'mesocosm' experiments of TNO (Netherlands Institute of Applied Science) (pers. comm. Scholten). With respect to the second method, above, it is proposed to employ a species used in the laboratory as well as sticklebacks. Field results with the laboratory species can then be compared with laboratory results. The sticklebacks are studied in cages, allowing for frequent observation. There is considerable experience with cage trials employing fish (Shires & Bennet, 1985; Zischke et al., 1985). In the Netherlands, however, there is little experience with cage trials employing sticklebacks, so that methods must still be developed. The present guideline proposal therefore has a provisional character. When employing caged sticklebacks, it is important to guarantee a continual supply of food (pers. comm. Van Urk); cages should not therefore be overpopulated.

Studies have been performed in natural waters as well as in specially designed experimental ponds and so on. In the Dutch context, we are most concerned with the most exposed surface waters, i.e. ditches in agricultural regions.

Experimental conditions

The main category of ditch we are concerned with are the smaller drainage ditches between fields. This proposal is based on enclosing part of a ditch by means of impermeable barriers, to prevent the compound from spreading. This implies some disturbance of the natural situation: the circulation pattern is discontinued. However, since no dilution or removal of the compound can occur, a worst-case situation is thus created.

It is recommended to perform the trial using crops giving the highest risk of exposure. In general, greatest pesticide drift occurs in fruit growing and sylviculture. When a field trial with fish is called for, it will therefore usually be in this setting.

Application

Because the aim of a field trial is to assess the impact of practical spraying operations, the compound should be applied to the field bordering on the ditch. The maximum recommended dosage is applied, as well as four times this amount, the latter simulating a worst case situation in which the fields on both sides of the ditch are sprayed as well as reflecting variations that are likely to occur during practical spraying operations.

With the described method of application, the ensuing surface-water loading may vary. Weather conditions play a major role; in addition, the exact application procedure is especially important in this trial. Variations in distance to the ditch, e.g. in wedge-shaped fields, may result in relatively large differences in exposure. When performing the trial, therefore, due allowance should be made for weather conditions and application itself should be performed with great accuracy.

The concentration of the compound in the water must be established, either by direct measurement or by adding a marker (e.g. a dye) to the compound. Determination of water concentration is very important for establishing a causal relationship between the presence of the compound and any changes in the fish. If, through unforeseen circumstances, there is no exposure, the compound may be added directly to the water. In this case, a quantity can be added that leads to a calculated PEC (predicted environmental concentration) under practical conditions as well as four times this amount. However, extrapolation of the results to practical spraying conditions will involve a degree of uncertainty.

Observation

Fish and (a)biotic environmental factors

Besides growth and development of the fish (populations), a number of physical, chemical and biotic parameters are also monitored. On the one hand, differences in e.g. food availability will lead to differences in fish growth; on the other, effects of the pesticide on the fish may lead to an increase in, say, water fleas. The proposed guideline therefore prescribes sampling of invertebrates, at least groupwise and semi-quantitatively.

Long-term surveillance of fish can be problematic; although caged fish may be relatively easy to monitor, this is less true of the free-swimming fish in the experimental ditches. Fish may be sampled by means of an electric shock; fish introduced at the start of the trial can thus be caught again at the end. However, the question arises as to whether all the fish have indeed been sampled; as an additional measure, part of the ditch might be pumped dry to ensure quantitatively valid sampling. There does not seem to be a more sophisticated alternative. It is for this

reason that we recommend studying cage-held sticklebacks in addition to sticklebacks swimming free in the ditches. In addition, eggs and hatchlings might be introduced into the ditch to investigate effects on various stages of the life cycle. In the case of the natural fish population, it is also difficult to make an accurate determination of the initial situation; qualitative sampling is not easy. It is therefore recommended to evacuate all ditch compartments prior to the trial, e.g. by the electric shock method. Sticklebacks can then be introduced into the ditches, preferably those individuals earlier retrieved, but possibly supplemented by fish from another local source. In terms of age composition, the population should be balanced and reflect that occurring in the ditch at the time of the trial.

Compound

After application of the compound on the adjacent field, the concentration in the water must be measured regularly. Measurements should be performed frequently during the first few days after treatment (several times a day); later monitoring frequency can be reduced.

Proposal: FIELD TRIAL GUIDELINE FOR FISH

1. EXPERIMENTAL CONDITIONS

1.1 Choice of experimental organism and crop

Trial with three-spined sticklebacks Gasterosteus aculeatus and a species employed in laboratory trials that can survive in Dutch ditches. Select an experimental site having a soil type on which the compound will be applied in practice. Select a crop requiring the highest recommended dosage and resulting in the highest percentage drift.

1.2 Experimental conditions

Perform the trial in ditches bordering on the fields to be sprayed. Field plots are treated simultaneously with the compound under review and with water or with another innocuous carrier substance similar to the compound under review. Effects are evaluated in the adjacent ditches.

1.3 Other requirements

The experimental ditches should be representative, at least on a regional scale, i.e. of average dimension and with conventional use and management regimes. Perform the trial under weather conditions favouring exposure of the water body (moderate wind), avoiding extremes of weather (rain, severe drought, hard wind or no wind).

Prior to the trial, evacuate all fish from the ditches. Then introduce (free-swimming) sticklebacks into the experimental compartments, as well as caged sticklebacks and individuals of the species employed in the laboratory trial.

1.4 Experimental design

Plot size

In the experimental ditches, define 25-m compartments, treating the adjacent field with the compound under review.

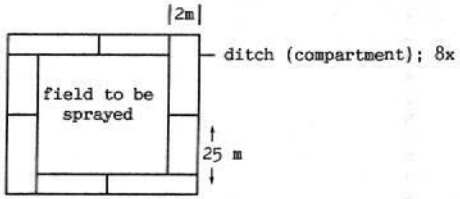
Plot arrangement

For the plot arrangement, see the figure. The arrangement comprises eight ditch compartments, for a single application. Each treatment requires a single plot. The whole trial is performed in duplo. The proposed plot arrangement is such that, under all weather conditions, there is always maximum exposure in one of the ditches. Other arrangements satisfying the same conditions are also permitted.

2. APPLICATION

2.1 Compounds

Apply the compound being assessed according to the manufacturer's guidelines, in a formulation used in everyday practice.



Test plot

2.2 Equipment

Apply the compounds using normal equipment, according to standard practice.

2.3 Dosage

Apply the maximum recommended dose of the formulation and four times this amount. If measurements show that the water is not exposed, add directly to the ditch the quantity calculated to give the PEC (predicted environmental concentration) ensuing from one and four times the maximum recommended dose.

2.4 Spraying schedule

Carry out spraying at a time at which the compound is normally applied. If possible, spray the various duplos simultaneously, but at least within a two-hour period, in order to avoid differences in weather conditions.

3. OBSERVATION

Methods and frequency

For their territory, a pair of sticklebacks requires at least 1-1.5 m (pers. comm. Leeuwangh). No more than 16-25 pairs can therefore be held in a 25-m ditch. It is advised to introduce 20 adult sticklebacks into each compartment, in addition to the caged sticklebacks and laboratory species.

In each compartment, place 4 cages for the sticklebacks and for the laboratory species: 20 juveniles in each cage. The cages are made of gauze; in size, they should be at least 1x1 m and as deep as permitted by the ditch. On each sampling date, remove one fish from each cage. Eggs

and/or hatchlings can also be introduced into the ditch using special cages, enabling the percentage of hatched eggs and hatchling development to be monitored.

Sample the caged fish once weekly during the first month post-treatment and then once every 2 weeks. To enable a direct comparison of the field results with results from laboratory trials, it is recommended to carry out additional sampling 4 weeks post-treatment. Sample the free-swimming fish at the end of the season. Measure the weight and size of all sampled fish. During sampling, also assess the general condition and similar parameters; for sub-lethal criteria, reference can be made to the results of the laboratory trials. To provide additional experimental data, residues in the fish may also be measured, but only when dead fish are found. If ecological effects are suspected, the algae and/or macrofauna may also be sampled. For the former, the reader is referred to the proposed algae trial. The macrofauna can be sampled using a standard net (Beltman, 1983). Identification down to the species level is not necessary, but must be accurate enough to reveal any major changes in the dynamics of species consumed by fish.

Also sample daily, at fixed times: temperature, dissolved oxygen, total CO_2 content, organic carbon, phosphate, nitrogen, pH, dissolvable CO_2 and conductivity. Measure the pesticide concentration three times daily during the first week post-treatment; subsequently, make these measurements at the same time as biological monitoring is performed. When the compound is no longer detectable, reduce the sampling frequency.

4. VALIDITY

Test results are not valid if results are unclear, or if there is high mortality in the blanks. The trial is also invalid in cases where there were already significant differences in the measured parameters between the plots before the trial was started.

COST

Test characteristics:

A	B	C	D	E	F	G	H
species	aspect	type	dosage	blank	repl.'s	samples	sampling
e n	c r	f c	hp 4hp')	b t	2	8	10

') if ditch is not exposed: PEC of hp and 4hp

The above codes are explained on the last page of this report.

	net working days	material expenses
1 materials	5	
- test organisms		1,000
- test plots		2,000
- field equipment		500
2 sampling (4x2x8x10)		
- test organisms	15	
- other biotic parameters	( )	
- abiotic parameters	10	
3 parameter measurement		( )
- test organisms (640x)	20	500
- other biotic parameters	5	( )
- residue measurements	20	4,000
- abiotic parameters	10	500
4 data interpretation	10	( )
5 completion of test form	5	n.a.
total working days	about 100	n.a.
cost estimate (subtotal)	Dfl. 75,500	Dfl. 8,500
overall cost (very rough estimate):	Dfl. 84,000	

( ) depreciation cost materials still to be included
n.a. not applicable.

Appendix 4.10.1 Review of existing field trial guidelines for fish

Concrete guidelines for field trials with aquatic organisms were not found. Field trials were mentioned in several procedures, however:

United States (EPA, 1982) A field study is desirable if the existence of a hazard is indicated by other tests. For procedures, reference is made to several handbooks and specific studies that 'can provide useful background information for conducting a simulated or actual field study ...'.

United Kingdom (MAFF, 1986) Working Document 7.1 merely mentions that field trials with aquatic invertebrates may be necessary under certain conditions.

FAO (1985) Field studies (incl. enclosure studies) with Daphnia sp. and fish is only necessary if prior laboratory testing gives rise to doubts. Field trials may allow conclusions to be drawn about recovery and interactions between species. Methods are under development.

OECD (1987) Indicates that field studies may have an important role to play. Possibilities mentioned include the introduction of artificial substrates and caged organisms. At the same time, the limitations of field studies are recognized.

Appendix 4.10.2 Review of field studies involving fish

Grzenda et al. (1962) Study of the effects on pond fauna in a peach orchard; parathion, particularly, is used in large quantities in peach growing. There was no untreated control. In addition to a number of physico-chemical parameters, pesticide residues were extensively monitored. The bottom fauna was collected using an Ekman dredge: 15 grabs per sampling session. Zooplankton was sampled with a 3-l flask; a total of 15-46 l was sampled and filtered in the field using a plankton net. Significant differences pre- and post-treatment were found for insects and midge larvae; this was in contrast to results for oligochaeta. In terms of numbers and species composition, no effects were found on zooplankton. The impact on fish was considered debatable: one species, a large-mouth black bass Micropterus salmoides (introduced to the pond), disappeared, while a sunfish Lepomis macrochirus survived. On the basis of laboratory tests, no unambiguous conclusion could be drawn about the role of parathion.

Apperson et al. (1978) Study of the side-effects of Chaoborus astictopus control with diflubenzuron, various quantities of which were added to rectangular breeding ponds (0.06-0.2 ha, 3-5 m deep). A larger lake (18.6 ha, 5 m deep) was also treated. The larvae of C. astictopus were sampled using mud samplers, the adults being collected with traps at the water surface as they flew out. Zooplankton and algae were sampled with a net. Fish were also netted at certain intervals; they were weighed and measured and their gut content was conserved and analyzed for diet. The dry weight of a 10% subsample was measured. Water fleas were found to be affected, although the effect varied widely from species to species. Fish were found to switch to other food sources, but did not appear to be affected by this change. One surprising result was that the fish no longer consumed copepods after treatment, not even when these had recovered.

Crossland et al. (1982) In England, sugarbeet and potato fields were sprayed with cypermethrin; in France, the same treatment was applied in vineyards. In England, the effects were studied in adjacent ponds and in France in two streams and in a drainage ditch. In the laboratory, cypermethrin is highly toxic to aquatic organisms. Compound deposition was measured on flat discs. Residues in fish were measured; the zooplankton and macrofauna composition were assessed. No effects were found. Because worst-case situations were chosen, no effects are to be anticipated in other surface waters.

N.B. In the Dutch context, the situation might differ: ditches are generally narrower and shallower than the waters investigated in this study, so that i) the whole surface is contaminated and ii) the compound is diluted in a smaller volume of water. Moreover, ditches are usually exposed over their entire length (and sometimes on both sides).

Crossland (1984) Three experimental ponds were exposed to methyl parathion, with three serving as controls. For methods, see Crossland & Hillaby (1985). The macrofauna was sampled by drawing a net 5 metres through the water, 3 times for each sample. The bottom fauna was sampled

by 'vacuuming' an area of 20x20 cm. Rainbow trout Salmo gairdneri were bred prior to the experiment. 25 fish (4.4-4.9 g) were introduced into each pond. After 11 weeks, an electric shock was administered to sample and weigh the fish. As was anticipated from laboratory tests, water fleas and copepods (Cyclops) were severely affected by parathion, in contrast to fish. Other invertebrates were also affected. Recovery set in, however, and after 10 weeks there was little difference from the controls. With regard to the bottom fauna, effects on midge larvae were found, but snails and oligochaetes were unaffected. Fish grew better in the untreated ponds. Because no toxic effect was found in the laboratory, this was ascribed to a dietary cause.

Shires & Bennet (1985) Effects of aerial spraying of winter wheat with cypermethrin on aqueous fauna in ditches. Sampling sites were chosen adjacent to the field and upstream and downstream in the same ditch; during the study, however, no current was observed. The ditch had a high fish population. In a different ditch, measurements were also made adjacent to the field and at a distance of several hundred metres; here, the only fish were sticklebacks Gasterosteus aculeatus and eels Anguilla anguilla. Temperature, dissolved oxygen, pH, conductivity, chlorophyll content and dissolved particles were measured in the water. Pesticide deposition was measured on series of 12 sheets of aluminium foil (250x250 mm). For residue analysis, at each location water samples were taken consisting of four 200-250 ml sub-samples, mixed to one litre, to which 50 ml of hexane was added. Residues in fish were also measured: 10 days before treatment, 2 galvanized steel cages (0.5x0.5x1 m high, 10 mm mesh) containing 10 carp weighing about 10 g were placed in the ditch. On each sampling day, one fish from each cage was sampled. Bio-assays were performed on freshwater shrimps Gammarus pulex in ditch water. Zooplankton were sampled by collecting 8 columns (9 cm diameter and 50 or 30 cm deep) of water from each location and extracting the zooplankton by means of a sieve. Macrofauna were observed visually and sampled using a net (0.95 mm mesh) drawn through 10 m of the ditch. The behaviour of the fish (both caged and free-swimming) was also observed visually. A slight toxic effect was found in air-breathing boat bugs (Corixdae); effects were more pronounced in water mites. In the bio-assays with Gammarus pulex, mortality was observed during the first 2 days post-treatment. No effects on fish were observed, nor were residues found. Zooplankton populations fluctuated so widely that any effects were masked. Deposition measurements showed that a substantial pesticide fraction was deposited on the leeward and windward ditch banks: 29% and 9%, respectively. In the ditch itself, these figures were 3% and 6%, respectively.

Zischke et al. (1985) In experimental channels, it was investigated whether a toxic limit concentration calculated on the basis of laboratory data adequately protects the aquatic biocoenose. One channel was exposed to the calculated level, one to 3 times and one to 9 times this level; one served as a blank. The channels were 520 m long and made up of 30.5 m mud-bottomed pools alternating with 30.5 m gravel-bottomed, faster-flowing runs. To these channels, river water was added that was continually contaminated with pentachlorophenol. Grids were placed between the compartments to keep the fish in place. The water was sampled twice weekly and analyzed for pesticide. The following parameters were also monitored: dissolved oxygen, pH, temperature, alkalinity, hardness,

ortho- and total phosphate, ammonia, total nitrogen and BOD. Traps were used to sample micro-invertebrates; these were set in the evening and removed in the morning, the organisms being trapped during nocturnal migration. Benthic invertebrates were sampled with the aid of steel containers (0.02 m^2) filled with channel substrate and let into the channel bottom. Invertebrate drift was also sampled. Reproduction of the snail Physa gyrina was studied by counting the egg capsules deposited on an artificial substrate. Differences were assessed by means of variance analysis (significance: < 0.05). About 450 rock-basses Pimephales promelas and about 55 sunfish Lepomis macrochirus were introduced into the channels, some of which had been anaesthetized beforehand and weighed. Fifteen of the latter species were placed in a cage (1.3x0.9x0.9 m). Three times weekly, the number of dead fish was counted. The growth of 50 of the former species was monitored weekly. Egg deposition was monitored 3 times weekly by sampling 25% of 112 artificial substrates. Larvae were also sampled by monitoring the drift. At the end of the season, all fish were removed, counted and measured; 10% were weighed. Micro-invertebrates (including chironomids and water fleas) were found to be affected by the middle concentration, with greatest abundance found in the control. Snails were severely affected by the highest concentration; under these conditions, there was also fish mortality; however, effects were also found at other concentrations. It was thus concluded that the calculated limit concentration did not provide adequate protection of the aquatic biocoenose.

Goodman & Cripe (1987) Describes cages for studying effects on aquatic organisms. Cages of various size and nets of varying mesh are used, depending on the kind of organism involved. The cages are made of halved polypropylene pots, with nylon netting stretched between these 'lids'.

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Symbols used for characterization of field trials

(see also page 57 of the main report)

A species used:
- e = explicitly studied species
- l = locally occurring wild flora/fauna
- n = non-indigenous flora/fauna, but resistant to (a)biotic conditions in the Netherlands
- w = wild flora/fauna

B aspect of organism on which test is focussed:
- a = numbers
- b = biomass or production/growth
- c = condition, incl. any deformities
- h = behaviour
- p = population dynamics
- r = reproduction
- s = species composition
- x = post-mortem examination

C type of test:
- f = field study sensu stricto
- c = employing cage(s) and/or enclosure(s)

D dosage (of formulation):
- hp = highest practical dosage
- 4hp= four times highest practical dosage

E blank / reference material:
- b = experiment including blank/placebo situation
- t = experiment including well-known toxic compound

F replicates
- 1 = single experiment
- 2 = in duplicate
- 4 = in fourfold

G/H number of samples per sampling operation(s) during entire field trial

- - -