Framework for field trials for side-effects of pesticides

Jong, F.M.W. de

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SUMMARY

In the present registration procedure for pesticides, a large number of laboratory toxicity tests and mathematical models are available and are used for risk assessment to underpin standards-setting on toxic chemicals for protecting ecosystems. However, hardly any field studies are being carried out to investigate the occurrence of side-effects in the field. This paper will show when field trials are necessary, and what their specific contribution can be.

First (Chapter 2) an overview of the present procedure is given, with particular attention being paid to the role field trials have played to date. An overview is given of the field trials used in the current Dutch procedure for compound assessment. Attention is paid to the effects that the recently adopted EU legislation will have on the registration procedure and to the role of field studies. The general principles of the EU concerning pesticide approval are characterized by the disappearance of the former 'moderate hazard' assessment. For the aspects being assessed, only one standard exists; if this standard is exceeded no authorization shall be granted, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable effects occur after use of the plant protection product according to the proposed conditions of use.

In Chapter 3 the potential role of field trials is identified. Field studies can considerably improve the predictive and protective capacities of the registration procedure on three points: 1) validation of the starting points and models of the procedure, 2) field trials aimed at the predicted effects before registration, and 3) field trials aimed at the outcome of the procedure after registration.

The first point aims at validating the premises and substance of the models used to calculate predicted exposure and toxicity and the resultant effects. Attention is also paid to the ecological relevance of the test species used. The second point concerns the use of field trials as an element of the registration procedure. An overview of the methods of field validation is presented, distinguishing between semi-field studies and full-scale field studies. The third point concerns post-registration monitoring and incident registration.

This chapter also considers the occurrence of indirect side-effects and a suggestion is made for incorporating these effects in the registration procedure.

Chapter 4 considers how compound properties (such as toxicity and persistence), usage data (such as formulation) and the environment receiving the compound can provide an indication of where effects are likely to occur. A field trial can then be conducted at that place (compartment, habitat) and with that organism group where effects are most likely to occur.

In Chapter 5 it is shown which concrete species should be subject of the field trials, for both the aquatic and the terrestrial environment. For the species selected it is also indicated whether field trial methods or guidelines already exist.

Chapter 6 deals with assessment of the results. General requirements such as experimental conditions, application and observations are considered. Next, the points requiring a more

technical assessment, such as the statistical significance of the results, are treated. At the end of this chapter a number of aspects which may be of use in interpreting the results are discussed.

It is concluded (Chapter 7) that priority should be given to one-off validation of starting points and models. By doing so, the predictive power of the procedure will be improved, and the need for field trials as part of the procedure will diminish. Furthermore, the safety factors used at present might be able to be reduced.

For the limited number of cases in which field trials will be needed as a part of the registration procedure, guidelines should be available for a range of field trial methods.

It is proposed that a commission, within the Board for the Authorization of Pesticides (CTB), be designated, charged with the assessment of field trials. This commission could, in consultation with the applicant, decide which field trial should be conducted and under what conditions, thus to avoid a situation whereby the results of a field trial cannot be well interpreted.

Post-registration field trials do not constitute part of the EU procedure. We propose to make post-registration monitoring part of the procedure only in cases where a pre-registration field trial does not yield a clear result. In such cases a compound could be approved, on the proviso that post-registration monitoring be carried out. The aforementioned commission could decide on an acceptable form of monitoring.

In a number of cases pesticide side-effects may only come to light after use on a practical scale. If this is the case, the results of post-registration field studies should be fed back to the approval procedure.

In general, it is concluded that field studies will improve the registration procedure for pesticides considerably, however. A well-validated procedure, combined with post-registration monitoring, should be able to offer protection against the occurrence of most side-effects.

1 INTRODUCTION

1.1 Background and motivation

Around the world the increasing demand for food combined with technological development have led to an intensification of food production. This intensification has resulted in large-scale monocultural agricultural production, which, together with the demands on product quality, has resulted in an enormous increase in the use of pesticides.

In the Netherlands, for instance, annual agricultural pesticide use stands at about 20-21 x 10^6 kg active ingredient (a.i.) (MJP-G, 1991), amounting to an average of 14 kg/ha yearly. This high usage is due to the very intensive use of land in the country, resulting, *inter alia*, in the need to apply soil disinfectants, especially in areas without crop rotation. Soil disinfectants account for a relatively large proportion of Dutch pesticide use. Another reason for this high consumption figure is substantial pesticide use on non-food crops such as flower bulbs and in greenhouse horticulture.

The compounds are used within the agricultural target areas. In the ideal situation the compound should reach the target organism, have its intended effect and decompose quickly into harmless compounds. In practice, however, a certain proportion is dispersed to the surrounding environment (water, soil, groundwater and atmosphere). In arable crops in the Netherlands this proportion may be up to 25% and in greenhouse areas up to 55% (*MJP-G, 1991*); a major proportion (80%-90%) of this mass enters the atmosphere as a result of drift or volatilization. The disposal of pesticides can lead to high concentrations in the environment outside the treated parcels (De Jong *et al.*, 1995). Measurements of pesticide concentrations in surface waters throughout the Netherlands indicate that, for all kinds of pesticides, standards are exceeded at 55%-60% of the sampling points (Muilerman & Matser, 1994). In agricultural areas, quite a number of pesticides are regularly shown to be present in rain (Heemraadschap Fleverwaard, 1993; Provincie Zuid-Holland, 1994). Pesticides have also infiltrated the groundwater (Van Haasteren, 1993).

Both within the treated plots and in the surrounding area the pesticides can contact non-target organisms, and side-effects (negative effects on non-target organisms) are therefore extremely likely. Two types of side-effects (Fig. 1) can be distinguished (*cf.* AEDG, 1994).

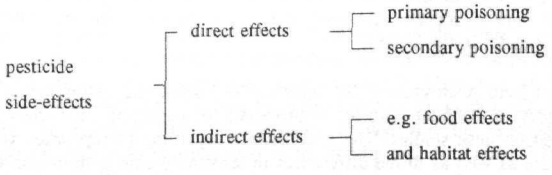

Figure 1 Categorization of pesticide side-effects.

First, there are the direct side-effects resulting from a substance's toxicity to an organism. These effects may be either primary or secondary. Primary poisoning occurs when the active ingredient has a deleterious impact not only on target organisms but also on non-target organisms. Secondary poisoning occurs at a higher trophic level, with lower-level organisms acting as intermediaries. This type of effect occurs mainly with persistent pesticides. Second, there are indirect side-effects: non-toxic effects on species of concern following, *inter alia*, from changes in the food chain (e.g. disappearance of a prey species) or changes in habitat (e.g. disappearance of vegetation).

Since 1986 CML has been studying these side-effects, commissioned by the Dutch Ministry of the Environment (VROM). In a series of desk studies, side-effects on vertebrates (De Snoo & Canters, 1990), invertebrates and aquatic fauna (Canters *et al.*, 1990) and fungi and vascular plants (De Jong *et al.*, 1992) have been investigated.

The main result of these studies is that, despite the legislative procedure in force, there are many indications that the use of pesticides has ecological side-effects. In the first place, over the past twenty years there have been regular reports of pesticide-related incidents affecting birds, mammals, fishes and honeybees (Spierenburg *et al.*, 1991; CUWVO, 1990; De Snoo & Canters, 1990). In the second place, in 1987 it appeared that water from the Netherlands' greenhouse horticulture area had to be diluted thirty times before water fleas could survive in it (Working Group "Effects ...", 1988). A correlation between water-flea survival and the pesticides content of the water was demonstrated in the same area in 1989 (Canters *et al.*, 1990). In the third place, an overall decline in the number of individuals and species in agricultural areas has been reported for flora, fauna and fungi (Bink *et al.*, 1994; Musters & Weinreich, 1990). Calculations by De Jong *et al.* (1995) predict side-effects of the use of pesticides in agricultural areas on areas with high natural values. It is concluded that pesticides, together with other stress parameters, play a significant role in the general decline in biodiversity in the Netherlands (MJP-G, 1991).

Some of the side-effects occurring are due to accidents, agricultural misuse and intentional poisoning of birds, for example (Spierenburg *et al.*, 1991); others, however, are caused by normal use and do not appear to be satisfactorily predicted by the risk-assessment procedure in force. Although the procedure provides protection against most side-effects, it can be concluded that the present, laboratory-based procedure does not protect against all side-effects. In an international context, the Dutch admission procedure is not inferior to that in force in other industrialized countries.

Recently, a harmonization of pesticide legislation has taken place at the European level (EU, 1994). The European legislation and its consequences for the Dutch legislation are discussed in detail in this report.

The lack of field research and the uncertainties concerning extrapolation of results from the laboratory to the field have led to proposals for designing field trials (De Jong *et al.*, 1990) based on desk studies. The uncertainties regarding extrapolation relate to dispersal and exposure as well as to the differences in sensitivity among individuals and species. In addition, other stress parameters may influence sensitivity in the field. As a follow-up to these desk studies, in 1991 the environment ministry commissioned CML to conduct a four-year field study to investigate the scope for field trials.

In that study field bioassays were developed with Duckweeds *Lemna spp.* and *Spirodela polyrhiza*, larvae of phantom midges *Chaoborus spp.* and amphipods *Gammarus spp.* for the aquatic environment, and with Oilseed rape *Brassica napus* and Annual meadow-grass *Poa annua*, caterpillars of the Large white butterfly *Pieris brassicae* and decomposition of dried Chinese cabbage *Brassica oleracea* leaves in litterbags for the terrestrial environment. The results of this field research are presented in De Jong & Bergema (1994).

1.2 Objective and problem formulation

Based on the existing information on the occurrence of side-effects, as stated above, it can be concluded that the approval procedure must be improved. On three points field studies could considerably improve the predictive and protective capacities of the procedure: 1) validation of the starting points and models used in the procedure, 2) pre-registration field studies, and 3) post-registration field studies. Validation of the starting points and models should be carried out independently of the admission procedure and individual compounds. Pre- and post-registration field studies should be carried out for individual compounds, in the case of a specific need (*cf.* Gezondheidsraad, 1994).

This report discusses the role and potential of these types of field studies. A framework for field trials is presented, comprising, *inter alia*, the role of the field trials in assessing pesticide side-effects, the conditions governing when field trials should be conducted, and the premises for field trials. Another subject is the interpretation of the results of field trials, including both statistical interpretation and ecological interpretation. In general, field trials should yield clear guidance. For this reason, in the case of moderate hazard or uncertainty it is important that field trials be conducted in such a way that the results are clearly interpretable. The basic point of departure is that the legislation procedure should be able to predict and prevent the side-effects as efficiently as possible.

At this point it should be noted that this study is aimed at ecotoxicological side-effects. The results should thus be viewed in the same perspective. 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.

1.3 Report design

Chapter 2 of this report summarizes the role of field trials in the present procedure; in Chapter 3 the potential role of field trials for assessing the side-effects of pesticides within the framework of the legislation procedure is discussed. In Chapter 4 it is identified which field trials are needed for assessing the side-effects of pesticides. Chapter 5 gives an overview of the concrete field trial methods needed and the methods presently available. Chapter 6 deals with interpretation of the results and in Chapter 7 the conclusions of this study are presented.

2 PRESENT PROCEDURE

International pesticide registration procedures seek to assess adverse effects using usage data, compound properties and data concerning (eco)toxicology. In such procedures, tiered systems and decision trees are used (EPPO, 1993; BBA, 1993), with further testing being required in cases where a previous test indicates a potential risk. In this report we take as an example the Dutch procedure, which is of comparable design (Van Vliet, 1992).

The Dutch policy concerning pesticides should be viewed within the framework of Dutch policy concerning chemicals. Here, the concept of progressive standard-setting has been adopted (Hekstra, 1991). Substances with an environmental concentration above the negligible risk level are bound to flexible quality standards, which are tightened up stepwise within a certain timetable to the negligible risk level as a target value. The Dutch policy plan concerning pesticides (MJP-G, 1991) is aimed at a 50% reduction in the use of pesticides in the year 2000 compared to the average use over 1984-1988, subdivided into soil disinfectants (68%), herbicides (45%), and others (35%). Together with emission abatement measures, emissions to the environment should decline by substantially more than 50%. The aims for emission are: 50% reduction for air, 75% for soil and groundwater and 90% for surface water. The goals for use seem to be realistic and the target for 1995 (35% reduction) had already been achieved in 1993. The emission targets constitute a larger problem, however, and the 70% emission reduction target set for 1995 has not been met (Moorman, 1995).

2.1 Risk assessment

In the Netherlands a pesticide is approved for use once it has been established with a reasonable degree of confidence that it is effective for the purpose in question and that neither the compound itself nor any conversion products have any harmful side-effects. Harmful side-effects are taken to include: effects on the health of the public, users or food, and effects on soil, water or air or animals, plants or part of plants whose maintenance is desirable, to an extent which is unacceptable (Reform of Pesticide Act, 1988). Separately, standards exist for compound properties such as persistence and bio-concentration factors. In 1994 uniform principles for the evaluation and authorization of plant production products were adopted by the European Communities (EU, 1994). Criteria for persistence, leaching and aquatic toxicity have been incorporated in Dutch legislation (Anonymous, 1995). Further criteria will be incorporated in 1995.

For assessing the impact on non-target species, manufacturers must comply with certain requirements concerning toxicity studies on a limited number of species. After manufacturers have presented research results to the Board for the Authorization of Pesticides, the compound's properties are assessed. The predicted exposure of organisms is estimated, based on usage data (prescribed dose, application method and formulation) and compound properties (such as persistence and mobility) and compared with the toxicity data (NOEC, LD_{50} and LC_{50} values). The calculated concentration is then compared with the toxicity of the formulation to several groups of organisms, and - for calculation of exposure via food - the bioconcentration factor, lipophility and other factors are also considered. This com-

parison provides an idea of the anticipated direct side-effects. For the compound properties persistence in soil, leaching to groundwater and bioconcentration factor, European standards have been incorporated into the Dutch legislation (Anonymous, 1995).

Models are in use for calculating the environmental concentration: the SLOOTBOX model (Linders *et al.*, 1990), for instance, is used to calculate the concentration in ditches adjacent to treated parcels. At the moment an integrated model, TOXSWA, is being developed for the aquatic environment, including drainage and run-off. This model can be used to estimate the long-term exposure. For the soil the PESTLA model (Boesten & Van der Linden, 1991) is used to calculate the pesticide content of the upper layer of the soil and the leaching to groundwater. Meanwhile, this model has been combined with other models to estimate leaching and accumulation from Dutch soils (Tiktak *et al.*, 1994).

At present, for the aquatic environment, laboratory toxicity data on algae, Daphnia and fishes are a standard requirement. For the terrestrial environment this holds for mammals (rats, for prediction of human toxicity), birds and, in certain cases, earthworms, honeybees and other beneficial organisms (CTB, 1994). Terrestrial non-target plants are not part of the procedure. Data may perhaps be derived from the efficacy test, which in any case yields data on effects on the crop. For herbicides, data for a number of target species are available.

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.

Based on the predicted exposure and the toxicity data, the potential risk of the compound with respect to nature and the environment is estimated. Considerable efforts have been made to improve this risk assessment (e.g. Traas *et al.*, 1989; Van Straalen & Denneman, 1989). Models for secondary poisoning exist, and can be taken into due account (Romijn *et al.*, 1993; Jongbloed *et al.*, 1994; Gorree *et al., in press*). At present, in the Netherlands the maximal tolerable risk for a compound is reached if the concentration of the compound is equal to the estimated concentration at which for 95% of the species the NOEL is higher than this concentration (Van Straalen & Denneman, 1989). Of course, only a limited number of acute and/or chronic data are available. In the risk assessment procedure safety factors are incorporated: if the variance of the NOEL values of different organisms is high, or only a limited number of NOELs are available, the safety factor is increased. The model has been improved on several points, concerning representativeness and safety (Van den Berg & Bodar, 1991). If chronic toxicity values are available for only three or fewer organism groups, the EPA method is adapted (EPA, 1984). In this case fixed extrapolation factors are used (1000, 100 or 10). The choice of the factor depends on the quantity and quality of the available toxicity data. At present, several risk assessment methods for various categories of substances are integrated into one assessment scheme: the Uniform System for the Evaluation of Substances (VROM, 1994).

If a compound is approved, usage criteria are laid down. Approval may later be withdrawn if new studies indicate that the compound has a greater, or a different, impact on

nature and the environment than originally anticipated, or less hazardous alternatives become commercially available (Mandersloot, 1993).

2.2 Field trials

The present procedure is based mainly on laboratory toxicity testing and mathematical modelling (see § 2.1), and hardly any validation has taken place. In the Netherlands validatory studies are currently being conducted in mesocosms in the field (Van Wijngaarden, 1993; Bowmer *et al.*, 1991).

In the registration procedure, field trials aimed at assessing side-effects are not yet standard practice. Additional data from cage and/or field trials are only prescribed for assessing hazards to honeybees *Apis mellifera* (CTB, 1994), if the ratio between the highest recommended field dosage in grams per hectare and the LD_{50} is between 50 and 2500. At first, cage trials are prescribed; if significant effects are found, a field trial is deemed necessary (Van Vliet, 1992). 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, 1992). It should be noted that these field data are required only if the pesticide is to be applied on crops which are visited by bees (CTB, 1994).

For other faunal groups, 'field' trials should be conducted for the beneficial arthropods *Encarsia formosa* and *Phytoseiulus persimilis*, according to EPPO guidelines 142 and 151 (EPPO, 1989 and 1990), respectively. These trials are aimed at greenhouse crops, however.

Additional studies under field conditions may also be required 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. No standard field trial guidelines exist. Also, in the case of wash-away, field studies may be required. Criteria for these studies are being developed.

For other groups, assessment of the toxicity of a pesticide under field conditions may be required as "supplementary data" and "in certain cases" (CTB, 1994). Supplementary data may be requested if a need is indicated by replies to other questions, by the nature of the application or by data on the behaviour of the pesticide in soil or water. The application form states merely that "it is most important that the experimental method and conditions are accurately described. Guidelines for these studies can, if necessary, be drawn up in consultation with Board experts", i.e. experts of the Board for the Authorization of Pesticides (CTB) (CTB, 1994). The form also refers to Working Document 7/1 of the British approval procedure (MAFF, 1986). When a pesticide is claimed for integrated control, study of the hazards to beneficial insects and mites is also prescribed. The IOBC tests used for this purpose may in part consist of field trials (IOBC, 1988).

In the Uniform Principles of the EU concerning the evaluation and authorization of plant production products (EU, 1994) it is stated that field trials can be used to prove that no unacceptable impact on the viability of exposed species occurs, in the case that risk assessment has predicted an unacceptable risk. In this case, therefore, field data are no

longer used to tip the scales in case of doubt, but are used to prove harmlessness in the case of a predicted risk.

To gain an idea about the use and impact of field trials in the registration procedure, Table 1 summarizes the available data concerning field trials. These data were gathered from the 'environmental evaluations' of approved active ingredients which have been published by the Board for the Authorization of Pesticides (CTB). In these evaluations, in a number of cases the results of field research are mentioned, including the outcomes.

Table 1 *Actually conducted field trials mentioned in Dutch environmental evaluations; n.r.* = no remark; * = because of lack of field data assessed as hazardous for bees (source: Board for the Authorization of Pesticides).

Active ingredient	Field trial conducted	Outcome of field trial	Directions for use
INSECTICIDES			
acephate	bee	hazard for bees	bee hazard
azinphos-methyl	-		
carbaryl	-		
*chlorfenvinphos	wood mouse	treated winter cereal/effects on chol. activity.	n.r.
	bee	hazard for bees	n.r.
chlorpyrifos	-		
deltamethrin	earthworm	no effect	n.r.
	aquatic, 2 x	mortality of crustaceans, fishes, algal growth	hazard to aqu. org.
	bee (more studies)	some effects, no hazard concluded	n.r.
demeton-S-methyl	-		
diazinon*	-		
fenitrothion	-		
fenpropathrin*	-		
fenvalerate*	pond, invertebrates	large effects on invertebrates, no effect on snails or worms	hazard to aqu. org.
fonofos	-		
formothion	-		
methidathion	-		
methomyl	cage study birds	no effects	
omethoate	-		
oxydemeton-methyl*	-		
parathion-methyl	-		
phosalone	bee	no hazard for bees	n.r.
phosphamidon	-		
phosmet*	-		
pirimiphos-methyl	-		
pyrazophos*	-		
sulfotep	-		
triazophos	-		

Active ingredient	Field trial conducted	Outcome of field trial	Directions for use
HERBICIDES			
aclonifen	-		
amitrole	-		
asulam	-		
carbetamide	-		
chlormequat	-		
chloroxuron	-		
chlortoluron	-		
cyanazine	-		
cycloate	-		
cycloxydim	bee (tent)	no effect	n.r.
dalapon	earthworm, centipede, mite, springtail	no effect	n.r.
daminozide	-		
desmetryn	-		
dicamba	-		
dichlobenil	-		
difenoxuron	-		
diflufenican	-		
dikegulac-sodium	-		
dinoterb	-		
ethephon	-		
hexazinone	-		
lenacile	-		
methabenzthiazuron*	earthworm, mite	no effect after 6 & 12 months	n.r.
	enchytraeids	effect after 6 & 12 months	n.r.
	springtail	effect after 12 months	n.r.
	soil respiration	sometimes effect after 90 days	n.r.
	different soil studies	variable results	n.r.
metolachlor	-		
metoxuron	-		
monolinuron	-		
pendimethalin	-		
prometryn	-		
propazine	-		
terbutryn	-		
trifluralin	fish	spinal abnormalities	n.r.
FUNGICIDES			
fentin hydroxide	-		
fluazinam	-		
iprodione	-		
metiram	-		
propiconazole	-		
sodium dimethyldi-thiocarbamate	-		
thiram	nitrification	effect found	n.r.
triforine	-		

From Table 1 it can be concluded that field trials have been conducted for only a limited number of compounds. In most cases the outcomes of the field trials are reflected in the directions for use. In one case (chlorpyrifos) a hazard for bees was found in the field trials, but no hazard is indicated in the directions for use. Only in the case of honeybees is it clear why the field trials have been conducted (a moderate hazard or uncertainty is indicated on the basis of the laboratory trials). In the other cases, the criteria for conducting field trials are not clear.

The incorporation of the EU directive is not likely to cause any substantial change in the frequency of field trials. In the case of honeybees, for instance, under the terms of the new directive "no authorization shall be granted if the hazard quotients for oral or contact exposure are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybees" (EU, 1994). It is to be expected that a manufacturer will conduct a field trial only if he expects there to be no hazard. So, in spite of the disappearance of a second absolute 'unacceptable risk' value it is not to be expected that for these compounds more field data will become available. This expectation can be underpinned by Table 1. Here we can see that in a number of cases where a hazard was predicted on the basis of laboratory studies this hazard was found in the field trial as well.

The Dutch pesticide approval procedure also provides for the use of a pesticide for experimental purposes (so-called experimental exemption) (Mandersloot, 1993). Approval for such exemption might also include field testing but, again, standard criteria for conducting field trials are lacking.

3 POTENTIAL ROLE OF FIELD STUDIES

In this chapter the potential role of field studies for the registration of pesticides will be defined. As stated in Chapter 1, we distinguish three major functions of field studies: validation of starting points and models used for risk assessment, pre-registration field trials as part of the procedure and post-registration field trials. These three types of field studies will now be discussed successively.

3.1 Validation of starting points and models in the procedure

At present, pesticide side-effects are predicted on the basis of a comparison between the predicted exposure of a compound and the NOEC or LC_{50} or LD_{50} values for the test organisms, using mathematical models (see Chapter 2). Standards are derived from these models. These standards have a considerable impact on the use and legislation of certain compounds. To what extent these standards reflect the 'real world' is an issue of interest to policy makers, among others, and it is becoming increasingly important to validate the starting points and cut-off criteria of the registration procedure (Notenboom & Van Beelen, 1992; Gezondheidsraad, 1994). A number of differences between the laboratory and field may engender a need for validation: for example, the comparability of species (or populations) between lab and field, the heterogeneity of populations in the field, differences in exposure and the occurrence of indirect effects (Tamis & De Jong, 1992). Table 2 shows three aspects of importance for assessing pesticide side-effects: usage, exposure and the effects themselves. For each aspect, consideration will be given to what assumptions are made, what models are used and where a need for validation exists. Table 2 indicates what aspects are part of the present procedure, what aspects are validated and where a need for validation exists.

Usage

The usage data constitute the first point of validation. At present, a compound is assessed on the basis of the *recommended* dose, frequency etc., assuming *good agricultural practice*. In *actual agricultural practice*, however, there may be considerable deviation from all these aspects. In the case of glyphosate in knapsack sprayers, usage was found to be 0.1 to 6 times the recommended dose in practice (De Snoo & Wegener Sleeswijk, 1993). In the Netherlands, the actual use of pesticides has been studied by means of interviews (Berends, 1988). In that report it was concluded that actual usage will not generally be more than twice the recommended dose. To cope with this variation in spraying procedures, a safety factor could be incorporated in the tests and models. For the Dutch situation, there exists an overview, albeit incomplete, of the ranges in dosage, frequency of use and spraying equipment used. With respect to the ranges in weather conditions and the distance the farmer keeps from the crop edge, both of great importance for the dispersal of pesticides, no validation has taken place. It is recommended to investigate these aspects under practical conditions, an exercise that can only be conducted after registration. This kind of validation should be repeated regularly. As users' knowledge and control systems improve, practical use will then gradually approximate the recommended dose.

Table 2 Topics requiring validation in the registration procedure; +/- = partly; n.a. = not applicable.

	starting points			predicted effects pre-registration		procedure post-registration	
	present model	validated	need for validation	validated	need for validation	validated	need for validation
Usage							
- dose	+	+	+/-	n.a.	n.a.	+	+
- frequency	+	+	+/-	n.a.	n.a.	+	+
- spraying equipment	+	+	+/-	n.a.	n.a.	+	+
- weather conditions	+	-	+	n.a.	n.a.	-	+
- distance from crop edge	+	+	+/-	n.a.	n.a.	+	+
Exposure							
- emission routes	+	+/-	+	-	+/-	-	-
- environmental conc.	+	+/-	+	-	+/-	+/-	+
- concentration in food	+	+/-	+	-	+/-	+/-	+
Effect							
Direct							
- primary poisoning	+	+/-	+	+/-	+	+	+
- secondary poisoning	+	-	+	-	+	+	+
Indirect							
- food	-	-	+	-	+	-	+
- habitat	-	-	+	-	+	-	+
- behaviour	-	-	+	-	+	-	+

Predicted exposure

The second element of the procedural validation concerns the predicted exposure. To predict the exposure, the concentration of the compound in the environment or in the diet of organisms is calculated. For this calculation, emission routes are modelled, based on the compound properties (for abbreviations: see appendix) K_{om}, $K_{s/l}$ and DT_{50} values, the retardation factor (Rf), Henry's law constant and the BCF (Linders *et al.*, 1994). Field validation should be used for answering specific questions, in order to improve the predictive capacity of the models (Vighi & Calamari, 1990). Several aspects are dealt with below.

Emission routes

With respect to emission routes, it can be stated that pesticides can reach the environment via the air (vapour, drift or dust (De Jong *et al.*, 1995; de Heer *et al.*, 1985; Goselink *et al.*, 1993) and via the soil, by run-off or leaching (drainage included) (Van Beersum, 1990; Steenvoorden *et al.*, 1990). Pesticides may also become bound to soil particles.

For emissions to the air, fixed drift percentages are used in the emission model, differentiating between a number of crop types, for example 1% for low crops and 10% for fruit-growing (Linders *et al.*, 1994). These data are based on one-off measurements only (De Heer *et al.*, 1985). De Snoo (1994) measured deposition at several distances from a treated parcel using water-sensitive paper. He found relatively large deviations (3-4 times higher) from the percentages used in the registration procedure, especially at higher wind speeds ($\geq$ 5 m/s). Internationally, considerable efforts have been devoted to measuring pesticide drift deposition and its effects (McCartney *et al.*, 1990; Cooke, 1993; Van Ripke & Warnecke-Busch, 1992). The results of these studies can be used to validate the Dutch models.

Several studies indicate the possibility of effects of vapour drift (De Jong *et al.*, 1995; Breeze, 1988; Elliot & Wilson, 1983; Cooke, 1993). For vapour drift, reference is currently made to international procedures and guidelines. In these guidelines, persistence in the air is a key criterion (BBA, 1993). In the Netherlands, a model for short-range pesticide transport has been developed by TNO (Huygen *et al.*, 1986). This model has been validated partly for fruit-growing and emissions from glasshouses (Baas & Huygen, 1992; Baas, 1994).

A particular emission route is involved in the case of granules and treated seeds. In the laboratory, it is possible to determine the toxicity to birds and mammals only (Mineau, 1993). However, the number of granules or seeds that will be available on the surface and the behaviour of the animals in the field (some birds or mice are quite selective in searching for seeds in the rows) cannot be predicted in the laboratory. Field observations of the number of seeds on the soil surface and the grit uptake of birds can improve the prediction considerably (Tamis *et al.*, 1994). A quite different problem concerns spillage of treated seeds. The occurrence of spillage can make an accurate risk assessment useless.

Concentration in environment and food

In the Netherlands the SLOOTBOX model (Linders *et al.*, 1990) is used to calculate the

concentration of a compound in ditches adjacent to treated parcels. Behaviour in the aquatic environment is assessed on the basis of such characteristics as the compound's persistence and degradation products. As already stated, however, pesticides are found on a large scale in surface waters, indicating the poor protective capacity of the present procedure. It is not at all clear whether the concentrations found are a result of normal use or of incidents such as spilling during tank-filling. A solid validation of the SLOOT-BOX model, combined with a study of other emission routes, will therefore be necessary to trace the causes of this problem. The available concentration measurements might be used for this validation. The incorporation of drainage and run-off into to TOXSWA model might improve the predicting capacity of aquatic exposure; validation of this model will be desirable as well, however.

For behaviour in the soil, a number of compound properties are required, such as persistence, mobility and degradation products (CTB, 1994). The PESTRAS model (Tiktak, 1994) calculates accumulation in soil and leaching to groundwater. Here, again, validation would improve the model's accuracy and predictive capacity.

For the terrestrial environment, additional parameters such as Daily Food Intake are used. On this point, toxicity can be well predicted in the laboratory. In the field, however, factors such as food pattern and availability of food can play an important role (De Reede, 1982). Other differences, for example in the calorific value of food and in assimilation efficiency, occur as well. Kenaga (1973) studied the relation between food consumption and body weight of different species and ages of birds. For several aspects a field validation could be conducted to increase the predictive value of the models.

However, even in cases where the concentration of a compound can be readily predicted, uncertainties about the exposure of organisms may still remain. Field bioassays can improve the accuracy of exposure prediction.

Direct effects

In the laboratory, it is only possible to study such toxic effects as survival, growth and development and reproduction. However, minor differences in behaviour or temporary growth inhibition (in the case of plants) can have a severe impact on species composition. A classic example of the effects of behavioral effects is the case of temephos and the Fiddler crab. On the basis of lab testing, no effects were predicted. In the field, however, a very small effect on retreat behaviour led to increased predation (Ward, 1978). In the case of plants, the microcosm studies of Marrs *et al.* (1991) indicated a shift in species composition as a result of pesticide drift. Therefore it is recommended to also investigate these more subtle toxic effects.

Laboratory tests are very suitable for the ranking of compounds on the basis of their toxic effects under highly standardized conditions. In the field situation, however, there is great variety in conditions: genetic variability, variation in age, and a variation in environmental circumstances such as temperature, weather conditions, soil, water quality, etc. For these reasons, it can be questioned whether lab tests can ever be sufficiently representative at all. On the other hand, it can be argued that the laboratory tests aim at worst-

case situations, with specific attention being generally paid to sensitive life stages, for instance. However, it should be validated whether the laboratory tests indeed reflect worst-case conditions. As a first step towards validation, the laboratory test organisms could be exposed under field conditions (Denneman & De Bruijn, 1992), using cages, for instance. Using post-registration data, it could be validated whether the lab tests do indeed protect against effects in the field.

At present the test species are chosen mainly on the basis of laboratory demands (rearing possibilities, suitability for lab research, etc.). Moreover, internationally accepted species are most generally used, although these are not necessarily found in local situations. In the Dutch procedure, for instance, three fish species are mentioned (*Poecilia reticulata*, *Onchorynchus mykiss* and *Brachydanio rerio*), none of which are native in the Netherlands.

Table 3 *Suitable groups of species for assessing pesticide side-effects*

functional & taxonomic group	aquatic sediment	water	soil	soil surface	vegetation
Producers					
algae		+			
vascular plants		+		+	
Herbivores					
molluscs		+		+	+
unsegmented worms	+	+	+		
segmented worms	+				
crustaceans	+	+			
mites			+	+	+
insects	+	+	+	+	+
fishes		+			
birds		+			+
mammals			+	+	+
Carnivores					
unsegmented worms	+	+	+		
segmented worms		+			
spiders & mites		+	+	+	+
insects	+	+	+	+	+
amphibians	+	+			
reptiles			+	+	
fishes	+	+			
birds		+		+	+
mammals		+	+	+	
Decomposers					
bacteria	+	+/-	+	+	
fungi	+	+/-	+	+	
unsegmented worms	+		+		
segmented worms	+		+		
crustaceans	+	+/-		+	
insects	+	+/-			

For a fundamental approach, for the most important emission and exposure routes a representative of each and every group of organisms fulfilling an important function (e.g. the food chain: primary production, herbivory, carnivory and decomposition) should be taken into account in the procedure. Furthermore, organisms from different taxonomic groups should be selected, i.e. organisms with different morphologies. Because pesticides can reach all environmental compartments (water, underwater sediment, soil, vegetation), all these compartments should be represented by the test species. Therefore, species should be chosen according to the following fundamental criteria:

1. Different taxonomic groups should be represented.
2. Different functional (ecological) groups should be represented.
3. All emission routes should be covered.

Table 3 lists species groups for the aquatic and terrestrial compartments based on these criteria. Of course not every species group needs to be taken into account for every compound, as in the present procedure. The compound's properties, means of use, etc. will focus suspicions of effects in a certain direction (compartment, type of species, etc.).

We suggest validating the representativeness of the presently used test organisms for local species and for other species groups as selected in Table 3 for a number of compounds with different modes of action. Only if these species are not found to be representative on the basis of the three criteria mentioned, should other species be selected. In 1978, already, Kenega studied the representativeness of species for acute toxicity. Leaving flora and fungi out of his scope, Kenega concluded that the rat, one species of fish and daphnia were found to be the best indicators of acute toxicity to a wide variety of species.

For secondary poisoning a few models exist in the Netherlands (Gorree *et al., in press*; Van de Plassche, 1994). These models have not been validated, however. When validated, a model should be adopted in the procedure.

Indirect effects

Indirect effects (food and habitat effects) do not constitute part of the procedure at all. It is feasible to predict these effects, however, proceeding from data on compound properties, use and toxicity (De Snoo *et al.*, 1994). Validation of these predictions cannot be done on a one-off basis, however. Nonetheless, it is proposed to incorporate the indirect effects in the procedure (see Fig. 2). As is the case with occurrence of direct side-effects, indirect side-effects could be predicted in the procedure. The likelihood of indirect effects occurring can be estimated on the basis of i) spectrum of action, ii) scale of use, iii) overlap of habitat, and iv) efficacy:

i) *Spectrum of action*: If a pesticide has a very specific mode of action (killing one or several species only), its spectrum of action gives no reason for suspecting indirect 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. The following criteria can be distinguished: the feeding habits of species, critical periods in species' life cycles, the influence of natural fluctuations and the persistence of the effect. If an ecological function (such as decomposition or pol-

lination) becomes impaired, at least on this point clarity should be obtained before approval is granted.

ii) *Scale of use*: We propose using the term 'widespread use' for cases where approval is requested for application on crops covering an area ≥10,000 ha (approx. 0.5% of arable land in the Netherlands). 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.

iii) *Overlap*: If there is a large degree of overlap between the area in which a pesticide is to be used and a certain habitat type (*e.g.* ditch banks), there is a potentially large hazard to this habitat. For this habitat, then, it is likely that indirect side-effects will occur. Likewise, if the area in where a pesticide is to be applied overlaps certain types of landscape (*e.g.* polders), there may be a risk of indirect side-effects in such areas. In these cases the 10,000 ha criterion does not apply. When connected areas are treated, the occurrence of indirect side-effects will have to be examined on a case-by-case basis.

iv) *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. 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 indirect side-effects if it has a broad spectrum of action, or if it is used on a large scale.

The internal weighing of the criteria could be based on a multi-criteria analysis, resulting in a minor or a (serious) hazard for the compound.

Figure 2 indicates the role of field trials within the procedure and the place that might be given to indirect side-effects in the procedure. Apart from the procedure, environmental surveillance programmes and incident registration might be carried out. Results can have effects on the registration of a pesticide (see § 3.3). This kind of field research is not seen as a part of the procedure, however.

Of course the procedure in Figure 2 only indicates the ecological side-effects. Other aspects should be taken into account for a final assessment of a compound; the US-EPA even indicates that 'as the economic benefits of a chemical increase, the standard for significant regulatory action is higher. For example, significant economic benefits may not be outweighed by risks unless those risks are very high, very widespread, or involve especially valued species or habitats.'

3.2 Pre-registration field studies

In the present procedure the probability of direct side-effects occurring is calculated (see Chapter 2). The results of the procedure are (EU, 1994):

1. There is a *minor hazard*; in this case, approval is recommended and no (pre-registration) field trials are required to assess direct side-effects.
2. There is a *(very) serious toxic hazard* to non-target organisms; because of the anticipated direct side-effects, approval is not recommended. In the case of a very serious hazard, a field trial to demonstrate these side-effects is unnecessary and, for ethical reasons, even undesirable.

A serious hazard may be indicated directly during initial assessment, but there may also be specific exposure hazards involved: granular formulations may be picked up by birds, for example, resulting in very high exposure.

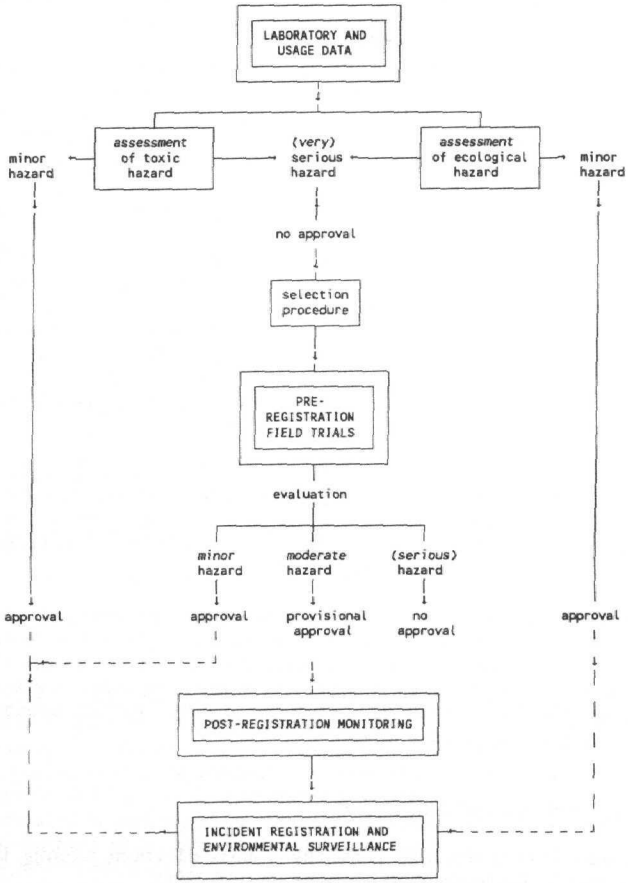

Figure 2 Proposed procedure for assessing the ecotoxic hazards of pesticides.

In some cases, differences between the laboratory and field (e.g. environmental factors, behaviour, food patterns) may make it impossible to predict the effects well. Other indications of side-effects - experience in other countries, for instance - may also indicate a serious hazard. 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 new formulation or a new application.

In all these cases, safety factors will be introduced, which, in a number cases, will lead to a serious hazard. In this case, in accordance with the Uniform Principles concerning pesticides of the European Union (EU, 1994), field trials can be conducted by the applicant to prove "clearly through an appropriate risk assessment that under field conditions there is no unacceptable impact on the organisms concerned of the plant protection product according to the proposed conditions of use".

In the case of very toxic substances, it is very unlikely that they will appear to be harmless in a field trial, so field data will probably be provided in the case of the former 'moderate hazard'. In the US too, a policy change occurred in 1992 (AEDG, 1994). The registration decision is no longer to be based on pre-registration field trials, but on other sources of information, such as laboratory data or incident reports. Field studies will only be required under unusual circumstances, such as a new mode of action or a new class of chemicals.

Indirect side-effects cannot be investigated in the laboratory at all. As already stated, these effects can be predicted based on compound properties and usage data. If these kinds of effects are to be expected, a procedure comparable to that for the toxic effects is proposed. These trials will be directed towards food organisms or the habitat of the species for which the indirect effects are anticipated. These kind of side-effects are not mentioned in the 'Uniform Principles' of the EU at all.

A number of methods can be used to conduct pre-registration field trials. We distinguish between semi-field studies and full-scale field studies (De Jong *et al.*, 1990). Semi-field studies are studies in which some kind of manipulation has taken place; in full-scale field studies (parts of) existing ecosystems are studied. Both types are illustrated below.

Semi-field studies

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

Bioassays

The abundance of one or more species enclosed in a highly restricted space is artificially increased (De Jong & Bergema, 1994). Examples include small underwater cages containing water fleas or fishes, beehives placed in cages, or potted plants. Bioassays are intermediate between field and laboratory tests. Two types can be distinguished:

i. The medium (e.g. water or soil samples) is taken from the field and tested with standard organisms in the laboratory.
ii. Standard organisms from the laboratory are used in a field situation in enclosures in the field medium.

Bioassays have the advantage of being relatively controlled; on the other hand, there still remain differences with the field situation.

Experimental ditch trials/plot trials
A new ecosystem is created by digging experimental ditches or ponds or by creating trial plots. In most cases, these test set-ups are spatially isolated for the species to be studied.

Enclosure trials
An existing ecosystem is enclosed spatially. This may involve the use of nets to isolate parts of a lake, or a fence placed around parts of a field. This category also includes the use of beehives, because in practice the bees' movements are limited to the field in which the hives are placed.

Full-scale field studies

This type of study focuses on the effects of pesticides on species occurring naturally in an existing ecosystem, which may be natural, semi-natural or cultivated (agricultural). The ecosystem to be studied is integrated with its surroundings, with no form of artificial isolation. In a full-scale field study, parts of the ecosystem, such as one species or a limited number of species or processes, can be studied as well as interactions among them. Several sample methods are described in the literature (*cf.* Fite *et al.*, 1988; Somerville & Walker, 1990).

To yield readily interpretable results, a full-scale field study requires a detailed lay-out. A full-scale field study is very intensive and therefore expensive. Such a study should be conducted only if there are clear suspicions of certain effects which cannot be traced in any other way.

In all types of field studies it will be necessary to measure exposure time and concentrations. This will be necessary to underpin a dose-effect relationship.

Choice of trial type

The sequence of field trial types presented - bioassays, experimental plots, enclosures, and full-scale field studies - reflects increasing representativeness but decreasing scope for controlling conditions.

Bioassays are suitable for tracing short-term toxic side-effects. Bioassays can be carried out as part of an enclosure or plot trial. Bioassays are especially suitable for validating predicted exposure. In the procedure, an environmental concentration is predicted. By bringing sensitive test organisms to the field, it is possible to validate whether effects do indeed occur under variable environmental conditions. Furthermore, bioassays are suitable for testing effects on a single species under field conditions. In a bioassay different life stages or ages can be studied, too. By using different bioassays with different species, effects on different functional groups can be studied.

Trials in enclosures and experimental plots allow the interactions among species of different functional groups to be investigated, because a number of species can be studied together. The mobility of the species is limited, however, which may influence the

exposure pattern (e.g. food choice). In enclosures, effects on local organisms can be studied. In an experimental plot, more factors are controlled, however.

In a full-scale field trial, the effects on organisms in their normal environment can be studied, unhampered by experimental conditions. This is the only way to trace indirect effects in the field situation.

The choice of trial depends partly on the type of organism to be studied. For organisms active over a large area, a semi-field trial may not be 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 full-scale field study is required, to ensure realistic exposure dynamics, among other reasons. For ecological effects, too, a full-scale field study is required.

3.3 *Post-registration field studies*

Following a positive decision on a given compound, there may be a need for post-registration monitoring, comparing the data obtained in laboratory and field trials with data from actual practice (e.g. combined use of pesticides). In the US, post-registration monitoring is also used to assess the efficacy of mitigation strategies (AEDG, 1994). A distinction is made between monitoring (planned, active sampling of populations at risk) and incident registration (studies in response to reported mortality) (Greig-Smith, 1990).

Monitoring

Monitoring may focus on the concentration of a compound in environmental compartments or in organisms (compound monitoring), or on the organisms themselves (effect monitoring). Compound monitoring may, for instance, aim at studying the effects of emission abatement measures, or at assuring the protection of groundwater or sensitive areas.

Effect monitoring is a method whereby groups of organisms are studied over a longer period of time. It can therefore be used to trace long-term side-effects and to ascertain the harmlessness of large-scale control operations. The selection of the organisms and parameters monitored is crucial for the impact of this type of field study. Post-registration monitoring methods and techniques which can be used for vertebrates include, for example, avian surveys, casualty searches, nesting studies, bioassays, radio-telemetry, animal behaviour studies, cholinesterase assays and residue measurements (*cf.* Bairlein, 1990). Detailed descriptions of how to measure the relevant parameters in vertebrates and invertebrates are given in Somerville & Walker (1990). Monitoring can thus be conducted at the full-scale field level, but may also make use of enclosures or bioassays.

Monitoring can be part of the registration procedure, in cases where effects are suspected that cannot be traced before a (provisional) registration. It is conceivable that the compound might be registered, provided there is a post-registration monitoring programme focusing on certain effects (see Fig. 2). An example might be a casualty search after the use of treated seeds. Within the framework of the EU Uniform Principles, we suggest that post-registration monitoring should be required in cases where a field trial does not yield a clear result.

In most cases, however, monitoring will be part of governmental environmental control programmes designed to measure environmental quality. In such a monitoring programme, however, it might appear that a certain compound has an effect on environmental quality; in this case these results can have an impact on the registration of the compound (see below). The Avian Effects Dialogue Group (AEDG, 1994) distinguishes between environmental surveillance and targeted monitoring. Environmental surveillance (including incident registration) is in this case a more passive process, while targeted monitoring is aimed at the anticipated effects of a certain compound.

Incident registration

Incident registration can be regarded as a special kind of monitoring, viz. monitoring of victims of pesticide poisoning. At present, incident registration is used for vertebrates and honeybees only, because larger animals are more readily found and honeybees are watched by the beekeepers. For other faunal groups it cannot be expected that incidents will be reported representatively. Incident registration has a warning function. It may provide information on certain aspects not studied in controlled experiments, e.g. unforeseen hazards, effects under unusual conditions, effects on rare species, and pesticide abuse, as in the Incident Investigation Scheme in use in the UK (Fletcher *et al.*, 1991) or in the system proposed by the US Avian Effects Dialogue Group (AEDG, 1994). In the Netherlands coordinated incident registration has been discontinued, for financial reasons. On an *ad hoc* basis agencies such as the Central Veterinary Institute (birds), the Ambrosiushoeve (bees) and the Association of Water Boards (aquatic organisms) gather some information of incidents. Incident registration can also bring to light secondary poisoning and combined effects. It is therefore important that a good incident registration procedure should exist, and the causes of an incident be able to be traced.

Incident registration will not be aimed at specific compounds, and in this respect is not part of the registration procedure for specific compounds.

Impact of post-registration data

Any new results obtained in the post-registration monitoring phase should be compared with the data from the laboratory and/or pre-registration phase, to gather supplementary information on direct and indirect effects. If the post-registration tests indicate the occurrence of effects, this may provide a motive for conducting more specific field or laboratory studies.

Knowledge of post-registration monitoring data might furthermore be used in the regular re-evaluation of compounds and could lead to label changes. In the case of significant adverse effects coming to light, cancellation of the approval of the registered compound should be considered (*cf.* Brassard & Rieder, 1993). Urban (1990) illustrates the importance of field data for validating laboratory data or for completing risk assessment.

4 REQUIRED FIELD TRIALS

In Chapter 3 the role of field trials in the approval procedure and the need for such trials is indicated; furthermore, various different types of field trials are specified. Once the desirability of a field trial has been established, the question arises of which trial (which type, which organism) should be conducted. In the following, it is elaborated which trial should be chosen, 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.

Field trials for validation of the models or starting points of the procedure should be very specific in relation to the questions to be answered. Therefore no general procedure can be given. This chapter focuses on pre- and post-registration field trials.

The Uniform Principles of the EU (1994) prescribe that field trials can be conducted to prove harmlessness in the field when the risk assessment predicts a serious hazard. Therefore, the kind of effect to be studied in pre-registration field trials is already partly specified. Many choices remain, however, such as the choice of environmental compartment, crop, habitat, species etc. In De Jong *et al.* (1990), a procedure for identifying the anticipated effects is given. Below, this procedure is given in an updated version. The compound characteristics, usage data and data on the 'receiving environment', are used to indicate where and which field trials should be conducted. The same data can help direct post-registration field trials as well.

Provisionally at least, the field trials focus 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.

4.1 Compound properties

The properties of the compound provide indications of the effect or mechanism on which testing should be focused. Table 4 summarizes these relationships for the properties considered most important. Below, the properties are classified in terms of (inter)nationally accepted classes, which can be used to assign a relative weight to a potential effect.

Toxicity and type of action

Toxicity should be tested for birds, algae, Daphnia and fish, honeybees, beneficial arthropods, earthworms and non-target soil organisms (EU, 1994). Standards for the toxicity/exposure ratio are set. If this ratio falls below these standards, no authorization shall be granted unless it is clearly established through an appropriate risk assessment that under field conditions there is no unacceptable impact.

Therefore a field trial can be conducted by the applicant if there is a high risk of a compound reaching one of the above-mentioned organism groups. In this case it is proposed to conduct the field trial with a number of related species, or species living in

the same compartment. In the case of a risk for birds, a field trial should be conducted with at least five European vertebrate species, and in the case of algae, with five aquatic plant species. In the case of Daphnia, five aquatic invertebrates could be tested, etc.

Table 4 Specification of anticipated effect based on compound properties.

property	mechanism	specific effects anticipated
toxicity & type of action	primary poisoning	toxic effect on related organisms/ processes
	secondary poisoning via increased availability or anomalous behaviour of target organism	toxic effect or food effect on predators of target organisms
persistence	accumulation in environment	organisms in soil/aquatic sediment
bioconcentration factor	accumulation via food or environment	toxic effects on organisms at end of food chain
mobility	dispersal in environment	groundwater, surrounding ecosystems, particularly ditches
efficacy	complete eradication of target organism	food or habitat effect on predators, flower feeders/pollinators, organisms dependent on target organisms
spectrum of action	eradication of broad spectrum of food organisms, habitat destruction	food or habitat effect on predators of target organisms or habitat-dependent organisms

Table 5 Specification of effects based on target organisms.

intended action	organisms at risk
bactericides	prokaryotes
virucides	prokaryotes
fungicides	fungi
algicides	algae
herbicides	plants
nematicides	unsegmented worms
molluscicides	molluscs
acaricides	mites & spiders
insecticides	insects
rodenticides	mammals

The intended action of a pesticide can also be regarded as a compound property, the target organisms providing an indication of likely side-effects; this information can then help to select the test organisms. Table 5 categorizes the various types of pesticides on the

basis of target organisms. If a toxic side-effect is anticipated, side-effects should be anticipated primarily on non-target organisms from the same group and occurring in the same environmental compartment as the target organisms.

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.

Persistence

In the Dutch government's general administrative order on Environmental Approval Criteria for Pesticides (Anonymous, 1995), a product is approved if:

- half-life < 90 days
- soil-bound residues after 100 days do not exceed 70% of the initial quantity; mineralization velocity is not less than 5% within 100 days
- these criteria are not applicable if the applicant can prove that the compound and its decomposition products do not accumulate and have no effects on diversity and richness of non target organisms, and the sum of the concentration of the compound and its decomposition products is so low that after two years the MTR (maximum acceptable level at which 95% of the species is protected) is not exceeded.

If a no authorization is granted, owing to these values being exceeded, the applicant can conduct a field trial to prove that in a field situation these standards are not exceeded. We suggest conducting these field trials under realistic conditions on several (e.g. five) soil types, representative for Europe and for the crops on which the pesticide is to be applied.

Bioconcentration factor

In the Uniform Principles of the EU concerning pesticides it is stated that no authorization shall be granted if the BCF for vertebrates is greater than 1. Terrestrial organisms are exposed mainly via food. In general, organisms at the end of the food chain are often K-species, and are relatively slow to recover when there populations have been reduced. Therefore these organisms are generally more affected by compounds with a high BCF (Moriarty, 1990), depending, of course, on the mode of action.

In the EU-directive the BCF limit for the aquatic environment is set at 1000 for products which are readily biodegradable or 100 for those which are not readily biodegradable. In the aquatic environment organisms will be exposed mainly via the water. As for the terrestrial organisms, organisms at the end of the food chain are likely to suffer most from compounds with a high BCF.

It is concluded, therefore, that a field trial to prove the harmlessness of a product with a high BCF should be conducted with species at the end of a food chain; of course the mode of action should be taken into account in choosing the test organisms. By choosing

these organisms, the occurrence of bioaccumulation through the food chain is taken into account as well.

Mobility

For mobility, standards are set for leaching to groundwater (Anonymous, 1995). If the predicted concentration of a compound in soil water exceeds certain standards, authorization is not granted unless it is proved that, through some kind of decomposition process, the concentration in groundwater is below these standards. In general, compounds with a high water solubility (> 1000 mg/l) or a high evaporation rate (vapour pressure Pa > 1) (*cf.* Van Gestel, 1984) have a greater risk of reaching the surrounding environment and surface water. Therefore these compound properties could give an indication as to the compartment or place were a field trial should be conducted.

Efficacy and spectrum of action

If the efficacy or spectrum of action point to the need for field testing for ecological side-effects (see Chapter 3), trials should be focused on organisms dependent upon the target organisms for food or habitat. If a need for field testing is indicated by other compound properties, then efficacy and spectrum of action may form grounds for conducting field trials with non-target organisms related to the target organisms.

4.2 Usage data

Table 6 Specification of effect based on pesticide formulation and application method.

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

Usage data, such as formulation or mode of application, may be useful for further specification of where to conduct a field trial. Table 6 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.

4.3 Receiving environment

The nature of the 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 effects to certain groups of organisms in a general sense (Table 7).

Table 7 Specification of anticipated effect based on environmental compartment of compound application.

compartment	treatment	species at risk
water	ditch	aquatic flora/fauna/
(+sediment)	ditch bed	ecosystem
soil	soil fumigation	soil fauna and ecosystem
	ditch bank treatment	riparian and aquatic flora
		aquatic fauna and ecosystem
crop	crop treatment	fauna bound to non-target vegetation
	row treatment	ditto
	seed treatment	soil fauna
	defoliation	vegetation-bound fauna
		non-target vegetation
	weed control	ditto; also species dependent on affected habitat
animals	pest control	birds and mammals
		predators of affected organisms
indoors	soil treatment	possibly via leaching
	greenhouse treatment	ditto

The ecosystem or type of area (Table 8) may focus a field trial directly on certain communities, enabling a further specification of the indications obtained from Table 7. An important aspect to be considered here is the rarity of the species or communities concerned.

Table 8 *Specification of effect based on area or ecosystem 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

4.4 Resumé of the required field trials

In the previous sections it was indicated how the data available at the time of application for approval can be used to indicate where and with what organism group a field trial should be conducted. 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.

5 CHOICE OF TEST ORGANISMS

In this chapter concrete test species are selected with reference being made, where possible, to existing test methods.

First, for each group of species mentioned in Chapter 3, suggestions are made for concrete test species. Although with time there will be increasing emphasis on the ecosystem approach, based on the use of groups of species, we here present individual species, thus staying in line with current legislative procedures and laboratory tests, which focus mainly on individual species. In choosing species the following criteria are employed:

1. The test species should be fairly abundant in agricultural areas; this is important for the extrapolation of test results to the real field situation, and determines the choice of concrete species.
2. The test organisms should not be extremely insensitive to pesticides in general; data on the sensitivity of the organisms have been obtained mainly from the literature. There may, of course, exist large differences among species and within one species among different compounds. In general, however, there are fairly general ideas about which species occur in polluted conditions, and these species should not be used for assessing side-effects of pesticides.
3. Species should be appropriate for field trial research. Here, too, several literature sources have been used. In cases where guidelines or well-documented trials exist, these could be used as an important additional criterion. Also, species should preferably be used in international procedures.

Next, for the species selected it is set out which field trial methods are currently available. In this context, an examination was made of the methods employed by a number of international organizations: OECD, IOBC, EPPO, FAO, Council of Europe and EU, and by various national organizations, *viz.* in the United States, England, Germany and The Netherlands.

5.1 Aquatic test species

Hardly any concrete guidelines for field trials were found in the literature. In 1991 two workshops were organized that focused at aquatic studies: a Workshop on Aquatic Microcosms for Ecological Assessment of Pesticides (Anonymous, 1992) and a Meeting of Experts on Guidelines for Static Field Mesocosm Tests (SETAC, 1991). Both workshops resulted in guidance documents.

The guidance document for microcosms describes general methods for constructing microcosms, monitoring their ecological characteristics, treating them with test pesticides, and analyzing the results. The basic microcosm design is an outdoor tank approximately six to ten cubic metres in volume, containing water, sediment, and aquatic communities including fish. Microcosms bridge the gap between simple laboratory test systems and full-scale field studies. All kinds of aquatic organisms can be studies: phytoplankton, zooplankton, periphyton, macroinvertebrates and fish.

Odum (1984) proposed the term mesocosm to describe bounded and partially enclosed, outdoor experimental units that closely simulate the natural environment. Since the two workshops obviously did not exchange definitions, mesocosms are taken to be all outdoor experimental systems. Here again the methods are described extensively, from design, composition and characterization, through statistical design and treatment, to end-points and sampling and data handling.

For the aquatic environment it can be concluded that guidelines for these types of semi-field studies do exist. For full-scale field studies no guidelines were found. This picture was confirmed at a European workshop on Freshwater Field Tests (Hill *et al.*, 1994).

Table 9 shows the various different groups of species that are suitable for assessing pesticide side-effects. Proceeding from these species groups, test species are then suggested below.

Table 9 Relative score of groups of species on criteria on which test species for the aquatic environment are selected.

functional & taxonomic groups	direct exposure		part of functional group	suitability for field studies	suitability for field trial
	sediment	water			
Producers					
algae	-	+	+++	+	+++
vascular plants	-	+	+++	+	+++
Herbivores					
molluscs	-	+	+++	+	++
unsegmented worms	+	+	-	+	-
segmented worms	+	-	++	+	+
crustaceans	+	+	++	+	+++
insects	+	+	++	+	++
fish	-	+	+	+	+
birds	-	+	+	-	-
Carnivores					
unsegmented worms	+	+	-	-	-
segmented worms	-	+	-	-	-
spiders & mites	-	+	+	+	+/-
insects	+	+	+++	+	+++
amphibians	+	+	-	+	-
fish	+	+	++	+	++
birds	-	+	++	-	-
mammals	-	+	-	-	-
Decomposers					
bacteria	+	+/-	+++	+	++
fungi	+	+/-	+++	+	+++
unsegmented worms	+	-	+	-	-
segmented worms	+	-	++	+	+
crustaceans	+	+/-	-	+	+
insects	+	+/-	-	+	+

Primary producers

Table 9 shows that both algae and vascular plants are relevant. Both are important primary producers, especially in the shallow waters and ditches of the Netherlands. In surface waters, besides, algae are a major food source for all kinds of herbivores, and plants are also important as a habitat or substrate for a number of species.

For vascular plants, tests should be performed with representative species. Since hardly any data are available on the sensitivity of aquatic vascular plants to pesticides, it is recommended to use several species. No standard methods are available, however. Barrett & Wade (1988) discuss the problems involved in field testing with vascular plants rather than presenting a ready-to-use method.

Duckweeds are sensitive to herbicides (Grossman *et al.*, 1992) and extremely suitable for measuring the direct effects of pesticide drift, as a validation of predicted drift (De Jong & Bergema, 1994). Effects are easy to measure using floating compartments (De Groot *et al.*, 1987). Duckweeds are less representative for vascular plants living in the water column, however.

Algae can be divided into two groups: periphyton (epiphytic, for instance *Stigeoclonium* sp.) and phytoplankton (planktonic, for instance *Scenedesmus* sp.). In the ditches of the Dutch polders, periphyton is quantitatively the most important group. Phytoplankton, however, is an important food source for filter feeders. Therefore, field trials should be available for both groups. For periphyton, methods involving assessment of algal growth on microscope slides are well known (Hamilton *et al.*, 1987; De Jong & Bergema, 1994). For phytoplankton, field methods are now available; a method using an alginate matrix with immobilized algae cells is being developed (Bozeman *et al.*, 1989; Faafeng *et al.*, 1994). In the case of algae, it is relevant and possible to conduct the bioassays with not just one species, but with the overall group. If the species composition is additionally studied, any impact on individual species can be traced as well as effects on overall species composition.

Herbivores

Crustaceans appear to be the most suitable group for testing effects on aquatic herbivores. As a matter of priority, therefore, a field trial method should be developed for this group. Molluscs and insects may be suitable as well.

As filter feeders, water fleas *Daphnia* sp. and copepods *Cyclops* sp. are important consumers in the aquatic environment and, besides, both are important food sources for other organisms. Comparing the two species, it can be observed that water fleas are used as standard organisms in lab trials. A lot is therefore known about these organisms (see, for example, NEN, 1980), and comparison between the lab and field will thus be easier if water fleas are used. Moreover, water fleas are generally very sensitive to pesticides (Mayer & Ellersieck, 1986), so priority should be given to a field trial with water fleas. In a number of studies in the Netherlands, bioassays with water fleas in glass jars in

ditches have been used (Anonymous, 1990, De Jong & Bergema, 1994). This method could be developed further into a standardized field trial.

Molluscs are also important herbivores. Their sensitivity, however, is questionable: even in severely contaminated ditches, where most other groups of organisms had disappeared, molluscs survived (Working group "Effects ...", 1988). Therefore, no priority should be given to this group of species.

There is hardly any experience with herbivorous insects in the water column, except in stream studies. In the Netherlands, however, most water is relatively stagnant. Because insects will have already been included with the carnivores, though, no priority is given to field trials with herbivorous insects.

The most common herbivorous 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 carried out by RIZA (Netherlands Institute of Inland Water Management and Waste Water Treatment), in which sublethal effects (jaw deformities) and lethal effects were found in midge larvae (Van Urk *et al.*, 1991). There are other ongoing projects involving the use of midge larvae in laboratory and mesocosm studies (Eijsackers & Bosma, 1989). Therefore, development of a field trial with these organisms is recommended.

Carnivores

A field test with insects is needed primarily because of the hazard of insecticides. Since the larvae of aquatic insects are generally more sensitive than the adults, a test with the former should be developed. By choosing predatory insects, this aspect of the ecosystem can also be covered. In moving waters, methods aimed at invertebrate drift do exist (Kreutzweiser & Kingsbury, 1987; Cuffney *et al.*, 1984) and are standardized to a certain degree (Kreutzweiser & Capell, 1992). These tests focus on species present in the local situation, however. For a more standardized trial, concrete species should be selected. For reasons of size, a test with the larvae of predatory beetles or nymphs of predatory bugs could be developed, for instance, although 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). Furthermore, the larvae of *Chaoborus riparius* appear to be sensitive and suitable for bioassay research (Bergema & Rombout, 1994; Helgen *et al.*, 1988). Since there is little experience with other groups, further studies are needed.

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, some experience has already been gained with water mites (Canters *et al.*, 1990).

To include vertebrates, it is useful to include a fish species in a field trial. Sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius* are representative of ditch predators and

they are also abundant, especially in smaller ditches. Since *Gasterosteus aculeatus* has disappeared from the bulb-growing area in the Netherlands, this could indicate that this species is sensitive to pesticides (oral comm. Sevenster, Dept. of Ethology, Leiden University). For moving waters, tests with fish do exist (Kingsbury & Kreutzweiser, 1987). For the Dutch situation, however, it should be noted that fish species are relatively sensitive to low oxygen levels, which are rather normal in the Netherlands, certainly locally. This aspect therefore renders fish species unsuitable for cage studies, for instance. With Dutch species, tests have been performed in experimental ditches (Deneer, 1994) and an early warning bio-alarm system with *Leuciscus idus* has been set up (Hendriks & Stouten, 1993).

Aside from 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) to develop a field trial. For a review of laboratory and field research on pesticide side-effects on amphibians, see Harfenist *et al.* (1989).

Decomposers

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: *cf.* Heath *et al.*, 1966). A preliminary laboratory experiment using litterbags in glass jars showed an effect of captan on litterbags (De Jong & Bergema, 1994). By varying the mesh of the litterbags, the effects on different organism groups might be studied. Another possibility is to use cotton strips, in which the loss of tensile strength is assessed (Harrisson *et al.*, 1988).

Unsegmented worms

In the benthic environment, unsegmented worms (e.g. nematodes) 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.

Segmented worms

Of the segmented worms (Annelids) living in aquatic sediments, the Oligochaetes (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 IBN (Netherlands Institute for Forestry and Nature Management).

Crustaceans
A test involving *Gammarus* sp. and *Asellus* sp. is recommended, possibly proceeding from the work being done at the Staring Centre (Brock *et al.*, 1992). Our own research (Canters *et al.*, 1990, De Jong & Bergema, 1994) also points to the suitability of these organisms for a field trial.

5.2 Terrestrial organisms

In Table 10 the different criteria are quantified for each group of terrestrial species.

Table 10 Relative score of groups of species on criteria on which test species for the terrestrial environment are selected.

functional & taxonomic groups	direct exposure			part of functional group	suitability for field studies	suitability for field trial
	in soil	on soil	on vegetation			
Producers						
vascular plants	-	+	n.a.	+++	+	+++
Herbivores						
unsegmented worms	+	-	-	+	+	+
molluscs	-	+	+	+	+	+
mites	+	+	+	+	+	+
insects	+	+	+	+++	+	++
birds	-	-	+	+	-	-
mammals	+	+	+	+	-	-
Carnivores						
unsegmented worms	+	-	-	++	-	-
spiders & mites	+	+	+	++	+	++
insects	+	+	+	++	+	++
birds	-	-	+	++	-	-
mammals	+	+	-	++	-	-
reptiles	+	+	-	-	-	-
Decomposers						
bacteria	+	+	+	+++	+	++
fungi	+	+	+	+++	+	+++
unsegmented worms	+	-	-	+	+	+
segmented worms	+	-	-	++	+	++
crustaceans	-	+	-	+	+	+

n.a. = not applicable

Producers

In the terrestrial environment, vascular 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, among other things, the wild flora of agricultural areas is under severe pressure, as discussed in the Netherlands' National Environmental Policy Plan (NEPP, 1989). Development of a field trial with vascular plants is therefore urgently required. This can be based on existing trials with this group of organisms, *viz.* efficacy tests (EPPO, 1989b) and the EPA guidelines (EPA, 1982; 1986a,b). At a SETAC meeting in 1992, there was a session on plant testing (Aldridge *et al.*, 1993) where it was concluded that plants play a critical role in ecosystems and should thus be protected from the adverse effects of pesticides. Ecologically relevant groups or species should be chosen.

For studying the side-effects of pesticides, two groups of plant species would be suitable: i) common or threatened species that are sensitive to pesticides, and ii) very sensitive species, giving the greatest chance of finding side-effects. The choice of test species is elucidated below.

For selecting test species of the first type, the studies of Marrs *et al.* (1989, 1991) should be mentioned. They studied the side-effects of drift on plants of nature conservational interest. There studies show *Lychnis flos-cuculi*, *Prunella vulgaris*, *Digitalis purpurea*, *Cardamine pratensis* and *Medicago lupulina* to be most sensitive to side-effects of herbicides. *Medicago lupulina* is very common in the Netherlands; therefore effects are already to be expected. *Lychnis flos-cuculi* and *Cardamine pratensis* are annuals and occur in a more open habitat; they therefore appear to be suitable test species.

As a very sensitive species to herbicides, tomato *Solanum lycopersicum* is mentioned (Breeze 1988, Breeze & van Rensburg, 1991). Oilseed rape *Brassica napus* is also mentioned and appears to be even more sensitive (Eagle, 1982). Besides, oilseed rape has become naturalized in the Netherlands (Van der Meijden *et al.*, 1983). Therefore, this species seems to be more suitable for use in a field trial.

For a monocotyledonous species, *Poa annua* seems to be suitable. This species is sensitive to air pollution in general (oral comm., Van der Eerden, Research Institute for Plant Protection, IPO) and can be used in bioassays (De Jong & Bergema, 1994).

Herbivores

Unsegmented worms

Nematodes form a relatively diverse group of organisms, fulfilling various functions in the ecosystem (Bongers, 1988). A variety of plant-parasitic nematodes exist and in a number of cases these are the target organisms of pesticide applications. In the context of the efficacy testing 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 molluscs and they may, in

principle, be subject to 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 with slugs seeems desirable.

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 focuses 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.

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. An internationally adapted guideline exists (EPPO, 1992). Honeybees are not the most representative herbivores, however. Therefore, in addition, a field trial with caterpillars is suggested (Davis, 1993; De Jong & Van der Nagel, 1994). Caterpillars of the Large white butterfly *Pieris brassicae* are suggested as test organisms, being both sensitive to insecticides and easy to handle in a field bioassay.

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 laboratory trials, seed-eating birds are generally used. In field trials, herbivorous and carnivorous species might be studied. Because the field methods do not differ essentially, the two groups are dealt with together.

In the selection of test species, the Avian Effect Dialogue Group (AEDG, 1994) suggests working with 'focal species'. These species could include representative species, high-risk species and/or surrogate species, the latter if the species of concern itself is unavailable for direct study. These starting points lead to a number of aspects which will have to be taken into account when selecting test species: sensitivity, exposure, abundance, ease of study, habitat and range, history of incidents, similarity of taxonomy and field/laboratory verification.

In developing trials, use can be made of the general bird-inventorying techniques developed and used in the Netherlands (Hustings *et al.*, 1989). For a field trial method, reference is made to the British guideline (MAFF, 1986) and to the EPA guideline (Fite *et al.*, 1988). Dingledine & Jaber (1990) have reviewed a number of field methods. Radiometry can enhance the reliability and usefulness of field trials, but costs increase proportionately (OECD, 1988).

Carnivores

Unsegmented worms

For nematodes, reference is made to the remarks made when discussing herbivores.

Sensitive nematodes with longer life cycles do exist and could be suitable as indicators (Bongers, 1988).

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 (Free University, Amsterdam) and LUW (Wageningen Agricultural University; *cf.* Van Straalen & Everts, 1989), IBN and PD (Van de Bund, 1980) and developments in Hungary (pers. comm., Oomen, PD). A simple whole-soil bioassay aimed at heavy metals and based on micro-arthropods is proposed by Sheppard & Evenden (1994).

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 (carabid and staphylinid beetles) can be taken (MAFF, 1986). Use can also be made of the work at IBN 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).

For beneficial arthropods, cage, tent and field trials to assess mortality, behaviour and loss of (predatory) function are available (OECD, 1988). 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 (EPPO, 1990)
5 ichneumon fly *Encarsia formosa* in greenhouses (EPPO, 1989)
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. In Germany guidelines are available for two groups of species, *viz.* beneficial arthropods in arboriculture (BBA, 1981) and predatory mites in viniculture (BBA, 1986).

Spiders and 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 Wageningen Agricultural University (Everts, 1990; Jagers op Akkerhuis, 1993).

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 as part of the procedure. If effects are anticipated, limitations can then be imposed on use in situations where exposure is likely, or user guidelines adapted accordingly.

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 IBN 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. Also the EPA guideline can be used (Fite *et al.*, 1988).

Decomposers

For studying effects on decomposition, fungi appear to be most suitable; bacteria and segmented worms seems to be suitable as well (Table 10).

Fungi

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

Soilborne fungi play a dominant part in the decomposition process. Many studies are concerned with the effects of pesticides on mycorrhiza-forming fungi (Menge, 1982; Trappe, *et al.*, 1984; *cf.* De Jong *et al.*, 1992). However, almost all studies deal with effects inside the target area, and are concerned with the mycorrhizal fungi on the crop. In these cases it is the degree of root infection after a pesticide treatment that is assessed. Field bioassays with mycorrhizal fungi have not been found in the literature. Laboratory studies appear to be poorly representative for the field situation (Unestam *et al.*, 1989). Termorshuizen (Wageningen Agricultural University, LUW) suggests the use of leek *Allium porrum* in the field. This plant can then be used to detect the number of mycorrhizal fungi in the soil. However, this method is likely to involve many difficulties. Firstly, detection of mycorrhizal fungi is rather time-consuming; secondly, the number of mycorrhizal fungi and their dispersal pattern in different types of soil in the Netherlands is likely to vary and depends on soil fertility. Therefore, further studies will be needed to investigate the potential for such a field trial.

Another possibility is the use of mushroom-type fungi. Studies of effects on the mycelium are not known, and no methods were found in the literature. Consequently, this type of species was not chosen.

It is also possible to measure decomposition itself. Two methods are worthy of mention: the use of cotton strips and the use of litterbags. Both methods are described extensively

in the literature (Harrisson *et al.*, 1988; Heath *et al.*, 1964, 1966). In the cotton-strip method the loss of tensile strength is assessed and in the litterbag method the decomposition of organic matter is measured. Both methods seem to be suitable. For a litter decomposition field trial, reference is made to a British guideline (MAFF, 1986). For studying litter decomposition, field trials are even stated as being the only practicable approach, laboratory simulation being impossible (OECD, 1988).

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 German soil microflora test (BBA, 1987).

(Un)segmented worms

For the nematodes we refer to the section on herbivores, above. Among the 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 BBA in Germany (OECD, 1988) and that already in force in the UK (MAFF, 1986), incorporating the developmental work of RIVM (Van Gestel, 1991) and other studies (Ebing *et al.*, 1984).

Side-effects in the soil are primarily to be expected within the target area. Outside the target area, however, the repeated use of fungicides can lead to a deposition of low concentrations (De Jong *et al.*, 1995). Some indications have been found for the occurrence of side-effects of such deposition (De Jong & Bergema, 1994). In general, however, it will be very difficult to trace side-effects of soil processes outside the target area, because of the low exposure, combined with the large number of organisms contributing to the decomposition process. Any effects on one group of organisms will easily be covered by other groups.

6 ASSESSMENT OF FIELD TRIAL RESULTS

In this chapter various aspects of field trial assessment will be elaborated. First, a number of general requirements for all kinds of field trials will be treated (§ 6.1); then assessment in a more technical sense will be discussed (§ 6.2); the Chapter concludes by considering the interpretation of field trial results (§ 6.3).

6.1 General requirements

The field trials must meet a number of general requirements which, though seemingly trivial, will be stated explicitly for the sake of completeness; a number of them will be elaborated below. 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 (untreated or treated), 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 as far as possible be excluded. 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. Extreme environmental conditions should be avoided.

Experimental conditions

Crop

For the field trial, a crop is chosen for which the highest exposure of the organisms studied has been calculated or is to be expected. This need not automatically be the same crop for the terrestrial and the aquatic environment or for different groups of organisms.

In the Dutch situation, in the aquatic environment the Dutch polder ditches will be exposed as a result of drift, run-off and leaching. The amount of drift depends, *inter alia*, on the dosage, so in practice the crop for which the highest dose is prescribed will often lead to the highest drift. However, the crop type and the mode of application also influence drift percentages. With fruit-growing, for example, high drift percentages are to be expected; if an application is filed for use of the compound in fruit-growing, therefore, this crop should presumably be included in the field trial.

For the terrestrial environment, the crop chosen will depend on the manner in which the organisms studied are exposed. In the case of the hazard of treated seeds to birds, for example, the fact of whether seeds are available to and eaten by birds is far more important than the dosage of the active ingredient per hectare. Therefore, for each compound and for each expected effect it should be determined whether it is necessary to select a certain crop. In any case, the situation (crop, application method, formulation) in which the highest exposure of the non-target organisms is predicted should be included in the field trial.

Species

In a field trial, it is of great importance to determine effects on species actually found in the area of study. In the Dutch situation, these should always include species that are common in the Netherlands, or species that have been shown to be representative. For a comparison between laboratory and field, it would be desirable for the same species to be used. Therefore, it should first be studied whether the species presently used in lab-testing are representative of the field situation in the Netherlands (see Chapter 3). Then, if these species are moreover suitable for use in the field, they should preferably be chosen. The choice of test species has been elucidated in detail in Chapter 5.

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

Untreated control

In all cases, the exposed plot should be compared with an untreated plot. This untreated plot must have undergone the same mechanical operations, must bear the same crop, and also, for instance, be treated with a substance resembling the formulation used, e.g. water or granules. If indirect effects are anticipated, it may be necessary to distinguish between direct and indirect side-effects. In that case, a second 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 organism studied. 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.

Treated control

In addition to an untreated reference, a 100% treated control is also often desirable. In the terrestrial environment the 100% treated control is the treated crop itself. If the field trial is aimed at side-effects inside the treated plot, an extra treated control is not needed. If a field trial is aimed at side-effects outside the treated plot, a control inside the treated plot may be desirable, to discover whether the 100% dose has any effect.

For the aquatic environment, too, it may be necessary to know whether a 100% dose has any effect. To this aim, an enclosure can be used and treated with a 100% dose to assess whether this maximum dose has any effect. Only then does it make sense to study the effects of much lower exposure rates, such as those due to drift, for instance.

Apart from a 100% treated control, if the effects of a compound on the test organisms are unknown, it may be useful to arrange a test plot treated with a pesticide having the same intended action as the test compound 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. This could be the case with organisms that are exposed by direct contact, for instance, when these organisms are inactive and hidden at the time of application, because of certain weather conditions, for example. In the case of mammals, birds and fish, such a 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 effects. 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, or one generation of the test organism. 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.

The minimum duration is the period after which effects in the treated control become manifest. An experiment may be ended if no new effects are found in the exposed control, while the experiment may be of longer duration if recovery is part of the research question.

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 two times the maximum dose prescribed, thus incorporating a worst-case situation in 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 twofold 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 two 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.

Formulation and method of application

The use of a certain formulation or method of application may be 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 focused on those situations in which exposure will be highest.

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 a different context. A 100% load 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.

Weather conditions
Weather conditions, in terms of temperature and relative humidity, should be those under which the compound in question will normally be applied. If effects other than those on the treated plot are being studied, there should be a moderate wind, not less than 4 m/s, in the direction of the outside units being exposed. Extreme weather conditions should be avoided at any times.

Observations

The methods of observation depend primarily on the effect anticipated. In general, mortality or changes in the number of organisms will be the first parameter to assess. However, growth, pupation, moulting, etc. can also be assessed, depending on the organism and the anticipated effect. Effects should be monitored from the moment they become measurable in the treated control.

In a field trial, it is always essential to know whether the experimental organisms are actually exposed. Measurement of pesticide concentrations or depositions in the exposed environmental compartments and in the organisms tested should be part of the standard procedure, as these data can provide support in establishing causal relationships.

6.2 Technical assessment

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. Below, a number of points of importance in evaluating test results are distinguished.

Requirements
In general, the requirements mentioned in Section 6.1 should first be fulfilled. Field trials that do not meet these requirements may be valid, but their scope will be limited. Thus, the validity of such field trials depends on the question to be answered.

Statistical significance
Effects must be demonstrated with 95% (two-sided) certainty. This condition places high demands on the test method. In a number of cases, it will be necessary to determine the variation in the field prior to the trial. Using these results, the number of repetitions

required to obtain 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 10% differences from the untreated control.

Effects in the untreated control

In laboratory tests, mortality in the untreated control 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 untreated mortality in the field. In several places in the literature, a field mortality rate of 15% in the untreated control is quoted as being acceptable (e.g. EPPO, 1992).

Effects in the treated control

In a field trial a 100% treatment does not necessarily have to lead to a 100% effect. If the aim of a field trial is to trace the side-effects within the treated plot, no extra treated control need be used, and only the differences between the treated and the untreated plot need be established. In the case of the side-effects of lower dosages, in ditches or outside the treated plot, for example, it is necessary for the 100% treated plot to show a clear effect. Otherwise, it is unlikely that effects will be established at lower dosages. The effects to be expected at 100% treatment level can be derived from the results of laboratory studies. If lab studies show only minor effects with a 100% treatment, there is no use investigating the effects of much lower dosages in the field.

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 focused 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.

6.3 Interpretation of field trial results

At this point it is assumed that the technical requirements have been fulfilled and a field trial is deemed valid. If no effects are found, interpretation is clear: a minor hazard exists. The same is true if very clear and serious effects are found in a field trial; in this case there is a very serious hazard. In these cases, the organization charged with the task of pesticide assessment can use these results in the evaluation of the environmental aspects compared to other aspects. Because test species are, *inter alia*, chosen on the basis of their representativeness, the effects on these organisms can be interpreted at higher organizational levels.

The problem, of course, arises when the results of a field trial are not that clear. How, for instance, should a growth reduction of 25% or an increased mortality of 10% be interpreted? In this case we could indicate a moderate hazard; this, however, will not bring the assessor any further than he was after the laboratory trials. In the following, we

will deal with a number of aspects which can be of help in assessing the results of field trials. A number of standards will be proposed, on the basis of which it can be decided whether a hazard is to be deemed minor or serious.

First, it should be stated that the effects found are obviously those towards which the field trial is directed. The field trial was selected by following a procedure to identify the most relevant trial, with relevant parameters. This means that at this point the effect parameters, and the trial itself, are no longer a point of debate, thus limiting the points of assessment considerably. Thus, only the magnitude of the effects should be assessed, and not their nature.

For vertebrates, it is defensible that any mortality implies a serious hazard. This is supported by the Avian Effects Dialogue Group (AEDG) of the EPA. This group agreed that bird mortality can be an adverse environmental impact and should be documented for pesticide regulations. This also holds for population effects, where a population effect is a sustained change in the composition (e.g. age structure, genetic) or size of a local, regional or national population. For a threatened or endangered species, the loss of an individual is always an impact of concern (AEDG, 1994). Here, there is a problem when sublethal parameters are being assessed.

In general, effects at the population level will be the standard and the problem is how to translate a sublethal effect (on individuals) to a population effect. The US Avian Effects Dialogue Group indicates that it is especially in this field that research is still needed (AEDG, 1994).

In the Uniform Principles concerning pesticides (EC, 1994), it is stated that field trials can be used to indicate that no unacceptable effect or impact occurs. Here again, it is not indicated when an effect is unacceptable.

Although these problems exist, we would like to propose two criteria, a spatial and a temporal, to give an idea about how to assess the results of a field trial. However, further research is absolutely necessary to flesh out and validate these criteria.

Decline inside and outside target area

As a starting point, we assume a long-term (*i.e.* over a 5-10 years period) protection target: no negative trend or significant decrease relative to the preceding five or ten years for the individuals of a priority species. Based on this assumption, with respect to effects on populations of priority species (a decline in density, for example) we propose the following criterion for the consequences of pesticide use within the target area. A maximum decline of 5% in a population in areas with general environmental quality and no decline at all in areas with special environmental quality[1]. Outside the target area, there should be no decline at all in population densities or natural functions. For vertebrates, we could add the requirement that outside the target area no effects at all are acceptable.

[1] General and special environmental quality are terms used in Dutch environmental policy to characterize the ecological quality of particular areas.

In the Netherlands, substance policy is based on risk prevention. For ecological effects, the point of departure is that for 95% of the species there should be no negative effects (VROM, 1991). Models for predicting side-effects are based, *inter alia*, on this criterion. Long-term toxicity data should be available for at least four species for risk prediction to be reliable. Following from this criterion, it is proposed to study in a field trial at least four species within the group of concern. The results of this field trial could be used as inputs for the models again.

A point of discussion is the group of species for which 95% should be protected. We propose to choose the group for which a hazard is predicted. For instance, in the case of a hazard for Daphnia, a field trial should be aimed at the protection of aquatic invertebrates, and at least four aquatic invertebrates should be part of this trial.

Duration of effect and recovery

It will also be necessary to define, in the relevant legislation, the minimum period of time within which effects may occur. As pesticide use is frequently seasonal, it is realistic to set (TCB, 1990) the requirement that, in areas having general environmental quality, within a certain period following application of the compound populations of priority species must have returned to their original density, pesticide concentrations fallen to the NOEC level and natural functions been restored. In the Dutch Environmental Quality Objectives, a two-year period is currently set (Anonymous, 1995). It should be borne in mind that ecosystems are dynamic and it is not always certain that the species of concern will recover to their original density, as a result of changes in species composition, changes in concurrence, food availability, etc. Consequently, in some cases effects cannot be demonstrated until a year after application.

If, despite all attempts, the results of a field trial do not yield a clear distinction between a minor and a serious hazard, a more procedural solution can be formulated. In such cases, it is suggested to give provisional approval to a pesticide, on the proviso that environmental monitoring be undertaken to elucidate the aspects that remain unclear.

7 CONCLUDING REMARKS

In this report a number of points have been elaborated on which field trials might play an important role in relation to the registration of pesticides. Of course, the procedure will be rendered more extensive by adding field trials. The aim, however, should not be to aggravate the procedure with a series of field trials, but to improve it.

Priority should therefore be given to one-off validation of starting points and models. By doing so, the predictive power of the procedure will be improved, and the need for field trials as part of the procedure will be diminished. Furthermore, it might be feasible to reduce the safety factors used at present.

The Uniform Principles of the EU concerning pesticides assign a distinct place and function to field trials within the procedure: field trials can be conducted to prove the harmlessness of a product after the laboratory-based procedure has indicated a (serious) hazard. It is up to the applicant to decide whether the costs of field trials balance the expected benefits.

In every case, it should be investigated whether a field trial is the most efficient way to gain a clear answer with regard to the expected risk. For specific questions, concentration measurements combined with laboratory trials might also yield clear-cut answers. Because, in general, such laboratory trials are cheaper, have less variation and greater reliability, these kind of trials can be conducted where possible. The difference from a field situation is considerable, however. It should therefore be assessed on a case-by-case basis whether the results of such trials are to be deemed acceptable.

For the limited number of cases in which field trials will be needed as part of the registration procedure, guidelines should be available for a range of field trial methods. The number of concrete and internationally accepted guidelines is relatively small, though. Many initiatives towards field trials have been taken, however, again in an international context. In some cases these are separate studies, of which elements could be used when conducting a field trial, while in others guidelines have already been drawn up. There is a long way to go, however, before such guidelines are accepted by an international organization.

It can be questioned whether it is desirable to draw up strict guidelines. As stated in Chapter 4, a number of aspects relating to a compound and its use give direction to a field trial. In general, however, the principles of Chapter 6 should be adhered to. Because of the new function of field trials within the EU procedure, the assessment of trial results is crucial. It is, however, the applicant who has to prove the harmlessness of his product.

It is conceivable that a commission within the CTB be designated, charged with the assessment of field trials. This commission could, in consultations with the applicant, decide which field trial should be conducted and under what conditions. This would avoid a situation whereby the results of a field trial cannot be well interpreted.

For the Dutch situation it is concluded that, except for honeybees, hardly any field trials are being carried out as part of the registration procedure. In a number of cases, a

compound is deemed dangerous for honeybees because of a lack of field data. At a number of points in the Dutch procedure, field trials are mentioned as a possibility, but for most groups no concrete guidelines exist, and it is indeed unclear whether field trials are conducted at all.

Post-registration field trials do not constitute part of the EU procedure. We propose to make post-registration monitoring part of the procedure only in cases where a pre-registration field trial does not yield a clear result. In this case, a compound could be approved, providing post-registration monitoring is carried out. The aforementioned commission could pronounce upon the kind of monitoring deemed acceptable.

In a number of cases, pesticide side-effects may come to light only after use on a practical scale. If this is the case, the results of post-registration field studies should be fed back to the approval procedure.

Post-registration field trials could play a role within the framework of governmental monitoring programmes (CCRX, 1993). At present, a number of pesticides are found in surface wafers on a large scale in the Netherlands. The sources of these pesticides are currently being studied and the results of these studies may have consequences for the use (application, formulation or even approval itself) of the compound in question.

Field studies will improve the registration procedure for pesticides considerably, however. A well-validated procedure, combined with post-registration monitoring, should be able to offer protection against the occurrence of most side-effects.

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APPENDIX

List of abbreviations.

BCF Bioconcentration Factor: ratio of a substance concentration in fish to the concentration in water at steady state.

DT_{50} time in which 50% of the parent compound has disappeared from soil or water by transformation

EC_{50} median Effective Concentration: concentration resulting in a 50% change in a parameter relative to the control, or concentration at which a particular effect is observed in 50% of the organism population relative to the control

Henry's law constant: water-air partition coefficient; the ratio between the concentration of a substance in water and its partial pressure in the gas phase

$K_{s/l}$ soil-water partition coefficient; sorption coefficient

K_{om} sorption coefficient divided by the fraction of organic matter in soil

K_{ow} octanol-water partition coefficient

LC_{50} median Lethal Concentration: a statistically derived concentration that can be expected to cause mortality in 50% of animals exposed for a specified time

LD_{50} median Lethal Dose: statistically derived single dose that can be expected to cause mortality in 50% of exposed animals

NOEC No-Observed-Effect Concentration: the highest concentration without adverse effects

PEC Predicted Environmental Concentration: the expected concentration in an environmental compartment, calculated using a model

Rf Retardation factor: the distance moved by a substance relative to the distance moved by the water front