TERRESTRIAL FIELD TRIALS FOR SIDE-EFFECTS OF PESTICIDES

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Summary

This thesis discusses terrestrial field trials to detect side-effects of pesticides, and concentrates on the roles of field trials in pesticide registration procedures and in research into the nature and extent of side-effects of pesticides in the Netherlands. The study was conducted at the Centre of Environmental Science at Leiden University (CML). Most of the results have been derived from a number of projects carried out by CML for the Ministry of Housing, Spatial Planning and the Environment (VROM).

The thesis consists of five parts. Part 1 comprises only Chapter 1, which defines the research area and formulates the basic questions underlying the research project. Part 2 is concerned with the place of field trials in the registration procedure. It includes Chapter 2, which provides a framework for the place and role of field trials in the registration procedure, and Chapter 3, which presents decision schemes for selecting the appropriate trial, and discusses the basic principles of the implementation of such trials and the evaluation of their results. Part 3 reports on the development of specific field bioassays for plants (Chapter 4), caterpillars (Chapter 5) and decomposition (Chapter 6). Part 4 uses the results of the bioassays and other available field data to estimate the effects of pesticides for the whole of the Netherlands; Chapter 7 does this for droplet drift and Chapter 8 for vapour drift. Part 5 (Chapter 9) draws some conclusions, discusses the findings and provides recommendations.

Part 1: Introduction

The Introduction shows that registration procedures have in the past largely relied on laboratory tests and models to assess the side-effects of pesticides on non-target organisms. Pesticide registration procedures were harmonised for the whole of the EU in 1994, which means that products now have to satisfy the requirements laid down in European legislation. The European procedure includes a significant role for field trials. The procedure assesses pesticides (or their specific applications) on the basis of the laboratory data referred to above. If the product does not comply with the relevant standards, it will in principle not be admitted, unless the applicant can provide an adequate risk assessment showing that the product does not produce any unacceptable effects under field conditions. Field trials are an important tool to assess whether a product will lead to unacceptable effects in such conditions.

The Introduction also describes how pesticides are regularly found in all environmental compartments in the Netherlands. Although little field research is being conducted into the effects of pesticides on non-target organisms, there is evidence that such effects do occur, although there is no information on the nature and extent of such effects. While the place of field trials in the current registration procedure is clear, it is not clear which field trial should be used for which problem, how such trials should be designed, which requirements they have to meet and how their results are to be evaluated. The present thesis aims to evaluate the potential role of field trials in pesticide registration procedures, their design, implementation
and interpretation and the use of field trial results to assess the nature and extent of pesticide side-effects. It tries to achieve this aim by answering the following questions:

1. Which role can and should field trials play in the European registration procedure for pesticides? Can a framework be constructed which clearly describes the place and role of such field trials, with a decision scheme describing which trials should be used for which aim?
2. How should field trials be designed, under which conditions should they be implemented and how should results be interpreted? Are such field trials feasible, and what is the applicability of the results?
3. How can information from field trials and related research be used to assess the type of side-effects of pesticides, and the magnitude of such side-effects in the Netherlands?

These questions are discussed in Parts 2, 3 and 4 of the thesis, respectively.

Part 2: Field trials and the registration procedure

Framework for field trials

Chapter 2 presents a framework for field trials, designed on the basis of the international literature on this subject and a number of existing registration procedures. In principle, field trials can be used in three parts of the registration procedure:

i) the validation of models used and assumptions made in the registration procedure;
ii) the verification of the occurrence of effects in the pre-registration stage; and
iii) the evaluation of the registration procedure as a whole in the post-registration stage.

i) Validation of the principles of the procedure involves a number of aspects:

Use
An important aspect is the dosage and the method of administration. It is assumed that the user complies with regulations in the sense that he does not exceed the recommended dosage and meets the requirements for administration, such as weather conditions and the use of emission reducing measures. These aspects are of great importance as admission is based on exposure estimates, which are combined with the product’s toxicity for non-target organisms. Survey data collected by Statistics Netherlands (CBS) show that farmers do not always comply with the instructions for the use of pesticides. Usage varies considerably, and recommended dosages for a number of products are regularly exceeded. Incorporating this information in the registration procedure should allow a more realistic estimate of the dosages actually used.

Exposure
Estimates of exposure levels are largely based on models, which are continually being refined. Such models need to be validated in order to assess how realistic the predicted exposure levels are. For example, validation of droplet drift measurements has already led to adaptation of the drift percentages used by the Dutch body responsible for the authorisation of pesticides (College voor de Toelating van Bestrijdingsmiddelen, CTB). Vapour drift, the most important emission route, is also being increasingly investigated, and models are being validated by means of measurements at various spatial levels. Although, unlike the terrestrial
environment, refined exposure models are available for surface water, the fact that pesticide residues are still frequently found in surface water shows that the use of these models in itself provides insufficient protection against the dispersion of pesticides to the surface water compartment. Validation of these models, whether for the terrestrial or the aquatic environment, can lead to improved models or to the addition of new emission routes that had so far not been included.

**Effects**

Whereas effect studies in the laboratory generally concentrate on direct toxic effects, such as those on individual survival and reproduction, sublethal effects can also have a major impact on the survival of particular species in natural ecosystems. A minor change in behaviour, for instance, could lead to increased predation and, in the case of plants, to shifts in abundance rates. Another important point is that the organisms currently used in tests have often been selected for purely pragmatic reasons, such as ease of cultivation and the fact that certain tests are internationally accepted. This means that the environmental effects of pesticides in the Netherlands are sometimes assessed using organisms that do not even occur in the Netherlands. The minimum option for validating this selection is to compare the sensitivity of such organisms with that of indigenous Dutch organisms. But if the registration procedure is to provide information about the effects on ecosystems, test organisms must be selected on the basis of much more basic criteria:

1. The test organisms must be representative of the various taxonomic groups.
2. The test organisms must be representative of the various functional groups.
3. The various emission routes must be covered.
4. The test organisms must be relatively sensitive.
5. The test species must be natural inhabitants of farming areas.
6. The selection must be adapted as much as possible to international procedures.

Currently, indirect effects (that is, effects mediated by food or habitat) are not included in the procedures, though they ought to be.

ii) After models and assumptions have been adequately validated, it might be asked whether pre-registration field tests would still be needed to test the predictions. They might still be required, however, since conditions in the field might differ from those in the laboratory to such an extent – for instance as regards environmental circumstances, behaviour of test organisms or feeding patterns – that the actual effects in practice are considerably different. In addition, certain effects, such as those mediated by food or habitat, which cannot be adequately assessed in the laboratory, and for which field trials have a useful role to play. Field studies can be divided into semi-field studies and full field studies. Semi-field studies, such as bioassays, mesocosms or experimental fields, are more closely controlled, but are also less representative. Bioassays are especially suitable for examining short-term effects, and particularly for validating predicted exposure levels, while mesocosms or experimental fields allow interactions between various species to be studied. Full field studies are completely identical to the practical situation and the organisms to be studied are not restricted in their behaviour.

iii) Post-registration field trials may involve monitoring, with the aim of detecting any unexpected effects, or specific field studies initiated on the basis of specific suspicions. The latter situation may also include field studies related to preliminary admissions, which are conditional upon proof that no unacceptable effects occur in practice.
The chapter ends with the assertion that the construction of a framework is not intended to further complicate and enlarge the registration procedure, but actually to allow field trials to be used much more efficiently.

**Basic principles of analysis and interpretation**

Chapter 3 starts by indicating specifically how knowledge of the characteristics of products, such as their toxicity and mode of operation, as well as knowledge of the methods of administration, such as full field spraying or the use of granules, may be used to specify the expected effects. Taking such information into account from the very start allows one to predict effects more accurately and to use field trials much more efficiently.

A number of basic principles are then formulated for the design of field trials. These relate to the conditions under which trials can be implemented, in terms of the presence of particular crops, the choice of test organisms, the presence of an untreated control plot or a positive control (i.e., a plot treated with such a high dosage that effects are definitely expected) and the duration of the trial. It also discusses the methods of administration of each product and the detection of the effects.

Chapter 3 further discusses the evaluation of the effects, for which it formulates a number of criteria, including statistical reliability of the findings and comparisons with the effects in untreated and positive controls. Finally, this chapter reviews the important topic of the effect size that is regarded as acceptable or unacceptable, distinguishing between the treated parcel itself and the non-target areas. Temporary effects on populations of non-target organisms within the treated parcel itself are regarded as acceptable, provided they recover within a reasonable period of time (e.g., one year), and provided the area is then left in a state which allows it, in principle, to be used for other than agricultural purposes. Outside the target area, it may be questioned whether any effects are acceptable. This is a topic of debate which is treated in greater detail in Chapter 9 of the thesis.

**Part 3: Development and applications of field bioassays**

Bioassays were developed using plants, caterpillars and decomposition, in order to test whether it was indeed possible to develop practical field trials on the basis of the principles outlined in Part 2. These three groups of organisms were chosen because they represent completely different ecological functions.

**Bioassays using plants**

Chapter 4 describes the plant bioassays, which used the dicotyledonous species *Brassica napus* and the monocotyledonous species *Poa annua*. These species were chosen because they are very common in the Netherlands, and because there is evidence that they are particularly sensitive to herbicides and can be successfully handled in bioassay set-ups. After various designs for bioassays had been tested in a greenhouse setting, the following optimised method was eventually chosen: *B. napus* plants were grown in trays comprising 80 small wells. After the plants had grown for about two weeks, and the first real leaves had appeared, they were transferred to the field for the spraying. Twenty plants were harvested before
spraying started, to check whether any differences between the trays might have developed during the growth phase. Such differences were never found. The trays were placed in a 5 x 10 m test plot which was to be sprayed, as well as at distances of 2, 4, 8 and 16 m downwind of the test plot. Wind speeds during the tests ranged from 3 to 6 m/s. A further tray was situated at a large distance (> 500 m) from the test plot. Two hours after the spraying, the trays were returned to the cultivation rooms, where 20 plants were harvested after 7, 14 and 21 days. Wet and dry weights were determined. The \textit{P. annua} tests used 80 x 15 cm window boxes, each of which was sown with a fixed amount of 0.075 g of seed, in three sections. The boxes were once again used in spray tests after two weeks, and plants were harvested in three portions after different periods. The deposition rate of the spraying fluid was determined using water-sensitive paper.

The bioassays used glyphosate, bentazone and diquat. Considerable differences were found between species as well as between pesticides. \textit{P. annua} was found to be much less sensitive to diquat and bentazone than \textit{B. napus}, while the sensitivity of the two species to glyphosate was comparable. The distance at which 50\% growth inhibition was found after glyphosate spraying was between 5 and 6 m from the test plot for both species. Diquat only led to a 50\% growth inhibition at this distance in \textit{B. napus}. Repeated bentazone sprayings resulted in great variations in the effect on \textit{B. napus}, 50\% growth inhibition being found at a distance of 3 m in one experiment, against 12 m in another.

In some of the experiments, growth inhibition was even found at the furthest sampling point (16 m from the sprayed test plot), even though no deposition was measured there with the method we used (approx. 0.01\% of the field dosage). The bioassays were able to detect 10\% differences with the untreated controls, with a 95\% statistical reliability.

The use of an untreated and a positive control was found to be essential. The untreated control was needed to measure the rate of growth inhibition in the exposed setting relative to that in the untreated state. The positive control (sprayed with the recommended field dosage) was necessary because in some experiments even these positive controls showed only minor growth inhibition. In such cases it is obvious that either the product used is not very toxic to that particular species, or there were circumstances that prevented most of the effects.

The plant bioassays thus showed that effects on the growth of two common plant species in the sensitive stages occurred up to at least 16 m from a treated test plot.

\textbf{Bioassays using caterpillars}

Chapter 5 reports on the caterpillar assays, which used \textit{Pieris brassicae}. This is a common species in the Netherlands, which is known to be sensitive to insecticides and is easy to breed. A number of laboratory and greenhouse experiments were conducted, as well as two field tests. The field bioassays used caterpillars aged 2 – 3 days. It was our intention to place 10 caterpillars on each cabbage plant (\textit{Brassica oleracea}). One of the experiments involved 5 plants per treatment while another used 8; in one experiment, the number of caterpillars available was only sufficient to place 6 of them on each plant. The caterpillars were placed on the food plants just before the start of the experiment. Two hours after the exposure, the plants were covered with gauze and returned to the cultivation room. The numbers of caterpillars were counted every 2 – 3 days, and the numbers of pre-pupae and pupae were counted after about 3 weeks. It was found that nearly all of those caterpillars that developed
into pupae also hatched. The field bioassays were conducted only with diflubenzuron, a compound that inhibits the formation of chitin, which means the caterpillars cannot complete their moulding process and die. The insecticide pirimicarb was only used in greenhouse experiments. The design for the field test layout, including controls, was the same as that used in the plant bioassays.

The diflubenzon field tests revealed significant effects on survival at distances up to 8 m from the sprayed test plot. Exposed caterpillars that survived were found to take longer to pupate, but after pupation there were no differences between the exposed and non-exposed caterpillars in pupal weight (which is a measure of the likelihood of hatching and of the butterflies’ physical condition). The greenhouse test with pirimicarb found significant effects at dosages of at least 2% of the field dosage.

The caterpillar bioassays thus showed that insecticide effects on the sensitive stages of a common species were found at distances of up to 8 m from a sprayed test plot.

**Decomposition bioassays**

Bioassays based on decomposition (Chapter 6) were initially carried out by assessing the breakdown of organic materials in *litterbags*. The tests used 20 x 20 cm nylon bags with 40 µm mesh, containing 20 discs of dried cabbage leaf. These litterbags were buried at a shallow depth, exposed to the pesticide after one day and retrieved after one week, after which their dry weight was determined. The level of variation among the discs within one bag was found to be minimal, allowing even very small differences between the bags to be detected in a statistically significant way.

In practice, however, correlations with distance and exposure were only found in one experiment, in which an orchard was sprayed with captan; the effect was found up to a distance of 10 m from the sprayed plot. The effect was no longer found when the experiment was repeated in an experimental field.

Since these litterbag experiments yielded no unequivocal results, another experiment was conducted, in which diluted soil materials were added to agar-filled petri dishes. As in the other bioassays, these dishes were placed in and around an experimental field shortly before it was sprayed. This yielded a clear correlation with the distance, with effects being found at distances of 2 and 4 m from the sprayed plot. The agar used in this experiment was not selective for fungi or bacteria. Since both of these groups play an important part in the decomposition process, this method would seem to offer good opportunities for tracing the effects of low doses of fungicides on organisms involved in decomposition.

**Part 4: Nature and extent of the side-effects of pesticides**

**Effects of droplet drift**

The effects found in the bioassay experiments have been used in Chapter 7 to estimate the side-effects of droplet drift of pesticides in the Netherlands, using the data on droplet drift provided by IMAG. Droplet drift models were constructed and used to evaluate three scenarios: the recent past (1998), the current situation (2000-2003) – incorporating certain
drift-reducing measures – and the near future, assuming optimised use of drift-reducing measures. These include particularly the introduction of unsprayed and crop-free zones around the fields and the use of low-drift nozzles. Subsequently, the numbers of fields sprayed with herbicides, fungicides and insecticides were estimated, yielding some indication of the total surface area being exposed to certain levels of each of these pesticide groups. This information was then combined with data from the bioassays, resulting in an estimate of the area of land where the $EC_{10}$, $EC_{50}$ or $EC_{90}$ levels are being exceeded. The calculation related to the linear elements which usually border the sprayed fields: ditch banks, road verges and hedgerows.

The outcome of the calculation showed that, in 2000, the $ED_{50}$ level for herbicides was being exceeded in 44% of the surface area of linear elements bordering the fields, and 6% of these elements even exceeded $ED_{90}$. The $ED_{50}$ level for insecticides was being exceeded in 40% of the linear elements area, and $ED_{90}$ in 19%. The corresponding percentages for fungicides were 23% and 9%. After the introduction of new drift-reducing measures, like wider unsprayed zones and air-assisted spraying equipment, $ED_{90}$ levels would no longer exceeded anywhere, and $ED_{50}$ levels only in 1% of the linear elements for herbicides and in 3% of the elements for insecticides. $ED_{50}$ levels for fungicides would no being longer exceeded anywhere.

A similar calculation was made for nature conservation areas situated near farmland. It showed that if drift-reducing measures were taken, the $ED_{50}$ levels for the year 2000 would no longer be exceeded anywhere, although the $EC_{10}$ level would still be exceeded in 16% of the conservation areas. The introduction of further measures would eliminate this as well.

**Effects of vapour drift**

The effects of vapour drift (Chapter 8) were studied using four products as examples, including two herbicides (atrazine and MCPA), one fungicide (captan) and one soil disinfectant (MITC, the active moiety of metam-sodium, which has a nematicidal, fungicidal and herbicidal effect). Dispersion calculations were based on the crops for which these products are most commonly used. Two methods of calculation were applied, one calculating dispersion from the sprayed parcel over short distances (up to 2 km), and one using a regional model in which a number of parcels were used as the source and a conservation area as the target.

In addition, a literature search was used to collect information on the effects of low concentrations of the above compounds on non-target plants and fungi. The search yielded very little information, so that a rough estimate had to be made on the basis of what little data was available. Since no data at all was found for MITC, the value of NOEL was assumed to be equal to 0.1% of the field dosage.

Comparisons between the calculated deposition rates and estimated sensitivities show that atrazine and MITC can be expected to exert effects within a 500 m distance from the sprayed parcels. At regional scale, effects can particularly be expected for products that are slowly degraded. Regional scale depositions of MITC in particular can be expected to be of the order of the NOEL.
Part 5: Conclusions, discussion and recommendations

Part 5 of the thesis (Chapter 9) draws conclusions, discusses the findings and provides some recommendations.

From Part 2 it is concluded that:

Field trials can play a significant role in the pesticide registration procedure: they can be used:

- to test the assumptions and models incorporated in the registration procedure used by the regulatory authorities, especially regarding the conditions of use, the predicted exposure of non-target organisms and the predicted effects of the pesticides on non-target organisms,
- for pre-registration testing by the applicant of the occurrence of predicted effects; for this aim a selection procedure for field trials has been developed, allowing types of field trials to be selected on the basis of information from laboratory toxicity studies, from compound properties and from the type of effects to be expected,
- for post-registration validation and monitoring by the applicant or by the relevant authorities, in which monitoring or surveillance can be used to validate the registration procedure as a whole, using actual field data.

It can be concluded from Part 2 that the role and place of field studies in the pesticide registration procedure can be clearly defined. A procedure has been drafted which allows the appropriate type of test and test organism to be selected. It is recommended to include this procedure in the registration procedure. In addition, applicants should present a protocol to the CTB before they initiate field trials, making it clear in advance that the findings of such trials will actually provide a decisive answer to the question whether the potential effects will materialise.

From Part 3 it is concluded that:

In order to test the applicability of the constructed framework, actual field bioassays have been developed and conducted for species from different ecological groups: vascular plants (Brassica napus and Poa annua) as primary producers, invertebrates (Pieris brassicae) as herbivores and decomposition is studied using litterbags and micro-organisms:

- Bioassays with B. napus and P. annua appear to be very suitable to demonstrate side-effects of herbicides at low deposition rates, until 0.01% of the actual field dose, at a downwind distance of 16 m from the treated plots.
- Bioassays with P. brassicae larvae appear to be suitable for tracing the effects of insecticides at low exposure rates outside the target area until 0.5 % of the actual field dose at a downwind distance of 8 m from the treated plot.
- Litterbag bioassays with fungi showed relatively small and short-lived effects (<10% inhibition on decomposition). In one experiment, effects of captan treatment of an orchard could be traced up to 10 m from the treated plot. Differences of 10% with the untreated control could be traced. These results could, however, not be repeated on an experimental plot. Measuring the effects on the soil micro-organisms directly, by using soil micro-organisms in agar, revealed larger impacts and has a better potential for use as a bioassay. This method was only applied once, however, and needs to be developed further.
In part 2 general requirements for field trials have been formulated regarding the experimental design (including the test species and endpoints used, the control tests used and the required number of trials), the application of the pesticides (including the dosage and mode of application) and the interpretation of the results (including the level of significance and variation in untreated controls). These requirements form the prior conditions for conducting field trials, and have been validated for the measurement of the effects of pesticide drift using bioassays.

The bioassays developed in the course of the present project indicate that it is definitely possible to set up field trials for ecologically relevant groups of organisms, using organisms that commonly inhabit farming areas in the Netherlands. Although bioassays are relatively cheap and easy to use, they can only be used for a limited range of applications, particularly in situations where there are uncertainties about actual exposure rates in the field. Other types of effects, particularly indirect effects, require other types of tests.

From Part 3 it is concluded that:

The results of the bioassays have been extrapolated to the level of the Netherlands as a whole, in order to estimate the type and extent of the effects of droplet drift.

- The results show that in the year 2000, until a maximum of 44% of the total surface area of linear elements such as ditch banks, verges and hedgerows ED_{50} was exceeded. The effectiveness of a scenario for the abatement of pesticide droplet drift impact on adjacent parcels results in a more than tenfold improvement.

- For conservation areas in farming regions, in the year 2000, only the ED_{10} level for herbicides appears to be exceeded in 16% of the surface. With the above measures the ED_{10} level would no longer be exceeded either. This means that the planned government policy would appear to be quite effective in reducing the effects of pesticide droplet drift.

In addition to droplet drift pesticide vapour drift may also affect biodiversity.

- Calculating the vapour drift rates of the four pesticides studied (atrazine, captan, MCPA and MITC (the active conversion product of metam-sodium) shows that atrazine, captan and MITC exceed the NOEC at shorter distances from the parcel.

- At larger distances, of the order of kilometres, effects on vascular plants and fungi can be expected, especially from compounds like MITC, which degrade slowly.

The extrapolation of available field data about effects of pesticides on non-target organisms yielded the occurrence of short-term acute toxic effects on sensitive parameters, with effects being greatest close to the treated parcels. Since one quarter of the surface area of the Netherlands consists of arable land, even short-distance effects can affect large parts of the Netherlands.

Extrapolation of the bioassay data to a national scale shows that only one of the potential emission routes in the Netherlands may be expected to have considerable effects, but also that drift-reduction measures appear to be effective. One striking feature is that, on the one hand, there is ample evidence of pesticides actually being emitted to the environment and exerting certain effects there, as is shown for instance by the bioassay experiments reported on in the present thesis, while on the other hand very little information is available on the actual effects of pesticide use on natural ecosystems. A thorough, large-scale field research project, combined
with a systematic analysis of all available data, would be required to evaluate the nature and extent of the side-effects caused by current pesticide use.

It is remarkable that, although the Dutch Pesticide Act was introduced as long ago as 1962, the meaning of the phrase ‘effects deemed unacceptable’ is still not fully defined. The law does not stipulate which effects are deemed acceptable or unacceptable, and in what circumstances. The evaluation of the results of field trials clearly requires such definitions. In current practice, there is extensive debate on the acceptability of certain effects which are limited in size, area and time. The government’s criteria are unclear, as is the significance of such effects for the long-term functioning of ecosystems. The results of the bioassay experiments described in the present thesis clearly show that spraying does lead to certain effects outside the fields actually being sprayed. The debate about the acceptability of effects could be resolved by regarding all effects outside the target area as unacceptable. This could then be taken into account in the registration procedure and in instructions for the use of particular pesticides. The calculations of the drift effects show that effects outside the target area caused by this emission route can be considerably reduced with the help of measures that are practicable in everyday agricultural practice.