SIDE EFFECTS OF PESTICIDES ON TERRESTRIAL INVERTEBRATES AND AQUATIC FAUNA

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ON

TERRESTRIAL INVERTEBRATES AND AQUATIC FAUNA

Kees J. Canters
Geert R. de Snoo
Frank M.W. de Jong
Jaap van der Linden

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Centre of Environmental Science
Leiden University
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Kees J. Canters, Geert R. de Snoo, Frank M.W. de Jong and Jaap van der Linden

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Foreword to the second edition

The first edition of this report - the second report of the project "Side effects of Pesticides" - was sold out within two months. Because there were still many requests for this report, we decided to produce a second edition. We used the opportunity to incorporate at several places the most recent information. Furthermore, the editing has been improved and typing errors and inaccuracies have been corrected.

Kees J. Canters, Geert R. de Snoo, Frank M.W. de Jong and Jaap van der Linden

Leiden, July 1989.

Foreword to the English translation of the second edition

In Canada there was again - as in the case of our first report in the "Side effects of Pesticides" project - a opportunity to make an unedited translation, which is now published here by courtesy of the Secretary of State of Canada and the Canadian Wildlife Service. We especially wish to thank dr. Pierre Mineau, Head, Pesticide Evaluation of the National Wildlife Research Centre in Ottawa. He asked for the translation, which was made by Pieter H. Barkema and formed the basis for this English edition of our report.

Drs. H. Bezemer did a fine job by correcting this translation, also in a short time. We also like to thank Miss. H.F.M. Dullaart, J. van der Peet-van Loon and M.M. van Rijn-van der Scheer, who typed in the translation and the last corrections in a short time.

In this report many names of Dutch institutes are mentioned, including their abbreviations. These abbreviations are explained in Annex 6, which also contains the names in Dutch of the institutes.

Kees J. Canters, Geert R. de Snoo, Frank M.W. de Jong and Jaap van der Linden

Leiden, November 1990.

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SUMMARY

SIDE EFFECTS OF PESTICIDES ON TERRESTRIAL INVERTEBRATES AND AQUATIC FAUNA

SUMMARY

This summary describes the objectives, methods, results and conclusions of the research done by the CML in 1988 on the side effects of pesticides on terrestrial invertebrates and aquatic fauna. The summary can be read as a separate unit. For detailed results, background info, sources and references we refer to the report itself.

INTRODUCTION (Chapter 1)

In 1987, the Department of Housing, Physical Planning and Environment (VROM), Directorate-General for the Environment (DGM), Substances and Risk Management Division, commissioned the CML to study the side effects of chemical pesticides in large-scale use in agriculture on terrestrial invertebrates and aquatic fauna. This was a follow-up on a former study about the side effects on vertebrates done by the CML for the same department.

The problems that led to this study
The reason for the research was the fact that the former study showed that in determining the side effects of pesticides on vertebrates the invertebrates, as intermediate variables, always were of great importance, incl. the possible side effects on those invertebrates themselves. In addition, during 1987 information became available about some surface waters in certain areas being ecological dead, and also about some alarming developments in the North-East Polder and Eastern Groningen (Province).

Objectives (1.1)
1. Taking inventory of the recently concluded and current research in The Netherlands about the side effects of chemical agents in large-scale use in agriculture on terrestrial invertebrates and aquatic fauna, and scrutinizing relevant parts of the registration procedure in this respect.
2. Creating a reference and evaluation framework for this kind of side effects.
3. Give an overview of the nature and extent of these side effects in The Netherlands; where possible and making sense partly based on own field research.
4. Making recommendations for i) future research, ii) improving the registration procedure and iii) general policy measures to be taken.

Method (1.2)

Desk research (1.2.1)
The data required were gathered by i) holding interviews, ii) doing literature research and iii) organizing a workshop. The data from the interviews were mainly used to get an idea of the actual nature and extent of the side effects. Furthermore, those interviewed were asked their opinion about the functioning of the registration procedure. Literature research was done to get an overview of the nature and extent of the side effects. In addition, it gave information for the overview of

the research in The Netherlands. Various aspects of the registration procedure were raised in the workshop. The results were used in obtaining an overview of the functioning of the registration procedure and to get an impression of the desirability of a number of proposed alternatives and additions.

Field research (1.2.2)
The field research was aimed at tracing the side effects observable in the field and at further developing the methods used. It was attempted to correlate the data on the crops or the pesticides used and the fauna groups present. Terrestrial invertebrates were sampled in the Haarlemmermeerpolder; sampling was done in various crops and at various levels of use of pesticides. The aquatic fauna was sampled in a number of regions among various crops. Besides this inventory research, bio-assays were done with a number of species.

RESEARCH AND REGISTRATION IN THE NETHERLANDS (Chapter 2)

Research (2.1)
In the overview of research in The Netherlands, a distinction was made between research by institutes (incl. university groups and water quality managers), research programs, commissions and working groups and direct departmental activities. The inventory of the research efforts on the side effects of pesticides was divided in effects on the terrestrial environment, on the aquatic environment, and the effects via the air respectively.

Registration procedure (2.2)

Present registration procedure (2.2.1)
The present registration procedure includes in some components the side effects on terrestrial invertebrates and aquatic fauna.

Aquatic fauna
Short term toxicity research has to be done for each pesticide, with crustaceans and at least one species of fish. Long term toxicity research must be done only when there is a good chance of exposure and when effects on reproduction and embryo development are to be expected, and also involves crustaceans and fish.

Terrestrial invertebrates
Data on bees are required if the agent is used on flowering crops and plants which are visited by bees. For agents, claimed to be suited for application in integrated crop protection schemes, data must be supplied on the effect "on useful arthropods, the maintenance of which is desirable, insofar as they are involved in the application situation". Short term toxicity research with earthworms is required, unless it can be shown that the agent will not get into the soil, and long term research is required if there is a chance of long term exposure.

Criticism on the registration procedure (2.2.2)
The study shows that in general the present registration procedure is thought insufficient to bring the side effects of the use of pesticides to light. The most important items of criticism are:

1. The use of single species tests and their interpretation

The disadvantages of single species tests are the low representativity of the field situation, and the fact that no insight is obtained into the effects on interactions. It was proposed to supplement the test with simple, standardized food chain research. Field research is largely lacking at the moment, so an expansion of this kind of research is certainly desirable. The present single species tests work satisfactorily in comparing the agents (ranking), partly because of the internationally agreed standardization.

2. The test organisms used

The criticisms are concentrated on the following four aspects:

i) Only those organisms are tested, which are useful from an agri-economic point of view (bees, earthworms, useful insects and mites), but not all of them and not always either;

ii) The test organisms used in the present procedure have not been selected on the basis of exposure. This is especially important for the terrestrial environment. It is indicated though that probably the differences among species are smaller than the differences between the lab and field situation;

iii) The tests have too little ecological background. Consequently, not sufficient relevant ecological information can be derived from the tests for risk control measurements. The aquatic organisms used are considered more representative of their environment than the terrestrial species. Still, in the aquatic environment as well, more attention needs to be paid to chronic toxicity research;

iv) There are too few tests available, especially for the terrestrial environment. The results of fundamental research are needed, in selecting suitable new terrestrial test organisms.

Other criticisms

- The lack of sufficient attention to ecological side effects
- The non-transparency of the registration procedure as a whole
- Too little standard research of the terrestrial environment
- Too little input by the water quality managers in the registration procedure
- The lack of a central incidents registration and processing
- The lack of checking for side effects afterwards
- No attention to illegal use
- New agents with less/fewer side effects do not have an advantage in the registration procedure (costs and/or time)
- The registration does not compare agents
- The assessment of synergism of agents is not involved in the registration
- A paradoxical situation in the registration procedure: if a substance is not toxic in a first global test, no further research takes place
- In the re-evaluation of substances, by the Netherlands Institute for Public Health and Environmental Protection (RIVM), data from open literature are not used except when supplied by the manufacturer.

Conclusions (2.3)

Terrestrial environment

In general it can be stated that very little is known as yet concerning the side effects of pesticides on terrestrial invertebrates. For the time being, a coherent approach in the area of side effects of pesticides is still lacking - in spite of the existence of large research programs. The

research is aimed mainly at useful organisms, such as bees and spiders. Research on other animal groups is done less systematically.

Field research
Field research on effects is the research area which has remained farthest behind. The field research by the department of Toxicology at the Agricultural University of Wageningen (LUW) within the framework of the Stimulation Plan Toxicology Research constitutes a positive exception here. At the moment, no research is being done on the duration or the nature of the side effects, or the scale on which they occur.

Tests
Lacking for the terrestrial environment is comparative research with species which can be used as test organisms. The fact that the ecological knowledge of various species is insufficient plays an important part here.

Other points of attention
More attention must be paid to research aimed at solutions, e.g. the reduction of emissions from agriculture, including the implementation of the measures resulting from this in normal farm management.

Aquatic environment
In the aquatic environment much research is being done by the separate water quality managers and by institutes such as DBW/RIZA (Governmental Waste Water Treatment Institute), RIVM and IOB (Pesticides Research Institute). On the basis of the number of joint projects it can be stated that co-operation in this area is reasonable to good. There is also a structured approach, in view of the multiplicity of aspects which are being researched jointly (inventory of usage of chemicals, incidents studies, toxicity research in the lab, model ecosystems in the form of test ditches, effect measurements in the field).

Data processing co-ordination
The co-ordination too of the processing of data concerning incidents, chemical measurements and the selecting of sample sides is gradually getting off the ground. In view of the high concentrations found in various locations, such a research effort is certainly justified.

Field research
Field research on effects is still lagging behind at the moment. To get an idea of the scale of effects in The Netherlands, expansion is certainly desirable; up to now this research mostly takes place in a limited number of regions. The recently found substances still have to be incorporated in the measuring programs of the individual water managers. Often, still only chlorinated hydrocarbons are being measured. In bringing these things about, especially the costs play an important part, besides availability of sufficiently accurate methods.

Air
The research on effects of pesticides on ecosystems via the air has lagged behind. For some time, the RIVM has been doing concentration measurements. Field research aimed at the effects is totally lacking, however. Direct side effects of pesticides (herbicides, fungicides and soil disinfectants) via the air on vegetation and fungi can certainly be imagined, as well as indirect side effects on e.g. the vitality of

forests, through the effects on mycorrhiza as a result of fungicides. Such assumptions justify a greater research effort in this area.

EVALUATION OF SIDE EFFECTS (Chapter 3)

On the basis of the Pesticides Act from 1962 a chemical is allowed only if no unacceptable harmful side effects are to be expected from the chemical or from its conversion products. Harmful side effects are, among other ones: The damaging of soil, water or air, or of animals, plants or parts of plants whose maintenance is desirable, to an extent which is unacceptable. This study is aimed at the side effects of pesticides on terrestrial invertebrates and aquatic fauna. As up to now little experience has been gained with the interpretation of this kind of effects, a reference and evaluation framework is created for this.

Use of pesticides (3.1)
In total, on an annual basis ca. 18,000 tons of active substance are used (1987). Almost half of this consists of soil disinfectants (47%), followed by fungicides and herbicides (each apr. 23%); insecticides are relatively little used (apr. 3%). In the recently published Memorandum "Environment Criteria" it is indicated, among other things, what the norms are and will be for the occurrence of pesticides in the environment. Some important points here are: i) the LC50-value for water organisms must not be exceeded, ii) ditto for earthworms, iii) at least 95% of the species in an ecosystem must not experience any harmful effects, iv) half-life period less than 2 months, and v) individual pesticides in ground water less than 0.1 µg/litre and total of pesticides less than 0.5 µg/litre.

Effects and environment quality (3.2)
In evaluating the side effects, the general environment quality and the social objectives related to this serve as a reference framework. Further detailing of the general environment quality takes place from two angles: i) basal requirements set by specific functions of the area, and ii) general characteristics to maintain the multifunctionality of the area. The following social objectives are distinguished: i) the health of people (≈ national health), ii) the usage functions, and iii) the nature value. It is attempted to deduce the interference with these social objectives from the side effects on terrestrial invertebrates and aquatic fauna. Further, for specific environment quality the points of departure are specific biocenosis, species, or forms of use restricted to certain areas and/or specific environment conditions.

In determining the quality of the environment, not only the present, actual use of a certain area is paid attention to, but also the use of contiguous areas and possible other use at a later time.

"Species whose maintenance is desirable" (3.3)
For the general quality of the environment, an optimum ecological functioning is of great importance. This can be known by the presence of indicators of two important basal ecological processes, in which specifically invertebrates are involved: i) decomposition (by saprophages such as earthworms and isopods), and ii) regulation by the mesofauna (by microbivores such as mites and springtails as well as by predators such as spiders and ground beetles).

With regard to specific environment quality, for agriculture the presence of useful organisms to prevent pests is important (ichneumon wasps and predatory mites, but other predators as well). For certain crops the presence of bees is important too, as flower pollinators. As regards nature value, the susceptibility of protected species should be paid attention to. This susceptibility is determined not only by the toxicity and exposure of chemicals, but also by place of occurrence. Regarding this last aspect, attention is paid to those species, whose occurrence primarily lies in the agrarian area. This applies not only to those invertebrates themselves, but also to invertebrates constituting an important food source for (protected an/or) rare vertebrates, such as e.g. the badger, who depends on earthworms for an important part of his food.

"Damage to an inacceptable extent" (3.4)
Since a qualitative approach alone of the effects on social objectives is not sufficient, it is necessary also to provide a point of reference in a quantitative sense. To be able to set norms for the extent of the effects on species whose maintenance is desirable, it is proposed to check whether it is possible, with regard to numbers occurring, to use ecological background values as references. One can think here of numbers occurring in a series of types of biotope, running from drifting sand and agricultural soil on the one hand, via heath and pasture to brush and bush and finally to forest on the other hand. The advantage of the setting of such quantitative norms is that not just the presence or absence of a species is paid attention to, but also the minimum and maximum numbers.

Tests (3.5)
To operationalize the use of the species selected on behalf of the registration procedure, various forms of test are available. These can be classified as follows:

1. Test to be done in the lab
a. Toxicology research with standard species under totally controlled circumstances; often single-species tests, but multi-species tests also come under this.
b. Bio-assay in the form of: check the reaction of standard species in medium brought from the field to the lab.

2. Tests to be done in the field
a. Bio-assay in the form of: place caged standard species in the field, and check behaviour/mortality.
b. Taking inventory of relevant species(groups).

Conclusions (3.6)
The reference framework of the general environment quality appears to offer enough reference points to allocate more than only factual significance to the side effects of pesticides on terrestrial invertebrates and aquatic fauna. It is possible, starting from the general environment quality, to interpret the possible side effects so that they can be allocated significance in terms of the effects on the usage functions and nature values.

TERRESTRIAL INVERTEBRATES (Chapter 4)

Literature, opinions of experts and incidents (4.1)
For by far the most part, research on side effects is done with agriculturally useful organisms (earthworms, bees and predators/parasites). The side effects for these groups are discussed. Further, information is given about side effects on some other species groups.

Earthworms (4.1.1)
Little is know about the toxicity of fungicides for earthworms. It was found though, from toxicity as well as field data, that specifically benzamidazols are (very) toxic, often with long recovery times occurring in the field. Insecticides vary in toxicity. Little is known about herbicides. Indirect effects of herbicides can occur though through changes in the quantity of vegetation and organic material covering the soil. Of the soil disinfectants, the fumigants are extremely toxic for earthworms. Nematicides applied in granular form are less toxic than fumigants. There are also some very toxic ones among these though (aldicarb e.g.). The molluscicide methiocarb, at dosages used in practice and applied as spray-powder, is very toxic for earthworms.

Bees (4.1.2)
In general, fungicides are not very toxic for bees. Almost all insecticides are (very) toxic for bees. This is confirmed by the many incidents. The acyl-ureum compounds, bacteria preparations and sulphides constitute an exception. Tin compounds and some chlorinated hydrocarbons as well are less toxic. No toxicity data were found concerning herbicides and soil disinfectants. Some incidents are known though with MCPP, paraquat and diquat.

Predators and parasites (4.1.3)
In general, fungicides are little toxic or not toxic for predators and parasites. An exception is the organic phosphor compound pyrazophos. For most predators and parasites, insecticides are moderately to (very) toxic. Not toxic are acyl-ureum compounds, bacteria preparations and tetradifon. Insofar as data on herbicides are available, these turn out to be in general little to not toxic. Exceptions, however, are dinoseb, and for certain species also bromefenoxim, fenmedifam and to a lesser extent, the anilids. No data were found about the toxicity of soil disinfectants.

Butterflies (4.1.4)
Butterflies have declined rapidly in recent decades. However, no toxicity data were found, or incidents with butterflies tracked down. Further, as seen from an agricultural point of view, caterpillars of butterflies are among the pest organisms and are therefore purposely controlled.

Springtails and mites (mesofauna) (4.1.5)
Toxicity data on springtails and (saprophagous) mites have not been systematically gathered; the only information we obtained came from those interviewed. Soil disinfectants probably have the most effect on these soil invertebrates. In addition, however, toxic side effects from insecticides and fungicides are also possible. Herbicides are probably not very toxic. Ecological side effects, as a result of the vegetation dying off, can occur though; a possible toxic side effect can be overruled, in

that case. With use of fungicides as well, indirect side effects are possible.

Snails (4.1.6)
Incidents are not known and research on effects has not been done; toxicity data too are almost totally lacking. A number of (phytophagous) species is purposefully controlled, e.g. Arion rufus and Mylax species.

Discussion (4.1.7)
Invertebrates are affected in their occurrence by a large number of factors. The relative seriousness of pesticides should not be overestimated here. In general, habitat changes are deemed more important than the toxic side effects of pesticides. Pesticides can contribute to these changes in habitat though.

Field research (4.2)
In an orienting way, two biology students have been doing field research in the Haarlemmermeerpolder. The following objectives were set:
1. Getting an idea of side effects of pesticides on terrestrial invertebrates in a number of crops and at various levels of pesticide use.
2. Developing a method for tracing the side effects of pesticides by means of sampling invertebrates.

Method (4.2.1)
The research was aimed at the relationship between various levels of pesticide use and the occurrence of various groups of soil vertebrates and was done in three crops: potatoes, sugar beets and wheat. It was assumed that the abundance of a certain group of invertebrates in a certain crop depends not only on the crop-specific use of pesticides, but that the crop also has a direct effect on the invertebrates occurring in a field (a.o. through the differences in vegetation structure). It was found to be possible to distinguish, within one crop, differences in the level of pesticide use.

Results (4.2.2)
Differences were found in the invertebrate fauna between fields with different levels of pesticide use; this even turned out to be possible at the high taxonomic level used (orders and families). It should be noted here though that other factors too can have an effect on the fauna. A survey showed that specifically the factors fertilizing and tilling varied. Consequently, further research on the relative contributions by these factors is desirable.

The biggest differences were found in mites, potworms, cantharids, centipedes and ladybirds (for all these groups applies: smaller numbers at higher pesticide use) and in two-winged insects and rove beetles (for which applies: larger numbers at higher pesticide use). Springtails occur in the soil in larger numbers at higher pesticide levels, but are also found on the soil in large numbers at lower levels. At higher levels, relatively more predators occur, at lower levels more herbivores and detrivores. Among the potato fields, the most individuals and largest number of groups are found in organic farming.

Discussion (4.2.3)
The terrestrial field research had an orienting character. Thus, many methodical aspects remained under-exposed. However, the method used

appears to have so much potential for tracing the side effects of pesticides on invertebrates that further research on the various methodical questions appears to make sense.

Conclusions (4.3)
In a general sense it is concluded that - although on the basis of literature data, data from experts and data on incidents, for some species groups a picture emerges about the toxicity of many pesticides- only very fragmentary information is available on occurrence of side effects in the field. Toxicity data on non-useful organisms are largely lacking, let alone research data on how these animals react to pesticides under field conditions.

The field research has shown that there are differences in the presence of species groups at various levels of pesticide use. In principle, the method used offers the possibility of tracking the side effects of pesticides under conditions in practice.

AQUATIC FAUNA (Chapter 5)

Literature, opinions of experts and incidents (5.1)
Apart from the herbicides, most pesticides are (very) toxic for fish. Consequently, relatively many incidents with pesticides are known in which fish mortalities occurred, in which specifically the many incidents with endosulfan stand out. No data are available about reptiles. For amphibians, incidents are known with frogs (endosulfan) and water salamanders (methyl bromide). Much less is known about invertebrates. As is to be expected, in general insecticides are (very) toxic.

The concentration of only a relatively small number of chemicals in the surface water is regularly determined. As the chemicals which are being measured are found relatively frequently, chances are good that other chemicals too are present at harmful concentrations. In view of the great toxicity of many pesticides, side effects on aquatic fauna are therefore certainly expected.

Problems are occurring in the Westland, the North-East Polder and the (former) peat districts. There is, however, no reason to assume that no problems are to be expected in other large-scale agricultural areas, where larger-scale measurements are as yet not being taken, such as Zeeland, West-Friesland, the clay regions in Friesland and Groningen, and North-Western North-Brabant. Crops under glass too appear to be capable of producing relatively many problems.

Field research (5.2)
In view of the fragmentary picture and the lack of a method for evaluating the effects in the field, we did our own field research. The following objectives were set:
1. Getting an idea of the side effects of pesticides in a number of crops and regions.
2. Developing a method for plotting out the side effects of pesticides, by means of sampling the aquatic fauna.

Method (5.2.1)
The aquatic fauna was sampled in various regions: the Westland, Haarlemmermeerpolder (the polder near the city of Haarlem which used to be a lake), the environs of Aalsmeer and the North-East Polder (in the former Zuiderzee, now called IJsselmeer = IJssel Lake) and in various crops: potatoes, sugar beets, wheat, crops under glass and controls. A number of other factors were also measured, such as the width of the ditches, the presence of vegetation and some physical-chemical variables, including sum (total) parameters of pesticides.

Results (5.2.2)
The width of a ditch turns out to be of great importance in the occurrence of species groups. Since the vegetation cover also differs here (in the narrower water courses it is taller than in the wider ones), the effects of these factors cannot be separated. For further processing we to splitted up the set of data into two groups: narrow (under 3 meters) and wide (over 3 meters).

Ways of growing
The following differences were found among the various ways of growing crops: in the wide greenhouse (area) ditches, flatworms, springtails and beetles occur significantly less than in the control (reference) ditches, while springtails also occur less in wide arable field ditches. Also, leeches, amphipods and semi-aquatic bugs barely occur in wide greenhouse (area) ditches; these differences are not significant, however. When the numbers of groups are compared, we find that in the wide reference-ditches significantly more groups occur than in the arable field ditches and greenhouse (area) ditches.

In the narrow greenhouse (area) ditches, semi-aquatic bugs and beetles occur less than in the references. Amphibians on the other hand occur the most in the greenhouse (area) ditches. Leeches occur more in the greenhouse (area) ditches than in the arable field ditches; there is no significant difference with the references, however.

Further, the following differences can be seen: in the reference-ditches, 10% of the groups does not occur, or occurs in only one reference. All other groups occur in at least two reference-ditches. In the potato ditches, on the other hand, 55% of the groups do not occur, or occur in only one ditch. In the beet and wheat ditches this applies to 30% of the groups, and for the greenhouse ditches to 25% of the groups. A number of groups occur everywhere; for these groups it would probably make sense to use a lower taxonomic level. It concerns here the oligocheate worms, water fleas, ostracods, copepods, corixid bugs, mosquito larvae, snails and fishes.

Way of growing as related to other factors
A number of times, correlations were found among a number of other factors measured (mostly vegetation cover) and the occurrence of the species groups. In those cases in which a correlation was found between the way of growing and the species groups as well as between another factor and the species groups, we checked whether there was a connection between the factor way of growing and the other factor. This turned out not to be the case, so that it can be assumed that an effect of the way of growing on occurrence of the species groups does exist.

Region
In the greenhouse ditches in the Westland, fewer species groups occur than in the references. For the ditches in the environs of Aalsmeer it would appear that specifically the wide greenhouse ditches have a poorer fauna. The narrow reference-ditches have a richer fauna than the ditches among crops and greenhouses. In the Haarlemmermeerpolder, fewer species groups occur per crop and in the references as well, than in the other regions, but no differences are found between the references and the other ditches. In the North-East Polder no negative effect of tilling on the fauna composition was found.

Chemical contents
In practically all samples demonstrable quantities of cholinesterase-inhibiting agents were found. The highest values were measured in greenhouse ditches in Westland; these went as high as 22.4 µg/litre (the norm in the Indicative Five Year Environmental Program (IMP) is 0.5 µg/litre). In some ditches in the environs of Aalsmeer too, relatively high contents were found. No correlations were found among cholinesterase inhibition and occurrence of species groups.

In part of the samples the concentration of a number of chlorinated hydro-carbons was determined. In the samples checked from the Haarlemmermeerpolder and the Aalsmeer and Westland regions, contents were found up to a maximum of 215 ng/litre; in all cases the norm for the basic quality of surface water was exceeded. No correlation could be shown among the chemical contents and the occurrence of species groups.

Discussion (5.2.3)
In almost all ditches examined, representatives from the various trophic levels were present. For this aspect, consequently, basis quality is maintained, in contrast with the chemical contents. On the other hand though, in a number of cases it was a matter of only one or a few species per trophic level.

Bio-assays (5.3)
By means of bio-assays, the toxicity for a number of organisms was investigated of water collected at the same places where the fauna sampling was done. The bio-assays were done with water fleas (Daphnia magna), water mites (Unionicola minor and Mideopsis orbicularis) and isopods (Asellus aquaticus). The following objectives were set:
1. Checking the suitability of bio-assays with various species in detecting side effects of pesticides.
2. Checking the trouble spots found in the preceding stage of the study.

Method (5.3.1)
For the bio-assays water samples were collected during three rounds. To exclude mortality for reason of lack of oxygen, air was put through the samples for a few minutes. The pH, chloride content and phosphate content of the samples were measured, so that the effect of these factors could also be checked.

Results (5.3.2)
High mortality percentages (up to 100%) were found in the water from small ditches in the greenhouse area of Westland. Further, high mortality was observed in the water from two wide arable field ditches near Aalsmeer. In one pasture ditch at Aalsmeer too, increased mortality was

observed. For Asellus aquaticus and the water mites, in addition to the mortality measurement after 48 hours, the death percentages after 24, 120 and 192 hours were also measured. For Mideopsis orbicularis no mortality was observed at all. For Unionicola minor mortality did occur after 120 hours. In two greenhouse ditches from Westland some mortality occurred as well as in a beet ditch from the Haarlemmermeerpolder. For Asellus aquaticus the same picture was observed.

Discussion (5.3.3)
It was found that there are great differences in sensitivity between the water fleas and the other organisms tested. Possibly the presence or absence of certain groups of chemicals plays a part here. In a control experiment with parathion it was found e.g. that water fleas are much more sensitive than water mites and Asellus aquaticus. We were able to correlate the content of cholinesterase-inhibiting substances with the mortality of the water fleas. With a content of over 3 µg/litre of cholinesterase inhibitors mortality is always over 50%. The chlorinated hydrocarbons measured always stayed well below the LC-50 values of these substances for invertebrates. The endosulfan concentration found in Westland though can definitely be toxic for some fishes.

In the bio-assays, water fleas turned out to be strongly indicative of the cholinesterase-inhibiting substances content. This connection was not found in the field, however, for water fleas occur even in the ditches with the highest contents. Possibly the species used in the experiment, Daphnia magna is more susceptible to cholinesterase inhibition than other species. Mites and isopods appear not to be hampered in their occurrence (and in the bio-assays) by high contents of cholinesterase-inhibiting substances; these groups could be susceptible to other groups of chemicals, however.

Conclusions (5.4)
The available data show that there is a fragmentary picture of the seriousness of the side effects of pesticides on the aquatic fauna. Research on the relationship with other interferences (ditch cleaning, level control, discharges, etc.) has not been done thus far. With the fauna sampling method used, in principle, differences in the occurrence of fauna groups with various (ways of growing) crops can be traced. This shows that a number of groups already differentiate at a high taxonomic level. Although significant differences were found in only a limited number of cases, it is striking that in general more groups occur in the references-ditches than in the other ditches. Particularly in the potato ditches and greenhouse ditches, fewer species groups are occurring.

In one greenhouse ditch near Aalsmeer the water quality was so bad that water mites and isopods did not survive the bio-assays. Water fleas could not survive bio-assays in water from the greenhouse ditches in Westland. In Westland high concentrations of cholinesterase-inhibiting substances were found, which turned out to be correlated with the mortality of the water fleas. No correlation was found with the mortality of isopods and water mites.

Since high mortality occurred in all three sample rounds, one can speak of a permanently poor water quality in these ditches. The water flea Daphnia magna is well suited for tracing high contents of cholinesterase-inhibitors. Asellus aquaticus and Unionicola minor are possibly suited

for tracing other (groups of) pesticides. Mideopsis orbicularis is less suitable for tracing pesticides in surface water.

CONCLUSIONS AND RECOMMENDATIONS (Chapter 6)

Conclusions (6.1)

Research in The Netherlands (6.1.1)
In the terrestrial environment, relatively little research is taking place; especially field research on effects has remained well behind. In the aquatic environment, relatively much research is being done at the moment. However, field research on effects is remaining behind here too, and expansion is desirable. The research on side effects of pesticides via the air on ecosystems and their surroundings has hardly got off the ground. For some time now, chemical content-determinations are being done, but field research on effects is lacking.

The registration procedure in The Netherlands (6.1.2)
In general, the registration procedure at present is deemed insufficient for bringing to light the side effects of the use of pesticides. There are many points of criticism, the most important ones being:
1. The use of single-species tests and their interpretation. Multi-species tests and possibly a field test would appear to offer more perspectives.
2. The present test organisms: only species considered useful from an agricultural-economic viewpoint are tested. Ecological background knowledge, showing how the tests are related to effects on the ecosystem, is lacking. In addition, specifically for the terrestrial environment, too few test species are on hand.

Evaluation of side effects (6.1.3)
In evaluating the effects, the general environment quality, the specific environment quality and the social objectives set are used as a reference framework. Determining the general environment quality is possible from two angles: i) basal requirements set by specific functions of the area, and ii) general characteristics on behalf of maintaining the multifunctionality of the area. With regard to the latter aspect, specifically optimum ecological functioning is important. With regard to the soil, this can be checked by the presence of indicators of important basal ecological processes. The side effects can also be related to specific environment quality. To be able to establish norms for the extent of the tolerable effects, it is proposed to investigate whether it is possible to take the numbers of selected species occurring under daily circumstances in a limited number of types of biotope as a reference point.

Side effects on terrestrial invertebrates (6.1.4)
The knowledge existing at the moment is insufficient to be able to provide an overall overview. It is clear though that soil disinfecting, done large-scale in The Netherlands, constitutes a real threat to the ecosystem. Terrestrial field research has shown that doing fauna sampling can make sense in tracing side effects of pesticides. The use of chemicals appears to effect the fauna composition.

Side effects on aquatic fauna (6.1.5)
The literature shows indications of the presence of pesticides in surface water leading to problems for the fauna. Our own field research and the bio-assays have confirmed this; the methods developed concerning this, with further elaboration and supplementing, can make a valuable contribution here. Repeatedly great problems arise in greenhouse market gardening. Other ones are encountered in fruit, potato and mushroom growing.

Recommendations (6.2)

Proposal for optimising the registration procedure (6.2.1)
To solve the criticisms raised, a procedure is proposed based in part on the existing procedure and in apart on our own ideas. It consists of 3 phases:

1. Laboratory data to be supplied by applicant
   The most important addition to the present procedure is that the number of test organisms be expanded on a limited scale, viz. with species to indicate the side effects on the social variables.
2. Evaluation of these data by the CTB (Pesticide Registration) Commission)
   In the evaluation it is attempted to estimate the possible risk the agent can cause in the field. It is proposed to expand this with an extra component, in which i) a comparison is made of the risks of the new agent and those of existing, already registered ones, and ii) attention is paid to the risks of the agent in relation to the total of agents used and other interferences in the field.
3. Doing of a specified field test by the applicant
   The field research is done standard for all substances to be registered. It is checked in the field, under circumstances in practice, whether toxic or ecological side effects occur with a significant effect on the usage functions. On the basis of the results of the field research, the agent is or is not submitted by the CTB for registration. On the basis of this submission, it should be possible to register objections, by the manufacturer as well as by other organizations and private persons.

Proposals for designing a field test protocol (6.2.2)
No standard procedure exists for field research as indicated above. In a follow-up study we shall pay attention to this. The components of this research are indicated below.

As the first component, tests are mutually arranged with regard to purpose, set-up, feasibility and results. Also, further selection is done of the indicators of effects on usage functions and nature significance. The usefulness of these tests is then checked against the literature, personal views, and the opinions of experts. The results are worked into a rough protocol.

The second component comprises empirical research, to check in how far the proposed protocol is useful. Use can be made of test fields, where one can manipulate dosages, formulating and test conditions. Bio-assays can be located in the ditches adjoining such test fields. On the basis of results obtained, a definite proposal is made for a field test protocol, which can be used in the registration procedure for pesticides.

Proposals for an integral incidents registration system (6.2.3)
During the interviews and the workshop it was repeatedly emphasized that for determining the nature and extent of side effects in the field a properly functioning, integral incident registration is required. The procedure here proposed has the following components:

- a network of field observers (police, veterinarians, hunters, etc.) to make observations;
- a central reporting-point to pass along observations to;
- causes-analysis at the reporting-point, on the basis of incoming data;
- risk evaluation on the basis of the causes-analysis;
- taking of measures if desired and needed (e.g. in the form of revoking or amending the registration concerned);
- and finally, regular evaluation of data received and measures taken during a certain period.

Proposal for an environment quality test (6.2.4)
The objective of this is to check in how far environment quality in the agrarian areas is affected by the use of pesticides and by other interferences. The test is aimed at evaluating the effects of already registered agents in a certain region and is also, in principle, suitable for much wider application (other substances, but other interference as well). By means of empirical testing it is checked in which way and by which interference plants, animals and ecosystems are affected. This is judged by the extent to which usage functions and nature significance are affected.

The result of the test is that statements can be made about the environment quality of the area examined; in addition, a cause(s) analysis is taking place. It is also checked which interference(s) contribute to what extent to the effects indicated. If the damage is unacceptably great, seeking other alternatives is proceeded with, such as using other agents, modifying or revoking the registration, or taking compensating measures. Finally, the measures taken are examined as resorted their effectiveness.

Policy recommendations (6.3)

Pesticides Act
As regards the registration of pesticides, one might think of reformulating art. 3. This should give a wider interpretation of the organisms the maintenance of which is desirable. Explicit expansion to non-target organisms could be considered as well. Clearer co-ordination with other legislation seems to be desirable.

Research
In view of extent of use and of the degree in which pesticides end up in the environment, it is important to (keep up) do(ing) extra research efforts in this direction. Points of attention here should be reducing the use and emissions of pesticides, but also field research concerning the effects and research on evaluation aspects.

Crops and regions
In concentration areas for certain crops, such as those in greenhouses, and potatoes, fruit, flowers, bulbs and mushrooms, the problems come to the fore most clearly. Consequently, research and remedial efforts should be particularly aimed at these regions. Here, one should guard against choosing "end of pipe" measures.

Emissions
As in most agrarian areas the standards of basic quality are exceeded for cholinesterase inhibitors and for chlorinated hydrocarbons. This is a very alarming fact and certainly justifies great(er) attention from policy e.g. in the form of suitable measures to limit emissions.

(Chemical) Agents
A number of (groups of) agents require specific policy attention, on the basis of their toxicity and/or incidents observed. We are thinking here of the benzamidazols, organic phosphor fungicides, dithiocarbamates, dinitro-alkylphenols, and in a more general sense, of insecticides and soil disinfectants. The data on the toxicity of pesticides are not at all complete, however, and even with regard to the 20 agents used most in The Netherlands data are available to only a very limited extent.

Species Groups
With regard to general and specific environment quality, in connection with the effects on the multifunctionality c.q. the usage function agriculture and nature significance of the use of pesticides, more policy attention is required for side effects on the following groups:
General environment quality:
- terrestrial: microbivore, saprophagous and predator species
- aquatic: detritus eaters and predators (mesofauna)

Specific environment quality:
- agriculture: useful organisms and organisms with a long generation time and/or wide-ranging movements.
- forestry: higher plants and fungi
- fisheries: eel (and/or pike or pickerel), three-spined stickleback, and species diversity
- nature: protected terrestrial invertebrates and aquatic fauna with accent on occurrence in the agrarian area, specifically the rare species.

SIDE EFFECTS OF PESTICIDES ON TERRESTRIAL INVERTEBRATES AND AQUATIC FAUNA

1. INTRODUCTION

In 1986-87 the Centre of Environmental Science of the University of Leiden (CML), on behalf of the Department of Housing, Physical Planning and Environment (VROM) (Directorate-General for the Environment (DGM), Substances and Risk Control Division), did a study on the side effects on terrestrial vertebrates of chemical agents in large-scale use in agriculture (see: de Snoo & Canters, 1990). As a follow-up, in 1987 the same principal gave an order to CML for research on the side effects on terrestrial invertebrates and aquatic fauna. These two studies are indicated respectively as phase 1 (= terrestrial vertebrates) and phase 2 (= terrestrial invertebrates and aquatic fauna) of the project side effects of pesticides.

The results of phase 1 can be summarized as follows:
- In general, a great deal is known about the toxic side effects[1]; the registration procedure is entirely aimed at this kind of side effects.
- Ecological side effects are hardly and certainly not systematically considered, although at the moment it is precisely here that most side effects are expected.
- Certain groups of pesticides, such as soil disinfectants, and fungicides and herbicides to a somewhat lesser extent, are in such large-scale use that for that reason alone, more research on the ecological side effects is considered desirable.
- There are many scattered research efforts, in which co-ordination and co-operation, apart from direct contacts among researchers themselves, are not optimal.

Besides these results, a number of recommendations came out of phase 1 about the registration procedure and about research to be done. For the latter, 19 research proposals were generated at the time. At a workshop, held in May 1987, of specialists in the field of side effects of pesticides, the following proposals received high priority:
- doing research in field margins, aimed at raising nature values in the agrarian areas
- more attention for incidents registration and illegal use
- setting up a side effects of pesticides data base
- improving co-ordination and (inter)national co-operation, inc. the setting of priorities for research to be done to fill lacunae in knowledge.

Phase 1 also showed that in determining side effects of pesticides on vertebrates, the invertebrates, as intermediate variables (see also: Chapter 3), always turned out to be very important, as well as the possible side effects of pesticides on those invertebrates themselves. Further, in the course of 1987 information became available on some

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[1] A toxic side effect is taken to mean the direct influencing of an organism by a pesticide, including secondary poisoning; these effects can be of a lethal as well as of a sub-lethal nature. An ecological side effect is taken to mean the indirect influencing by a pesticide, e.g. through elimination of food organisms or by affecting the habitat.

surface waters in certain regions being "ecological dead" (cf. CCRX, 1988), and other worrisome information as well, such as that from the North-East Polder and Eastern Groningen (for details see: 5.1). These things lead to follow-up research (phase 2 of the side effects of pesticides project). This would look specifically at the side effects of pesticides on aquatic fauna, especially the invertebrates. To complete the picture, side effects of pesticides on terrestrial invertebrates would also have to be studied.

The Crop Protection Guide (Gewasbeschermingsgids, van Rijn, 1987) already gives some information, albeit very restricted, on the side effects of pesticides of registered agents on invertebrates and aquatic fauna:
- the toxicity for water organisms (symbols),
- danger for bees (symbols), and
- danger to earthworms (at only 3 spots in the guide).

In general this information concerns only toxicity indications, with related use restrictions in connection with the risks. No data are included in the Crop Protection Guide on ecological side effects of pesticides. In view of this minimal information, the objectives of phase 2 were formulated generally and broadly; for obvious reasons (congruency, logic, comparability, etc.), connections were made with the objectives of phase 1 of the side effects of pesticides project (see: de Snoo & Canters, 1990).

1.1 Objectives

1. Taking inventory of recently concluded and current research in The Netherlands on side effects of pesticides in large-scale agricultural use on terrestrial invertebrates and aquatic fauna, and looking at relevant parts of the registration procedure.
2. Creating a reference and evaluation framework for these side effects of pesticides.
3. Giving an overview of the nature and extent of these side effects of pesticides, including where possible and sensible, field research.
4. Making recommendations about i) improving the registration procedure, ii) research to be done, and iii) general policy measures to be taken.

The following remarks are made regarding these objectives:

1. Overview of research and registration procedure
For this purpose, it must be indicated what research is being done in The Netherlands at the moment, and in how far results of research abroad are relevant for the Netherlands situation; in addition it should be indicated where the highest priorities are for further research. From the study of the present registration procedure, points result on which this procedure is deemed insufficient and/or could be improved, with specific reference to field research.

2. Reference and evaluation framework
In evaluating the nature and extent of side effects of pesticides, an approach is used in which these effects are expressed in the extent to which functions for the society are affected. Besides looking at the effects on these sectoral functions (= usage functions), as they apply

e.g. to the sectors agriculture, fisheries or recreation, we also consider the effects on national health, nature and landscape.

3. Nature and extent
To make the scope of study not too broad, in consultation with the principal the following delimitation was chosen: terrestrial: concentrate on invertebrates, and attention to vegetation, fungi and bacteria only insofar as these play a part as intermediaries from chemicals to invertebrates; aquatic: attention to those areas where it may be assumed the side effects of pesticides used in agriculture are greatest, i.e. including the (small) ditches, but excluding the large rivers and the marine environment; thus in principle no attention to emissions into large rivers but special attention to emissions in(to) the agricultural areas.

4. Recommendations
From the results in objectives 1, 2 and 3 should be derived where lacunae in knowledge exist, and whether this concerns a lack of factual knowledge (e.g. number of deaths, toxicity, exposure or auto-ecology) or lack of insight into backgrounds or methods (e.g. operating mechanisms or extrapolation problems from lab to field and vice-versa). From this, recommendations should result for improving the registration procedure, research to be done and policy measures to be taken.

1.2 Methods

To reach the objectives sub 1.1, in phase 2 desk research and field research was done. The methods used are described in short in the following sections. If needed, details are given in the respective chapters.

1.2.1 Desk research

The data needed here were gathered by: i) holding interviews, ii) doing literature research and iii) organizing a workshop. Further details below.

Interviews

The discussions with experts on (side effects of) pesticides took the form of interviews held at the institute of the scientist concerned (for a list of the people see: Annex 1.1). In some cases a phone conversation was sufficient. Interviews usually lasted some 1½ hours.

The interviews had the following structure:
- The first questions were aimed at the research and other activities in the area of pesticides by the researcher(s) concerned and the institute, inc. possible co-operation arrangements with other institutes, such as e.g. within specific research programs.
- Subsequently their opinion was asked on nature and extent of the side effects, also as related to other interventions in the agrarian area. Here, we also asked about supposedly problematic areas, with the accent on (ways of growing) crops, chemicals and animal species; finally we inquired about the possibly known incidents.

- The next point was inquiring about the background research deemed necessary concerning side effects, such as new promising aspects as related to (ways of growing) crops, chemicals and locations.
- Subsequently the registration procedure was discussed, specifically inquiring about possibly existing difficulties in the present procedure and about suggestions for solutions for these.
- Finally specific points were inquired into, depending on the expertise of the interviewer and/or the field of the institute concerned.

The results of the interviews were always laid down in internal notes, which in a number of cases were submitted to those interviewed for approval. The information obtained was used in various places in implementation and reporting, especially to get an idea of actual nature and extent of the side effects and of the research in The Netherlands, including their correlation.

Literature search

To get an overview of existing literature on side effects of pesticides on terrestrial invertebrates and aquatic fauna, the following sources were consulted (see also: de Snoo & Canters, 1990):

BIOSIS file
This was searched by computer (on October 26 and December 1 1987) for relevant magazine articles, books and reports on Symposia. The search profile was drafted with the help of Drs. J.A. Smit of the University of Leiden. The computer search covered the period 1978-87 and provided some 700 references. This output was supplemented with references already gathered in the literature search for phase 1 in 1986.

TNO/SCMO project file
To track down research recently concluded and current in The Netherlands, inc. relevant publications, a printout was provided in Dec. 1987 of the TNO/SCMO projects file. This was supplemented with material from the inventory of ecotoxicology research currently in The Netherlands (Murk et al., 1987) and with information on special research programs.

The relevant literature will be mentioned in various places in the report. The information is dealt with more explicitly, however, in chapters 4 and 5, viz. in the discussion of nature and extent of the side effects of pesticides on respectively terrestrial invertebrates and aquatic fauna, and in chapter 2, in giving the overview of current research in The Netherlands.

Workshop

On 29 April 1988 a workshop was organized in Utrecht on the pesticides registration theme. This was participated in (by invitation) by experts from government, research institutes and industry. Annex 1.2 lists the names of the participants. Three speakers had been invited to lecture, viz. dr. M. Fletcher (Incidents Registration and illegal use in England); dr. Ch.W. Heckman (Field research on side effects of pesticides in West Germany) and ir. J.W. Everts (Extrapolation from lab tests to field situation).

The participants had previously been given a number of items, on which their comments were requested. The following subjects were raised at the workshop:

- the evaluation and reference framework
- the registration procedure in a general sense
- the lab tests
- the field tests, and
- the field research to be done in 1988 by CML.

In the afternoon discussion, on the basis of a number of specific questions, it was attempted to take inventory, as well as possible, of the (various) opinions on the subjects mentioned. Also, each participant was given the opportunity to give his/her views in writing. The general conclusions of the workshop were obtained by asking each participant umbrella-type question. The results were used to get an overview of the functioning of the registration procedure and to get an impression of the desirability of a number of proposed alternatives and supplements, such as new lab tests, incidents registration and field tests.

1.2.2 Field research

This was aimed at tracing side effects of pesticides observable in the field and developing c.q. evaluating the methods to be used in it. It was attempted to make connections among the (way of growing) crops respectively the use of chemicals and the groups of fauna present. In the field research a comparison was also made of newly gathered data and existing data(bases).

Terrestrial

For the terrestrial environment, the epigeal and edaphic soil fauna was sampled on a number of fields in the Haarlemmermeerpolder. The sampling was done in three different crops and with different chemical usage. The choice of the sampling points in the Haarlemmermeerpolder was such that there were differences among the farmers in intensity of chemical use. Thus, the effects could be compared of respectively no use of chemicals (organic farmer) and 60%, 80% and current (= 100%) use of chemicals. To facilitate finding a possible correlation between occurrence or not of invertebrates and pesticides - by countering "noise" - a number of other factors (type of soil, manner of tending the crop, and quantity and kind of fertilizing) was also involved in the research.

Aquatic

The aquatic fauna was sampled in various regions (Westland, Haarlemmermeerpolder, environs of Aalsmeer and in the North East Polder), in various crops (potatoes, sugar beets, wheat, greenhouse crops). An essential part of the set-up of this component is that ditches not affected by pesticides were also sampled, so that there was a reference. To exclude effects from other factors as much as possible here too, a number of other factors in the aquatic environment were measured, such as the width of the ditches, the presence of vegetation and a few physical-chemical variables, including sum (total) parameters of pesticides.

The data gathered in the field were also compared with existing inventory data. To be able to judge whether the differences found in the invertebrates are correlated with water quality, bio-assays with water fleas, water mites and isopods were also done.

1.3 Setup of the report

This report is structured as follows. Chapter 2 gives an overview of recently concluded or current research in The Netherlands on side effects of pesticides on terrestrial invertebrates and aquatic fauna. It also gives a short overview of the present registration procedure, dealing with difficulties indicated.

Chapter 3 goes into a specific and important difficulty, viz. the lack of a framework within which the side effects can be placed to be able to arrive at an evaluation; a proposal is made concerning this. In Chapter 4 an overview is given of nature and extent (in The Netherlands) of side effects of pesticides on terrestrial invertebrates and the same thing in Chapter 5 for aquatic fauna. The report concludes with conclusions and recommendations (Chapter 6).

2. RESEARCH AND REGISTRATION IN THE NETHERLANDS

This chapter consists of 2 parts. In the first part an overview is given of the research in The Netherlands on side effects of pesticides on terrestrial invertebrates and aquatic fauna (2.1). The second part (2.2) goes into the registration procedure for pesticides used in The Netherlands, and a number of aspects are discussed in detail.

2.1 Research

For the inventory of current or recently concluded research in The Netherlands the following sources were used:
- projects file of TNO-SCMO (printout 1987/88, key word "pesticides"
- Murk et al. (1987): "Ecotoxicological Research in The Netherlands"
- research programs of institutes, boards and commissions
- information obtained during the interview rounds.

The results of the inventory are shown below, subdivided into i) relevant research, by institutes (2.1.1), ii) research programs and the work of commissions and working groups (2.1.2) and iii) direct departmental activities (2.1.3).

2.1.1 Institutes

An idea is given in short of the research institutes in The Netherlands involved in the research on side effects of pesticides on terrestrial invertebrates and aquatic fauna of the type of research (aimed at effects or incidents), approach used (lab, field or desk research) and where possible, the groups of animals or chemicals concentrated on. If known, the name(s) of the researcher(s) concerned are also given. First the university institutes are dealt with (2.1.1.1), then the non-university research institutes (2.1.1.2) and finally the water quality managers (2.1.1.3).

2.1.1.1 University groups

Agr.Univ. Wageningen (LUW) - Toxicology
Terrestrial: Field and lab research on the effects of pyrethroids on spiders. The field research is done in the Flevo Polder, in colza and grain fields. Developing a spider test (Everts, Jagers op Akkerhuis).
Aquatic: In the past, research in test ditches; this was taken over by other institutes, including the IOB. Now developing a tubifex test and research on exposure of fishes and snails.

Agr.Univ. Wageningen (LUW) - Nematology
Terrestrial: Evaluating biological soil quality by means of the nematode fauna as related to substances alien to the environment (Bongers).

Leiden University (RUL) - CML
Terrestrial: desk and field research on effects on invertebrates (Canters, de Snoo, de Jong, van der Linden).

Aquatic: desk and field research on effects on aquatic fauna; also bio-assays with water fleas, isopods and water mites (names as above).

Utrecht University (RUU) - Toxicology
Aquatic: Toxicological lab research (QSAR-analyses) of effects on organic phosphor compounds and organic tin compounds on fishes (Hermans, Seinen).

Free University Amsterdam (VU) - Ecotoxicology
Terrestrial: lab research on effects of dithiocarbamates on an isopod-fungus system (= multi-species test), incl. test development with this system; also model development (Joosse, van Straalen).

Free University Amsterdam (VU) - Theoretical Biology
Aquatic: model development for extrapolation from lab to field situation (Kooyman).

2.1.1.2 Non-university institutes

CABO (Centre for Agro-Biological Research)
Aquatic: lab research by means of micro-ecosystems on the effects of herbicides (Butijn); now concluded.

DBW/RIZA (Inst. for Inland Water Management and Waste Water Treatment)
Aquatic: lab and field research on effects on algae, water fleas, mosquito larvae and fishes. Also test development, incident studies; as well as surveys among contract sprayers and inventories of the use of pesticides (van der Gaag, van Urk, van Beersum, Wagemaker).

Experimental Station 'Ambrosiushoeve'
Terrestrial: lab and field research with bees, also bio-assays (van Heemert).

Experimental Station 'Schuilenburg'
Terrestrial: lab and field research on predators and parasites (ichneumon wasps, predatory mites) in relation to possibilities for integrated control (Blommers).

IB (Institute for Soil Fertility)
Terrestrial: Completed research on the effects on soil organisms in connection with factors such as tilling. (Lebbink).

PAGV (Research Station for Arable Farming and Field Production of Vegetables)
Terrestrial: Experimental Farm "Developing Farm Management Systems" (OBS) near Nagele (a.o.): Comparison of a common and an integrated management system, focusing on the economical cost-effectiveness yet with some attention to side effects of pesticides (cf. de Jonge, 1988).

PD (Plant Protection Service)
Terrestrial: effect research on natural enemies of pest organisms, micro-arthropods and bees (just desk research with bees).
Also test development (Oomen); Van de Bund is still working with data gathered in recent years on the relationship occurrence of soil vertebrates and (mainly) chlorinated hydrocarbons.

RIN (Research Institute for Nature Management)
Terrestrial: lab research (biological background research) on effects on worms, nematodes and micro-organisms; in the past on soil arthropods such as springtails and isopods, also as related to herbicides (Ma, Doelman, van Capelleveen).
Aquatic: lab research by means of micro-ecosystems (bacteria, algae and water fleas); recently a test was developed for sub-lethal effects on water fleas (Kersting).

RIVM (Neth. Instit. for Public Health and Environmental Protection)
Terrestrial: lab research effects on earthworms and nematodes; also test development with these species (Van Gestel, Schouten).
Aquatic: lab research on the effects on water fleas and fishes; also concentration measurements in surface waters; model-ecosystems (same as those of the RIN) and model-developing (Canton, Greve, Minderhoud, Slooff, de Zwart).

TNO (Applied Research Organization)
Terrestrial: incident studies and effects research with worms; also emissions to air (de Kreuk, Adema, Huygen).
Aquatic: lab research on effects on water fleas (Adema).

Winand Staring Centre (before January 1 1989: IOB in co-operation with ICW)
Terrestrial: Incident studies of substances in soil and emissions from sprayers (Leistra, de Heer).
Aquatic: Incident studies and effect research, i.a. on biological availability and extrapolation from lab to field situation; algae research (also perifyton), water fleas, fishes, and small-scale with isopods, inter alia with micro-ecosystems and test ponds and ditches; improving of tests; concentration measurements in drain pipes (de Heer, Leeuwangh, Leistra).

2.1.1.3 Water Quality Managers

These include water control boards, polder boards and provinces. They take biological samples in the field and do concentration measurements as well.

Special mention goes to the Rhineland Polder Board research in the Haarlemmermeerpolder and in the (flower)bulb region (Klapwijk), the Polder Board of Delfland in the Westland (van der Wal) and that of the West-Overijssel Water Purification Board in the North-East Polder (Bekooy, Jol). They are looking specifically for the presence and possible side effects of pesticides.

The Municipal Water Utility (of the City of) Groningen (Flentge) last year measured pesticides in the Drentsche Aa (River). Recently, this enterprise together with some other institutes, has begun to measure pesticides under conditions in practice and at farm scale. The objective, among other things, is to compile a mass balance.

Concentration measurements are also being done within the Project Integration of Environment Measurements of the Province of South-Holland and biological samples are being taken (PIMM, 1987).

Finally, the Union of Water Control Boards (UvW) should be mentioned. This co-ordinates the registration of the incidents reported by the water quality controllers (de Vries). Shortly, the UvW, in co-operation with the Water Control Boards will make a recommendation for the measuring of pesticides deemed relevant, by region and (way of growing) crops.

2.1.2 Research programs, commissions and working groups

Besides the research dealt with in 2.1.1, there are also a number of specific research programs, commissions and working groups, the activities of which (partly) concern the research areas aforementioned. The most important ones among these are elucidated here in short.

Stimulation Plan Toxicology Research (Department of Education & Science)
Objectives (cf. Koeman, 1986):
- developing the discipline: ecotoxicology
- developing toxicity tests as alternatives for animal experiments
- attention to the aspects: work (labour) and health
- promote optimum co-operation among research groups, government (specific departments) and business
- the connection between toxic substances and chronic diseases as related to nutritional and living customs.

Institutes involved are i.a. RIN, LUW (Toxicology, Nature Management and Water Purification Groups), IOB and ICW; the latter two institutes, together with 'Stiboka', became part of the new Winand Staring Centre from January 1 1989.

Various current research programs, i.a.:
- LUW-Toxicology: Quantitative prediction of the effect of pyrethroids on indicator species, particularly terrestrial non-target arthropods (Everts, Jagers op Akkerhuis) and around the theme "Evaluation of lab test for predicting the fate and effects of pesticides in freshwater ecosystems"
- IOB: Experimental pond and ditch research (Leeuwangh);
- LUW Nature Management: "Ecotoxicological effects of an insecticide in a laboratory meso-scale cosm" (van Vierssen).

Spearhead Program Soil Research (Departments of O&W, VROM, L&V and WVC)
Objectives: stimulating knowledge development for implementing a policy offering perspectives for more fundamental solutions for the medium and long terms of soil problematics. The aim is that in principle, processes in the soil be able to continue to occur, so that the soil retains the capacity to properly perform its various functions, in the long term as well, as well as to retain the properties of the soil which are important for its various possible functions (PCBB & PCTB, 1988).

Organization: Advising by Programming Commission Basic Knowledge Soil Research (PCBB) and Programming Commission Technique Development Soil Research (PCTB); various projects are underway at research institutes.

Within the soil research Spearhead Program, six lines of research are distinguished:

A. Theory-forming and synthesis
B. Evaluation methods for contamination and soil deterioration on the basis of organisms
C. Basic research on susceptibility and possibilities for recovery by the soil
D. Weighing methods for cleanup and preventive measures to be taken
E. Clean-up and preventive techniques
F. Economic and management aspects.

Within research line B, especially the developing of ecological tests is of interest. Concrete research efforts concerning this cannot be found in the annual report for 1987 (Spearhead Program Soil Research, 1988). Research being done in this framework on the (side) effects of pesticides:
- field experiments on the effect of nematicides on the occurrence of Rhizoctonia (Bollen, LUW)
- the effect of nematicides on interactions among soil organisms and pathogenic fungi of potatoes and sugar beets (Bollen, LUW)
- theoretical-biological supporting and integration of research on the functioning of soil ecosystems in a form of "current" and "integrated" tilling (Brussaard, IB).

Technical Commission Soil Protection (TCB) (Department of VROM)
Objectives: i.a., gathering registration criteria from the point of view of the soil system.
Developing of ideas within TCB concerning this is considered extremely desirable by VROM itself. To this end, late in 1987 a working group was formed which was given the following points of attention (written communication, De Bruin, TCB):
- Evaluation from point of view of soil organisms and soil ecosystems (micro-biological activity; soil organisms; side effects via food chain; recovery and re-colonization).
- Physical-chemical aspects (equilibrium distribution of soil - soil moisture - organisms; soil structure and leaching into groundwater).
- Effectiveness of applications (unintentional spreading; dosage and frequency of application).

Pesticides Registration Commission (CTB) (Departments of VROM, L&V, SZ and WVC)
For a general description of the activities of the CTB we refer to the report in phase 1 of the side effects of pesticides project (de Snoo & Canters, 1990; but see also: 2.2). Within the support group "Environment" a number of ad hoc working groups have been active since fall 1987, charting specific problems and forwarding solutions.

Objective of ad hoc working groups: improve a systematic evaluation; look into mutual correlations of relevant aspects, ending up with an evaluation method, with certain variants. Ad hoc working groups:
- "Behaviour in the soil" (Boesten, Dutman, van der Linden, Linders and Loch)
- "Toxicity for water organisms", formerly "Risks for water organisms" (Canters [per 010189: de Snoo], Canton [per 010189: Roghair], van der Gaag, Jobsen [per 010189: de Heer], de Jong and Leeuwangh)
- "Behaviour in water" (van der Gaag, Bruggeman, de Heer, Linders, van der Meent and Struijs)
- "Toxicity for vertebrates"
- "Influences on soil organisms".

A couple of working groups have met a few times in the course of 1988, and reported on the results to support group "Environment".

Within the framework of the CTB activities, a complete re-evaluation is being done (by RIVM i.a.) to check whether the registrations in force, specifically those of a large number of "old agents", should be revised c.q. revoked, as in time the environmental requirements have been stepped up and there is a backlog in their implementation.

Agriculture Advisory Commission (LAC) (Department of L&V)
Within the LAC there is active i.a. a Working group Pesticides in Surface Water (IOB, VKA, RIVM, DBW/RIZA, Union of Water Control Boards, West-Overijssel Water Purification Board, Polder Board of Delfland):
Objective: Provide an insight into the occurrence, as well as the effects on aquatic ecosystems, of pesticides in surface waters; pay attention as well to the significance of the presence of pesticides for the production of drinking-water from surface waters.
Tasks: i) Develop a chemical monitoring program for surface water and identify high-risk agents, ii) develop proposals for a biological monitoring program in surface water (van Doorn, Mol, Canton, van der Gaag, de Vries, Vasse, van der Wal, de Heer and Leeuwangh). In the recent past the research was concentrated mainly in the greenhouse area in the Westland. In 1989 research will also be done in the fruit and potato crops in the North-East Polder (oral comm. Leeuwangh, van der Gaag and Jol).

National Council for Agricultural Research (NRLO) (Department of L&V)
It can be concluded from the annual report over 1987 of the NRLO (1988) that attention to (the side effects of) pesticides is greatly scattered. In 1987 a Task Group Effects and Side Effects of Pesticides was founded though (secretary: de Waard). Within its area, this task group handles the following two subjects:
i) way of working and effects of pesticides, and ii) environmental effects of toxic organic substances. Under the first item, the task group wants to try to unite the researchers working in this area in a Contact Commission, with as objective the promotion of research on new types of pesticides. In addition, there is the Contact Commission Optimization of Application Techniques - mainly aimed at above-ground techniques - in which possibly the now separately operating Working Group Application Techniques will be included.

In the latter field is active the Working Group Ecotoxicological Aquatic Systems, the participants of which are largely those involved in the experimental pond and ditch research (see also: the Winand Staring Centre in 2.1.1.2 and the Stimulation Plan Toxicology in this section).

Health Council (Department of WVC)
In the report Ecotoxicology, "Views of 31 people involved" (Murk, 1987) an overview is given of the manner in which a number of Netherlands ecotoxicologists look at their area of expertise. After an expose of the views and the methods in ecotoxicology research, and current applied and pure ecotoxicology research, the touchy policy points are entered into. The following ones are elaborated further:
- the setting of standards
- environmental measurement networks
- threats to ecosystems, and
- international aspects.

From 31 views, a large number of research recommendations result (21 in total), a striking characteristic of which is that research would have to be concentrated mainly at the ecosystems level. Further elaboration in the form of implementation at institutes is lacking here too, however.

Recently, a commission of the Health Council also produced an advisory report on the ecotoxicological risk evaluation of substances (Health Council 1988). In this advice, three (Netherlands) extrapolation methods are compared and evaluated.

Council for Environment and Nature Research (RMNO)
In "RMNO Multi-Year Vision 1987" (RMNO, 1988) the RMNO presents i.a. *top* recommendations on the basis of indicated needs for research. When looking at pesticides, research on "the designing of integrated control systems for diseases and pests in agriculture" gets such a mention. Although it is also indicated i.a. that increasing use of pesticides leads directly or indirectly to a decline of flora and fauna, no explicit consequences are tied to this concerning research to be done.

Advisory Council on Science Policy (RAWB)
In the "Advice concerning Ecotoxicology" (RAWB, 1987) given on the occasion of a "Strategic Conference on ecotoxicological research" (April 9 1987) with i.a. as objective "better utilization of research results in setting standards for substances potentially dangerous to the environment", the following fields of research come to the fore:

I. Analyzing the (social) protection objects and objectives and their operationalizing
II. Developing methods for risk evaluation and setting of standards, i.a.:
   - on the basis of the literature, setting up tentative methods of risk evaluation, testing them and adjusting them if required
   - differentiation in standards in relation to functions for the society, abiotic/biotic characteristics, and types of antropogenic stress)
III. Evaluating methods for risk evaluation and standard-setting:
   - extrapolation of effects found in one species, toward other species
   - extrapolation of exposure at protocol level toward effects at ecosystem level
   - extrapolation of effects at single species level toward effects at ecosystem level
   - extrapolation of effects on an organism in a certain stage of its life cycle toward another (more or less sensitive) stage
IV. Developing testing methods on behalf of policy
V. Improving field knowledge
VI. Attention for environment-chemical aspects of ecotoxicology.

A shortcoming in this RAWB report is that, although integration of disciplines is advocated, no proposals are made for such integration, and also the inter-disciplinary institutes themselves are not given the floor, let alone were involved in elaborating these items of attention.

Co-ordination Commission for the Measurements of Radioactivity and Xenobiotic Substances (CCRX) (Departments of WVC, L&V, SZW, VROM and V&W)
The CCRX has as its task to attune the systematic measuring programs carried out by the various governmental institutes and to co-ordinate the

exchange of measured data among these institutes. To this end, the commission compiles overviews of the distribution of substances (incl. chlorinated hydrocarbons and cholinesterase-inhibiting substances) throughout the entire biological environment. In addition, for the more incisive evaluations overview reports are made, such as e.g.: "Evaluation report cholinesterase-inhibiting substances in The Netherlands" (CCRX, 1985).

2.1.3 Direct departmental activities

Department of Agriculture and Fisheries (L&V)
Memorandum **"Toward a task-setting multi-year plan for crop protection"** (draft, October 1987), about which in February 1989 Minister Braks (L&V) supplied further information to the Lower House:
Objective: In the year 2000 agrarian management (crops, operations, trade, marketing) and administration directed at the green spaces will have to be such that the requirements for optimum crop protection have been integrated as much as possible into operations and management. Also, maximum use should then be made of people- and environment-friendly crop protection methods, which fit in with sustainable and profitable agricultural systems.

The multi-year plan, which has to be ready in 1989 and can be considered the successor to the memorandum "Crop Protection in The Netherlands" (Lower House, Session 1983-84, 18 100, Chapter XIV, 2, Annex IX) will have four angles of approach:
- reducing the causes of infections in the crop (requirement-setting crop protection);
- reducing the consequences of crop protection measures (consequence-reducing crop protection);
- developing and optimising the instruments for crop protection (innovating crop protection);
- an effective, correlated implementation strategy of the plan (condition-creating crop protection).

Department of Housing, Physical Planning and Environment (VROM)
Uniform evaluation system new substances (DGM-RIVM)
Objective: By making optimum use of existing data, to signal potential risks from new substances to people and the environment at as early a stage as possible, making use of estimating systems, models and databases (Roghair, 1988). This evaluation system is not specifically aimed at pesticides though. Also worth mentioning in this connection is the risk brochure, now in preparation "Handling risks" (DGM), to which we shall return in Chapter 3.

Department of Housing, Physical Planning and Environment (VROM)
Project Ecological Compatibility (Substances) (DGM)
Objective: Putting coherence into environmental policy on the subject of the evaluation of material effects on ecosystems (also as a result of physical interference and climate change) to assess the ecological conditions for sustainable use of the biosphere, incl. developing instruments required to implement the policy, on the one hand by limiting the causes (source oriented policy) and on the other hand by measures to repair or compensate for damage to ecosystems (= repairability).

Five components are distinguished within the project:
1. Tuning of uniform evaluation system within DGM (see: previous page)
2. Risks of calamities for ecosystems (see: previous page)
3. Soil test development
4. Extrapolation factors
5. Increasing insight into fundamental characteristics of ecosystem structures and processes.

As a general note regarding this project we remark that the validation in the field of research results still appears to remain under-exposed. We shall come back to this in the following chapters, and in the recommendations in chapter 6.

2.2 Registration procedure

Following the research in The Netherlands, in this Section the Dutch registration procedure for pesticides is discussed. In 2.2.1 the present procedure is summarized. In 2.2.2 specific points of criticism are raised, and possible solutions indicated - partly mentioned by those interviewed. In the concluding Section 2.2.3 the more general criticism of the registration procedure is advanced.

2.2.1 Present registration procedure

The components of the registration procedure concerning the side effects of pesticides on terrestrial invertebrates and aquatic fauna are shown below. The data are taken from the application forms for registration of agents, part H.: "Toxicity for organisms occurring in the environment" (CTB, 1987). It should further be noted that part H is continually adjusted as e.g. upon the recommendations by the ad hoc working groups of the CTB (see: 2.1.2).

H1 Toxicity for birds
As this is not relevant in this study, we do not go into this here and refer to De Snoo & Canters (1988).

H2 Toxicity for water organisms
H2.1 Short-term toxicity research
Data should be supplied for any pesticide.
- EC50 algae, 96 hrs.
- LC50 and/or EC50 crustaceans, 48 hrs.
- LC50 and/or EC50 at least one fish species, 96 hrs.

Long-term toxicity research
Data to be supplied when big exposure chances exist for water organisms; this can also be requested when effects on reproduction and embryo development are to be expected.
- NOEC crustaceans; criteria: mortality and reproduction
- NOEC (juvenile) fishes; criteria: embryo development, mortality, behaviour and growth.

The test duration depends on the life cycle of the animal and the parameter to be investigated.

H3 Hazards for bees and other useful insects and mites

H3.1 Hazards to bees

This is determined not only by the toxicity but also the dosage, manner of application, special properties of the agent and the crop to which an agent is applied. Data on bees are mandatory if the agent is used on flowering crops and plants frequented by bees.

- LD50 (oral and contact) for stage at greatest risk (usually adult workers).
- If LD50 values in relation to highest recommended field dosages give reason to, cage and/or field test data are also required.
- Depending on specific manner of application and characteristics of the agent, other data may also be deemed required.

H3.2 Hazards to other useful insects and mites

With agents for which application in integrated crop protection schemes is claimed, data must be supplied on "the effects on useful arthropods, whose maintenance is desirable, insofar as these are involved in the application situation".

H4 Effect on soil organisms

H4.1 Effect on nitrification

Effect on soil microflora and related enzymatic processes. Nitrification is important from an agricultural point of view as well, for reason of the nitrate-feeding of the crop, and from the environment point of view for reason of possible nitrate leaching. Data can remain limited to soil treatment agents.

- Measurements in at least two types of soil.
- Further research under field conditions can be required in cases of fairly long-term influencing of nitrification.

H4.2 Toxicity for earthworms

- Acute toxicity for earthworms, 14 days; data are required for all active substances, unless it is shown that the agent will not get into the ground.
- Sub-acute toxicity: 14-28 days, criteria: mortality, behaviour and reproduction; these data are required when a chance exists of long-term exposure (frequency of application, persistence), or can be requested when the results of acute toxicity tests give rise to this or if there are indications of effects on reproduction.

H5 Effects on treatment of waste water

As not relevant in this study, we shall not go into this. Micro-organisms are raised though in chapter 3, insofar as they play a role as intermediaries.

H6 Data on bio-accumulation in water organisms

Data are mandatory when the nature of the substance, the persistence and frequency and manner of application give occasion. For most organic substances, sufficient insight can be obtained from octanol/water-distribution co-efficient or from the water solubility of the substance. For other substances, species-accumulation can be requested.

H7 Supplementary data

H7.1 Secondary poisoning

Only for birds and mammals. Data can be required when organisms are

killed with the agent which at time of application are much eaten prey for birds (insects e.g.).

H7.2 Research on toxicity effects under field conditions
Can be required in certain cases.

2.2.2 Criticism of test components and possible alternatives

In this section the points of criticism concerning the registration procedure are given, as submitted along large lines at the workshop "Registration". Where possible, solutions and/or alternatives will also be given. To an important extent, the criticisms are derived from interviews with experts in The Netherlands. In addition, data from the literature and ideas of our own are included. The data about the aquatic environment are partly derived from the ad hoc group Toxicity Water Organisms of the CTB. The criticisms of the test concerns an enumeration of sticky points and remarks by experts and our own ideas; insofar as possible, alternatives are also mentioned.

H2 Toxicity for water organisms
H2.1 Short-term toxicity testing
General: Whether all species should be taken equally seriously and whether e.g. it should count that algae recover after 2 days already, is a point of discussion. Any elucidation besides the toxicity data is welcome, however. Data from pond studies, which give an impression of the effect on an ecosystem, are also important.

Algae test
Criticism: Not advanced.
Alternative: Periphyton test (Leeuwangh). The effect of pesticides on the growth of algae is equal to the oxygen production/consumption of very differently composed periphyton. This test is also suited for investigating sublethal effects.

Daphnia test
Criticism: Raising-problems and reproducability; so far though, the raising-problems apply to all test organisms.
Alternative: Artemia test (Persoone & Zorgeloos, University of Gent, Belgium); no raising-problems with these.

Fish test
Criticism: At the moment, a four-day LC50 (but with daily measurements) is maintained.
Alternative: There is a preference for a 14-day LC50 with day-values.

H2.2 Long-term toxicity testing
Criticism: i) it is indicated that few data are supplied or requested about long-term toxicity testing. Long-term toxicity testing appears desirable when a) a large system is simultaneously stressed, b) the stress is long-term, or c) specific effects on reproduction etc. can be expected; ii) often a test with Daphnia magna can suffice (reproduction); iii) only mortality and no sub-lethal effects are traced.
Alternatives: Daphnia food intake test (Kersting): cheap test, also suited to examine sub-lethal and ecological (food)effects.

H3 Hazards for bees and other useful insects and mites

H3.1 Hazards for bees

Bee test

Criticism: the test is done only when application of the agent on flowering crops and plants is envisaged, and thus exposure of bees can occur.

Alternatives: i) make the test standard-required, with bees being the model for the group of flower-frequenting insects; ii) other flying insects, e.g. flies, which can be easily raised.

H3.2 Hazards for useful insects and mites

Useful insect and mite tests

Criticism: i) test aimed only at detecting non-dangerous agents which can be usable in integrated crop protection schemes; ii) the test is not very suitable for determining the toxicity of an agent and thus not very suitable either for allowing or not allowing an agent on the basis of this toxicity. The test is done with only a one-time residual exposure to the formulated substance, with only the highest applied concentration being tested.

Alternatives: i) during the interviews an LD50 test was suggested; ii) do the testing with species which are also relevant for open-air crops; iii) do the test as a standard requirement.

H4 Effect on soil organisms

H4.1 Effect on nitrification

Criticism: none advanced.

Alternatives: other sum (total) processes, such as soil respiration or enzyme activities (see also: 3.3.1).

H4.2 Earthworm test

Criticism: i) the earthworm test (Van Gestel; Ma) is insufficient for covering the side effects of pesticides on the entire soil fauna; ii) from the point of view of exposure, the earthworm test is suitable only for testing effects of persistent agents staying in the soil for a fairly long time and with which the worms can get into contact. The epigeal soil fauna is exposed in a different manner and a representative from this group would also have to be used as a test organism; iii) worms are inactive in winter, vary in occurrence in space (depth) as well as in time.

Alternatives: i) spider test (Everts), spiders turn out to be good indicators (sensitive to pesticides, can be monitored all year and also exposed in the field). Disadvantage at the moment: too expensive, costly raising method; ii) Nematodes test (Bongers), however, here too the exposure disadvantage; iii) other soil arthropod species, further research is required for this. Possibly the tests developed in the IOBC framework (to determine the effects on useful organisms in this connection, specifically predators such as ground beetles) may be useful here. One can also think of tests with springtails; a test for this group was recently developed by Kisz & Bakonyi (written comm. Oomen).

H6 Bio-accumulation test

Criticism: i) bio-accumulation data can also be derived from physical/chemical data; ii) nothing is done with the data from the test; iii) in the elucidation, no limit is indicated at which testing is required. In aquatic ecosystems, the rapid secretion inhibits accumulation

and thus the possibility of secondary poisoning is slight (unless terrestrial predators or seals are present). It is noted here though that a) in a hunger phase stored fat is used up and this can lead to "exposure"; b) in fish during vitellogenesis stores are used up, which stored substances can lead to effects on eggs; d) too little is known about secondary poisoning.

Alternatives: as a limit for testing, a P-octanol value of 5 or less could apply.

2.2.3 General points of criticism

Besides the criticisms on the separate test components - and the alternatives indicated - those interviewed also mentioned more general criticisms. These were advanced and discussed at the workshop "Registration", on April 27, 1988 in Utrecht. An enumeration of the most important ones follows. Many will be dealt with further in later chapters.

First it should be noted that **by far the majority of participants in the workshop thought the present registration procedure insufficient to bring the side effects of the use of pesticides to light.** The two most important criticisms here are:

1 The use of single species tests and their interpretation. Disadvantages of the single species tests are the poor representativity for the field situation, and not obtaining insight into the effects on interactions, in food chains e.g.. Consequently, extrapolating the data from single species tests in the lab toward the level of the biocenosis in the field gives problems. A sticky point in this connection is the biological, standardized food chain research (= multi-species tests). Field research is largely lacking at the moment and expansion is certainly desirable. The present single species tests are acceptable for comparing the agents (ranking), partly for reason of the standardization internationally.

2 The tested organisms used. Criticism of the present test organisms is concentrated on the following four aspects:

   i) Only those organisms are tested which, as seen from an agri-economics point of view, have a certain use (bees, worms, useful insects and mites) but not all of them, and not always either. It is considered desirable to link the side effects of pesticides to the reference framework "consequences for functions for the society via indicator species" (see: Chapter 3).

   ii) The test organisms used in the present procedure were not chosen on basis of exposure. A supplement to present tests is well possible by making more use of functional groups in relation to manner of exposure. This is of particular importance for the terrestrial environment. It is indicated though that the differences among species are probably smaller than the differences between lab and field situation.

   iii) The tests have too little ecological background. On the basis of the present single species tests, no insight is obtained into the "ecological toxicological profile" of an agent. Thus, insufficient relevant ecological information can be derived from the tests for the risk control aspect. Also, mutual correlation among the tests is lacking. The aquatic organisms used are considered more representative for their environment than the terrestrial species. Still, in the aquatic environment too,

there should be more attention paid to chronic toxicity research. There further was a plea to expand the test conditions (sub-lethal effects).

iv) There are too few tests on hand, specifically for the terrestrial environment. It is necessary to have on hand the results of fundamental research, now still lacking, for selecting suitable new test organisms for this compartment.

Other items of criticism are:

- Ecological side effects
  The lack of sufficient attention to ecological side effects. No attention is paid in the present procedure to possible occurrence of such effects.
- Non-transparency
  The registration procedure as a whole is very non-transparent. It is not clear what decisions are based on; decisions often appear to have a strongly ad hoc character. In many places a wish for standardization is expressed.
- Standard testing
  At the moment, in the terrestrial environment, standard testing of the toxicity of the agents takes place only for earthworms. The other tests are done only if the agent has a special application or time of application.
- Water quality managers
  These, or the Department of V&W, are hardly involved in registration; the Department of L&V on the other hand, appears to be over-represented. An applicant for registration can register an objection to *non-registration; for someone else to register an objection against* a registration is not possible.
- Incidents registration
  This is not done centrally; hardly any attention is paid to mammals and fishes, and certainly not to invertebrates. A well documented and managed overview of all incidents with pesticides is also missing.
- Checking for side effects later-on
  Although in practice high concentrations of pesticides are found in the surface water and in the soil, there is no checking for side effects later-on.
- Illegal use
  In registration no attention is paid to occurrence of possible illegal use; inspection as regards adherence to instructions for use is largely lacking.
- Better (chemical) agents
  New agents, and better ones with a view to minimizing side effects (selective ones e.g.) do not get onto the market because the costs incurred in supplying the data required for an application and registration are too high. This problem occurs mainly with the small-scale crops. The fact that developing these agents is already barely feasible economically also plays a part here.
- Alternative treatments
  In the registration, no account is taken of alternative treatments: what is better e.g., spray once with a persistent agent, or ten times with a short-term agent.
- Synergism
  Synergism of agents is not involved in the registration. Toxic as well as ecological side effects of using several agents are not taken into account.

- Paradox in registration procedure
  There appears to be a paradox contained in the registration procedure: if a substance is not toxic in a first global test, no further detailed investigation takes place. This does appear desirable though, as otherwise subtle effects will not be found.
- Open literature
  In the re-evaluation of substances by the RIVM, only manufacturers' data are used. Data from open literature are not used, unless supplied by the manufacturer. Formerly the RIVM did do its own literature research, but now, with the "catching-up operation", in which the registration of by far most substances used in The Netherlands is re-examined, it does not!

2.3 Conclusions

Below we first give conclusions about the research (2.3.1), first of all terrestrial (2.3.1.1), then aquatic (2.3.1.2) and finally air (2.3.1.3). The more general conclusions around registration procedure are found in 2.3.2.

2.3.1 Research

At the moment, many diverging projects are running in The Netherlands, aimed at the (side) effects of pesticides. These vary from background research and theory-forming to test-development and risk evaluations. In a general sense it can be stated about the research in The Netherlands that it is housed at various departments, and within very different research frameworks (even within one department). Thus, a structural approach is more difficult. Better co-ordination of what policy managements want to, can and must e.g. in the form of semi-annual consultation, could already bring much improvement in our view.

2.3.1.1 Terrestrial environment

In the terrestrial environment, much less research is done. In general it can be said that developments in this compartment appear to lag behind those in the aquatic area. There seems to be a less coherent approach to side effects of pesticides, in spite of the existence of large research programs.

So far research is aimed mainly at "useful organisms", such as bees and spiders. Data about other animal groups are gathered much less systematically (see also: Chapter 4). In this framework, the research on soil organisms, in contrast with that on vegetation-bound organisms, is bedded-in in larger research programs and is getting more attention now. For the terrestrial environment there is no broadly set up comparative research on usable test organisms (see also: conclusions about the registration procedure, 2.3). The fact that the ecological background of various species is not completely clear can possibly play a part here. Also, in The Netherlands little attention is paid to terrestrial micro-ecosystems and semi-field-experiments. Effect-oriented field research is the area with the most lag. That of LUW-Toxicology in the framework of Stimulation Plan Toxicology Research is a positive exception. At the

moment no research is being done on the scale on which side effects occur, and on their time duration and seriousness. Finally, more attention will have to be paid to solution-oriented research, aimed at reducing emissions from agriculture, the developing of alternatives and the compatibility of measures with (farm) management.

2.3.1.2 Aquatic environment

In the aquatic environment at the moment relatively much research is being done by the separate water quality managers and by institutes such as DBW/RIZA, RIVM and IOB. On the basis of the number of joint projects it can be stated that co-operation in this area is reasonably intensive. There is also a reasonably structured approach, at least in view of the large number of aspects being investigated (taking inventory of substance use, incidents studies, toxicity research in the lab, model ecosystems in the form of experimental ditches, effect measurement in the field). Co-operation with respect to processing, the data on incidents, chemical measurements, and co-ordination in selecting measuring-points also appear to be getting well of the ground. In view of the high concentrations found in various places, such a research effort is an absolute necessity.

Effect-oriented field research (biological sampling of water courses related to the use of pesticides) is still lagging at the moment. Expansion is certainly desirable to get an idea of the nature and extent of the (side) effects occurring in The Netherlands (up to now, often limited to certain regions). It is also desirable to investigate the possibility to use existing data (fauna inventories e.g.) or to adapt certain research programs so that the data obtained can be used for tracking side effects of pesticides (in the Province of North-Holland e.g.). Also, inclusion of the recently demonstrated substances in the measuring programs of the separate water managers should happen in the short term. So far, often only the chlorinated hydrocarbons were measured. Also, a solution needs to be found for the accompanying high costs.

2.3.1.3 Air

The research on side effects of pesticides via the air on ecosystems and the environment has lagged behind. For some time measurements have been done by the RIVM. Effect-oriented field research is lacking though. Direct side effects of pesticides (herbicides, fungicides and soil disinfectants) on vegetation and on fungi are well possible. But also indirect side effects on e.g. forest vitality, via a decrease of mycorrhiza as a result of fungicides, an be imagined. Such suppositions certainly appear to justify a greater research effort in this area. We can think here of taking inventories (nature areas within agricultural areas), but also of bio-assays in the field with fungi, lower and higher plants, at certain distances from fields.

2.3.2 Registration procedure

The most important conclusion concerning the registration procedure is that by far the majority of participants in the workshop thought the present registration procedure insufficient to bring the side effects of pesticides to light. Here, the two most important items of criticism are:

- The use of single species tests and their interpretation
- The test organisms used (only useful organisms, no selection on the basis of exposure, insufficient ecological background knowledge and too few terrestrial tests available).

In addition, a large number of other criticisms came to the fore, such as:
- the lack of sufficient attention to ecological side effects,
- the registration procedure as a whole being non-transparent, and
- the lack of checking of the occurrence of side effects later-on.

In Chapter 6 a number of proposals are made to meet the objections indicated with respect to the registration procedure.

3. EVALUATION OF SIDE EFFECTS

In the Pesticides Act from 1963 it is made mandatory that before a substance is allowed onto the Netherlands market, first the soundness and side effects of that agent be investigated. An agent is allowed only if no unacceptable harmful side effects of the agent or of its conversion products are to be expected. Harmful side effects are i.a.: "The damaging of soil, water or air, or of animals, plants or parts of plants whose maintenance is desirable, to an extent which is unacceptable." (Pesticides Act, art. 3).

The acceptability of damage is an arbitrary point in the registration of a pesticide, as it is not immediately clear what is desirable to maintain and from which point of view this maintenance is defined. But also when seen from a specific viewpoint, such as that of nature conservation, uncertainties can still exist. With few exceptions, invertebrates do not have protected status in The Netherlands and thus have no direct relevance for policy, for which reason acceptability of damage is difficult to gauge. Vertebrates, on the other hand, often have a much more clear (protected) status and thus they are directly relevant for policy, c.q. the acceptability of damage to a species can be weighed against the maintenance of a species.

As indicated by the title of this report, this study is aimed at side effects on pesticides on terrestrial invertebrates and aquatic fauna. In this chapter, we see the maintenance of which species from these two groups is desirable, and why. It is also indicated, how the possible effects on the continued existence of these species from pesticides can be determined by means of tests.

We proceed as follows. First, in general terms, we deal with the use of pesticides in agriculture (3.1). Then we check from which points of view the maintenance of species can be desirable. Two approaches are used here: i) the species are seen in relation to the use functions an area has, and ii) the species are seen from the point of view of their nature significance. Before that, however, first in 3.2 the relationship with environment quality is established. The further elaboration (3.3) is limited to terrestrial invertebrates and aquatic fauna while attempting to trace those species, the presence or absence of which says something about the (un)suitability for the various functions or which directly or indirectly represent an independent nature significance. The result is a selection of species deemed relevant which can be used in the registration procedure or in evaluating environment quality in general. After in 3.4 a proposal is made about quantitative norm setting of the side effects on pesticides on terrestrial invertebrates and aquatic fauna it finally indicated how the use of the species selected for the registration procedure c.q. the environment quality can be operationalized by means of tests (3.5). To this end, the tests are first described and it is indicated how they are or can be used in the registration procedure. Finally, the use of the selected species on behalf of the general and specific environment quality is summarized in a concluding section (3.6).

3.1 Use of pesticides

In Fig. 3.1 a number of important activities are enumerated which are related to production and use of pesticides. For all of these activities, effects on the environment are imaginable.

Examples of activities and resulting effects are:
- production: emissions in current production (bentazon in the Rhine [River] at BASF) and in accidents (Sandoz, Basel); but also in bulk storage at depots (Volgermeer Polder)
- transport: accidents with ships, trucks, etc.
- waste management: cleaning of sprayers, barrels, etc. (see also: the Intermezzo on page 26).

This study concentrates on the effects which can originate when using pesticides in agriculture, as by far the biggest quantity of these ends up in the environment. Attention is concentrated particularly on the non-intentional effects, the side effects.

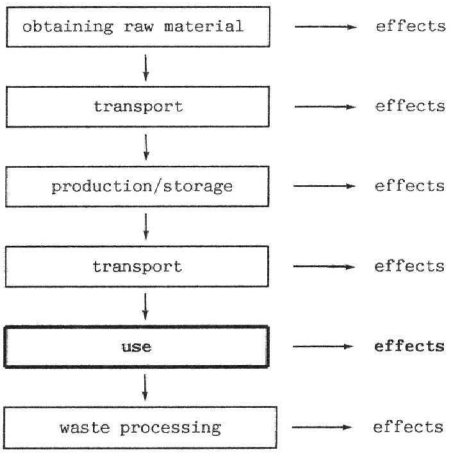


Figure 3.1 Diagram of activities in the field of pesticides.

INTERMEZZO

In the Westland market gardening in greenhouses has been wrestling for years with the problem of waste water strongly contaminated with pesticides. At the discharge points, great fish mortality is occurring regularly. The pesticides are now, at least in part, broken down in the ditches, the polder water and the 'Nieuwe Waterweg' between

Rotterdam - Hook of Holland or are drained off into the North Sea- which there too can lead to undesired effects - but also attach to the mud on the bottom of the waterways. Often this leads to greatly exceeding the basic quality standard for surface water from the IMP Water (Indicative Five Year Plan Water), by as much as a factor of 500 (van Haasteren, 1988). The plans existing here for a new drainage system, in which the waste water is gathered and removed to the New Waterway of the North Sea do not merit recommendation, however, as they present no real solutions. A development which perhaps offers a better perspective is the Environment Action Plan Greenhouse Market Gardening of the 'Landbouwschap' (Agriculture Board) (Volkskrant [newspaper], December 23 1988), under which the crops are grown in a closed water usage system, and each greenhouse is provided with its own water treatment installation. Since the crops are grown on substrates, a new problem occurs then, viz. the processing of the growing mountain of wet glass- and rock-wool, in which residues of pesticides are often present.

The present use of pesticides (in 1987) amounts to 18.000 tons active substance and can be described as follows (see also: Table 3.1): soil disinfectants are used a great deal (almost half the total quantity), followed by fungicides and herbicides (both just under a quarter). Insecticides are relatively little used (2.7%). For data from preceding years, we refer to the IMP-M 1987-1991 (Indicative Five Year Plan 1987-1991). When the total of active substance used is compared with the area in agriculture in The Netherlands, we find that some 25 kg of active substance is used per hectare. The twenty agents used most in The Netherlands, on the basis of quantities of active substance, are: atrazin, bentazon, captan, dichloropropene, dinoseb, fentin acetate, glyphosate, copper oxide, mancozeb, maneb, MCPA, mecoprop, metam-sodium, metamitron, methyl bromide, mineral oil, sodium hypochlorite, coal tar oil distillate, TCA and zineb (Lower House, Session 1987-88, Annex: p. 1366).

soil disinfectants	8,423,220	(46,7)
fungicides	4,070,202	(22,5)
herbicides	3,911,925	(21,6)
insecticides	498,186	(2,7)
other	1,183,723	(6,5)
total	18,087,256	(100)

Table 3.1 Use of pesticides in agriculture in The Netherlands, in 1987 (in kg active ingredient); between brackets: percentual contribution (after Berends, 1988; source: NEFYTO).

In using pesticides in agriculture, other emissions or interventions in the environment also occur. These can all lead to effects. Thus, besides the emission of the substance itself, use of pesticide can also lead to soil disturbance (by the tractor with spraying equipment), emission of the carrier substance and/or additive, etc. In general, this type of

secondary interventions has only limited consequences for the environment. Regarding the carrier substance and/or additive it should be noted, however, that the effects will not always by slight. In the use of pesticides themselves, depositions in the form of gasses, liquids and solids can be imagined, to the air, soil/groundwater, surface water and biota.

Regarding the use and side effects of i.a. pesticides, on 26 Jan 1989 the memorandum "Environment criteria concerning substances to protect soil and water" was submitted to the Lower House (Session 1988-89, 21 012, 1-2). In this, the latest state of affairs is described regarding policy on substances alien to the environment (incl. pesticides) and is also indicated what the standards are or will be for the deposition of this type of substances. As the emphases here are different from the present registration procedure (see: 2.2) the following was derived from it:

Toxicity

* water organisms: LC50-value must not be exceeded for algae, water fleas and fish species (see: registration procedure, 2.2);
* soil organisms: LC50-value for earthworms (incl. a safety factor to be further defined) must not be exceeded and the effects of soil disinfectants on nitrification are checked to get a general picture of these agents (see also: 2.2); a criterium for these agents will be further elaborated;
* ecosystems: risk approach according to Health Council (1988) (see: Intermezzo, next page); supplementary to this, the NOEC (semi-chronic) in long-term exposure shall not be exceeded.

Exposure

* persistence: maximum one year, corresponding to a disappearance time of 50% = DT50 of 2 months, so that in principle accumulation is not possible; from the point of view of sustainable development, non-reversible effects must be prevented; effects are deemed harmful if they affect the multi-functionality; with respect to soil-bound residues, advice was requested from the TCB; additivity, specifically of persistent substances, is reason for extra care;
* mobility/transport/leaching and presence in the upper groundwater (the level of which, in model calculations, is constantly assumed to be 1 m below ground level): i) individual pesticides less than 0.1 µg/litre and ii) total pesticide content less than 0.5 µg/litre (this is also the European Community standard);
* (bio-)accumulation: accumulation in the topsoil shall not be such that two years after application the multi-functionality of the soil is still affected; substances with an octanol-water distribution coefficient of over 1000 accumulate significantly in organisms and should therefore not be allowed.

INTERMEZZO

According to the memorandum "Environment Criteria" (1989), the criteria and standards to be used will probably connect with the risk approach now under development at VROM. In a fourth draft of the risk brochure "Handling risks" of VROM-DGM, now in preparation, various risks are defined. Besides an individual risk and a group risk, the collective risks for

ecosystems are also defined: "the chance that a function of the ecosystem is affected". For determining this, the point of departure is a model-based approach, i.c. the use of extrapolation factors on the results of standard tests (cf. Health Council, 1988). From this results the maximum permissible level. This level corresponds to that at which 95% of the species does not experience harmful effects. In tracking risks from substances alien to the environment it is often assumed that the maintenance of species is a guarantee for the protection of ecosystems. In this connection, the Health Council investigated 5 methods for doing a risk evaluation: all 5 turned out to be based on this principle. A disadvantage of this approach is not only that all usage functions are comprised under the function of the ecosystem - so that no differentiation can be made - but also that the standard is strongly quantitative, and for the time being does not take into account specific requirements set from a certain usage function (such as e.g. the maintenance of protected species). In addition it is assumed in this that for the protection of ecosystems a protection at the level of the structure (species composition in a qualitative and quantitative sense) also offers sufficient guarantee for protection of the ecological functioning.[2] In view of the discussion now occurring about pattern- and process-oriented nature management and the resulting changes of policy in the direction of more process-oriented nature management, this point of departure is less obvious.

3.2 Effects and environment quality

In a speech on the occasion of the introduction of an improved agricultural sprayer on December 27 1988 in Horst, minister Nijpels (VROM) indicated on which three pillars the present policy - at the level of emissions - is based:

- substitution of harmful substances (i.e. persistent and/or mobile and/or toxic for non-target organisms) by less harmful substances;
- reduction of emission by improving current application methods;
- decreasing use by promoting integrated methods and biological means.

This study is aimed at a specific aspect of the first pilar: how can the harmfulness of a substance be determined. Also, the characteristics named by the minister are not only relevant in connection with the toxic side effects, they can also be important in the occurrence of ecological side effects.

In the already mentioned memorandum "Environment Criteria", the objective for the policy for substances dangerous to the environment (incl. pesticides) is also put into words: "see [to it] that (in the long term as well) there are not harmful effects on people (health), animals, plants and ecosystems and environment functions (maintenance of e.g. drinking-water-producing function and agricultural function of the soil), this as

[2] Also, in the same brochure the negligible level of the risks for people and for ecosystems is set at 1% of the maximum pollution level (oral comm. Klein, NMF).

a component of a sustainable development". From this can be distilled three social objectives:
- national health
- the fulfilling of various forms of environment use (= sectoral functions/usage functions/environment functions) and
- nature significance.

Before going further into these social objectives (3.3) it is first indicated how the indicated policy relates to environment quality policy. As regards environment quality, mostly a distinction is made between general and specific environment quality. The IMP-M 1986-1990 means with general environment quality: "an environment quality such that the health and wellbeing of people and the maintenance of animals, plants and goods and forms of use in a general sense are guaranteed". Translated toward the soil, this means a quality such that the retention is guaranteed of the soil properties which are important for its various functions (= ***multi-functionality***). This multi-functionality can be further described as the principle that the soil must retain the potential to continue to properly perform its various functions. To this end, the properties essential for the various functions need to be protected. With multi-functionality there is a strong emphasis on the reversibility of certain interventions and the time involved. Translated toward the surface water, general environment quality means a quality such that at the location and elsewhere: i) there is no annoyance of the environment and the water does not look polluted, ii) a chance to live is offered to aquatic biocenoses, incl. higher organisms, and also ecological interests outside it are protected, and iii) offers opportunities for certain forms of human use (= **basic quality**). In basic quality, there is a strong emphasis on ecological functioning.[3]

The general environmental quality applies to all of The Netherlands and in principle, consequently, the requirements to be set must be met everywhere. It is possible though, with regard to global distinctions, such as e.g. high (= pleistocene) and low (= holocene) Netherlands, or fresh, brackish or salt, to set different requirements. For it is not sensible, realistic or desirable to have this type of requirements apply to all of The Netherlands. General environment quality can be further defined from two angles of approach: i) basal requirements set by specific functions, which have to be met everywhere (e.g. total pesticide content in groundwater always under 0.5 µg/litre) and ii) general properties on behalf of multi-functionality, which also must be met everywhere. The first angle is more source-oriented and linked to emission standards. The second approach is more effect-oriented; particularly this approach, also in view of the subject of this study, has been elaborated in 3.3.1, with attention paid to proper and healthy ecological functioning of ecosystems, as can be read out from the course of characteristic processes e.g. (or the presence of the species involved in them).

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[3] There is some misunderstanding around the idea of ecological functioning, also indicated as the ecological function, as though it concerned a usage function; to prevent confusion with these usage functions, we prefer the concept of ecological functioning, and the term ecological function is further not used.

The aforementioned IMP-M 1986-90 describes the specific environment qualities as the quality required for specific biocenoses, species or forms of use (in relation to certain areas and specific environment conditions).[4] For the surface water, in regard to specific environment quality and basic quality, a distinction is possible at three levels: the lowest, medium and highest quality levels. The lowest level corresponds to the basic quality, and the medium and highest levels to two levels of specific environment quality; the highest level corresponds to the description of the natural biocenosis of the surface water at the location CUWVO, 1988); at present this is often only the potential quality.

The specific environmental quality is area-oriented and is based on the destination or present use of an area. For this, on the basis of present use functions, specific requirements are derived, such as e.g. concerning the maintenance of protected plant and animal species. Not only present use is considered though, but also usage function(s) of contiguous areas, and possible other usage(s) at a later time. One can think here of a nature area beside an agricultural area, respectively of use as arable land followed by use as pasture. Use of an agricultural area as a drinking-water source area also comes under this; this then has as a result that requirements are set for the agricultural function by the source-of-drinking-water function.

3.3 "Species whose maintenance is desirable"

In the definitions of the general environment quality and the specific environment quality, clearly corresponding elements can be recognized, viz. the quality as related to i) people, ii) the use functions, and iii) the nature significance. For each of these three social objectives, it is examined in this section in how far effects on it from pesticides can be traced via the side effects on terrestrial invertebrates and aquatic fauna.

In selecting the species deemed relevant for this, a number of other aspects must also be watched, such as:
- exposure (in which compartment does the species occur; do not look at insects only, but also at earthworms, snails, spiders);
- the recovery time and lifespan (the slower the recovery, the better an effect can be demonstrated, and the longer the lifespan, the longer individual exposure is);
- the farming plan (i.e. which crops, fertilizers, pesticides, etc. (cf. van Straalen et al., 1985);
- feeding habits and behaviour.

In the following, we first deal with general environment quality (3.3.1) and then the requirements to be set per social objective, with regard to specific environment quality (3.3.2).

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[4] In the amendments to the adjusted Act on General Stipulations for Environment Hygiene, now in preparation, a chapter with environment quality requirements will be included; it is expected that the concepts afore mentioned will then be explicitly defined with regard to each other.

3.3.1 Indicators of general environment quality

As stated above, regarding general environment quality a distinction is made between basal requirements on behalf of specific functions and general properties on behalf of the multi-functionality set for processes and the species involved in them. As an example of basal requirements on behalf of specific functions we deal in short with the function as a source of drinking-water and the part which the aquatic fauna, c.q. the side effects on this group of organisms, can play in this. Subsequently we deal more widely with the general requirements for a good and healthy ecological functioning.

For the supply of drinking water, importance must be attached to the conditions of existence for fish (think e.g. of the rainbow trout Salmo gairdneri as a guardian of quality in use by the water managers) as an indicator of the water quality. On the other hand, however, invertebrates are often much more sensitive than fish. For the isopods applies that they, as decomposers, could be especially indicative of the quality of the water bottom. For the aquatic environment, three species (algae, water fleas and fish species) are already in use as test organisms. Whether the potential of other species (e.g. water mites and isopods; see: Chapter 5) for the use function drinking water can be realized needs to be further examined. Further, according to Leeuwangh (oral comm.), a periphyton test can be used instead of an algae test. Further, little is known as yet about the usefulness of a Artemia (crustacean) test as an indicator of too high a concentration of chemical agents.

General requirements for ecological functioning

Important processes in ecosystems, which can act as parameters for proper and sound ecological functioning, are the substance cycles such as those of carbon, nitrogen, phosphor and sulphur. To a great extent these occur in the soil. Especially in the carbon and nitrogen cycles, invertebrates play an important part; also, the macro-nutrients are involved here. These two cycles can be sub-divided into a number of part-processes, with which also the important soil processes are enumerated (cf. de Kruif et al., 1984):

carbon cycle	nitrogen cycle
- assimilation	- biological NO_2-binding
- consumption	- NO_3-uptake
- dissimilation	- NH_4-uptake
- fragmentation	- nitrification
- decomposition	- mineralisation
	- denitrification
	- precipitation and leaching

Although for soil invertebrates e.g. it cannot always be precisely indicated what function they perform in these processes, a number of indications can be given. Invertebrates from the terrestrial environment play either an important part as "doers of much work", the saprophages (e.g. in fragmenting and consuming organic soil material), or a part as a regulator (e.g. as a predator or parasite of other invertebrates; see e.g. also: van Straalen & Everts, 1989). It remains difficult though to indicate how great, under certain circumstances, the quantitative significance is of a certain species in a specific process. In addition,

depending on how the food supply changes, the functional role of an organism can change (Eijsackers & van de Bund, 1980). In the aquatic environment too, these two functions can be distinguished, viz. important consumer at a low trophic level (grazing, consuming phyto- and/or zoöplankton) or, at the higher trophic levels, predator. Further, of course invertebrates themselves constitute an important source of food for higher organisms; this is entered into further when dealing with the nature significance (3.3.2.3).

The ultimate choice of species and other biological parameters is strongly determined by the indicative and prediction value of test data obtained as related to the processes and patterns in and among ecosystems. Thus, at the RIVM, among various process characteristics, specifically substance cycles, sum (total) processes, and species parameters (growth and reproduction i.a.) are considered promising, and among pattern characteristics species abundance, life strategy, distribution of saprophages, herbivores and carnivores over the ecosystem and number fluctuations (Cleef, 1988).

Many soil-microbiological processes can be considered sum-processes, such as respiration and enzyme activities e.g. Eijsackers (1986) lists a number of advantages connected with the use of such sum parameters:
- reflection of heterogeneity of natural populations
- greater representativity of the natural situation than standard tests
- incorporation of the effect of biological availability.

The following processes specifically are of importance and usable:
- nitrification
- respiration (to be measured by CO_2- and/or O_2-content?)
- enzyme activities.

According to Van Straalen (oral comm.) it is not yet possible at the moment to select a few species of soil invertebrates for registration research. For this reason it is necessary to set up some broad research in which e.g. 20 species are examined for their usefulness. From those then, e.g. species can be selected from three different functional groups: i) microbivore species such as orbatid mites, with a long generation time, nematodes, specifically bacterivore species, and springtails), ii) saprophagous species (such as earthworms, isopods, land snails, e.g. Helix aspergus and Arion rufus - easy to raise - and myriopods) and iii) predators (such as ground spiders, rove beetles and ground beetles). These groups should consequently be represented in the tests.

A comparable reasoning can be applied to the aquatic environment, where besides the algae, water fleas and fish species, more attention would have to be paid to detritus-eaters (e.g. an isopod) and predators (e.g. a water mite (see also: Chapter 5).

3.3.2 Indicators of specific environment quality

In this section we trace which specific requirements are posed by the various social objectives on the specific environment quality when considering the use of pesticides in agriculture. The emphasis lies on tracing terrestrial invertebrates and aquatic fauna which can indicate this specific environment quality.

3.3.2.1 National health

The acute and chronic side effects of the use of pesticides on people are mainly of interest for relatively much exposed population groups, such as spray contractors, farmers and their relatives (incl. the children; cf. van den Berg, 1989) and other employees in the agrarian sector. Chronic exposure can also occur in residential areas close to intensive farming areas.

In general, invertebrates supply few directly usable data for tracing these side effects on the health of people. In this connection, toxicity tests with vertebrates (esp. mammals) are more suitable. Consequently, using invertebrates for this purpose is further left out of consideration. It will become evident in the following, however, that invertebrates can be used indirectly for the side effect on national health, e.g. in the usage function of production of drinking-water.[5]

3.3.2.2 Usage functions

In this study five social sectors were distinguished as being of interest to be looked at more closely, viz. agriculture, production of drinking-water, forestry, fisheries and recreation. More "urban" functions (related to housing and working) and infrastructure functions are not considered here, as it is obvious that this type of function is not directly affected by side effects of pesticides. These five are discussed below.

Agriculture

In this usage function, in relation to invertebrates there is often a distinction made between harmful, useful and indifferent species; as this can be used here too, we shall deal with it in short.

Harmful organisms
These are control targets (= target organisms) especially when the economic damage is deemed unacceptable. The fact whether a species is a target organism or a non-target organism can depend, besides on the fact from which angle one looks at it (nature conservation or agriculture e.g.) also on the life stage of a species: thus, caterpillars can be harmful to agriculture, while the maintenance of the imago of the same species (= the butterfly) can be a policy objective from the point of view of nature conservation.[6]

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[5] One possibility of making use of (the behaviour of) invertebrates is perhaps the tracing of possible residues on a crop, or determining the suitability of surface water as sprinkling water (in people's gardens e.g.); that the occurrence of too high residues of cholinesterase inhibitors is not just imaginary is evident e.g. from CCRX (1985).

[6] To solve this problem, in analogy with the Hunting Fund, a fund could be created to compensate for possible damage caused.

Useful organisms

These can be subdivided into 3 groups, viz.:

a. Species which can make a realistic economic contribution to crop protection; in this, especially the predators and parasites of harmful organisms are important. In current open-air agriculture and market gardening, the contribution by naturally occurring predators and parasites is not considered relevant. The many workings of the soil and rotating of crops are the cause of this (oral comm. Kooystra, Oomen & Jobsen, Plant Protection Service). Only in greenhouses and in fruitgrowing, in integrated control, are these organisms taken into account as much as possible and is their presence promoted, frequently by consciously intervening.

b. Bees, whose economic importance is now mainly in connection with pollinating a number of crops and thus for agricultural production. Annually some 2500 swarms are rented out for pollinating crops under glass (strawberries, gherkins, melons, etc.) and for outside crops, such as apple, cherry, pear and blackberry (written comm. van Heemert). Van der Vaart (1987), in making a rough estimate, arrives at some 460 million guilders annually added to agrarian production through pollination by bees. Up to now the presence of bees is taken into account only if the crop concerned - or the weeds present - are being visited by them. Experts estimate the loss of bee swarms in The Netherlands as a result of the use of pesticides as of the order of a few tenths of percents or a few percents (oral comm. spokeswoman VBBN in: van der Vaart, 1987) of the total number of bee swarms.

c. Soil organisms making a realistic economic contribution to soil processes important for agriculture, e.g. decomposition, structuring and aeration. (The importance of these species in general environmental quality was also mentioned in 3.3.1). The contribution by soil organisms to such processes is hardly taken into account within current operating systems in agriculture and market gardening. Only if zero tillage is used, is the role played by soil organisms deemed important (earthworms e.g.). In fruit growing and in raising cattle, however, earthworms are considered useful organisms. Van der Vaart (l.c.) estimates yield loss resulting from the loss of earthworms in growing fruit at several percents.

Indifferent species

The species remaining, after determining the harmful and useful ones, can be deemed the indifferent ones, from the point of view of agriculture.

It is evident from the foregoing that in current agriculture and market gardening on open soil there are only a few invertebrates which are considered useful and consequently should be protected from an agricultural point of view.[7]

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[7] The Useful Animals Act (1914) shows that early in this century these problems were regarded differently and even vertebrates were considered useful for agriculture. The purpose of the Act was to protect wild animals. When the interests of agriculture, market gardening or forestry were harmed by catching or killing useful animals, measures could be taken by General Administrative Measure to protect them. This Act was used for frogs, moles and hedgehogs. These animals were and are considered important in controlling pests. The Memo of Explanation also mentioned the shrew and all bats, as useful at all times in agriculture

This applies to the predators and parasites (not or hardly further differentiated for the time being though), bees and earthworms. It is therefore recommended to elaborate the usefulness aspect further, and give it a more explicit and broader content.

Tests
The IOBC (1988) has issued regulations for a number of tests with predators as useful organisms (see: Annex 3.1). This mainly concerns predatory mites (four species) and ichneumon wasps (five species) but also a predatory bug (Hemiptera), lace-wing fly, ground beetle, rove beetle and ladybird. In a further selection one can consider e.g. the ecology of these species (monophage - polyphage e.g.), biotope selection and distribution. Much research is being done at the moment, especially on the possibilities for the field tests.

When considering the ecology, we see the following (see: IOBC, 1988). With these useful organisms, it concerns, as stated, always predators; a number of these are polyphagous. In connection with a more generally valid interpretation it appears desirable to give preference to the less specialized species. Further, the species must occur in The Netherlands in the open field. One could think here of Bembidion pampros (a ground beetle often occurring in plowed land, polyphagous and wintering in field margins), Amblyseius finlandicus (a predatory mite often found in orchards) and Encarsia formosa (an ichneumon wasp in greenhouses).

Effects on future or other usage functions
Apart from the direct significance of invertebrates for the agricultural function, i.e. the actual present usage function, a part is also played by the fact that usage functions must be able to succeed one another in time (e.g. a subsequent crop), or that functions, which spatially are located one beside the other, must not preclude each other (of importance e.g. for the crop in the adjoining fields). We shall deal with this in short below.

For another, future agricultural function (changing to another crop) use can be made of the "Persistence Memorandum" (Lower House, 1985-6, 19 200, chapter XIV, 59: 1-3) and the memorandum "Environment Criteria" (see: 3.1) which assume a recovery time of 12 months maximum; by using this period, accumulation in the course of the years is prevented. This means that especially the continued existence of the species with a long generation time is important in measuring the effects of this usage function: attention must be concentrated on K-strategists. One can think here of overwintering invertebrates, such as isopods, predatory mites and certain butterfly species and overwintering females of e.g. spiders.

Concerning the side effects on the agricultural function of adjoining fields and areas one would naturally consider the range of the test organism. Especially flying species are of importance in this regard (bees, ichneumon wasps and possibly also butterflies and ladybirds). Ground beetles too can cover considerable distances (up to hundreds of meters) and thus are suitable as a species for tracking side effects of

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or forestry. There are now no Gen. Admin. Measures pursuant to this Act in force. At present the role of the Act has been partly taken over by the Nature Conservation Act (Lambers, 1985).

interventions occurring at some distance. Perhaps wolf spiders can also be used; they are known to range widely.

The surface water too is among the adjacent areas. In the agrarian area, specifically in ditches, watercourses and canals, it can be allotted the following agricultural functions (see also: CUWVO, 1988):
- maintaining an optimum water economy, incl. use for irrigation and as sprinkler water
- drinking-water for livestock
- acting as fences and separating fields and lots
- transport of harvest, livestock, implements, etc.

In the first function, besides a quantitative aspect, quality too plays an important part (see also: Intermezzo, page 26). Thus, one can imagine the use of surface or groundwater leading to damage to a crop and/or undesired residues on that crop. The presence of the three well-known water organisms (algae, water flea and fish species) can provide an indication of water quality in this connection too. For the second function as well (drinking-water for cattle) one can imagine that side effects occur through the use of pesticides; it is not suitable here though to use the aquatic invertebrate fauna as an indicator for these side effects, because other (toxicity) data, specifically of vertebrates, are more suitable for this.

Drinking-water supply

The usefulness of surface and groundwater can be endangered if the concentration of pesticides becomes so high that it cannot be made into drinking water. Thus this usage function is affected, specifically when obtaining water from under agricultural areas. Consequently, it is being found in The Netherlands to an increasing extent that as a result of pesticides the obtaining of groundwater in the agrarian area presents problems. In monitoring the quality of surface water, for quite some time aquatic organisms have been used in the form of bio-assays with water fleas and fish. Research by Jenner (KEMA, Arnhem) has shown, however, that various species of mussels too are extremely sensitive to substances such as tributyl tin oxide (under 5 ppb); this kind of substances is therefore used to counter encrustation of ships with these mussels i.a. (= repellant effect) (Linsen, 1988).

The use of invertebrates to determine water quality is aimed mainly at surface water. It has not been excluded that effects on the quality of the groundwater can also be monitored by making use of invertebrates.

Forestry

In forestry in The Netherlands little pesticide is used. This type of application does not have negative (economic) consequences for this usage. It is probable though that pesticides used in the agrarian area will reach forests by drifting, and possibly affect their vitality. It is worth mentioning in this connection that in rain water, in Germany as well as in The Netherlands, pesticides have been observed in demonstrable quantities (cf. van Haasteren et al.; 1987; CCRX, 1988). Specifically herbicides are important here, for forest vitality. However, indirect

effects of fungicides as well, via mycorrhiza, can easily be imagined. It is not considered excluded that in areas with intensive livestock keeping (Eastern North Brabant [Province] e.g.) it is a matter of synergism or at least additivity in the occurrence of side effects as a result of overmanuring and acidification on the one hand, and the side effects of the use of herbicides on the corn on the other. It is therefore important, in determining the effects on forest vitality, to examine the phytotoxicity for higher plants, also taking into account the correlation with the effects on the mycorrhiza.

Invertebrates appear to be less suitable in tracking side effects in forestry, as expected side effects are concentrated on mycorrhiza (by fungicides) and high plants (by herbicides). It is recommended, however, that a fungus test and a phytotoxicity test be developed for these biota. This could be done in connection with the already existing requirement to supply data about the soundness of an agent, in which, when it concerns herbicides, the effects on crops are also considered. Such a phytotoxicity test can also be used more in general within the framework of the registration procedure; at the moment only an algae test is required on the effects on plants.

Fisheries

From the point of view of commercial fisheries, a number of economically important fish species can be listed for fresh water; the most important ones are eels and to a lesser extent pike and pike perch. For sport fishing, in general an abundant supply and varied species composition are very important. In this connection, the following species are important e.g.: eels Anquilla anquilla, pike Esox lucius, roach Rutilus rutilus, rudd Scardinius erythrophthalmus, chub Leuciscus cephalus, bream Abramis brama, carp Cyprinus carpio, tench Tinca tinca, crusian carp Carassius carassius, perch Perca fluviatilis and pike perch Stizostedion lucioperca.

The following has been derived from a literature search by the Organization to Improve Inland Fisheries (OVB), oriented toward the large rivers (de Iongh, 1982). It was found that no clear conclusions can be drawn about the effects of chemical water pollution, incl. pesticides, in the form of population changes in a number of general species. On the other hand, as a result of accidents, clear effects have become known. In addition, in various species found in Rhine water, abnormalities have been found in the form of blunted noses, abnormal skeletons, enlarged livers and decreased growth; tumors were found not to occur frequently. Other demonstrated side effects finally are increased susceptibility to predators and to diseases.

Regarding commercial fisheries we propose to use the eel as a test organism (occurs generally, numerous, and economically of relatively great importance too). Choosing the eel means that specifically the water bottom compartment will be examined. It makes sense therefore to further examine whether a test with pike or pike perch can be developed (predators, which are also of economic importance). For reason of being easy to handle, a test with the three-spined stickleback can also be thought of, and is perhaps easy to introduce. Something obvious for sports fishing is

to make use of a diversity standard related to the average number of species occurring in a certain type of water.

Recreation

No side effects are known of the use of pesticides in the agrarian area on the usage functions related to the recreation function. Side effects on the nature recreation areas (e.g. via butterflies, dragonflies and other attractive, easily observed species) or on the number of fish - important sports fishing - are discussed under fisheries function and nature significance. The occurrence of side effects can certainly be imagined though, viz, when protected species decline, or disappear because of the use of pesticides. We shall come back to these species too at nature significance.

To determine the effects on the recreation functions one can also use species diversity, for the following reason. Although the significance of this parameter, from the point of view of nature conservation, is not always unambiguous, a high diversity has direct significance for the recreation function. The measure of diversity namely is a reflection of the complexity of an ecosystem and is often directly related to variation and variety in an area and thus determines, to an important extent, the suitability of an area for the recreation function.

3.3.2.3 *Nature*

To protect nature, policy can globally follow two directions: area-oriented or species-oriented. With the former approach, special landscapes can be protected e.g.: via the Nature Protection Act, the Natural Beauty Act or the Forest Act, but via specific Acts as well, e.g. the Middle-Delfland Act (cf. Cuperus et al., 1988). Policy memoranda and structure plans also offer connections here, such as the Relationship Memorandum (Anonymous, 1975) and the Structure Plan Nature and Landscape Conservation (Anonymous, 1986a). Additionally, one can think of the maintaining, arranging and developing specific ecosystems in the form of nature areas, where the objective is i.a. the continued existence of characteristic populations (meadow birds or waterfowl e.g.).

The second possibility is the protection of explicitly mentioned species. At the moment, only four invertebrates (crayfish Astacus astacus, stag beetle Lucanus cervus, large copper Lycaena dispar and Roman snail Helix pomatia) have protected status under the Nature Protection Act. The same Act also protects certain aquatic (fresh water) vertebrates: bullhead Cottus gobio, schneider Alburnoides bipunctatus, wheaterfish Misgurnus fossilis, spined loach Cobitis taenia, stone loach Noemacheilus barbatulus, minnow Phoxinus phoxinus, bitterling Rhodeus sericeus amarus, wels Silurus glanis, lampern Lampetra fluviatilis, brook lamprey Salmo trutta fario, terrapin Emys orbicularis and all frogs, toads and salamanders. It is expected though that within a foreseeable time the species-oriented Chapter V of the Nature Protection Act will be integrated in a new Act: the Flora and Fauna Act. The draft bill of this latter Act was ready end 1987. In view of future developments, present research is based on this draft bill. It indicates, in more general terms than the Nature Protection Act, which species should be protected. Thus, in principle all

indigenous mammals, birds, amphibians, reptiles and fishes will acquire protected status, unless otherwise stated and with the exception of the fish species coming under the Fisheries legislation.

For terrestrial invertebrates and aquatic fauna the region-oriented approach was never worked out in detail, while so far the species-oriented approach was used to only a very limited extent: only a (very) small number of invertebrates species has protected status at the moment under the Nature Protection Act. Now, to somewhat further elaborate these two approaches we shall proceed as follows. We shall take the species-oriented approach, and work it out further in three directions: i) protected species, ii) condition-creating species, viz. conditions of existence for other species, and iii) process species. The latter group of species was already dealt with at general environment quality. The former two groups and the use of the species belonging to them in the frame work of this study shall be further dealt with below.

Protected species

In the draft species list included in the draft version of the new Flora and Fauna Act (Anonymous, 1987b), certain insects, shellfish and mollusks are listed as species to be protected (see: Annex 3.2.; for a number of these species information about occurrence and status is lacking though).

For the terrestrial invertebrates it is indicated in which biotope type the accent of their occurrence lies (see: Annex 3.2a). It is evident that - in so far as data are available and complete at least - this is not, or hardly, in arable land. A number of species do occur in pasture though. When the occurrence of side effects is to be traced, one will have to look mainly for effects at a distance, i.e. in biotopes others than the arable land itself, in nature areas e.g. When looking at occurrence in the agrarian area in general, eight terrestrial invertebrates remain. With the exception of the brown skipper Erynnis tages and Silver-studded Blue Plebejus argus, they are all rare to extremely rare.

With respect to the aquatic fauna, the same reasoning can be followed (see: Annex 3.2b). Again we find that available information is limited; but here too a number of species have their main occurrence in the agrarian area. Of the aquatic species to be protected, 13 invertebrates and 7 fish species occur in the agrarian area, though it should be noted that most occur only locally in general. With the exception of the Beautiful Demoiselle Calopteryx virgo (= fairly rare), the invertebrates are rare to very rare; for the fish species the same thing applies for brook lamprey Lampreta planeri, minnow Phoxinus phoxinus, bitterling Rhodeus sericeus amarus and wels Silurus granis.

Particularly for above-mentioned rare species with their main occurrence in the agrarian area, it is important to take into account the possible toxicity of and the exposure to pesticides.[8]

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[8] It is further evident from the draft bill that especially vertebrates and only very striking and/or extremely rare invertebrates get attention in policy.

Condition-creating species
These are taken to be those species which are necessary for the continued existence of other species. In most cases it concerns important prey species of the protected species concerned.[9] It turns out, however, that insufficient information is available to indicate for most of the protected terrestrial invertebrates and aquatic fauna which species specifically serve as food.

mammals	breeding birds	amphibians and reptiles
Erinaceus europaeus	Tachybaptus ruficollis	Salamandra salamandra
Sorex araneus/ coronatus	Botaurus stellaris	Triturus alpestris
Sorex minutus	*Ixobruchus minutus*	Triturus cristatus
Meomys fodieus	Nycticorax nycticorax	Triturus helvetius
Crocidura leucodon	Ardea purpurea	Triturus vulgaris
Talpa europaea	Perdix perdix	Alytes obstetricans
Chiroptera	Phasiantes colchicus	Bombina variegata
Meles meles	Philomachus pugnax	Pelobatex fuscus
Lutra lutra	Limosa limosagodwit	Bufo bufo
	Chlidonias niger	Hyla arborea
	Cuculus canorus	Rana lessonae
	Alcedo atthis	Rana ridibunda
	Alauda arvensis	Rana cf. esculenta
	Riparia riparia	Rana arvalis
	Delichon urbica	Rana temporaria
	Saxicola torquata	Natrix natrix
	Cetti cetti	
	Cisticola juncidis	
	Locustella luscinioides	
	Acrocephalus schoenobaenus	
	Acrocephalus arundinaceus	
	Panurus biarmicus	
	Lanius collurio	
	Passer montanus	
	Miliaria calandra	

Table 3.2 Vertebrates with main occurrence in agrarian area and with a food packet mainly of terrestrial invertebrates and/or aquatic fauna; to all bird species listed applies that they show a declining population trend.

The vertebrates should also be involved in such an approach, however, as invertebrates form a very important source of food for many vertebrates. De Snoo & Canters (1988) give a selection of mammals with their main distribution in the agrarian and/or aquatic environment; for comparable data about the summer (brooder) birds, use was made of data from Bird

[9] It concerns here an ecological side effect in the form of a change in the availability of food; ecological side effects in the form of a change in habitat through effects on invertebrates are hardly expected, as such an effect is hard to imagine.

Protection Society (written comm.). De Snoo & Canters also indicate what the food packets of these species look like. From this can be derived which invertebrates are condition-creating for mammals and summer birds. These data have been summarized in Annex 3.2. With respect to mammals this means that in the agrarian area terrestrial invertebrates are condition-creating for all indigenous insect-eaters, incl. the bats, badger and otter. In many cases, these are rare and/or (very) threatened species (see also: Annex 3.2).

With respect to the birds, first a further selection was made, with as criterium a decreasing population trend. Subsequently, as for the mammals, the food packet was looked at. The species emerging from this selection are also shown in Table 3.2. Analogously, and making use of Bergmans & Zuiderwijk (1986), the amphibians and reptiles were also selected, for which the studied group of organisms is condition-creating (see: Table 3.2).

Finally, there is another separate group of condition-creating species to be distinguished. Besides the species playing an important part in the basal ecological processes, as described at general environment quality (see: 3.2), there are also more specific, "qualitative" processes to be distinguished, in which invertebrates play an important part (e.g. bees as pollinators). For proper ecological functioning, seen from the point of nature significance, this should be given separate attention. Flying insects too have so far been left out of consideration. This does not mean, however, that they are not susceptible to pesticides. In general, very little is known about this. In addition, many flying species spend part of their lives in the water or the soil, and then probably have a different exposure than in the adult phase.

3.4 "Damage to an unacceptable extent"

Since so far the effects on social objectives have only been discussed in qualitative terms, it is still necessary to find a quantitative connection. The proposal is to examine whether it is possible to arrive at the characteristically occurring numbers of certain animal groups or species i.e. the numbers present in a certain type of biotope under "normal" conditions. This could be based on a series running from drifting sand and agricultural soil on the one hand, via heather and grasslands to rough and bush and ultimately forest on the other hand. (Note: both former types are well comparable to each other, according to oral comm. from van de Bund).

The principle used here is shown in Fig. 3.2. Under average conditions, a certain species group is present in great numbers in e.g. arable land. With the use of a pesticide, this group is found to decrease greatly, even to below a certain minimum value, the norm in the form of a lower limit. For a species group b, the reverse was also shown in Fig. 3.2, i.e. the occurrence of numbers above a certain maximum value.

The advantage of this quantitative norm setting is that not only the presence or absence of a species is considered, but the numbers too, related to a minimum as well as a maximum. Perhaps it is also possible to develop in this way standard indices for the relationship/ratio among the numbers of certain functional groups, such as herbivore: predatory.

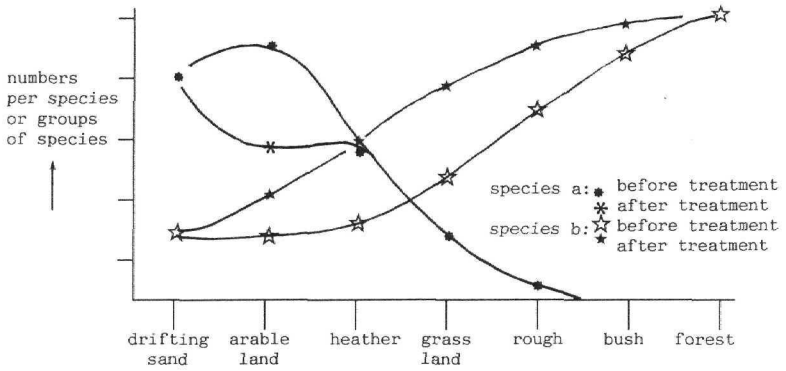


Figure 3.2 Occurrence of two species (groups) a and b in a series of biotope types from drifting sand to forest, to be used as reference or background values; through use of a pesticide, group a decreases to values lower than the "normal" spread, and group b gets higher values.

Regarding the size of the area affected - another quantitative aspect- applies that the upper limit corresponds to all of The Netherlands, while in principle the lower limit is formed by the area in which a population of a certain species can still barely occur. To make this more concrete: we are thinking of a level of scale between that of a parcel and of a polder. This corresponds approximately to land arrangement blocks. According to Den Hollander (oral comm.) this also fits in well with the Main Ecological Structure as recently published in the Nature Policy Plan (LNV, 1989). In practice this could be based at the level of crops, i.e. regions characterized by a certain crop or characteristic combination of crops. At this level, a "significant decline in the population of an affected species" is deemed undesirable (compare "significant impact assessment"); where significant means: the decline of the species after application of a chemical has not yet been rectified after one year.

3.5 Tests

Now that in 3.3 a reconnoitering has taken place concerning relevant species (groups) and other biotic parameters, in this section we indicate how these parameters can be made operational through tests. The use of data obtained via (toxicity) tests, within the framework of the present registration procedure, was already dealt with in 2.2. In the following we indicate what (other) types of test there are and how they can be used. The tests can be described and classified as follows:

1. To be done in the lab

a. Toxicology research specifically in the lab, i.e. under completely controlled circumstances. Intended to determine toxicity for a certain species. This often involves so-called single species tests, but multi-species tests and micro-ecosystems also fall under this category; one can think here of algae indices of mixed cultures (oral comm.

Ringelberg) and springtails - predatory mites or fungi - isopods[10] (oral comm. van Straalen); it remains very difficult though to deduce from single species tests anything more than the sensitivity of the species, but a single species test can possibly be used if a species is a good indicator for a certain function. Examples of this kind of test are: acute toxicity tests with an algae, water flea or fish species, but also (semi) chronic toxicity research with a species, with attention paid to reproduction, teratogenity and behavioral abnormalities.

b. Bio-assay in the form of: bring medium from the field to the lab, and look to the reaction of standard species (e.g. water flea) in that medium. It is also possible here to use species simultaneously, i.e. in the form of a multi-species test. Water samples as well as soil samples can be used for this. Examples are: the test done in this research with water fleas, water mites and isopod (see: Chapter 4); also tests with earthworms kept in soil samples belong to this form of test, as well as rainbow trout for monitoring drinking water quality.

2. To be done in the field

a. Bio-assay in the form of: placing standard species "caged" in the field and trace behaviour/mortality; here as well, a multi-species-test is not in advance. An example of this form of testing is the placing of sticklebacks inside a net in a ditch.[11]

b. Taking inventory of relevant species (groups), i.e. species, the presence or absence of which indicates that pesticides have caused harmful side effects. In principle, all inventories of certain groups of organisms can be counted as belonging to this type of test; several examples are given in chapters 4 and 5.

An important difference between the tests is, whether the data are gathered (largely) in the lab (1) or in the field (2). The difference between the two forms of bio-assay (1b and 2a) is therefore the place where the research is being done, c.q. the extent to which it is possible to control the conditions of the experiment. In principle, the test organisms used can be the same ones. Research on the occurrence of species (groups) (2b) occurs entirely in the field and, apart from setting traps etc., is not accompanied by extra interventions.

For actual application in the registration procedure, the bio-assay requires the combinations of factors as shown in Table 3.3. The difference between standard medium and sample from the field (in principle always

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[10] In this, the activities of isopods and the microbial activity with and without fungicide are examined; the activity of the fungus is looked at (CO_2-production) and the activity of the isopod (CO_2-production, eating activity and breakdown of organic material); in this way, the effect of the chemical on the interaction fungus-isopod can be traced (oral comm. van Straalen).

[11] A classic example of this form of tests is the canary which formerly went down with the miners to warn of the presence of mine gas: with even low concentrations of this gas, the canary died.

water or soil) is the presence or not of other contaminants, specifically pesticides. In general, clean soil will either have to be prepared artificially from clean soil constituents, or will be available only under special circumstances, such as the conversion from pasture to arable land or at farms which do not use pesticides.

Sample/treated	Yes	No
Standard medium	I	II
Sample from field	III	IV

Table 3.3 Combination of factors necessary for optimal carrying-out of bio-assays.

With respect to the usefulness of the species selected for tests, a number of practical criteria can be formulated (cf. van Eck & Hueck-van der Plas, 1984):

- availability of standard methods: handling of the animal must be reasonably possible (possible to raise it, short generation time and/or long lifespan; especially important is the juvenile phase and the reproduction phase).
- possibility to localize and recognize the organisms in the field (the so-called "prominent" species, oral comm. van de Bund)
- possibility to extrapolate the results to other species: something must be known about the sensitivity of the species to be expected, in relation to other species and groups of species
- general applicability in relation to the substances to be tested
- simplicity and low cost.

3.6 Conclusions

Starting from the premise that up to now no evaluation framework was available for evaluating side effects on invertebrates, we examine in this chapter whether it is possible to create such a framework. The general and specific environment quality was chosen as point of departure for this. It was found that possibly occurring side effects on terrestrial invertebrates and aquatic fauna can be used to trace effects on multifunctionality and nature significance. In evaluating these effects and side effects - in addition to scale level and time span - in relation to the general environment quality one can specifically pay attention to proper ecological functioning of ecosystems, i.e. the course of a limited number of basal processes. In relation to the specific environment quality we find that demands are made mainly by the agricultural function itself and from the point of view of nature significance.

The following species came to the fore more explicitly as indicators of effects on the usage functions and nature significance; the side effects of pesticides occurring in these species merit more and explicit attention on behalf of policy, in view of effects on environment quality:

General environment quality[12]:

* terrestrial environment:
  - microbivore species, such as orbatid mites, nematodes and springtails
  - saprophagous species, such as earthworms, isopods, land snails and myriopods
  - predators such as ground spiders, rove beetles and ground beetles
* aquatic environment:
  - detritus-eaters, such as isopods
  - predators within the mesofauna, such as water mites

Specific environment quality:

* agriculture:
  - useful organisms, such as bees and earthworms
  - explicit extension with other species considered useful in agriculture, viz. predators and parasites, such as ichneumon wasps and predatory mites, but other species as well qualify for this
  - more attention for species with a long generation time (in connection with long-term effects in relation with a long recovery time): overwintering isopods, predatory mites, butterflies and spiders
  - more attention for species with a wide range (in connection with effects occurring at a certain distance); flying species such as ichneumon wasps and ladybirds, but also certain beetle species and probably wolf spiders
* drinking-water:
  - freshwater mussels?
* forestry:
  - phytotoxicity for higher plants & fungi
* commercial fisheries
  - eels and/or pike and pickerel
  - three-spined stickleback
* sports fishing
  - diversity standard related to average number of fish species per type of water
* recreation
  - diversity standard related to e.g. average number of prominent species (see also: sport fishing and nature)
* nature
  protected species:
  - terrestrial invertebrates with main occurrence in agrarian area, specifically the rare to extremely rare species
  - aquatic fauna with main distribution in agrarian area, specifically the rare to extremely rare species
* condition-creating species:
  - more attention to condition-creating species; also of importance for vertebrates with their main distribution in agrarian area: insectivorous mammals (six species + all bat species) + badger and otter, approximately 25 summer brooder bird species with a

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[12] The use of other biotic parameters is not excluded; one can think of sum (total) parameters such as nitrification (already included in the registration procedure), respiration and enzyme activities.

downward population trend[13] and practically all indigenous amphibians and reptiles (17 species)

- more attention for flying insects, e.g. of importance for pollination.

[13] In a research into the possible relation between the use of a pesticide and this downward population trend, some agrarian species with a stable or upward trend should also be looked at.

4. TERRESTRIAL INVERTEBRATES

In this chapter we give an idea of the nature and extent of side effects of pesticides on terrestrial invertebrates. The data known to us now about side effects in the field have been gathered by animal group in 4.1. The following information was used:

- Incidents with pesticides in the field, insofar as mentioned by the experts interviewed or described in literature; we always attempted to trace which chemicals and which species were involved in these incidents.
- Opinions of experts about (groups of) chemicals which often cause specific side effects, incl. the circumstances under which these side effects occur (in which crops e.g.).
- From overview articles and handbooks an impression was obtained about the nature and extent of side effects in the field and the toxicity of the separate pesticides.

Supplementarily, we ourselves collected field data on a limited scale and in an orienting manner. This is reported on in 4.2. At the end of this chapter, some general conclusions are generated (4.3).

4.1 Incidents, opinions of experts and literature

Below we discuss the side effects of pesticides for some animal groups. We start with those we know most about, also in view of registration policy: earthworms (4.1.1), bees (4.1.2), and useful organisms such as predators and parasites (4.1.3). Besides, on a limited scale and insofar as material was supplied by those interviewed, some other groups are dealt with: butterflies (4.1.4), mesofauna, viz. springtails and mites (4.1.5) and snails (4.1.6). As far as possible, we deal with the side effects of fungicides, insecticides, herbicides and soil disinfectants. For the background of the toxicity data used in this chapter as well as the classification, we refer to Annex 4.1. In 4.1.7 the data obtained are discussed, also dealing with the seriousness of side effects of pesticides in relation to the (side) effects of other interventions.

4.1.1 Earthworms

The literature about the side effects of pesticides on earthworms was summarized by Ma (1982) and mainly concerns data under conditions in practice. The overview is given here, compressed and supplemented with new literature and remarks by experts. Although differences in sensitivity exist among earthworm species, the following deals with the group "earthworms". Insofar as could be traced, it concerned mainly data about the species Lumbricus terrestris and Eisenia foetida.

Fungicides

Annex 4.1 shows that few data exist about toxicity of fungicides for earthworms. It is clear though that benzamidazols are toxic to very toxic. Other fungicides appear less toxic. Field data too show that in practice benzamidazols are very toxic for earthworms; in grasslands and orchards drastic reductions in the earthworm populations were observed after spraying with benomyl and thiophanate-methyl. Leaf litter was no longer completely decomposed. In orchards with a program of spraying 7x

with 0.28 kg of benomyl/hectare, after 2 years all earthworm species have decreased in number. Lumbricus terrestris and Allobophora chlorotica had disappeared completely. Also with 0.28 kg of benomyl/hectare and 13x spraying in 1 year all earthworm species decrease in number. After 2 years, with the exception of L. terrestris, the populations were again at the normal level. It was expected that L. terrestris would recover after 3 years (Wright, 1977). In grassland, decrease in earthworm populations was observed after 3 years' application of 5 kg/hectare of benomyl. Within a year, 60-80% of the earthworm population recovered, except L. terrestris, this species did not recover, or very slowly. L. festivus, however, increased in number during this (Edwards & Brown, 1982). In addition it was found that benomyl is also used to kill earthworms on purpose, e.g. in the proximity of airports (Tomlin, 1981).

Van Gestel and Van Straalen (oral comm.) also indicate that earthworms are most sensitive to benzamidazols such as carbendazim and benomyl. Specifically in fruit-growing, where these agents are often used, they have also led to incidents with earthworm mortality.

Insecticides

Carbamates, such as carbaryl, carbofuran and propoxur are strongly toxic to earthworms. Under conditions in practice too, when applying carbaryl on grassland (2.5 to 4.5 kg/hectare), population reductions of earthworms were observed. When applying 4.5 kg/hectare of carbofuran to grassland, practically complete elimination of the population occurs. Only after a year one can speak of recovery. (NB: in growing sugar beets, 10 kg/ha is used.) Among the carbamoyl oximes, aldicarb is strongly toxic. Methomyl is less toxic, possibly this is caused by a low rate of uptake of this agent. Agents such as carbaryl, aldicarb and carbofuran also cause sublethal side effects (skin abnormalities e.g.). Organic phosphor compounds vary in toxicity. At practice-dosages, they appear relatively little toxic for earthworms. Certain chlorinated hydrocarbons, such as endrin and endosulfan, are strongly toxic to earthworms. No data are known about many other groups of agents.

Herbicides

According to experts, herbicides are in general little toxic to earthworms. So far, however, few data have been found about this group. From the groups for which data are available, herbicides appear to be little to not toxic in general. At practice-dosages too, alkane carbonic acids (such as TCA and dalapon), triazines (simazine) and phenoxy acetic acids (such as 2,4-D and MCPA) turn out to be not harmful. Only at high dosages have toxic side effects been observed from TCA.

Indirect side effects of herbicides can also occur through changes in the amount of soil-covering vegetation and organic material. Thus, after killing a grass vegetation with paraquat, certain species can initially increase in number. Application of TCA, atrazine and monolinuron on grassland caused a decrease in the number of earthworms; however, only with TCA was this independent of the reduction of soil-covering vegetation.

Further, a low toxicity of herbicides for earthworms is an essential condition for systems applying so-called zero tillage or direct drilling (minimal working of the soil). With this, the crop is seeded in soil which has hardly been worked and treated only with herbicides, in which

proper functioning of the earthworm population is necessary, especially the deep-digging species such as L. terrestris and Allobophora longa.

Soil disinfectants
Fumigants (= liquid chemicals; distribution in vapour form) are extremely toxic for earthworms. Examples are D-D, metam-natrium and methyl bromide. At practice-dosages too, these agents are very harmful. Relatively less toxic than the fumigants are the nematicides used in granular form, such as dazomet, aldicarb, methomyl and thianazine. Seen in the absolute, however, there are very toxic agents among these too (aldicarb e.g.).

Molluscicides
Very little is known about the effects of molluscicides on earthworms. At practice-dosages, methiocarb, used as a spray powder, is very toxic for earthworms. In granular form it is not very toxic though.

4.1.2 Bees

Fungicides
In general fungicides are little to not toxic for bees. The only exception are the organic phosphor compounds which are moderately toxic. Incidents with fungicides are not known.

Insecticides
Practically all insecticides are toxic to very toxic for bees, as to be expected. Only the acyl ureum compounds, bacteria preparations and sulphides constitute an exception to this and are not toxic. Also, tin compounds and some chlorinated hydrocarbons are less toxic.

It is evident from incidents too, that mainly insecticides and specifically the organic phosphor compounds give occasion for bee mortality. From information supplied by AID (oral comm. Valent) the following picture has been obtained of incidents which occurred in The Netherlands in 1985, 1986 and 1987:

- In 1985 29 complaints by beekeepers about abnormal bee mortality were received at AID, where according to the beekeepers themselves, pesticides may have played a part.
- In 1986 64 complaints came in. Investigations showed that in 50 of these the mortality was actually caused by pesticides. It concerned the agents: dimethoate, omethoate, azinphos-methyl, parathion, permethrin, bromophos, metidathion and mevinphos. The mortality often originates through blowing over of pesticides by the wind. The increase in complaints in 1986 is mainly caused by some 30 reports in August by beekeepers from the (formerly) peat lands. The mortality occurred at that time in bees flying on flowering weeds in potato fields. In these fields, at that time, plant lice were being controlled with agents permitted for that purpose: primicarb, dimethoate, parathion, thiometon and phosphamidon. In connection with this incident, legal use has now been changed so spraying of flowering weeds is no longer allowed (oral comm. Oomen).
- In 1986 near Bavel, heavy mortality occurred among bees, when 125 swarms died. A large-scale investigation was held by AID on this occasion. A total of 36 cholinesterase-inhibiting agents was investigated; probably none of these was the cause of the bee mortality, although aldicarb was found (oral comm. Valent). This concerned only

a very small quantity though and it is not probable that the mass death was caused by this (oral comm. Oomen).

- In 1987 the total of complaints was 26. In 13 cases the cause was poisoning by pesticides. The active substances causing death then were: paraquat, propoxur, azinphos-methyl, dimethoate, aldicarb, mevinphos, diazinon and bromophos-ethyl (written comm. Valent). Also, MCPP (on grassland: spray-killing the chickweed Stellaria media) and diquat (in peas: spray-killing the foliage) were mentioned.

According to Mrs. Vos of the Association for the Promotion of Beekeeping in The Netherlands (VBBN), in 1986 and 1987 near Nieuwvliet in the Province of Zeeland bee mortalities occurred with bromophos-ethyl and vinclozolin; at the time bees were gathering nectar in caraway seed. Vinclozolin, however, is not very toxic for bees, so that it is supposed the cause has to be sought in bromophos ethyl. It is possible that a wrong quantity was used (oral comm. Oomen).

Herbicides
No toxicity data were found for this group. Only in the Crop Protection Guide (van Rijn, 1987) at the dinitroalkylphenols and dinitroalkylphenylesters is there a warning for the danger of these agents to bees. As evident from the above overview of incidents, however, bee mortalities also occurred in The Netherlands in connection with the use of MCPP, paraquat and diquat.

For soil disinfectants no toxicity data for bees were found.

4.1.3 Predators and parasites

The toxicity data for the predators and parasites concern the following groups i.a.: hover-flies, ladybirds, ground beetles, ichneumon wasps, predatory mites and lace-wing flies (see further: Annex 4.1). In spite of the difference in sensitivity to be expected among these groups, we have attempted in the following to make statements, as much as possible, about the side effects on the total group of predators and parasites.

Fungicides
In general, fungicides are little to not toxic for predators and parasites. An exception to this is the organic phosphor compound pyrazophos: (very) toxic to moderately toxic. For certain species, carbendazim, propamocarb, thiram and mancozeb are also toxic.

Insecticides
For most predators and parasites, insecticides are (very) toxic to moderately toxic. Examples of strongly toxic agents are the carbamates, carbamoyl oximes, organic phosphor compounds, synthetic pyrethroids and pyrethrum. Within the carbamates group, pirimicarb is less toxic. Not toxic are acyl ureum compounds, bacteria preparations and tetradifon.

Herbicides
Few data were found about the toxicity of herbicides for predators and parasites. In general, the agents about which data are on hand appear to be not very to not toxic. Exceptions are, however, dinoseb and for certain species bromofenoxim and phenmedipham as well, and to a lesser extent the anilids.

Soil disinfectants
No data were found about the toxicity of soil disinfectants for this group of non-target organisms.

4.1.4 Butterflies

This group has declined greatly in the last decades in The Netherlands as well as elsewhere in Europe. Habitat loss is often mentioned as one of the most important causes. It is often stated that pesticides play a big part in this too, but evidence for this is hardly available (Heath et al., 1984). No toxicity data about butterflies were found. Neither are incidents with butterflies known in The Netherlands in relation to use of pesticides (oral comm. van der Made, LUW-Nature Management). The fact that the animals are prominent and probably are eaten very quickly may play a part here.

It can be noted here that from an agricultural point of view, butterfly caterpillars belong to the pest organisms and thus are purposely controlled (see: Chapter 3). An example of control with ecological side effects is that of the nettle Urtica dioica and field thistle Cirsium arvense. Many butterfly species depend specifically on these plant species for their food. Van der Made too (oral comm.) considers the near absence of butterflies from Dutch fields and pastures due to the practically complete absence of weeds from the fields as a result of the high growing pressures (lots of fertilizer), intensive working of the soil as well as weed control).

The butterfly inventory taken in The Netherlands in the 80's shows that 95% of the butterflies were found in areas where no pesticides were used (Geraedts, 1986). It should be noted here though that precisely areas where few species are expected (such as agricultural areas) were surveyed less intensively. The inventory also shows that a relationship exists between the occurrence of butterflies and the use of fertilizer: 87% of the butterflies are found in areas where no fertilizing is taking place.

Effects on butterflies can also be expected in places where the pesticides, as a result of drifting or incorrect application, end up outside the fields in ditches or on road shoulders e.g. Concerning the latter, butterflies still occur only on the wider shoulders: national and provincial highways (oral comm. van der Made).

The results of the Cereals and Gamebirds Research Project (in Britain) show that when keeping field margins free of pesticides, both the number of butterfly species as well as that of individuals increase greatly (from 17 to 21 and from 297 to 868 resp.) (Rands & Sotherton, 1986). According to the researchers, the increase is possibly caused by the greater food supply or more brood habitats for the imagines or by the greater numbers of host plants for the larvae.

4.1.5 Mesofauna: springtails and mites

Toxicity data about springtails and (saprophagous) mites were not systematically collected; we suffice with some statements by those interviewed (van de Bund, van Straalen and Everts). Soil disinfectants have probably

the most effect on these soil invertebrates. In addition, side effects from insecticides and fungicides are also possible. Because of their low toxicity, herbicides have probably few effects on these groups. Indirect side effects, as a result of dying off of the vegetation, can occur though. The soil fauna can increase in numbers because lots of organic material becomes available. In this way the toxic effect can be overruled. Such effects can also occur with the use of fungicides, when the fauna changes over to other sources of food (fungi dead, fauna starts eating bacteria or even plants: "man-made pests").

In general it is stated that saprophagous mites (opposed to predatory mites) and springtails are not that sensitive to pesticides. It was also found that both springtails as well as saprophagous mites are among the few groups of soil organisms frequently found in agricultural soils.

4.1.6 Snails

In RIN (1983) pesticides are indicated as "disastrous for land snails". A follow-up question to Butot (oral comm.) produced, however, that this statement is based on "general field knowledge". It is not known with which chemicals problems are occurring. Incidents are not known and effect-oriented research has not been done. Still, according to Butot, side effects are to be expected; there are hardly any snails in worked fields. A number of (phytophagous) species is purposely controlled. For snails eating detritus, food is largely absent in agriculture and market gardening, so that either the animals are not present or switch to other food. In many cases the animals then become phytophagous and thus a target for controlling. An example is Arion rufus. In stored items too, snails can develop into pests, e.g. Mylax species.

4.1.7 Discussion

In the preceding sections, the side effects of pesticides were discussed as though apart from other interventions in the fields. These other interventions can result from greatly varying activities, which latter can be placed in various social sectors. Below we deal in short with the seriousness of side effects of pesticides in relation to other interventions also specifically correlated with agriculture; other activities and interventions are too far outside the framework of this study.

In general it can be stated that the occurrence of a species is determined by (macro)factors such as climate, soil (physical/chemical properties and organic matter), crops and other vegetation, and geographical distribution of the species. In agriculture, at the micro-level, besides the use of pesticides, the following other agricultural interventions are of importance for the occurrence of species: plowing, seeding, manuring/fertilizing and allowing (the soil) to become less fertile, harvesting, mechanical weed control, irrigating and draining, and other forms of looking after crops c.q. disturbing the soil. These interventions can have consequences for the fauna, directly as well as indirectly (via an environmental factor as an intermediate step). In plowing, a direct effect can be that worms are killed, while for butterflies an indirect effect can occur because the host plant disappears.

In the case of most interventions it is not known how great the relative effects is in regard to the other interventions. It became evident from the interviews though that those interviewed thought the relative effect of working the soil (plowing) on invertebrates at least as great - and in many cases even greater - than that of pesticides (oral comm. van de Bund, van Gestel and Everts). This picture is confirmed by research abroad (i.a. Stinner et al., 1986). They found that the overall effect of working the soil and rotating crops on a number of non-target arthropods in grain fields was greater than that of pesticides. Still, a clear effect of pesticides was demonstrable.

If working of the soil is limited to a minimum (zero tillage), it is found that the soil fauna of such fields shows more and more similarities with that of grassland (oral comm. van de Bund). Because of greatly limited soil-working, macro-pores which originated through plant roots and earthworm activities, stay around longer. The consequently changed air/water ratio in the soil has as a result that the role of bacteria, protozoa and bacterivorous nematodes decreases, and the activity of fungi, fungivorous nematodes, fungivorous micro-arthropods (mainly springtails) and macro-arthropods increases. An additional phenomenon is that for reason of the minimal soil-working often more herbicides must be used to keep the weeds under control (oral comm. Brussaard). For the vegetation-bound invertebrates the effects of working the soil are probably less and only indirectly of importance.

In general it can be stated that habitat changes are considered more important than the toxic effects of pesticides, although in the agrarian environment pesticides too can contribute to this in a negative sense. Food changes are important only for a limited number of groups, as many soil invertebrates have a varied food packet and in case of a lack of food can switch over to other food sources (Eijsackers & van de Bund, 1980).

Finally, in regard to the relative seriousness of pesticides it should be noted that more and more often resistance occurs in pest organisms. In how far resistance also occurs in non-target organisms is not known. It is evident that in worked fields relatively few groups of invertebrates are present though. However, this can certainly also be interrelated with the earlier mentioned food and habitat factors or the other interventions. Possibly resistance will occur first in non-target organisms related to the pest organisms.

4.2 Field research

It is evident from 4.1 that for the Netherlands situation only a fragmentary picture exists of nature and extent of the side effects of pesticides on terrestrial invertebrates in the field. To supplement this picture, during 1988 field research was done in the Haarlemmermeerpolder in an orienting way. This was done by two biology students: Christa Groshart and Joost van Schijndel. A summary of their results follows below; for a detailed report, see: Groshart (1988) and van Schijndel (1988).

The research was aimed at the relation between various groups of soil invertebrates and various levels of pesticide use. The following objectives were formulated:

1. Obtaining an overview of the side effects of pesticides on terrestrial invertebrates in a number of crops and at various levels of pesticide use.
2. Developing a method for tracking side effects of pesticides by means of sampling invertebrates.

Three nationally important crops were studied: potatoes, sugar beets and wheat. The Haarlemmermeerpolder was chosen as the research area. It is relatively homogeneous with regard to type of soil, water economy and layout of fields, but also with regard to soil-working and farm management. The three crops studied are grown in the polder on a large scale. In addition, the agricultural advisors and a number of farmers were prepared to grant their co-operation.

In the Haarlemmermeerpolder mainly potatoes (23%), sugar beets (25%) and wheat (40%) are grown (Source: Agr. Advisory Service Haarlemmermeer). Crop rotation is practiced, with potatoes being grown on the same field only once every 4 years. On a smaller scale, grass seed, pulse, caraway and poppy seed are grown; dairy cattle are kept in a few places.

Before the field research, the possibility to involve data gathered by others in the research was examined. The information present in databanks from the European Invertebrate Survey turned out to be not detailed enough, however, with respect to distribution, so that it was not usable for transversal analysis by means of pesticides. In addition, agrarian areas were not or only rarely inventoried.

4.2.1 Method

It is assumed that the abundance of a certain group of invertebrates in a certain crop depends not only on the crop-specific use of pesticides (see: Annex 4.2) but that the crop (itself) too has a direct effect on the invertebrates occurring on a field (for reason of the differences in vegetation structure i.a.). Therefore, it does not make sense in this connection to only compare crops mutually. For this reason, a setup was selected in which, within each crop, differences in the level of pesticides use were looked for.

For each (kind of) crop, on the basis of interviews with agricultural advisors and farmers, a number of fields were selected concerning which it could be assumed that they differed in level of pesticide use. Five different farmers were prepared to co-operate. One of these has an organic farm, where no chemical pesticides are used. At two of the four other farms spraying is done more or less critically. The levels of chemical use of these two farms were estimated at 60% and 80% respectively. Besides the fields of these 5 farmers, one potato field in a vegetable garden was studied. for this it was initially assumed that no pesticides were used but later this turned out not to be the case. Ultimately the level of pesticide use was estimated at 80% maximum. A total of 13 fields were studied (see: Table 4.1).

In the study, the invertebrates living on (top of) the soil (epigeal invertebrates) as well as those living in the soil (edaphic invertebrates) were sampled. The fauna in the ditches alongside the fields was also studied; the results of this are discussed in 4.2.2.

crop way of growing	potatoes	sugar beets	wheat
non-critical farmer	2	2	2
critical farmer	1	2	2
biological farmer	1		
vegetable garden	1		

Table 4.1 Number of fields in the Haarlemmermeerpolder where sampling of terrestrial invertebrates took place, divided by crop and way of growing.

The sampling took place early in June with the following methods:
- The edaphic fauna was studied by taking 6 random samples in each field. These were dug up with a spade (area ca. 75 cm^2) to a depth of approximately 20 cm (total sample ca. 1500 cm^3). The fauna present in the soil was extracted by means of a Berlese funnel.
- In the spots where soil had been taken out for the soil samples, traps were installed for the epigeal fauna. These pitfalls were filled with formol and soap. Thus the animals falling into the traps were killed and preserved. The pitfalls stayed in place for a week.

All samples were examined in the lab and the animals occurring in them listed by name. They were classified down to the level of a number of main groups (orders or families), which with limited knowledge can be classified fairly quickly. This was done because it was supposed that even at the level of these main groups differences could be seen, and because further classifying to genus or species would take a lot of extra time. The six sub-samples were always elaborated separately. By means of two-factor variance analysis (Sokal & Rohlf, 1969; Dixon & Massey, 1969) and the rank correlation test of Spearman (Siegel, 1956) it was determined in how far the differences found between the crops and the pesticide levels were significant.

4.2.2 Results

In 4.2.2.1 the results are given about invertebrates living on the soil, and in 4.2.2. those about invertebrates living in the soil.

4.2.2.1 Epigeal fauna

The numbers of animals collected in the various fields with pitfalls are shown in Table 4.2. The following things can be derived from this table:
- The number of taxonomic groups and individuals, at the same level of pesticide use, is lowest in potatoes and highest in wheat.
- Within the potato fields, the largest numbers of individuals were found in the organic potato crop. The numbers occurring here are comparable to those in (non-organic) wheat.
- Among the organic potatoes more mites (Acaridae), centipedes (Chilopoda), springtails (Collembola) and larvae of ladybirds (Coccinellidae) are found than in all other (sprayed) fields.

- Within the sprayed fields at 60% pesticide use more cantharids, diptera and rove beetles Staphylinidae are found than at the higher levels of pesticide use.

Besides the taxonomic classification, one can also make a classification on the basis of ecological groups, i.e. a classification by the function the organisms perform in the ecosystem. Thus, we can divide the taxonomic groups into predators (Araneida, Chilopoda, Carabidae, Staphylinidae, Coccinellidae and Cantharidae) and herbivores and/or detrivores (Diplopoda, Collembola, Curculionidae, Aphidina, Isopoda and Diplura). No distinction is made here between herbi- and detrivores, as the Collembola cannot just be classed in one of the two groups, while numerically it is a very important group. A number of taxonomic groups contains representatives of both ecological groups and is therefore classified among the miscellaneous group.

pesticides level (+ crop)		predators	herbivores& detrivores	miscellan.	ratio p : h+d
organic	(p)	188	426	681	1 : 2,3
60%	(p)	63	63	29	1 : 1
	(b)	84	94	105	1 : 1,1
	(w)	125	255	317	1 : 2,0
veg. gard.	(p)	196	80	152	1 : 0,4
80%	(b)	257	93	175	1 : 0,4
	(w)	308	95	421	1 : 0,3
100%a	(p)	290	51	365	1 : 0,2
	(b)	300	34	94	1 : 0,1
	(w)	492	149	515	1 : 0,3
100%b	(p)	39	25	155	1 : 0,6
	(b)	104	83	118	1 : 0,8
	(w)	637	357	611	1 : 0,6

Table 4.3 Ecological groups, numbers of individuals found and the ratio between predators (p) and herbivores + detrivores (h+d) at various pesticide levels and crops; p = potatoes, b = sugar beets and w = wheat.

Table 4.3 shows which number of individuals in each of the ecological groups was observed. From this, the following comes to the fore:
- at low pesticide use, herbivores and detrivores are relatively numerous, and with high use there are relatively more predators;
- there are, however, large differences in this ratio between the two 100%-farmers;
- most herbivores and detrivores are found in organically grown potatoes

Epigeal fauna

group	Coll.	Acar.	Opil.	Aran.	Aphi.	Dipt.	Stap.	Cara.	Col.l.	oth.Col.
potatoes										
organic	393	100	5	78	30	204	63	6	2	370
veg.gard.	60	17	7	28	19	48	4	164	17	57
60	23	0	3	21	40	20	1	41	0	6
100a	34	0	3	69	17	220	79	140	0	142
100b	10	1	4	16	11	117	9	14	0	33
sugar bts										
60	50	0	6	32	41	53	5	38	0	44
80	89	0	2	55	3	87	11	190	1	83
100a	2	0	2	65	31	54	35	199	0	39
100b	70	0	5	52	11	62	25	27	0	51
wheat										
60	222	0	2	84	32	51	11	22	1	263
80	84	4	18	194	9	119	98	16	2	274
100a	133	0	4	114	13	204	358	20	3	300
100b	351	2	45	199	4	216	387	50	2	342

group	Cant.	Curc.	Chil.	Dipl.	Form.	Cocc.	Isop.	Diplu.	tot.ind.	tot.gr.
potatoes										
organic	0	0	13	0	0	29	3	0	1295	13
veg.gard.	0	1	0	0	6	0	0	0	428	12
60	2	0	0	0	0	0	0	0	155	9
100a	2	0	0	0	0	0	0	0	706	9
100b	0	0	4	0	0	0	0	0	219	10
sugar brs										
60	9	2	0	0	2	0	1	0	283	12
80	0	0	1	0	2	0	0	1	525	12
100a	0	0	1	1	4	0	0	0	428	10
100b	0	1	0	0	0	0	0	1	305	10
wheat										
60	4	0	0	0	0	6	1	0	697	12
80	0	0	0	0	1	0	0	2	824	12
100a	0	0	3	0	0	0	0	0	1156	11
100b	0	0	0	0	2	0	0	2	1405	12

Table 4.2 Number of animals collected in pitfalls in 13 different fields in the Haarlemmermeerpolder; six pitfalls were installed per field, and the total catch in them is shown.

From top to bottom, per crop, the level of chemical use is shown: organic = organic growing; 60, 80 and 100: (estimated) levels of chemical use (in %); a and b = farm a and farm b. The various groups are shown from left to right, plus total of individuals and total of groups. The following groups were distinguished (left to right): Collembola, Acardia, Opilionidae, Araneida, Aphidina, Diptera, Staphylinidae, Carabidae, other Coleoptera, Coleoptera larvae, Cantharidae, Curculionidae, Chilopoda, Diplopoda, Formicidae, Coccinellidae, Isopoda and Diplura.

Edaphic fauna

group	Coll.	Lubr.	Ench.	Dipt.ad.	Dipt.l.	Aphi.	Stap.	Col.l.	oth.Col.
potatoes									
organic	8	2	27	3	51	1	0	18	4
veg.gard.	1	8	21	0	95	0	0	4	1
60	6	1	71	2	5	0	0	0	1
100a	25	0	1	7	2	0	0	2	0
100b	11	0	1	3	15	0	0	3	0
sugar bts									
60	4	0	23	2	4	0	1	0	0
80	2	0	5	5	1	0	0	0	0
100a	5	1	1	6	4	0	0	0	0
100b	1	1	4	4	23	7	0	0	0
wheat									
60	4	0	3	0	2	3	0	4	0
80	9	0	1	4	5	0	1	4	0
100a	20	0	3	5	1	0	1	0	0
100b	24	0	0	7	29	0	6	1	3

group	Chil.	Dipl.	Aran.	Acar.	Isop.	Symp.	not id.	tot.ind.	tot.gr.
potatoes									
organic	1	0	0	34	0	0	0	149	10
veg.gard.	0	0	1	5	1	0	0	137	9
60	0	1	0	4	0	0	2	93	8
100a	0	0	0	2	0	0	0	39	6
100b	0	0	0	2	0	0	0	35	6
sugar bts									
60	0	0	0	1	0	0	0	35	6
80	0	0	0	0	0	0	0	13	4
100a	0	0	1	0	0	1	4	23	7
100b	0	0	0	2	0	0	0	42	7
wheat									
60	0	0	0	0	0	0	0	16	5
80	0	0	0	0	0	0	0	24	6
100a	0	0	0	3	0	0	0	33	6
100b	0	0	0	0	0	0	0	70	6

Table 4.4 Numbers of animals collected from the soil samples taken from 13 different fields in the Haarlemmermeerpolder; six sub-samples were taken per field, the total number of animals from which is listed here.

From top to bottom, per crop, the level of chemical use is shown: organic = organic growing; 60, 80 and 100: (estimated) levels of chemical use (in %); a and b = farm a and farm b. The various groups are shown from left to right, plus not identified, total of individuals and total of groups. The following groups were distinguished (left to right): Collembola, Acardia, Opilionidae, Araneida, Aphidina, Diptera, Staphylinidae, Carabidae, other Coleoptera, Coleoptera larvae, Cantharidae, Curculionidae, Chilopoda, Diplopoda, Formicidae, Coccinellidae, Isopoda and Diplura.

4.2.2.2 Edaphic fauna

The numbers of animals collected in the various fields by means of a spade are summarized in Table 4.4. From this table it can be deduced that:

- the greatest total numbers of individuals and groups are found in potatoes being grown organically;
- the greatest numbers of mites (Acardia) and beetle larvae are found in potatoes being grown organically, and
- in the fields with a higher pesticide use, more springtails (Collembola) and diptera (larvae and adults) and fewer potworms (Enchytraeidae) are found than in fields with a lower pesticide use.

Here too, the groups can be divided into predators (Chilopoda, Araneida and Staphylinidae) and herbivores and/or detrivores (Aphidina, Diplopoda, Collembola, Enchytraeidae, Lumbricidae and Isopoda). The predators were found in the soil in numbers too small to base statements on. The herbi-/detrivores are dominated by two groups: the Enchytraeidae and the Collembola. The former are more numerous at a low pesticide use, the latter at high pesticide use. Possibly it is a matter of ecological compensation here: at higher pesticide use, the ecological function of the one group is taken over by the other group. Possibly, the relatively large numbers of diptera larvae (for a large part herbi- and detrivores) on farm '100b' can also be explained in this way.

4.2.3 Discussion

The following notes should be made concerning the field research results described above:

- In the short research period it could not be entirely excluded that (part of) the differences found were caused by factors other than the toxic and/or ecological side effects of pesticides. Thus, the way of running the farms examined will differ on more points than just the use of pesticides. A survey showed that specifically level and manner of fertilizing and the frequency of working the soil of the organic farm differed greatly from the other farms. The differences between the two 100% farms can possibly also be explained (partly) for reason of other factors.
- Samples were taken at only one moment in the year, which very probably led to a non-representative picture. The recovery time after a spraying becomes a very important datum in this way.
- In the discussion of the results it is always assumed that the differences found are based on differences in the abundance of the groups on or in the fields. However, only those animals are found in the pits, which walk into them themselves; thus, the activity of the animals present also determines chances of catching them. Thus, groups of animals which move around a lot on the soil (ground beetles e.g.) will be over-represented in the samples relative to less mobile groups. It is also possible for the activity of a group of animals to differ from field to field, and that this causes differences in the numbers of animals landing in the pitfalls.
- It is not entirely clear what mechanism causes the change in the ratio between predators and herbi-/detrivores (see: Table 4.3). In itself, this is a very favourable picture for agriculture: the numbers of potentially harmful organisms are kept low by the use of pestici-

des, which is reinforced by an increase in the number of predators. One may wonder, however, what the predators live on, if their prey animals occur in such small numbers. Possibly more predation occurs among themselves.
- It would seem that when the potworms, which appear to be very sensitive to pesticides, decrease in number, their role as litter-eaters is taken over by the springtails and possibly also by the diptera larvae (ecological compensation). Eysackers & Van de Bund (1980) also indicate that the springtails are relatively insensitive to pesticides and can increase through their use.

Because of the limited extent of the study, it was not possible to go further into the problems and questions indicated here. The validity of the results would have to be looked into further on the basis of more extensive empirical field research. Also, improvements can certainly be made in the methods used on a number of points. One can think of sampling at several moments during the year, and of further identifying some groups (e.g. groups with a "diverse" choice of food).

4.3 Conclusions

In a general sense it is concluded that on the basis of literature data, data from experts and data concerning incidents, for some species groups a picture regarding the toxicity of many chemicals does exist, but that only a very fragmentary picture has originated of the occurrence of side effects in the field. Toxicity data on organisms other than useful ones are largely lacking, let alone it being known how these animals react to pesticides under field conditions.

Toxic side effects of pesticides under conditions in practice are found in earthworms from soil disinfectants (specifically fumigants), benzamidasols and carbamates. Populations decrease (very) greatly and recovery by species such as L. terrestris takes several years. On the basis of the toxicity of this group of agents, it is well possible that other soil invertebrates too, greatly decrease in numbers. In view of the fact that soil disinfecting is taking place on a large scale in The Netherlands, these side effects should not be under-estimated; they can form a real threat to the ecosystem.

Toxic side effects in the field have also been found in bees; these are mainly caused by organic phosphor compounds (insecticides). There are also suspicions though regarding certain herbicides. This aspect should be given more attention, also in view of the economic importance of bees (see also: Chapter 3).

The occurrence of direct toxic side effects from herbicides is not likely for the soil invertebrates. Even a positive effect is possibly (and temporarily?) not excluded, namely as the result of a greater supply of organic material. Ecological side effects through destruction of habitat or disappearance of host plants can certainly occur in a number of animal groups, such as butterflies. In other vegetation-bound invertebrates these ecological effects are also expected.

With the absolutely required care in view of the very limited extent of the research - the following general conclusions can be derived from the

field research. Differences in the invertebrate fauna between fields with a different level of pesticide use can be traced; also when identifying only to the level of main groups (orders and families). The biggest differences are found in mites, potworms, cantharids, centipedes and ladybirds (for all groups applies: smaller numbers at higher pesticide use) and for dipterous insects and rove beetles (for which applies: greater numbers at higher pesticide use). Springtails occur in the soil in greater numbers at higher levels. At a higher level of pesticide use, relatively more predators occur, at a lower level relatively more herbivores and detrivores. Within the potato fields, the greatest numbers of individuals and groups are found in organic farming.

Finally it must be concluded that the overview obtained of the nature and extent of the side effects of pesticides on terrestrial invertebrates probably still has (a great) many lacunae. The available information is so anecdotical and so spread in time and space, that obtaining a correct impression is considered hardly possible at the moment. The terrestrial field research has shown that taking fauna samples to trace side effects of pesticides can make sense. It should be noted though that it has not yet become clear whether other parameters, such as sum (total) parameters and ratio indices are not also good indicators of the occurrence of these side effects (see also: Chapter 3). The same thing applies to the use of bio-assays; no use could be made of these (yet) - in the terrestrial environment at least.

5. AQUATIC FAUNA

In this chapter a picture is given of the nature and extent of the side effects of pesticides on aquatic fauna.[14] In 5.1 have been gathered the data about side effects known to us at the moment as regards to fishes, amphibians and invertebrates, and a summarizing overview is given of the toxicity data available. Besides, during the research, already existing inventory data and some of our own were gathered. These were submitted to analysis, aimed at i) detecting sore points in regard to pesticide use, ii) tracing suitable test organisms, and iii) obtaining an overview of the nature and extent of the side effects in the field (5.2). Finally, research was done by means of bio-assays; with these it was attempted to establish a clearer connection between the occurrence of animal groups and water quality (5.3). In 5.4 the conclusions of this chapter are summarized.

5.1 Incidents, opinions of experts and literature

A picture of the nature and extent is obtained in the following sections for fishes, amphibians and reptiles respectively by means of literature, incidents and opinions of experts (5.1.1: fishes; 5.1.2: amphibians and reptiles; 5.1.3: invertebrates). The available toxicity data are summarized in Annex 4.1, including the background and classification used. As already many determinations of pesticide contents of surface water have been done, an overview of this is given in a separate section (5.1.4) and a relationship is established with available toxicity data. Finally, in 5.1.5 the results obtained are discussed.

5.1.1 Fishes

Toxicity

There are relatively many toxicity data about fishes. From the overview of these in Annex 4.1, the following can be deduced:

Fungicides
In general, fungicides are (very) toxic for fish. This applies specifically for captan, benzamidazon, copper sulphate, pentachlorophenol and organic phosphor compounds. For tolclofos-methyl and pentachlorophenol incidents are also known in which fish mortality occurred. Anilids are not toxic for fish.

Insecticides
Many data are available, which show that many insecticides are (very)

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[14] The aquatic fauna is often indicated with the term "macrofauna". Normally this is taken to mean the invertebrates larger than 1-1.5 mm. Small crustaceans and mites are not counted among these in general. As these have been included in our research, the term "invertebrates" is used, i.e. all invertebrates recognizable with the naked eye in a water sample. When fish and amphibians are also included, we speak of "aquatic fauna".

toxic for fish. Incidents are known with endosulfan and endrin. Only the acyl-ureum compounds are not toxic.

Herbicides
Although the toxicity of various herbicides varies greatly, it can be stated that in general herbicides are less toxic. (Very) toxic are bromopropylate and dinitro phenols. Incidents are known with dinoseb, use of which will be prohibited shortly. Not toxic are alkane carbonic acids, carbamates, phenoxy acetic acids and triazinons.

Soil disinfectants
In general, agents from this group are moderately toxic to very toxic. (Very) toxic are methyl isothiocyanate and aldicarb. An incident is known with metam-sodium; methyl isothiocyanate is the active form of metam-sodium.

Incidents

In Table 5.1 traced incidents with pesticides and fish mortality have been summarized.[15] The table gives a striking picture for a number of crops, chemicals and causes:
- A great number of times endosulfan has led to mass fish deaths, the striking aspect being that it involves various applications: emission of rinsing-water, spraying of fruit trees or use in a greenhouse.
- Most incidents concern emissions and/or spilling when rinsing or mixing up (solutions), or emissions from greenhouses. Fish mortality through normal use occurs less often.
- Few accidents have been observed in arable land areas.

These data can be explained as follows. Endosulfan is very toxic for fishes; locally, emissions lead to highly increased concentrations, immediately followed by local mortality. The same observations of mortality in arable farming areas may have several causes: in arable farming endosulfan is less used than in greenhouses and in fruit growing (in the Haarlemmermeerpolder for instance, the use of endosulfan is not advised; see: Annex 5.1); furthermore it is possible that there are few large fishes in the narrow or shallow ditches in arable field; consequently, a possible mortality has less chance to be observed.

5.1.2 Amphibians and reptiles

Incidents with reptiles are not known and only very sporadic toxicity data are on hand. In addition, in The Netherlands most reptiles occur in small numbers, only a few species of which occur regularly in the aquatic environment.

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[15] Only the incidents since 1985 have been included; accidents related to use, such as emissions of remainders and spills when filling-up, have also been included among the incidents; calamities (e.g. as a result of errors in production, or for reason of fire) are not taken into consideration.

Only two incidents are known with amphibians: mortality of frogs in the North-East Polder through endosulfan (written comm. Baars) and mortality of the small water salamander, probably through soil disinfecting with methyl bromide (Bergmans & Zuiderwijk, 1986). It is not clear whether this small number of accidents is because indeed little mortality is occurring or because the mortality is not noticed c.q. reported. Toxicity data about amphibians are only sporadically on hand.

pesticide	year	place	crop	cause
endosulfan	1985	South-Limburg	unknown	emission?
endosulfan	1986	South-Limburg	mushrooms	emission?
endosulfan	1986	South-Limburg	mushrooms	emission?
endosulfan	1985	Drenthe	market garden	leak in mixing tank
endosulfan	1987	Westland	greenhouse	illegal use
endosulfan	1987	Westland	greenhouse	emission?
endosulfan	1986	Maassluis	greenhouse	emission?
endosulfan	1986	Kralingen	market garden	emission?
endosulfan	1985	N.E. Polder	rose greenhouse	drain and rain
endosulfan	1985	N.E. Polder	fruit-growing	diffuse emission?
endosulfan	1985	N.E. Polder	fruit-growing	?
endosulfan	1986	Maasdriel	mushrooms	?
endrin	1985	Uithoorn	greenhouse	?
pentachl.f.	1986	Maasdriel	mushrooms	?
tolclof.m.	1986	Westland	?	emission spray liquid
metam natr.	1986	Westland	?	?
dinoseb	1987a	Drenthe	potatoes	mixing/rinsing?
dinoseb	1987b	Drenthe	potatoes	mixing/rinsing?

Table 5.1 Traced incidents with fish; sources: Havekes & de Vries, 1986; *oral comm. de Vries (UvW) and Baars (CDI).*

NOTE: At the moment, the Union of Water Control Boards is taking inventory among its members about the most recent incidents; an overview will be published soon (de Vries, in preparation).

5.1.3 Invertebrates

Toxicity

Only insecticides, and especially the organic phosphor compounds, have been thoroughly investigated (see: Annex 4.1); this group of pesticides is in general (very) toxic for aquatic invertebrates. Few data are known about fungicides and herbicides. The fungicides about which data are present, are often moderately to (very) toxic and the herbicides are usually moderately toxic. Especially the dipyridilium compounds are little toxic for invertebrates. Concerning invertebrates, no toxicity data are known of soil disinfectants.

Incidents

Incidents like a sudden mortality of invertebrates are not known. This is easily explained: dead invertebrates are difficult to see and furthermore are consumed or decomposed in a short time. There are some indications about the occurrence of effects on this group organisms; however, we must remark that the cause had not been unambiguously established.

- Ditches still containing only a few living snails and worms, which gave rise to strong suspicions toward methyl bromide and cholinesterase inhibiting substances (Anonymous, 1987a, oral comm. van der Wal, Polder Board Delfland). In this respect, one uses the term 'ecological dead ditches' (CCRX, 1988).
- The absence of invertebrates near a greenhouse area in the Rijnland Polder Board, where high concentrations of DDT and DDE have been found (oral comm. van der Does, Rijnland Polder Board).
- Ditches that may be affected by airplane sprayings with organic tin compounds, in potato fields in Groningen (Province) (van Haasteren, 1988). This kind of usage entails great risks for aquatic organisms (van Gestel, 1987).

A research done in the Westland in 1987, brought to light that some ditches did not contain beetles, bugs, dragonfly larvae, flatworms, springtails, and leeches (Working Group 'Effects ...., 1988). A relation with pesticides is probable, because of high contents of pesticides measured in these ditches, and because of the mortality of water flees observed in bio-assays with the same water (see also: 5.2 and 5.3). An indication of the possible existence of sublethal side effects is the presence of mosquito larvae with a higher percentage jaw abnormalities in arable farming areas compared to reference ditches (Faasen, 1988).

5.1.4 Concentrations of pesticides

In the following, those substances groups are dealt with, whose measured concentrations have been increased since 1985[16].

Here, only those measurements are given which show a striking increase relative to other measurements, or which entail a risk to the aquatic fauna. To this end, the concentrations were compared to the toxicity data from Annex 4.1. The cases in which actually fauna mortality was observed have already been mentioned in the preceding sections and are not repeated here. Sources used are: Bekooy, 1988; CCRX, 1988; oral comm. Flentge, Municipal Water Utility Groningen; van Haasteren, 1988; Polder Board of Delfland, 1987; Greve et al., 1986; Wammes et al., 1986; Canton et al., 1987; *oral comm. de Vries, Union of Water Control Boards; Working Group* "Effects ....", 1988; ZWO, 1988.

Fungicides

In Westland pyrazophos was found in 1987, which entails a risk, in view of the high toxicity for fish. In Drenthe a high maneb-content was found in 1986. As maneb is (very) toxic to fish, there is a risk. In the North-

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[16] Data from CCRX (1988) show that some pesticide groups occur in precipitation in measurable quantities; the effects of this on the contents in surface water are as yet unknown.

East Polder dithiocarbamates are found regularly; probably this is related to its use in growing potatoes. In general, agents from this group are (very) toxic to fish as well as to invertebrates. Carbendazim has also been found in surface water. This agent is toxic to fish as well as to invertebrates.

Insecticides

Measurements of cholinesterase inhibition are being taken on a fairly large scale. The IMP-standard is regularly being exceeded (005 µg/litre). A clear danger point is in the greenhouse garden market area of Westland. In 1985 contents were measured here up to a maximum of 405 µg/litre, and in 1986 even 2500 µg/litre once. In 1987 contents were encountered up to a maximum of 43 µg/litre. In bio-assays these contents caused mortality among water fleas. In any case, dichlorvos, heptenophos, parathion and triazophos were found as separate substances.

In the North-East Polder in 1986 (and in 1981), in drain water from carrot-growing, and in the surface water as well, high chlorfenvinphos contents were found; these are of the order of the LC-50 (96-hours) for fish. Also in 1986 diazinon, dichlorvos, dimethoate, ethyl parathion, sulfotep and thiometon were found in the North-East Polder in high concentrations.

Besides these substances, azinphos-methyl and malathion were also found in surface water. These too are very toxic.

The EOCl-content is regularly determined by various agencies. Part of the EOCl, for that matter, consists not of pesticides but of other compounds, such as PCB's. A number of pesticides from this group have not been allowed for use for some years already, but nevertheless high contents are still found regularly. The cause is probably the great persistence of these agents, so that they stay present in the environment for a long time. Agents from this group are (very) toxic for fish and invertebrates.

Herbicides

In 1985 and 1986 a high dinoseb content was found a couple of times in Drenthe (province). The cause of this is sought in spills when filling spray tanks, or when cleaning the tanks. In the Westland too a high dinoseb content was found in 1987. In view of the high toxicity of dinoseb for fish and of the incidents, one can speak of a high risk for fish. In Drenthe in 1986 a high content of atrazin and simazine was found, which probably means a less great risk in view of the moderate toxicity for fish.

Soil disinfectants

In the North-East Polder in 1986, as a result of cleaning soil disinfection machinery, dichloropropene and dichloropropane were found in ditches. Because not many toxicity data are known, the risk to aquatic fauna cannot be indicated. In the area of the Drentsche Aa (a brook) too, high concentrations of dichloropropene/propane are found regularly, which via the ground water cause problems in the preparation of drinking-water.

Methyl bromide too is regularly found in the surface water; this in any case is (very) toxic for fish.

5.1.5 Discussion

One of the most important problem points coming to the fore from the literature data, incidents and interviews, is that it is not possible to compile a reasonably complete and justified overview of the side effects on aquatic fauna in The Netherlands. Only a couple of incidental cases are known of side effects actually observed in the field. Hardly anything is known about recovery time either.

In general, only when mortality occurs or emissions are observed are pesticides measured in a targeted way. For this reason, the overview obtained is probably an underestimate of the occurrence of pesticides in surface water. In addition, the measurements largely occur in the big water courses. High concentrations in smaller water courses can certainly not be excluded.

The concentration of only a relatively small number of chemicals in the surface water is determined regularly. This is caused not only by problems with methods and finances, but also because the large number of chemicals used renders a complete analysis almost impossible. Sum (total) parameters do not exist for all chemicals either. As the chemicals which are (indeed) being measured (for) are being found regularly, there is a good chance that other chemicals too will be present. In view of the great toxicity of many chemicals, side effects on aquatic fauna are certainly expected. Also, at the present time, i.a. the Association of Water Control Boards is working at making available methods of analysis and a selection ahead of time of (groups of) chemicals, determining the content of which is deemed desirable (oral comm. de Vries).

The occurrence of specific invertebrate species partly depends on the vegetation present in the ditches (Higler, 1976). This leads to it that the occurrence of the species is directly or indirectly dependent on a large number of factors, such as the permanent presence or not of water, the dimensions of the ditch, the extent of being connected with or isolated from other ditches, type of soil, orientation of the ditch (exposure), and the welling-up or seeping away of the water (see i.a.: Gonggrijp, 1981). In addition, a few other very important activities are occurring, such as physical-chemical interventions (depositing fertilizer/manure, letting water in and out) and physical interventions (cleaning the ditch, dredging and water level control). So far, research on the effects of these interventions has not been done, specifically on their relative effects. At the moment, therefore, it cannot be indicated what the relative significance of the side effects of pesticides is as seen in relation to other factors and interventions.

5.2 Field research

Via targeted field research and by means of using existing data it was attempted to get a more accurate picture for a number of large-scale crops of the side effects on aquatic fauna occurring in the field. This research also stressed methods. The following objectives were formulated:

1. To obtain an overview of side effects of pesticides for a number of crops and regions.

2. To develop a method for tracing the side effects of pesticides by means of sampling the aquatic fauna.

Below, the experimental setup is shown in short. In 5.2.1 the method used is dealt with. In 5.2.2 the results are then presented, and subsequently discussed in 5.2.3

Setup
The field research was set up transversally (comparison in space) and was aimed in the first instance at establishing connections between the use of pesticides and the presence of fauna groups. Aspects of method also came to the fore, such as the sampling method, size of samples, number of repeats and level of detail of identification. The field research was targeted at a number of large-scale crops in the region (see: Table 5.2).

crop region	control	potatoes	sugar beets	wheat	greenhouses
Westland	x				x
Aalsmeer	x	x	x	x	x
Haarlemmerm. polder	x	x	x	x	
North-East Polder	x	x	x	x	x

Table 5.2 Overview of regions and crops sampled (x).

The premise was that the crops show differences in use of pesticides, and that it should be possible to find these differences back in the fauna composition of the bordering ditches. Annex 5.1 gives an overview of pesticide use in the Haarlemmermeerpolder and in greenhouse market gardening. This annex shows that indeed there are big differences in pesticide use. Naturally there are other differences between the crops as well, apart from pesticide use, specifically between the open-air crops and those in greenhouses (fertilizing, soil working, etc.). By comparing ditches bordering on a crop to reference ditches in the same area and by including a number of possibly interfering factors, it was possible to exclude as much as possible the effect of the other factors. In general, ditches between grassland fields in the same region were chosen as controls. Occasionally, ditches in parks had to be resorted to.

5.2.1 Methods

Data from several agencies were used and field data of our own were gathered. These two sources are discussed below.

Data gathered by other agencies

Data were used from macro-fauna surveys in North-Holland (province)(1981-86), Provincial Department of Public Works North-Holland), in the North-East Polder (1987, West-Overijssel Water Purification Board) and in the Westland (1986-87, Polder Board of Delfland). A condition for the use of these data is that it must be possible to link the samples to certain

locations	data from others		1st round july 1988		2nd round aug. 1988		3rd round sep. 1988	
	w	n	w	n	w	n	w	n
controls								
Aalsmeer	1981: 2	1	1	-	1	-	2	2
	1984: 1							
Egmond	1986: 1							
Haarlemmermeerp.	-	-	-	2	-	1	1	2
North-East Pold.	1986: 2	-	2	-	-	-	2	2
Schermer	1979: 2	-	-	-	-	-	-	-
	1986: 1	-	-	-	-	-	-	-
Westland	-	-	-	-	-	-	2	2
arable land								
Aalsmeer	1981: 1	1						
Haarlemmermeerp.	1892: 3	-						
North-East pold.	1986: -	1						
potatoes								
Aalsmeer	-	-	-	-	-	-	1	
Haarlemmermeerp.	-	-	-	2	1	1	1	2
North-East pold.	-	-	-	-	-	-	1	2
sugar beets								
Aalsmeer	-	-	1	-	1	-	1	2
Haarlemmermeerp.	-	-	-	5	-	2	1	2
North-East Pold.	-	-	-	-	-	-	1	2
wheat								
Aalsmeer	-	-	-	2	-	2	1	2
Westland	-	-	-	1	-	-	-	-
Haarlemmermeerp.	-	-	-	3	-	-	1	2
North-East Pold.	-	-	-	2	-	1	1	2
greenhouses								
Aalsmeer	1981: 1	-	-	2	-	1	2	3
Westland	1986: 1	-	2	4	1	3	2	3
Westland	1987: -	4						
Egmond	1984: 1	-	-	-	-	-	-	-
North-East Pold.	-	-	-	1	-	1	-	1
fruit								
North-East Pold.	1986: 1	-	1	1	1	-	-	-
flowers								
North-East Pold.	1986: -	1	-	1	-	1	-	-
market gardening								
North-East Pold.	-	1	-	1	-	1	-	-
total	17	9	7	27	5	14	20	33

Table 5.3 *Sampling points in field research and bio-assays; - = no sample, n = narrow water course (less than 3 meters), w = wide water course (over 3 meters), the figures indicate the number of sampling points. Bio-assays did not take place in the Haarlemmermeerpolder in the first round.*

crops. Because crop rotation is often practised and sampling takes place over several years, this is often not well possible in practice. In addition, many sampling points are in large water courses; because thereis quite a current here, linking to a specific crop does not make sense. Consequently, these ditches were classified at a more general level into reference ditches, arable land ditches and greenhouse ditches. Further, various sampling methods were used. At species level, the method can certainly play a part, but it is assumed that at the level of main groups the effect is negligibly slight. Also, the fact that the sample-taking occurred over several years becomes less of an objection with a group classification at a relatively high level. In view of these observations, these data must be used with the required caution.

Data gathered within this project

Our own data were gathered in three sampling rounds in (a total of) 67 ditches (see: Table 5.3). The first sampling round (June-July 1988) had an orienting character; a broad scala of crops was examined. The second sampling round (August 1988) was a repeat, with an adaption of the sampling diagram; in this round, however, the emphasis lay on doing bioassays (5.3). The third sampling round (September 1988) was aimed at a more limited number of crops, but with more sampling points per crop, so that a more complete overview was obtained for those crops.

Ditches
In selecting the ditches we always looked for ditches with the same crop on both sides; if there was no such ditch, ditches with the crop concerned on one side and on the other side a neutral surface (such as a road shoulder) were used. In general, the crops were grown on such a large scale, that in both directions from the sampling point to at least 100 meters distance the same crop was being grown. On a more general level, the wider water courses were classified into reference, arable land and greenhouse ditches.

Fauna sampling
In sampling a triangular dip net was used (base x height = 35 x 32 cm, 1 mm mesh). Per sub-sample, the net was dragged through the ditch shockwise over a length of one meter. The fauna found was classified in the field to the level of the following groups: flatworms, oligochaete worms, leeches, water fleas, copepods, ostracods, isopods, amphipods, water mites, springtails, mayflies, dragonflies, corixid bugs, back-swimmers, semi-aquatic bugs, mosquito larvae, beetles, snails, amphibians and fish. This classification was chosen because the groups used can be recognised relatively quickly, reliably and without aids in the field, which would also be a condition for a field test.

Sampling rounds
During the first two sampling rounds, one sample consisted of two subsamples, taken from two different habitats. In these two rounds, the quantitative occurrence of the fauna in the field was indicated as follows: not present; 1 specimen; a few (ca. 2-5) specimens; several (ca. 5-10) and numerous (more than ten). In the third round, five sub-samples were taken per sample, from all habitats present in the ditch in so far as possible. The sub-samples were processed separately. The objective here was to arrive at a conclusion concerning the minimum number of sub-

samples required. To be able to compare 5 sub-samples with other data, they were converted to the abundance throughout the entire ditch by distinguishing the following classifications, besides 'none' (0) and 'one specimen' (+):

1: few specimens: minimum of 2 specimens and maximum present in 5 sub-samples at 1 or a few specimens per scoop
2. several specimens: maximum 2 sub-samples with many specimens, or 5 sub-samples with a slight number of specimens per scoop
3. many specimens: maximum 3 sub-samples with many specimens, or 5 sub-samples with several specimens per scoop
4. very abundantly present: minimum 5 sub-samples with several specimens or 4 sub-samples with many specimens.

Pesticides

To be able to relate the occurrence of the various fauna groups to the presence of pesticides, the cholinesterase-inhibition, the chlorinated hydrocarbon content and the dithiocarbamates were determined in (part of) the samples of August and September. The measurements were done by or in co-operation with the RIVM (cholinesterase inhibition), the West-Overijssel Water Purification Board and the Polder Board of Rijnland (both: chlorinated hydrocarbons), and the Biopharmacy Group of Leiden University (dithiocarbamates).

Other factors

In addition to pesticides, there are a large number of factors which can affect the fauna composition of a ditch (see i.a.: Gonggrijp, 1981; Beltman, 1983): physical-chemical factors, management factors and biotic factors. First of all, it should be noted that a number of these factors mainly affect specific fauna species; in view of the group classification used the effect of these factors on the occurrence of entire groups is probably slight. Secondly, the effect of the other factors only becomes of importance for this research if either they are systematically correlated with the use of pesticides, or have a much greater (disturbing) effect than the pesticides. The groups of factors are dealt with separately below.

Physical factors

In general, wide ditches have an important water-removing function, so that the water quality at the moment of sampling is only partly determined by the bordering crop. In addition, the fauna composition is correlated with the width of the ditch (Beltman, 1983). Thus, in selecting the sampling points, a division into wide and narrow water courses was chosen. The limit was set at 3 meters, which also involves the function of the ditch (field ditch or drainage ditch for a larger area). As far as other factors (type of soil, upwelling/seepage) are concerned, there certainly are differences between the regions. Within the regions, however, these differences are less great, so that they play a lesser part in a comparison of ditches within a region. Current was observed only rarely and then mainly in the wider water courses. As current (often artificially caused) can occur at any moment, it was not possible to measure it systematically. This factor was not included and has possibly had an interfering effect on the results.

Chemical factors
To get some idea of the chemical factors, the pH, the chloride content and the phosphate content of the water were determined (see: Annexes 5.2 and 5.3) The phosphate content is a measure of the eutrophication of the ditches; a systematically higher stressing of the ditches which also have a higher pesticide content than the reference ditches could not be excluded in advance.

Biotic factors
The presence and composition of vegetation has a great effect on the composition of the fauna (Higler, 1976). Specifically in the 3rd sampling round therefore, for each sub-sample the vegetation composition was noted down, and the cover of submerged (under water), emergent (standing) and emerged (floating) vegetation was determined. The purpose in this was mainly to pin down the bigger effects; the classifications distinguish mainly between the extremes: very little, or lots of vegetation. The cover of the vegetation present was estimated and divided into the following classifications:
floating vegetation: under 5%: class 1; 5-50%: class 2; 50-90%: class 3; over 90%: class 4.
submerged and standing vegetation: under 10%: class 1; 10-50% class 2; over 50%: class 3

Management
With respect to cleaning and dredging it was assumed that per region these occur in a comparable manner and on about the same time. There was no reason to deviate from this.

5.2.2 Results

To investigate the effect of the various factors measured relative to each other, in principle a multivariate statistical technique would have to be employed. As we chose in favour of obtaining an overview, many samples were taken over a short time, with the fauna being divided into numerical classes. A disadvantage of this is that it not possible to apply multivariate analysis. It is possible though to apply tests to the data so obtained, thus examining the separate effect of factors.

In processing the data, a step by step approach is taken (see: Fig. 5.1). As initially a great effect was expected from the width of the ditches, it was first checked in how far such an effect could indeed be shown (5.2.2.1). In this, the width of the ditch must be seen as an estimator for a (whole) complex of factors, such as depth e.g. Subsequently, the effect of crop and pesticides was examined, also involving the other factors measured (5.2.2.2). In 5.2.2.3 it is indicated, by region, what the possible points of difficulty are. We start with processing the data from the 3rd sampling round, as in this round, in selection and sampling, a number of factors, such as width of ditch and vegetation, were measured systematically. In 5.2.2.4 the results are compared to results of earlier sampling rounds. In conclusion, we pay attention to the pesticides measured (5.2.2.5).

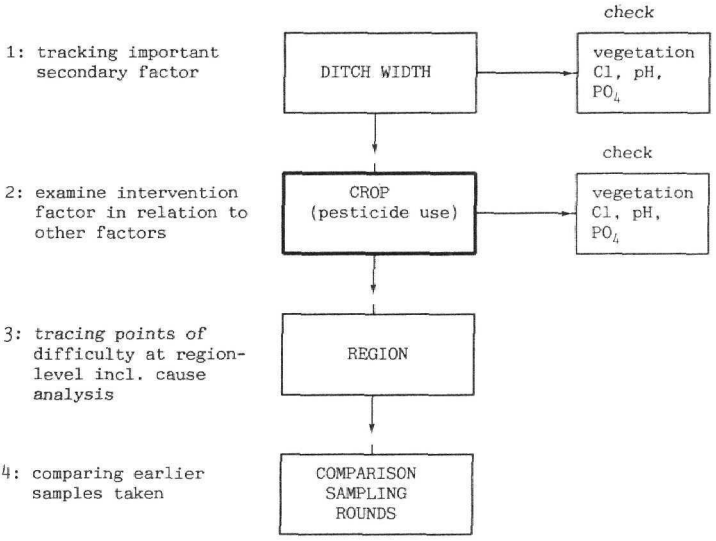


Figure 5.1 Step by step processing of the data.

5.2.2.1 Effect of width of ditch (step 1)

Fig. 5.2 shows the abundance classes of the fauna groups per ditch. The tests show that for 14 fauna groups there are significant differences between occurrence in wider and narrower water courses. However, of the other factors the cover of floating and submerged vegetation also differs; in the narrower water courses, both of these are significantly greater than in the wider ones. Thus, to investigate what is causing the differences in occurrence, the effect of the vegetation was also examined. It turns out that in beetles and snails, both of which groups occur more in the narrower ditches, there also exists a preference for greater cover by submerged vegetation. Consequently, which factor is actually causing the differences in these groups, can not be deduced from these data. The amphibians, which occur more in narrower ditches, show no correlation with vegetation cover. Occurrence of the groups found significantly more in the wider water courses (flatworms, oligochaete worms, water fleas, copepods, isopods, amphipods, mites, corixid bugs and mosquito larvae) shows no clear correlation with vegetation cover. It is *therefore obvious to assume for these groups that ditch width is an* important factor. Thus, the differences found constitute a good argument in favour of considering narrow and wide water courses separately in our further elaborations.

5.2.2.2 Crops (step 2)

Table 5.4 shows which species groups differ significantly in occurrence among different crops. For the wide water courses the following applies: flatworms, springtails and beetles occur less in the greenhouse area ditches than in the controls, while springtails also occur less in arable ditches. Also, leeches, amphipods and semi-aquatic bugs hardly occur in greenhouse ditches; the differences here are not significant, however. The total number of groups in the wide control ditches is significantly greater than in the arable land and greenhouse ditches.

species groups	wide water courses	narrow water courses
flatworms	control > greenhouse	
leeches		greenhouse > field
springtails	control > greenh., field	
semi-aquatic bug		control > greenhouse
beetles	control > greenh.	control > greenhouse
amphibians		control < greenhouse
total number of groups	control > greenh., field	

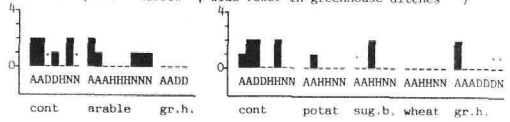
Table 5.4 Differences in occurrence of species groups among different ways of growing crops ($P \leq 0.05$).

In the narrow water courses semi aquatic bugs and beetles occur less than in greenhouse ditches. Amphibians, however, occur most in greenhouse ditches. Leeches occur more in the greenhouse ditches than in the field ditches; there is no significant difference with the controls though. In the other groups no significant differences occur. Besides these results, a number of (other) differences can be observed. In the potato ditches only 45% of the groups occur in at least 2 ditches. In the beet and wheat ditches this applies to 70% of the groups, and in the greenhouse ditches to 75% of the groups. In the control ditches 90% of the groups occur in at least 2 ditches. It would seem therefore that there are differences in occurrence among the various groups of ditches; specifically in the potato ditches many groups are absent.

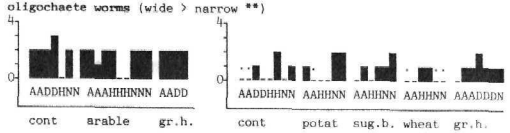
Figure 5.2 Occurrence expressed in abundance classes (see: pp. 78 & 79) of animal groups sampled in September 1988, per ditch; - = 0 specimens and . = one specimen (see also: 5.2.3); A = Aalsmeer, W = Westland, H = Haarlemmermeerpolder and N = North-East Polder; the differences in occurrences were checked against the Kruskal-Wallis one-way analysis of variance; * = differences significant at $P \leq 0.1$; ** ditto at $P \leq 0.05$.

WIDE NARROW

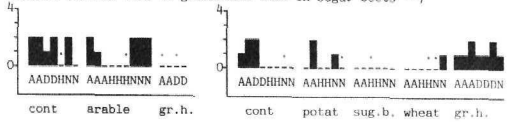
flatworms (wide > narrow *; wide fewer in greenhouse ditches **)



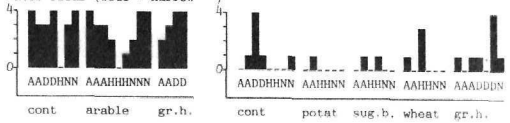
oligochaete worms (wide > narrow **)



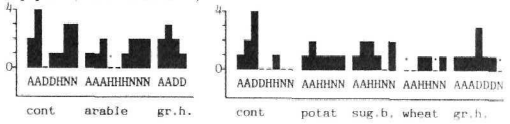
leeches (narrow more in greenhouse than in sugar beets **)



water fleas (wide > narrow **)

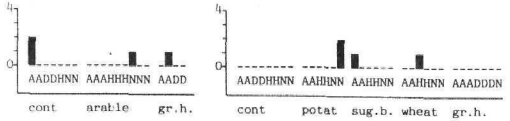


copepods (wide > narrow **)

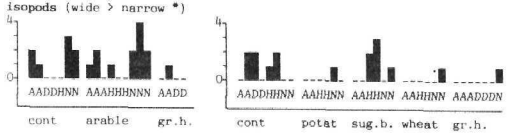


WIDE NARROW

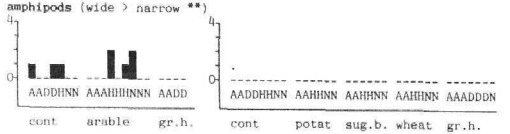
ostracods



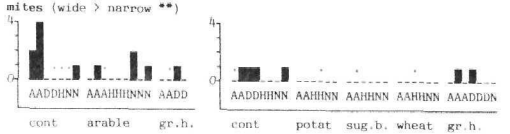
isopods (wide > narrow *)



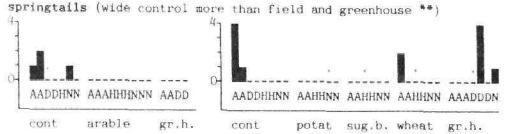
amphipods (wide > narrow **)

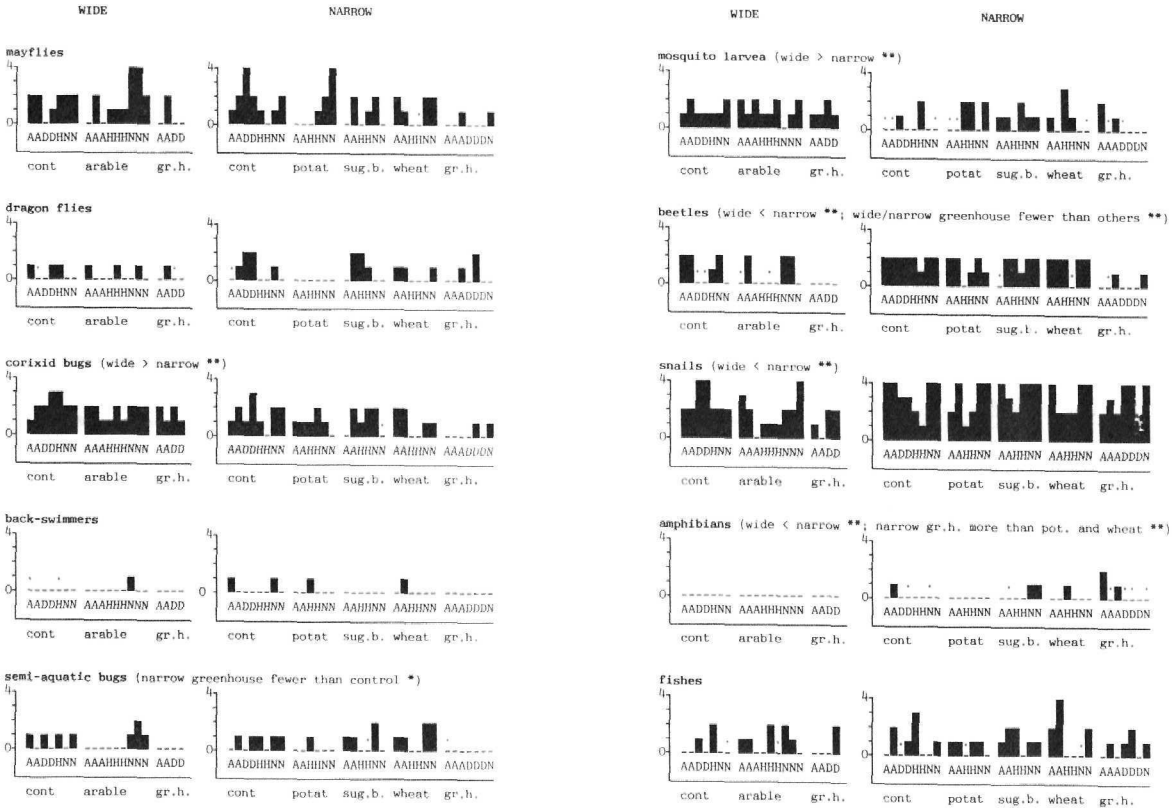


mites (wide > narrow **)



springtails (wide control more than field and greenhouse **)




WIDE
NARROW
mayflies
dragon flies
corixid bugs (wide > narrow **)
back-swimmers
semi-aquatic bugs (narrow greenhouse fewer than control *)
mosquito larvea (wide > narrow **)
beetles (wide < narrow **; wide/narrow greenhouse fewer than others **)
snails (wide < narrow **)
amphibians (wide < narrow **; narrow gr.h. more than pot. and wheat **)
fishes
4
0
AADDHNN AAAHHHNNN AADD
AADDHHNN AAHHNN AAHHNN AAHHNN AAADDDN
cont arable gr.h.
cont potat sug.b. wheat gr.h.

Verification

As a check on the results found, it was first examined in how far a correlation existed anyway between the other factors measured and occurrence of the species groups (Table 5.5). Subsequently the results were compared with the results in Table 5.4.

Effect of other factors

Table 5.5 shows that regularly positive connections are found between the cover of the floating, and especially submerged vegetation, and the occurrence of species groups.

	wide watercourses			narrow watercourses		
organisms	vegetation floating	vegetation submerged	PO_4	vegetation floating	vegetation submerged	PO_4
flatworm	+					
oligochaete						
leech				+	*	
water flea						
copepods			*		*	
ostracods					+	
isopod			-			
amphipods						
mite						
springtail	+					
mayfly			*		+	
dragonfly						
corixid bug						
back swimmer						
semi aq bug		+	-		+	
mosquito lar		*				
beetle					+	
snail						-
amphibian						
fish					+	

Table 5.5 Significant correlations between species groups and a number of factors; $P \leq 0.05$; + = positive correlation; - = negative correlation; * = a significant correlation, but not unequivocally positive or negative (see: text). Species groups with a significant correlation with the way of growing, are *shown in bold letters*.

The phosphate content is negatively correlated with isopods and semi-aquatic bugs (in wide water courses) and snails (in narrow water courses). In copepods and mayflies there is in the wide water courses a preference for the medium classes of phosphate content. With respect to the chloride content, it was found that great differences existed between the sampling points (see: Annexes 5.2 and 5.3). About three quarters of

the measured values are under 200 mg/litre, while in the Haarlemmermeerpolder peaks occur up to 1367 mg/litre. No significant correlations were found, however, between the chloride content and occurrence of species groups. Also, water fleas were not found in chloride contents of over 500 mg/litre. The pH of the ditches was measured in the lab preceding the bio-assays and varied from 7.31 - 8.62 (see: Annexes 5.2 and 5.3). No correlation was found with the occurrence of species groups.

Effect of vegetation and phosphate in relation to crop
In a number of cases (flatworms, leeches, springtails, semi-aquatic bugs and beetles) it was found that a correlation exists between crop and occurrence as well as between a measured factor and occurrence. In these cases, it was examined whether this factor differs among the various crops (see: Fig. 5.3).

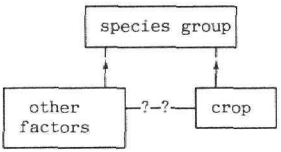


Figure 5.3 *Relationship between crop, other factors and occurrence of the species groups.*

A correlation between crop and the other factors is not found, not either when specifically looking at crops between which the species groups differed. This result renders it more probable that the differences in occurrence found are indeed caused by the differences between the crops.

5.2.2.3 Region (step 3)

In Fig. 5.4 are plotted the numbers of groups by region. In the figure all different species groups have been included in the same way. The results are discussed below, by region.

Westland
In Westland fewer species groups occur in the greenhouse ditches than in the controls. This difference is partly caused by the absence of mayflies, beetles and semi-aquatic bugs from all greenhouse ditches. In the wide ditches the flatworms are also absent. The causes for the absence of the species groups from the greenhouse ditches must very probably be sought in the pesticides content (see also: 5.2.5); the other measured factors did not systematically differ between the controls and the greenhouse ditches, and thus could be excluded as interference factors.

Earlier samplings, by the Delfland Polder Board as well as by ourselves, showed that no water mites are found in the greenhouse ditches. On the basis of this, water mites were used in bio-assays (see: section 5.3). In the third sampling round, this picture was not confirmed, however. In

regard to the sampling by another agency, it is not very probable that the sampling method plays a part. Thus, probably it is a matter of seasonal variation recovery or migration.

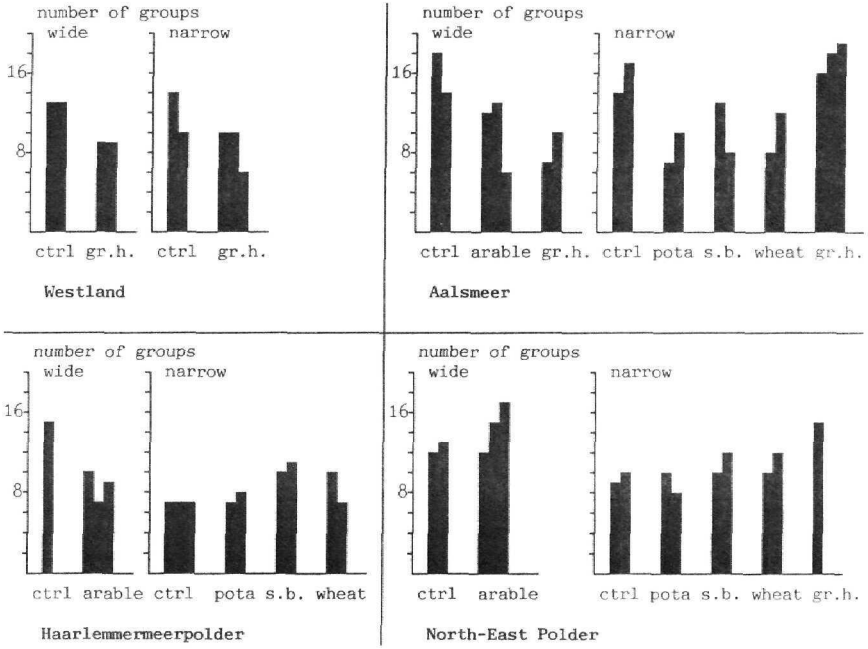


Figure 5.4 *Total number of species groups by region and type of ditch;* all groups distinguished were counted separately.

Haarlemmermeerpolder

Per crop and in the controls as well, fewer species groups occur than in the other areas. It was later found that one of the controls was not all that suitable: one of the banks had been treated with herbicides in early June. Fig. 5.4 shows, however, that the number of groups present in this ditch does not differ from the other controls. In the wide ditches there appears to be a difference between the control and the crop. Here, however, only one control was sampled, so that the results can be too accidentally based. The differences are caused by beetles and semi-aquatic bugs i.a.

In the narrow ditches there appears to be no difference between crop and controls. Two conclusions are possible: there is no effect by the crop and/or the pesticide use, or the sampling method is not suitable for showing this effect. Regarding the latter it can be noted that in a large-scale agricultural area such as the Haarlemmermeerpolder it is difficult to find unaffected controls. The controls used, with seven species groups each, can be characterized as low (number of species groups). In earlier sampling rounds, it had been attempted to establish a correlation between pesticide use on the field and occurrence of organisms groups in the ditch. Here too, no significant correlations were found. It was found though that in a ditch bordering a field with "60%" pesticide use (see: section 4.2) the most organisms were encountered.

Aalsmeer
Many species groups are present in the ditches around Aalsmeer, especially in the controls. This is probably partly caused by the area being relatively heterogeneous. Consequently, it was not hard to find controls here. When comparing the various crops, it would seem that specially the wide greenhouse ditches have a poorer fauna. Thus, flatworms, beetles and springtails hardly occur in greenhouse ditches, while in the wide greenhouse ditches leeches, amphipods and mites do not or hardly occur either.

The narrow reference ditches also have a richer fauna than the arable field ditches. The crops hardly differ among themselves though. For the narrow ditches, the differences cannot be ascribed systematically to specific species groups.

North-East Polder
In the North-East Polder no negative effect of the crops on fauna composition was found. In the wide water courses even more species groups occur in the arable field ditches. Regarding the narrow ditches it should be noted that one of the narrow controls had been cleaned shortly before the sampling (probably a few days). It was not practically possible to select a better control in short order, so that this ditch was included anyway.

In the first sampling rounds in the North-East Polder, orientingly ditches were sampled bordering on a market gardening field, a flower field and a fruit field. A poorer fauna composition was found in the flower field. Further research is desirable on the growing of flowers anyway.

5.2.2.4 Comparison in time (step 4)

The results of all sampling rounds together have been summarized in Annex 5.6. The annex provides the following picture: the differences in occurrence of the species groups between the crops are relatively constant over time, although looking at one moment in time incidentally leads to another conclusion. The cases in which the sampling at other times provides a clear complement to the results of the third sampling round (September 1988) are qualitatively indicated below:

- In the earlier rounds, mites do differentiate between the crops: they occur less in wide arable field ditches and in narrow potato and beet ditches than in the controls;

- water fleas occur less in the narrow potato, beet and greenhouse ditches than in the controls;
- isopods occur in the wide ditches mainly in the controls.

5.2.2.5 Pesticide contents

Cholinesterase inhibition

In practically all samples demonstrable quantities of cholinesterase-inhibiting agents were found (see: Annexes 5.2 and 5.3). The highest values were measured in two narrow greenhouse ditches in Westland. Here, in the water of one ditch, in August 22.4 µg/litre and in September 18.4 µg/litre was found, and in the second ditch 3.5 and 21.6 µg/litre respectively (the IMP-norm is 0.5 µg/l). In another narrow greenhouse ditch, in August alone a relatively high concentration was measured (6.4 µg/l), while in September a wide control ditch in this area turned out to contain relatively many cholinesterase inhibitors. Values of more than 3 µg/l were measured in a control ditch and a wide arable field ditch (with a drainage function for a greenhouse area) in the vicinity of Aalsmeer. No correlations were found, however, between cholinesterase inhibition and the occurrence of species groups.

Earlier too, specifically in the Westland, high concentrations were measured sometimes even up to 2500 µg/l1 (Delfland Polder Board, 1987). The concentrations found by us are in good agreement with the values measured by the Delfland Polder Board at the same points in 1987 (see: 5.3.2). Compared with these, in the North-East Polder the West-Overijssel Water Purification Board found very low concentrations (1988); the norm for the basic quality of surface water was nowhere exceeded here.

That the occurrence of high contents of cholinesterase-inhibiting substances is strongly related to the presence of greenhouses turns out to correspond to the use of these agents: it was found that in greenhouse market-gardening cholinesterase-inhibitors are indeed used in much greater quantities than in arable field agriculture (see: Table 5.6).

	arable fields			greenhouses					
	potatoes	s.bts	wheat	lettuce	tomato	cucumb.	pepper	roses	mums
tot. agents	15	5	2	10	12	16	13	87	220
chol.inhib.	-	-	0.2	2	2	7	9	27	47

Table 5.6 Use of chemicals (total dosage and dosage of cholinesterase-inhibiting substances in kg/hectare.year) in a number of crops in arable field agriculture and greenhouse market gardening; the data refer to the Province of South-Holland; - = negligible (source: Berends, 1988).

It is still not clear along which route the substances get into the water. Practically all greenhouses in the polder studied in Westland are hooked up to a central drainage system for drainage water, which should

render leaching of pesticides impossible. However, a number of enterprises, some of which are in the vicinity of the sampling points, has not (yet) been hooked up to this drainage system. It is also possible that emission occurs via another route, e.g. via the scrub-down drain, the rainwater conduit, the condensation gutter or the greenhouse cover (van der Linden, 1988).

Chlorinated hydrocarbons
In part of the samples of the 2nd round the concentration of a number of chlorinated hydrocarbons was determined (see: Annex 5.4). In these samples from the regions of Aalsmeer, Westland and the Haarlemmermeerpolder contents were found up to a maximum of 215 ng/l and in all cases the norm for the basic quality (median: 20 ng/l) for surface water was exceeded. Still, here too it was found that there was no correlation between chemicals content and occurrence of species groups.

Dithiocarbamates
Determining the dithiocarbamates turned out to be so problematic that no conclusions can be derived from this (see: Annex 5.5.).

5.2.3 *Discussion*

In this section the methods used (5.2.3.1) and the results concerning the species groups are discussed (5.2.3.2). In 5.2.3.3 we deal with the effect of the crops and in conclusion (5.2.3.4) the other factors are discussed.

5.2.3.1 Method

Catching method
The method used (scoop-net) is not suitable for all groups of organisms. Thus, bigger fish, which react instantly and move fast, are not caught, while bottom dwellers, such as worms and bivalves are sampled not very representatively. However, the method is suitable for most species groups, especially when also the various micro-habitats are sampled (see also: Beltman, 1983).

Number of sub-samples
In a field test, only those groups can be included which can be representatively sampled with a limited number of scoops. For most groups, five sub-samples is sufficient. Even with 5 sub-samples, however, it occurred that only one specimen of a group was caught while it is present in the ditch. For this problem, using a minimum occurrence limit can be a solution.

Controls and number of duplications
It was found to be very important to sample a number of good controls. Only then can it be proven that the absence of groups is correlated with the use of pesticides. Two controls and per region and per ditch width appears to be too few here, in view of the variation spread occurring among the controls, and the risk that controls have to be dropped. Four controls will give more certainty and are probably sufficient; we shall come back to this in a subsequent research phase. The number of duplications depends somewhat on the sampling objective. When it concerns the

comparing of crop groups, the same thing applies as for controls. When, however, it concerns an indication of the side effects in an individual ditch, then this ditch can very well be compared to the controls for the area concerned.

Time
Cleaning of the ditches was not included in the research as a factor. The composition of the fauna in newly-cleaned ditches gave a clearly poorer impression. Most ditches are cleaned out once a year, in the fall. This problem can probably be avoided by doing the sampling earlier, say at the end of August.

Identification
For this research, to meet the requirements for a field test (easy to carry out), a relatively high taxonomic level was maintained. For a number of groups, which do not differentiate at this level, it probably makes sense to use a lower level. It should be remembered here, that further distinctions will lead to lower numbers. Therefore, this makes sense only for groups occurring in greater numbers, such as oligochaete worms, leeches, water fleas, copepods, mayflies, corixid bugs, mosquito larvae and snails.

Method of elaboration
In this study, we chose to take many samples in a short time. Because of the classification, using a multivariate statistical technique was not possible. In a subsequent phase, a method will be worked out with which the effect of various factors can be separated better. At the moments, possibilities for this appear to be: numerical sampling, ordinal sampling in which, by means of a system of characteristic and sensitive species, statements can still be made, or possibly an intermediate form, in which only the species(groups) considered relevant are sampled numerically.

5.2.3.2 Species groups

Differentiating species
For flatworms, leeches, isopods, mayflowers and mites, differences are found among the crops. However, these differences are not found in all three sample rounds. Further study of the causes of this is desirable. Springtails, semi-aquatic bugs and beetles appear to be suitable without further ado for a field test in the form of inventories. Amphibians are found most in the greenhouse ditches; possibly the bank biotope plays a large part here. In arable fields it generally is a matter of bare and steep banks, while in the green house areas the banks are often flatter and have more growth. It is striking that the species groups living on the surface of the water differentiate so greatly. As the surface organisms are dependent on the surface tension of the water, possibly chemicals lowering the surface tension play a part.

Non-differentiating species
A number of groups (oligochaete worms, water fleas, copepods, ostracods, corixid bugs, mosquito larvae, snails and fishes) occur almost everywhere in large numbers. For these groups, possibly it makes sense to identify them further. A number of groups (amphipods, dragonflies and back-swimmers) occur so little that they appear not very well suited for use in a field test. Amphipods occur in large numbers in (formerly) peat pasture

ditches. The causes of the absence of this organism from the arable field and market gardening areas should be studied further. Also, it should be noted that only occurrence has been examined; it is probably possible to also involve other aspects, such as size, condition and reproduction in the study. Research on jaw abnormalities in mosquito larvae (see: Faasen, 1988) has shown that sub-lethal effects can also be observed in the field.

When these results are compared with data from others, the following is noticeable: In 1987 too, in Westland, flatworms, isopods, water mites and beetles are mentioned as possibly differentiating groups (Working Group "Effects ....", 1988). Mayflies and bugs are mentioned as well; within our study these groups do not differentiate. Concerning these groups we did have the impression though that it concerned only a few species, which occurred in large numbers, however. Thus, using a lower taxonomic level can lead to more differentiating here too. Caddis-worms too are mentioned as possibly differentiating; in all ditches together we only found a few specimens. The question is therefore whether the effects in arable land and market gardening areas are so great that caddis worms cannot (no longer?) occur here. Also, in studying ditches near orchards (see: Caspers & Heckman, 1981; Heckman, 1981) a strong decrease was found in beetles and mites. In addition, a decrease in caddis, bugs, dragonflies and mayflies is observed as well.

5.2.3.3 Crop

Although significant differences were found in only a limited number of cases, it is striking that more groups occur in the controls than in the other ditches. This can probably be attributed to the use of pesticides. It is, however, also possible that other factors too, such as fertilising e.g., also differ.

The results of the crop ditches can be interpreted in two ways: on the one hand it would appear that in these ditches, in spite of some reports otherwise, there is still always a certain minimum of fauna present; on the other hand, a number of groups are regularly absent from these ditches. When the various crops are compared among each other, it is noticeable in the first place that potato crops in general give a poorer composition of the fauna. For the greenhouse ditches it is noticeable that here, specifically in the first sampling rounds, there is a poorer fauna composition; in September, however, this difference from the other crops is smaller.

5.2.3.4 Other factors

Width, chloride and phosphate

Among the secondary factors measured, especially the width of the ditch and the phosphate content appear to be of importance. With respect to the width, an overall distinction between wide and narrow ditches appears to be sufficient. Correlations are found among species groups and phosphate content; to prevent an interference effect factor from this factor it has to be included. The chloride content appears to be mainly important to water fleas. When the sampling is aimed at water fleas it is recommended to examine the effect of the chloride content.

Vegetation cover
In the 3rd sampling round the vegetation cover was also included, per scoop. It was found that even in lengths of ditch without vegetation, and under very dense duckweed cover, still a few specimens of a group were caught. Because the scoops with 0 and 100% cover do incidentally deviate from the other sub-samples, for comparable sampling we advise to take samples in as many habitats in a ditch as possible.

Pesticide contents
No correlations were found between the cholinesterase inhibition and occurrence of species groups. It should be remembered here that high contents were measured only in a few ditches in Westland. Possibly other groups of chemicals do play a part. Measurements of pesticides remain required, however, to be able to establish possible correlations. The ditch bottoms were not investigated, but possibly they give a more stable impression of the stressing of the surface water with pesticides. In the bottom materials, the pesticides content can be many times higher than in the surface water (see: ZWO, 1988). In how far these contents can affect the presence of species groups will certainly have to be examined further.

5.3 Bio-assays

In connection with the field research bio-assays were done with organisms from the aquatic environment. In the lab, and while excluding as many interference factors as possible, the toxicity was investigated of water collected at the same places the fauna sampling was done. It is internationally customary that, with substances where toxicological risks can be expected for the aquatic environment, LC-50 tests are done with water fleas Daphnia spec. Usually Daphnia magna is used for this. A number of water fleas is added to a test vessel, in which there is a known concentration of the substance to be examined. After some time the mortality which has occurred in the vessel is determined, and compared to the mortality in a control vessel (blank). In this chapter we will examine whether this method can be adapted to tracking down toxic concentrations of pesticides in surface water.

Objectives here are:
- Testing the suitability of bio-assays as a method for detecting side effects of pesticides in the field.
- Checking bottlenecks encountered earlier in this chapter.

In 5.3.1 the methods are explained, followed by listing the data obtained (5.3.2), which are discussed in 5.3.3.

5.3.1 Methods

Water samples for the bio-assays were gathered during 3 rounds at the same points the fauna was sampled as well (see: Table 5.3). Most of the bio-assays were done with water fleas. Reasons for this were: these organisms, as plankton-eaters, have an important place in the aquatic ecosystem, they have a high sensitivity for many water-polluting substances, and internationally seen, much experience with water fleas has already been gained. The water fleas were obtained, in the first instan-

ce, from an aquarium shop. In a pre-experiment, they were found to be well able to survive in beakers with ditch water. Because, however, the condition of these water fleas turned out to be not optimum (see: results of first sampling round) and in addition several species were mixed up together, in later phases of the research Daphnia magna raised at the DBW/RIZA were used. Also, these were all under 48 hours old.

The bio-assays were carried out as much as possible in accordance with NEN-regulation 6501 (NEN, 1980). Standard bio-assays with Daphnia magna are done in duplicate with 25 water fleas on 250 ml of water. A standard 'water flea water' is used for this, to which the substance to be tested is added. Our research was set up differently; we used water from the field. Lake IJssel water was taken as the blank, as is usual at DBW/RIZA as well. Also, a number of control ditches from the test areas was included. To limit the amount of work, the bio-assays were done in duplicate with 10 water fleas per 250 ml of water.

According to the NEN-regulations it is not necessary to add food in the acute toxicity tests with Daphnia magna. However, algae populations will occur naturally in the surface water collected. The possibility exists that (in the long term) differences between the samples in the numbers of algae will begin to affect the survival of the water fleas. During the first series of tests it was found that after 48 hours no significant difference existed in survival of the water fleas in samples filtered over glass fibre and non-filtered samples (see: Annex 5.7). Still, after 140 hours growth and reproduction of the water fleas in non-filtered water appeared to have been greater than in the filtered water. As the tests were aimed at the effects after 48 hours, the samples were not filtered in the further tests. All samples were put through a (tea) sieve though, to prevent organisms from coming along in the water and interfering with the tests.

In part of the bio-assays water mites and isopods were used as well. These groups came to the fore from the first fauna sampling as possibly suitable indicator organisms for the presence of pesticides in surface water (see also: 5.2). The use of isopods as bio-indicators is experimented with elsewhere as well, on a small scale (Holdich & Tolba, 1981; Tolba & Holdich, 1981; Beurskens, 1986; Romijn, 1988; oral comm. Leeuwangh). Bio-assays with water mites were done with the species Unionicola minor and Mideopsis orbicularis. Of the first species, 5 and 10 specimens respectively were added to 250 ml of water, 4 specimens of the 2nd species (no duplicate). The water mites were supplied by the commercial firm Aquasense and had been caught by means of a light trap in the Maarsseveen Pond (see: Davids et al., 1980). Bio-assays with isopods Asellus aquaticus were done in duplicate with 5 isopods per 500 ml of water. These had been caught in a ditch in the immediate vicinity of Leiden. Water from this ditch was also taken, as a blank. The water mites and isopods were not given extra food during the tests.

Besides the pesticide content, other factors too can affect the survival of test organisms in the bio-assays. One can think here of the oxygen, and chloride content, and of the pH of the water. To exclude, as much as possible, mortality as a result of lack of oxygen, all samples of August and September were first aerated for a few minutes. The pH and chloride content of these samples were also determined. Of the September samples the phosphate content was measured as well.

To get somewhat more certainty about the sensitivity of the various species used relative to one another, a control experiment was done, in which the various species were exposed to water with various concentrations of parathion.

5.3.2 Results

In 5.3.2.1 the results from the 1st sampling round are given, and in 5.3.2.2 those from August and September. In 5.3.2.3, on the basis of various measured physical-chemical factors, it is examined what the most probable cause is of the mortality found.

5.3.2.1 First sampling round: June - July

On June 28 water samples were taken in the vicinity of Aalsmeer, and on July 5 in the North-East Polder and Westland. These different sampling sites turn out to be of importance for the interpretation of the results ultimately obtained.

number of sampling point and region	high mortality
control	
2. Aalsmeer	*
arable field	
3. Aalsmeer	*
greenhouses	
7. Aalsmeer	**
17. Westland	**
22. Westland	*
flowers	
26. North-East Polder	*

Table 5.7 Sampling points of 1st bio-assay round (June-July) where high mortality occurred among the water fleas; * = high mortality as compared to blanks, ** = very high mortality as compared to blanks.

In the experiment on June 28 the mortality in the blank water from a ditch deemed "clean", in the vicinity of Leiden, was found to be 15%. The mortality among the water fleas in water from the same ditch on July 5 was much higher, however (80%!). In water from other locations as well, supposed to be able to serve as blanks, the mortality on July 5 was high to very high. Consequently, the data from June 28 and 5 July cannot be directly compared. Therefore, a qualitative approach was adopted for this 1st round: it is only indicated at which sampling points a (clearly) higher mortality occurred than in the blanks. The results have been shown in Table 5.7 (for a complete overview see: Annex 5.7). This table shows

that high mortality occurs in the greenhouse areas of Aalsmeer and Westland. In the water from a wide arable field ditch near Aalsmeer and from a ditch bordering on a gladiolus field in the North-East Polder there also was increased mortality relative to the controls. In a grassland ditch from the Bovenkerker Polder finally (vicinity of Aalsmeer), selected as a control, the mortality percentage was higher than expected.

5.3.2.2 Second and third sampling rounds: August and September

On August 16 and 17 and September 19, 20 and 21 water samples were taken in the North-East Polder, Westland, The Haarlemmermeerpolder and the vicinity of Aalsmeer. First, the results of the bio-assays with water fleas are discussed. Then we deal with the results of the tests with isopods and water mites. Finally, correlations are established between the bio-assays and the physical-chemical parameters measured.

Water fleas

In Table 5.8, the mortality percentages of water fleas after 48 hours in the samples of August and September are compared to the June-July samples taken at the same spot. The use of water fleas of different origin and adapting the method used had as a result that in August and September no mortality occurred any more in the blanks and in a large part of the controls. Mortality was hardly measured in water from most of the other sampling points either. High mortality percentages are measured (again) in water from narrow greenhouse ditches in Westland and in the water from the wide arable field ditch near Aalsmeer. Further, mortality occurs in another wide arable field ditch near Aalsmeer in September while in the aforementioned Aalsmeer control ditch mortality is measured only in August The indication obtained in the 1st round, that the water fleas do not survive in water from the flower ditch in the North-East Polder, is not confirmed.

Taken overall, the 3 bio-assay rounds show a number of striking similarities. At 5 locations high mortality percentages were found more than once. Especially a number of ditches in the Westland greenhouse area come to the fore. In water from 2 of these locations, in bio-assays in 1987 too (see: Table 5.10) several times mortality of water fleas had been found (Working Group "Effects ....", 1988). In the water from a greenhouse ditch and some wide water courses near Aalsmeer too, water fleas cannot survive very well. It is striking that in almost all of these cases a large part of this mortality had already occurred within 24 hours (see: Annexes 5.8 and 5.9).

In August, parallel with the DBW/RIZA water fleas, the survival of the water fleas hailing from the aquarium shop was also examined. The results are shown in Annex 5.8. This shows that in many cases mortality among the commercial fleas is higher than among those from DBW/RIZA but not as high as in the June-July bio-assays. At 3 locations mortality among the commercial ones is clearly higher than in the blanks, viz. in a Westland greenhouse ditch, and in the (wide) arable field ditch and the control ditch near Aalsmeer. With this, the data obtained with the DBW/RIZA water fleas are confirmed.

location/sampling poin	june-july	august	september
blanks			
10.IJsselmeer	-	0	
31.Markermeer			0
1. Leiden	-		0
controls, narrow			
32.Aalsmeer			0
33.Aalsmeer			0-10
34.Delfland			0
35.Delfland			0-10
36.Haarl.meerpolder			10-20
37.Haarl.meerpolder			0
38.North-East polder			0
39.North-East polder			0
controls, wide			
2. Aalsmeer	+	0-60	0
40.Aalsmeer			0
41.Delfland			0
42.Delfland			0
43.Haarl.meerpolder			0
12.North-East polder	-		0
13.North-East polder	-		0
potato, narrow			
44.Aalsmeer			0
45.Aalsmeer			0-11
46.Haarl.meerpolder			0
29.Haarl.meerpolder		0	
47.Haarl.meerpolder			0
48.North-East polder			0
49.North-East polder			0-10
sugar beets narrow			
52.Aalsmeer			0-10
53.Aalsmeer			0
54.Haarl.meerpolder			0
55.Haarl.meerpolder			10-18
56.North-East polder			0-10
57.North-East polder			10-20
wheat narrow			
4. Aalsmeer	-	0-10	0
5. Aalsmeer	-		0
59.Haarl.meerpolder			0
60.Haarl.meerpolder			0
16.North-East polder	-	0	0
61.North-East polder			0-10
arable field, wide			
50.Aalsmeer			0
28.Haarl.meerpolder		0-20	0
51.North-East polder			0
3. Aalsmeer	+	100	10-20
30.Haarl.meerpolder		0	0
58.North-East polder			0-20
62.Aalsmeer			10-50
63.Haarl.meerpolder			0
64.North-East polder			0-36
greenhouses, narrow			
65.Aalsmeer			0
66.Aalsmeer			0
7. Aalsmeer	++	0	0
17.Delfland	++	100	100
21.Delfland	-	60-80	100
22.Delfland	+	100	0
23.North-East polder	-	0	0
greenhouses, wide			
67.Aalsmeer			0
68.Aalsmeer			0
20.Delfland	-	0	0
69.Delfland			0
fruit, wide			
24.North-East polder	-	0	
flowers, narrow			
26.North-East polder	+	0	
market gardening, narr			
27.North-East polder	-	0	

Table 5.8 Water flea mortality percentages in bio-assays after 48 hours; when duplicates differ, both % are shown. For comparison, the qualitative results of round 1 are also shown; only the samples examined have been filled out; - = no increased mortality, + = moderately increased mortality, ++ = greatly increased mortality relative to the controls.

However, in the two other ditches in Westland, where the DBW/RIZA water fleas largely died, mortality of the water fleas from the aquarium shop was not greater than in the control. Thus the results of tests with water fleas coming from different sources, must be composed cautiously. It is advisable, in the Netherlands, to use DBW/RIZA water fleas to reach a uniform standardization.

When these results are compared with those from the fauna sampling, we find that even in ditches with the highest mortality percentages water fleas do occur. A number of explanations can be raised for these (apparently?) contradictory research results. Perhaps the species used in the test, Daphnia magna, is more sensitive to pesticides than other species, so that a further classification into species in the field could give a clearer picture. Possibly the water fleas used in the bio-assays are more sensitive than the fleas living in the field. More explanations can be given, such as e.g. a short time stress or a last immigration of water fleas. It seems improbable though that the time period plays a part; in earlier sampling too (even in 1987), continuously, high mortality percentages were found. Thus, further research is desirable to find out the reasons for these discrepancies.

Water mites and isopods

As experiments with isopods as test organisms in bio-assays are only being done on a small scale as yet, and nothing is known as yet about the use of water mites for this purpose, a standard procedure for bio-assays with these species does not exist. Thus, it is not known at what moment the mortality should be measured. With Daphnia magna a period of 48 hrs is considered standard, while for other crustaceans and insects 96 or 120 hrs is customary. Thus, for isopods and water mites, in addition to mortality percentages after 48 hrs, those after 24, 120 and 192 hrs were also measured.

In the water mite Mideopsis orbicularis no mortality was observed at all, even after 13 days all animals were still alive. In the sample from an Aalsmeer greenhouse ditch this species did show abnormal behaviour though, the animals were not as active as in the other samples.

Fig. 5.5 shows how after various times the distribution of mortality percentages of Unionicola minor looks, by crop. The figure shows that after 48 hrs mortality occurred in only 3 samples: in water from a (blank) ditch in the vicinity of Leiden (5%), a beet ditch in the Haarlemmermeerpolder (22%) and an Aalsmeer greenhouse ditch (90%). In this ditch, no mites were found in the field either. After 120 hours mortality in this latter sample has increased to 100%. In a number of controls too, some mortality has occurred by then, while in the potato, beet and wheat ditches hardly any mortality has occurred at all yet. After 192 hours too, mortality has occurred in relatively more controls than in the other ditches. However, the mortality in the controls always stays limited to one of the duplicates (see: Annex 5.10). In a greenhouse ditch in Westland and a potato ditch in the Haarlemmermeerpolder, mortality does occur in both duplicates.

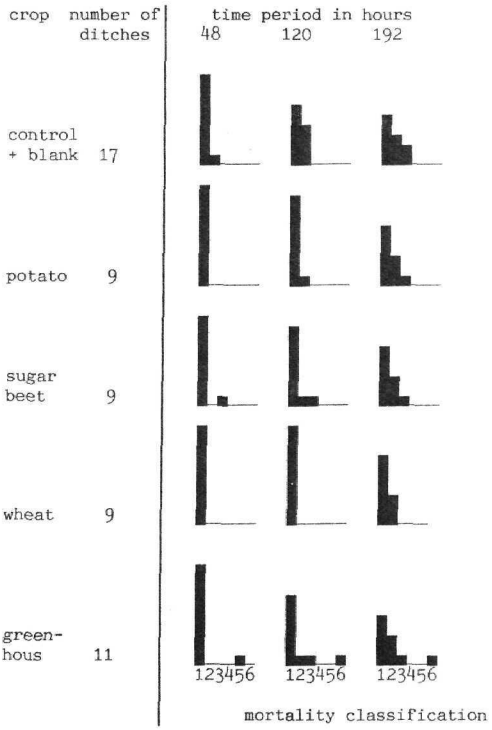


Figure 5.5 Relative distribution of mortality percentages of Unionicola minor by crop after different periods. The percentages after 24 hrs are equal to those after 48 hrs. Per ditch, always the average value for both duplicates was taken. The blanks form one group with the controls. Per crop, all ditches were placed in one of six classifications of mortality percentages: 1 = 0%, 2 = >0-20%, 3 = >20-40%, 4 = >40-70%, 5 = >70-100% and 6 = 100%. These classifications form the horizontal axis. The vertical axis shows the percentage of the total number of ditches in a certain crop within each classification (smallest square shown = 10%).

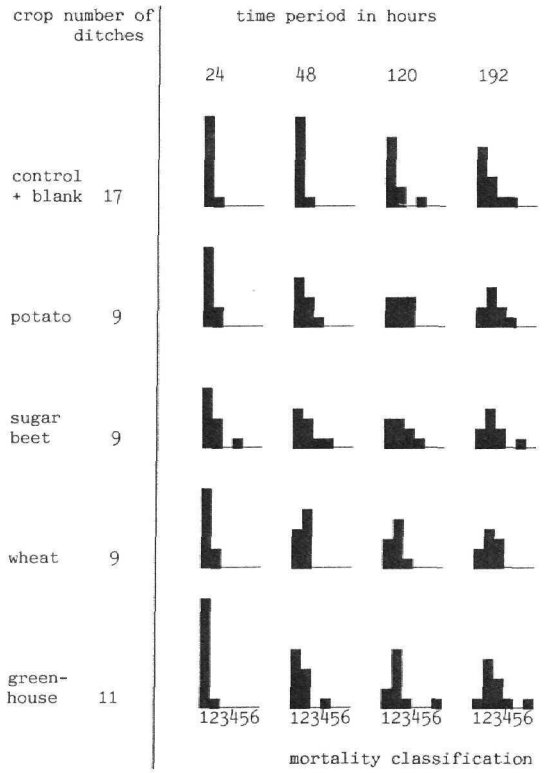


Figure 5.6 Relative distribution of mortality percentages of Asellus aquaticus by crop after different periods. The percentages after 24 hrs are equal to those after 48 hrs. Per ditch, always the average value for both duplicates was taken. The blanks form one group with the controls. Per crop, all ditches were placed in one of six classifications of mortality percentages: 1 = 0%, 2 = >0-20%, 3 = >20-40%, 4 = >40-70%, 5 = >70-100% and 6 = 100%. These classifications form the horizontal axis. The vertical axis shows the percentage of the total number of ditches in a certain crop within each classification (smallest square shown = 10%).

In Fig. 5.6 the mortality percentages are shown for the isopod Asellus aquaticus (see also: Annex 5.11). This figure shows that after 48 hrs already, a difference can be seen between the controls and the other ditches. In the controls, no mortality has yet occurred in a larger proportion of the samples after 48, 120 and 198 hrs. The highest mortality percentages are found in a greenhouse ditch near Aalsmeer and a beet ditch in the Haarlemmermeerpolder. No isopods occur in the Aalsmeer ditch, they do occur in the Haarlemmermeer ditch though. In this latter, however, no high pesticide contents are found. Among the controls, a relatively high mortality is found in the Markermeer water (in Lake IJssel).

Comparing the results for various species

Table 5.9 shows for which ditches, on the basis of the bio-assays, a suspicion can be harbored with respect the presence of toxic substances. It is striking that in the two Westland greenhouse ditches, where great mortality occurred among the water fleas, hardly any mortality occurred among the mites and isopods. The reverse occurs in an Aalsmeer greenhouse ditch, in which all mites and isopods died, while with the water fleas high mortality was measured only during the first round. In the beet ditch in the Haarlemmermeerpolder too, relatively high mortality occurred among mites and isopods without water fleas dying. This causes us to suspect that in these latter two ditches there were substances in the water other than in the Westland greenhouse ditches, concerning which the test organisms differ in susceptibility. In the other cases, the high mortality among water fleas occurred only during the first and/or second rounds, and no or only limited mortality was found among mites and isopods either.

sample number, location	round	1	2	3		
	crop	D.m.	D.m.	D.m.	U.m.	A.a.
2 Aalsmeer	ctrl	+	30	0	0	10
3 Aalsmeer	beet	+	**100**	15	5	20
54 Haarl.m.p.	beet			0	22	**60**
7 Aalsmeer	gr.h.	++	0	0	**100**	**100**
17 Westland	gr.h.	++	**100**	**100**	10	10
21 Westland	gr.h.	-	**70**	**100**	20	10
22 Westland	gr.h.	+	**100**	0	0	10

Table 5.9 Mortality percentages among different species in the bio-assays for those sampling points where high mortality occurred in one or more species; water fleas (D. magna - D.m.) after 48 hrs, mites (U. minor - U.m.) and isopods (A. aquaticus - A.a.) after 120 hrs.

5.3.2.3 Correlations among mortality in bio-assays and physical/chemical parameters

Annexes 5.2 to 5.4 show the results of the measurements done of some physical/chemical parameters of the water samples used for the bio-assays. Below we go further into the correlations which may possibly be established among the measured values and the mortality percentages in the bio-assays.

Acidity
All measured values lay within the range 7.31 - 8.62 (see: Annexes 5.2 and 5.3). This (slight) variation in the pH-values between the locations does not constitute an explanation for the mortality observed in the bio-assays. During the 2nd round, the pH was also determined in a few bottles after 48 hours. In all cases the pH had increased by an average 0.30. Thus, the mortality found was not caused either by possible acidification of the water during the experiment.

Chloride content
According to Leentvaar (1978) Daphnia magna occurs in water with a chloride concentration of up to 6 g/l. The values measured by us are far below this (see: Annexes 5.2 and 5.3). Thus, in the bio-assays with Daphnia no correlation could be established between chloride percentage and mortality. U. minor and A. aquaticus too were found to we well able to survive at high chloride contents.

Phosphate
No correlation was found between phosphate content (see: Annex 5.3) and mortality in the bio-assays. It is striking though that a number of times high contents go together with high cholinesterase inhibitor content. Possibly this is caused by emissions and/or leaching of water from greenhouses.

Chlorinated hydrocarbons
Although the basic quality norm was exceeded on a large scale, the values found (see: Annex 5.4) still remained well below the LC50 values for invertebrates; for some fish, the endosulfan concentration found can definitely be toxic. No correlation was found among the chlorinated hydrocarbon content and the mortality in the bio-assays.

Cholinesterase inhibitors
In general these are very toxic to aquatic organisms (see: Annex 4.1). Thus, for parathion (one of the most toxic agents in this group) an LC50 value is given for water fleas of 0.37 µg/l (Verschueren, 1983). It is consequently not fanciful to suppose a connection between the cholinesterase inhibitor content and water flea mortality in the bio-assays (see: Fig. 5.7).

It is clear that at cholinesterase inhibitor contents of over 3 µg/l often high mortality occurs. The picture is not quite unequivocal though: at two points with concentrations of over 5 µg/l e.g. no mortality occurs at all. A possible explanation may be that different cholinesterase-inhibiting substances are present, which differ in toxicity and/or biological availability, but which are all included in measuring the sum (total) parameter. At two sampling points in Westland the Polder Board also did bio-assays in 1987 (see: Table 5.10). This table shows that the

results are in good agreement, and that thus it is a matter of a fairly permanent stressing of this water.

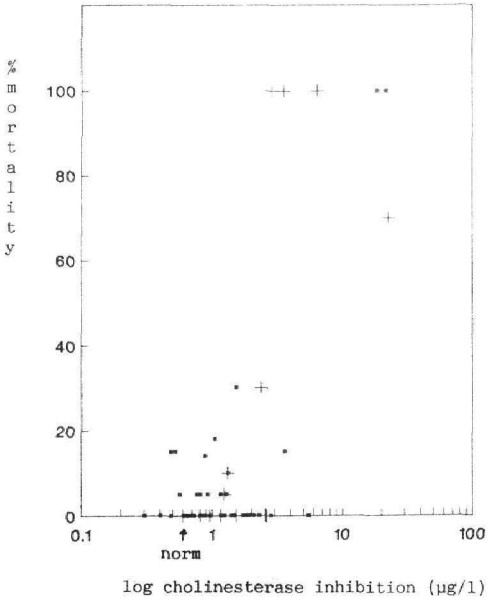


Figure 5.7 Mortality percentages of water fleas in the bio-assays plotted against the cholinesterase inhibitors content of the water concerned. Mortality in the 2 duplicates is always averaged. The bio-assays of August (+) and those of September (.) are represented by different symbols.

LC 50 values for water mites and isopods are probably considerably higher than for water fleas. Thus, in the case of parathion, for Asellus brevicaudatus (an isopod species which does not occur in The Netherlands) a 96-hour LC50 is given of 213 and 600 µg/l (Mayer & Ellersieck, 1986). This would explain why, in the samples with high concentrations of cholinesterase inhibitors, mortality among mites and isopods hardly occurred. Mites in the field too are not hindered in their occurrence by high cholinesterase-inhibitor contents. The literature (Tolba & Holdlich, 1981) shows that isopods too can survive well in polluted water, but that propagation and size of the animals are affected. Perhaps this is one of the causes of the absence of this group; also, agents from groups other than the cholinesterase-inhibitors might play a part. It should be remembered though that isopods were found in only a limited number of ditches, so that obviously other factors too affect their occurrence.

year	1987						1988		
date	18/8	15/9	29/9	13/10	10/11	8/12	5/7	16/8	19/9
% mortality D11	100	20	100	0	100	100	-	70	100
chol. inhib.	15,6	14,3	20,3	18,0	7,6	12,6		22,4	18,4
% mortality D12	58	84	0	0	0	0	+	100	0
chol. inhib.	5,6	<0,1	0,7	<0,1	0,1	1,3		6,4	1,4

Table 5.10 Mortality percentages of water fleas in bio-assays and cholinesterase inhibitors contents (in µg/l) at various times in 1987 (source: Working group 'Effects ....', 1988) and 1988 (our own data).

Parathion

To verify the results found above, a bio-assay was done, in which the test organisms were exposed to Markermeer water to which various concentrations of parathion had been added (see: Annex 5.12). Point of departure for the concentrations used was the LC50 value, known from the literature (Verschueren, 1983) for Daphnia magna. As it was supposed, ahead of time, that water mites and isopods would be less sensitive, a higher concentration was used for these species. The results are:

- even at the lowest concentration (0.18 µg/l) all of the water fleas were dead after 24 hours;
- the water mites Mideopsis orbicularis survived at all concentrations (0.18, 0.37 and 0.74 µg/l);
- the water mites Unionicola minor showed an increasing mortality percentage at increasing dosages;
- for the isopod, at the higher concentrations (200 and 600 µg/l) all of the animals were dead, while at a lower concentration (0.37 µg/l) half were still alive but hardly moved.

The picture arising from this agrees well with what the bio-assays showed: water fleas are very sensitive, isopod and mite Unionicola minor less sensitive and Mideopsis orbicularis not at all sensitive to the presence of toxic substances in the water. It is remarkable that all species (with the exception of Mideopsis orbicularis) react strongly to concentrations of parathion which are far below the cholinesterase inhibitors contents at which they were well able to survive in the bio-assays. The fact that in the water to which the parathion was added, there were already cholinesterase inhibitors occurring (Markermeer water: 0.64µg/l) cannot constitute an explanation for this. Possible explanations are though:

i) the agents found in the ditch water are less toxic or are taken up by the organisms to a lesser extent (lower biological availability) than parathion

ii) because of the low solubility of parathion, the concentrations at some spots in the test vessels (viz. at the bottom) were possibly higher than the average concentration.

Dithiocarbamates

Determining the dithiocarbamates produced such great method problems (see: Annex 5.5) that the results could not be used for interpretation. Other research (Wegman & Hofstee, 1981; Wammes et al., 1986; ZWO, 1988) has shown that dithiocarbamates hardly ever occur in surface water in measurable quantities. If anything is measured at all, the values are in the tens of µg/l and are far below the LC50 values for dithiocarbamates for Daphnia magna (van Leeuwen, 1986): for mancozeb, maneb and zineb, 48 hour LC50 values were measured of 1300, 1000 and 970 µg/l respectively.

5.3.3 Discussion

In a number of cases it is possible to relate the mortality in the bio-assays to high pesticide contents. This applies to the mortality of water fleas in a few ditches in Westland. High concentrations of cholinesterase inhibitors were found here. It is, however, not a matter of an unequivocal correlation between cholinesterase inhibitor content and mortality percentage. The high mortality percentages of water mites and isopods in water from a greenhouse ditch near Aalsmeer turn out to be not correlated with high contents of the group of chemicals examined by us. Possibly other groups of chemicals are involved here.

Regarding the test organisms used, the following was found. Mideopsis orbicularis did not work out well; this species is too insensitive to pesticides and survives where all other species die. Regarding Unionicola minor and Asellus aquaticus it can be stated that the results of the bio-assays with these species show strong similarities to each other and that thus, in further experiments, possibly one of these two would suffice. This in spite of the fact that the two species differ greatly qua their place in the ecosystem (the isopod is a detritus-eater and the water mite is a parasite/predator). The results with these two species do differ greatly from those with water fleas and constitute a good complement to those.

Concerning the usefulness of the method proposed by us, it was found that it works well for detecting toxic concentrations of pesticides in the surface water, provided other mortality factors can be sufficiently excluded. The lack of an unequivocal correlation between cholinesterase inhibitor content and the mortality in the bio-assays shows that for determining the extent of side effects in the field measuring this sum (total) parameter alone does not suffice. The presence of toxic combinations of in themselves not toxic (concentrations of) substances, or of substances which are not or barely measurable, can be established in this way in a quick and unequivocal manner.

In all cases, the cholinesterase inhibition measured in the North-East Polder and in the Haarlemmermeerpolder is relatively low (maximum 2.2 µg/l), also in comparison with measurements of other institutes (a.o. CCRX, 1988). In Aalsmeer a maximum of 5.4 µg/l was measured. In Westland high contents were measured regularly and in several ditches simultaneously.

The bio-assays show that the danger points occur mainly in the greenhouse ditches. The fauna sampling, however, also brings the arable land ditches to the fore as poorer. Possibly sub-lethal effects play a part here.

5.4 Conclusions

The literature shows that the presence of pesticides in surface water can lead to problems for the fauna. Our own field research and the bio-assays have confirmed this; the methods developed during this can make a valuable contribution, in further elaborating and filling-in, to tracking down the side effects of pesticides on aquatic fauna in the field. Big problems are occurring repeatedly in greenhouse market gardening. There are other danger points in the growing of fruit, potatoes and mushrooms. Below, the conclusions are given in greater detail, per approach employed.

Literature

On the basis of the literature, data from experts and incidents, we find that only a fragmentary picture originates of the nature and extent of side effects of pesticides on aquatic fauna in the field. This is in great contrast with attention to this fauna under lab conditions. Further investigation on the side effects in the field appears urgently required, also in view of alarming reports about "ecologically dead" ditches.

Relatively many cases are known of fish mortality caused by pesticides. A striking thing here is, that greatly divergent causes (emissions, but also normal use) have led up to the incidents. The agents which led to acute fish mortality hail specifically from the chlorinated hydrocarbon group (mostly endosulfan). However, fish mortality has also been observed with agents from other groups. In view of its high toxicity, the use of endosulfan involving any risk to the aquatic environment should be strongly advised against.

It can be concluded that in any case the Westland, the North-East Polder and the peat districts are problem areas. These, however, are the only areas where so far, on a fairly large scale, specific pesticide(s) (groups) have been purposely looked at. Consequently, problems can also be expected in other large-scale agriculture areas, such as Zeeland, West-Friesland, the Frisian and Groningen clay areas and North-Western North Brabant. Greenhouse crops too look like being capable of presenting many problems.

Field research

With the (simple and fast) method used, in principle differences in the occurrence of fauna groups among various crops can be detected. We found that a number of groups differentiate already at the high taxonomic level used by us. Other groups are less suitable for this. These latter groups can possibly be divided further. Then, it can still become apparent whether it is a matter of possible effects from pesticides.

In almost all ditches examined, representatives from the various trophic levels were present. Thus, in general, the basic quality is attained for this aspect. On the other hand, however, in a number of cases it was a matter of only a few species or even only one species per trophic level. Also, in a large number of cases, the basic quality limits for cholinesterase inhibition and chlorinated hydrocarbons are exceeded.

The use of pesticides appears to have as a result that certain species groups do not occur in the ditches. Indications were found that specifically narrow greenhouse and potato ditches have a poor fauna. In Westland and in the environs of Aalsmeer fewer fauna groups are found in the crop-growing (area) ditches than in the controls. In the Haarlemmermeerpolder relatively few species groups were found in the controls either; in the North-East Polder crop-growing appears to have less of an effect.

Bio-assays

The quality of water from the greenhouse area ditches in Westland was so poor that water fleas did not survive in bio-assays. In a greenhouse ditch from Aalsmeer water mites and isopods were not able to survive. In Westland high concentrations of cholinesterase-inhibiting substances were found; these concentrations were correlated with the mortality of water fleas. No correlation was found with the mortality of isopods and mites. The mortality cannot be explained on the basis of differences in some simple chemical variables, such as the chloride content, the acidity, the phosphate content or oxygen content of the water. As all three rounds show that increased mortality occurs in these ditches, it is a matter of permanently poor water quality.

The water flea Daphnia magna lends itself well for detecting high contents of cholinesterase inhibitors. Asellus aquaticus and Unionicola minor appear to be suitable for detecting other (groups of) pesticides. Mideopsis orbicularis turns out to be less suitable for detecting pesticides in surface water, if only the parameter mortality is measured.

6. CONCLUSIONS AND RECOMMENDATIONS

The results of the research described in this report lead to a number of conclusions and recommendations. The conclusions are mentioned in 6.1. For an amplification of the conclusions we refer to the individual chapters. The recommendations are described in 6.2. This final chapter is concluded by a list of attention points for policy (6.3).

6.1 Conclusions

6.1.1 Research in The Netherlands

The research in The Netherlands aimed at side effects of pesticides is housed with various departments and can be encountered within very different research frameworks. A structural solution for the existing lacunae in knowledge by means of specifically oriented research is made more difficult by this; creating specific research programs as a solution for this problem has as a disadvantage in any case that it entails many extra overhead-costs. A solution might be to distinguish between applied and fundamental research and to make available a yearly budget for these, during a medium long period.

Compared with the aquatic environment, relatively little research takes place in the terrestrial environment. The developments in this environment area trail those in the aquatic area, and there is not much of a coordinated approach concerning side effects of pesticides. Possibly the results of a number of fair-sized research programs will bring some improvement here in a few years. So far terrestrial research has been aimed mainly at "useful organisms". Data about other animal groups are gathered much less systematically. Still, research on soil organisms, as part of larger research programs, is getting more attention now; this in contrast with that concerning vegetation-bound organisms. A broadly set up comparative research on usable testing organisms is lacking, and little attention is paid to terrestrial micro-ecosystems or to semi-field experiments.

At the moment, a relatively great deal of research is being done in the aquatic environment. Co-operation among the researchers involved can be qualified as reasonable to good. There is also a goal-oriented approach, in which a large number of aspects is being examined. Also, the coordination of the processing of data about incidents (mainly with vertebrates) and concentration measurements is really getting off the ground. However, effect-oriented field research is lagging behind at the moment, and expansion is desirable, even required. Thus, it is desirable e.g. to research possibilities for the use of existing macro-fauna inventories, and to check whether certain research programs can be adapted so, that the data obtained can also be used for tracking down side effects of pesticides. Fitting the recently demonstrated substances into the measuring programs of the separate water control agencies has still to be done. Up to now, mostly only the chlorinated hydrocarbons are being measured. Finally, more attention will have to be paid to solution-oriented research, aimed e.g. at reducing emissions by agriculture, incl. the suitability of management measures which this entails.

The research on side effects of pesticides via the air on ecosystems and their components has lagged behind. For some time now measurements are being done, but here too, effect-oriented field research is lacking. Direct and indirect effects of pesticides (herbicides, fungicides and soil disinfectants) on vegetation and fungi can well be imagined. The real(istic) chances of these effects occurring, certainly justify a greater research effort in this area.

6.1.2 Registration procedure in The Netherlands

Those interviewed and the participants in the workshop, in a majority by far, were of the opinion that the present registration procedure in general insufficiently brings the side effects of pesticides to the fore. The two most important points of criticism raised were:

1. The use of single species tests and their interpretation. The present single species tests are satisfactory for comparing the agents mutually (ranking) - partly because of their internationally accepted standardization - but the most important disadvantages of these tests are the low representativity of the field situation and the related problematic extrapolation toward the level of biocenosis and ecosystem. Thus, with the single species tests it is not well possible to get an insight into the effects on interactions, such as in food chains e.g. It was therefore proposed to supplement the tests with simple, standardized multi-species tests and possibly a field test, in which then especially the ecological side effects would have to be examined. In any case, too little attention is paid to these latter effects now.
2. The present test organisms: only species which are considered useful from an agri-economic point of view are tested. For the terrestrial environment this normally means only a test with earthworms. Thus, an ecological background is lacking, which shows how the tests are related. In addition, specifically for the terrestrial environment, there are too few test organisms on hand. Also, the test organisms are not selected on the basis of exposure. Improving the present procedure is possible by making more use of functional groups in relation to manner of exposure; furthermore, one may expect that a more balanced weighing of interests in the registration procedure is possible by implementing the concept of multifunctionality (see further: 6.1.3). Regarding the aquatic environment, other interests than agriculture are taken into account, nowadays.

Other points of criticism are, among other ones:

- the too slight attention to inspection and enforcement of adhering to the instructions for use; this applies also to incidents registration and illegal use;
- insufficient input by water quality controllers into the procedure;
- the non-transparency of the registration procedure;
- the not being marketed of more selective pesticides, partly for reason of the high costs involved in registration;
- the lack of integral weighing of alternative crop treatment methods within a certain crop;
- not getting sufficient insight into synergetic effects, and
- not making use of the open literature in the re-evaluation of pesticides now being carried out by the RIVM.

6.1.3 Evaluation of side effects

Up to now, a framework within which the evaluation of side effects of pesticides on terrestrial invertebrates and aquatic fauna can be done, has been lacking. A proposal is being elaborated to make use of the concept "environment quality", general as well as specific, taking the effects on social objectives (national health, usage functions and nature significance) as points of departure.

For the general environment quality, the emphasis is on basal requirements set by specific usage functions, and in addition on proper ecological functioning, also to be motivated from the usage functions. Regarding this latter aspect especially, there appear to be good possibilities. We are thinking here specifically of a number of important ecological processes, such as the breaking down of organic materials, and the regulation of prey animal species by predators. It comes to the fore that side effects occurring on the group of organisms examined can, in principle, very well be used in this way.

Regarding specific environment quality, specifically the requirements set from the agriculture function and nature significance offer good tie-in points for evaluating side effects of pesticides on terrestrial invertebrates and aquatic fauna. Concerning the agriculture-function, special attention should be paid to useful organisms (but not just earthworms and/or bees, but also ichneumon wasps and predatory mites e.g.); concerning the nature significance, attention should be paid to protected - or shortly to be protected - species (incl. vertebrates depending on invertebrates for their food), to species which are important to the protected species, and to other special species, such as flying and/or flower-pollinating species e.g. In section 6.3 the species that deserve attention in this respect are enumerated.

6.1.4 Side effects on terrestrial invertebrates

Literature

In a general sense, it is concluded that on the basis of data from the literature, data from experts and data about incidents for a few species groups (mainly useful organisms) a picture of the toxicity of many agents does exist. The knowledge of side effects in the field is very fragmentary, however. Toxicity data about organisms other than useful ones are often lacking, let alone it being known how these animals, under field conditions, react to pesticides.

For earthworms toxic side effects of pesticides have been encountered in practice with soil disinfectants (specif. fumigants), benzamidazols and carbamates. Populations decrease (very) greatly, and recovery by species such as L. terrestris takes several years. On the basis of the toxicity of this group of agents, it is very well possible that other soil invertebrates are affected as well. In view of the fact that soil-disinfecting is occurring on a very large scale in The Netherlands, these side effects should and must not be under-estimated; they constitute a real threat to a diversity of agro-ecosystem and contiguous ecosystems.

Toxic side effects in the field have also been found in bees; these are mainly caused by organic phosphor compounds. There are also suspicions, however, about certain herbicides. This aspects should be given more attention, also in view of the part bees play in the production function of agriculture.

The occurrence of direct toxic side effects from herbicides on soil invertebrates is not probable. Even a positive effect is possibly (and temporarily?) not excluded, viz. as the result of a greater supply of organic material. Ecological side effects through destruction of habitat or disappearance of host plants will certainly occur in a number of animal groups, such as butterflies. These ecological effects are also expected in other vegetation-bound invertebrates.

Field research

The following conclusions can be derived from the research in the Haarlemmermeerpolder - with the absolutely required prudence, in view of the limited extent of the research. Differences in the invertebrate fauna were found between fields with a different level of pesticide use, even when identification takes place to only the level of orders and families. The greatest differences are found among mites, potworms, cantharids, centipedes and ladybirds (for all groups is valid: smaller numbers at higher pesticide use) and among two-winged and rove beetles (for which applies: greater numbers at higher pesticide use). At higher pesticide levels, springtails occur in the soil in greater numbers, but are also found on (top of) the soil at lower pesticide levels in great numbers. At higher pesticide levels relatively more predators occur, at lower levels relatively more herbivores and detrivores. Within the potato fields, the most individuals and the greatest number of groups are found on the organic farm.

Finally it is concluded that the overview obtained of the seriousness of side effects of pesticides on terrestrial invertebrates probably still has (very?) many lacunae. The information available is so strongly anecdotic and so spread out in time and space, that at the moment, acquisition of a complete picture is not deemed to be really possible. The terrestrial field research has shown that doing fauna samplings to detect side effects of pesticides can make good sense and in any case has turned out to be feasible. It should also be noted, however, that it has not yet become clear whether other parameters, such as sum (total) parameters and ratio-indices cannot also be good indicators of the occurrence of these side effects. The same thing applies to the use of bio-assays; we were not able to use them, at least not (yet) in the terrestrial environment.

6.1.5 Side effects on aquatic fauna

The literature shows that there are indications that the presence of pesticides in the surface water can lead to problems for the fauna. Our own field research and the bio-assays have confirmed this; the methods developed here can, with further elaboration and filling-out, make a valuable contribution in tracking down side effects of pesticides on aquatic fauna in the field. It comes to the fore that repeatedly big

problems occur in greenhouse market gardening. Other critical points are in the growing of fruit, potatoes and mushrooms.

Literature

On the basis of the literature, data from experts and incidents, it turns out that only a very fragmentary picture exists of the nature and extent of side effects of pesticides on aquatic fauna in the field. This is in great contrast with the attention given to this fauna under lab conditions. Further research on the effects in the field appear to be urgently required, also in view of the reports about "ecologically dead" ditches.

Relatively many cases are known of fish mortality caused by pesticides. It is striking that greatly divergent causes (emissions, but normal use too) have led to these incidents. The chemicals which have led to acute fish deaths hail specifically from the group of chlorinated hydrocarbons (mostly endosulfan). However, fish mortality has also been observed with chemicals from other groups. In view of its high toxicity, the use of endosulfan, entailing any risk to the aquatic environment, must be strongly advised against.

Field research

With the (simple and fast) method used, differences in the occurrence of fauna groups among various crops can be tracked down. We found that a number of groups already differentiates at a high taxonomic level. Other groups are less suitable for this. These can be split up further. Then, it can still become evident whether possibly it is a matter of effects from pesticides.

In almost all of the ditches examined, representatives of the various trophic levels were present, so the basic quality is attained for this aspect. On the other hand, in a number of cases it was a matter of only a few species, or only one species even, per trophic level.

The use of pesticides appears to have a result that certain species groups do not occur. Indications were found that specifically narrow potato ditches and greenhouse ditches have a poor fauna. In Westland and the environs of Aalsmeer in the ditches affected by crops fewer fauna groups are found than in the controls. In the Haarlemmermeerpolder relatively few species groups are found in the controls too; in the North-East Polder there are few differences between the crop ditches and the controls.

Bio-assays

The water quality in the greenhouse ditches in Westland was so bad that water fleas did not survive in it. Water mites and isopods could not survive in the water from a greenhouse ditch in the vicinity of Aalsmeer. In Westland, high concentrations of cholinesterase-inhibiting substances were found; the mortality of the water fleas was correlated with these concentrations. No correlation was found with the mortality of isopods and mites. The mortality could not be explained on the basis of some

simple chemical variables. As all three (sampling) rounds show that increased mortality is occurring in these greenhouse ditches, it is a matter of permanently poor water quality here.

The water flea Daphnia magna is well suited for tracking down high cholinesterase inhibitor contents. Asellus aquaticus and Unionicola minor appear to be suitable for tracking down the presence of other (groups of) pesticides. Mideopsis orbicularis turns out to be not that suitable for detecting pesticides in surface water, only if the parameter mortality is measured.

6.2 Recommendations

At various spots in the preceding sections recommendations have already been made, which flow from the research done. A number of recommendations, however, have not yet been mentioned. In the following sections these are elaborated in the form of a few concrete proposals, successively: the registration procedure (6.2.1), a field test for the registration procedure (6.2.2), the incidents registration - to keep "a finger on the pulse" after a chemical has been registered - (6.2.3) and finally an environment quality test (6.2.4).

6.2.1 Proposal for optimising the registration procedure

In 6.1.2 a number of critical points were indicated, in the present registration procedure. Solutions for a number of these are submitted in the following sections. The most important ones are:

1. The lab tests have insufficient prediction-significance for the field situation.
2. A number of aspects are not taken into in the registration procedure, viz.: ecological side effects, synergism of effects, the relationship with chemicals already used on the crop concerned, incidents and illegal use.
3. The registration procedure does not give enough insight for outsiders.

The first two points are inherent to the present setup of the registration procedure. Because mainly lab research is done, problems continue to exist with the extrapolation toward the situation in the field. Consequently it is also very difficult to make pronouncements about ecological side effects and synergetic effects. To solve (part of) these problems, it is proposed to include a field test (for details see: 6.2.2) in the registration procedure. To at the same time increase "insightability" of the procedure (see: 3 above), a (partly) new, staged registration procedure is described in the following, including the field research. Still, it concerns here a first draft, further working out of which is certainly still required. In addition, it should be noted that the proposed procedure concerns an extension of the present procedure, and was not designed to be able to evaluate new and selective chemicals faster and cheaper. In elaborating the procedure further, this should be paid attention to, (i.e.: pesticides that have a greater efficacy or have less side effects). In drafting the new procedure, the following conditions were applied:

a. The chemical for which registration is requested must be better than the ones already registered and in use for the same purpose;

b. the occurrence of side effects must be reasonable, relative to effectiveness and economic benefit from the chemical;
c. the standards which a chemical has to meet become the stricter, the larger the scale on which the chemical is used;
d. evaluation must be integral, i.e. must partly occur relative to the side effects of chemicals already used on the crop for other purposes; in this way synergisms are tracked down.

The procedure consists of three phases (see: Fig. 6.1):
1. the submission of lab data by the applicant;
2. evaluation of these dat a by the CTB;
3. doing of a specified field test by the applicant, the methods and norms for which have been established explicitly ahead of time.

Phase 1: Submission of lab data by applicant
The lab data requested for a chemical, required for evaluating the side effects on non-target organisms, are identical in principle to the data about toxicity as asked for in the present registration procedure (part H: toxicity to organisms occurring in the environment). An important difference is, however, that on a limited scale the number of test organisms is expanded with species which are to indicate the effect on functions for the society, or the replacement of certain species were replaced by other ones. As in the present situation, the data for this part are supplied by the applicant for registration to the CTB. Part H7.2: "Investigation of toxicity effects under field conditions" is dropped from this phase of the procedure, and returns in phase 3.

Phase 2: Evaluation of the data by the CTB
On the basis of the data supplied by the manufacturer in part H about toxicity and bio-accumulation, an evaluation of the chemical is done by the CTB **(phase 2a)**. This is done in correlation with the other data (such as the application(s) of the chemical and the physical-chemical properties) and is comparable with the present evaluation by the CTB. In this evaluation it is attempted to estimate the possible risks the chemical could cause in the field. For this, an estimated exposure of the organisms is used. This exposure is derived from the supposed emission and deposition factors.[17] For aquatic organisms, a distinction is made here between organisms in and outside the agrarian area (at 100 meters distance). For the time being, the deposition factors are derived from the drop drift (for the factors to be used see: Leeuwangh, 1984; Jobsen, 1987). In principle, however, the vapour drift, leaching (via drains as well) and run-off should also be included. Various authors give indications concerning this (*Wauchope, 1978; Leistra 1984a, b, c; Huygen et al., 1986a, b*). The deposition should be converted to concentrations per environmental compartment, to determine the exposure of aquatic and soil organisms. No good conversion method exists for organisms on the crop. This still has to be developed. Subsequently, by combining exposure and toxicity data, the risks for the species under examination are calculated. Finally, by means of calculations and/or using safety factors, extrapolation toward other kinds of ecosystems takes place (Cf. Health Council, 1988).

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[17] The ratio between the quantity of a chemical landing on the soil or the plant or in the water, per unit of area, and the dosage per unit of area.

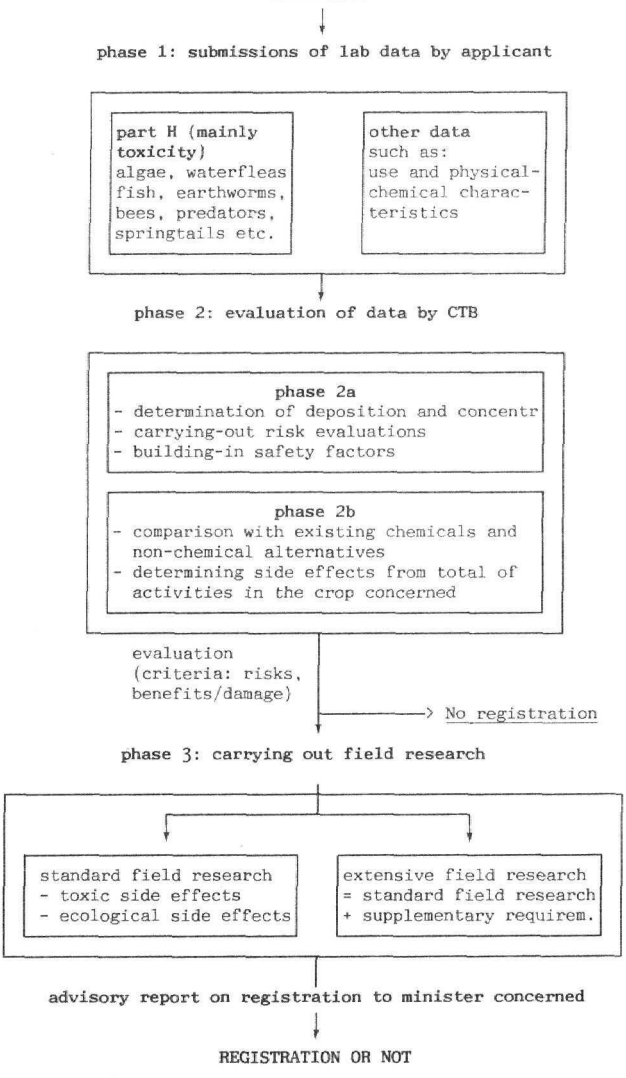


Figure 6.1 Set-up for an optimised registration procedure

In the present situation, with that, in most cases, the registration procedure is completed. However, because there are many (extrapolation) uncertainties in the aforementioned procedure, it is proposed to expand the evaluation with an extra component (**phase 2b**). In phase 2b it should be checked how the predicted risks of the new chemical stack up against existing, already registered chemicals used for the same purpose. Here, a comparison with non-chemical alternatives can also be made. Only if the new chemical is an improvement with regard to already registered chemicals can the chemical be further examined in phase 3 (field test) and can it qualify for registration. It is also possible - if feasible - that in phase 2 it is also checked how the predicted risks of the new chemical relate to the chemicals already used on the crop concerned (as well as the interventions), used for other purposes.

Finally, in this phase the open literature is consulted as well, and possibly (perhaps at the expense of the applicant) a motivated counter-expertise is carried out. Subsequently a choice is made, on the basis of the lab research and other data. In principle there are three possibilities, the chemical is:
1. not registered (no field test);
2. positively evaluated and subsequently field-tested;
3. positively evaluated and subsequently field-tested, with as extra condition some supplementary requirements regarding specially desired information.

Phase 3: Field test
The field test is done standard for all substances it is wanted to qualify for registration. The purpose is to examine whether in the field:
- toxic side effects occur with significant impairment of usage functions and/or nature significance;
- ecological side effects occur with significant impairment of usage functions and/or nature significance.

When after completion of phase 2 (evaluation by CTB) specific questions or gray areas still exist, supplementary requirements are set in addition to the standard field test, concerning information to be supplied. Here, one can think of stricter standards the chemical has to meet, e.g. when there are indications that the chemical will be used on a large scale (pursuant to the Act on Substances Endangering the Environment), as relatively small side effects, if they occur on a large scale, can still lead to an unacceptable situation.

During the field test, specific attention is paid to: i) the exposure of organisms in the field, ii) changes in food and habitat for the animal groups examined, and iii) the physical-chemical properties of the chemical under field conditions (for a more detailed description see: 6.2.2).

Upon completion of the field test, on the basis of the then available info, the CTB advises the minister concerned on whether to register the chemical or not. It should be possible to object to the decision, for the manufacturer (also to a registration the manufacturer considers too limited) as well as the consumer, as well as for private individuals and organizations (consumer associations and nature and environmental protection associations).

6.2.2 Proposal for designing a field test protocol

One of the most important difficulties in the registration procedure is the extrapolation from lab data to field conditions (see also: 6.1.2). For a solution, in principle two directions are open, viz.:

- carrying out risk evaluations, incl. the compiling of argued safety factors;
- doing empirical field testing for side effects, and pay attention not only to toxic side effects, but ecological ones as well.

Extrapolation research in various forms, making use i.a. of risk analysis, is being done at present by DGM (VROM, 1989), RIVM (Roghair, 1988) and DBW/RIZA (van der Gaag); as well, the Health Council (1988) has done research in this direction (cf. Kooijman, 1987; van Straalen, 1987; Slooff et al., 1986). In all cases it concerns theoretical modelling studies, that are based only for a limited part on empirical field research.

Various research organizations are also doing field research. Lacking here, however, is a sensible integral synthesis of the various and often greatly divergent methods and techniques, incl. a weighing of the relevance of the results for the registration procedure. Such a synthesis would have to lead to a coherent protocol, with which the (side) effects of pesticides under field conditions can be determined, and which can subsequently be used for registration policy. As well, the results of this synthesis should be checked in practice in view of feasibility and usefulness.

Besides the lack of this integration, another point is that in the present registration procedure, regarding terrestrial invertebrates, only organisms useful to agriculture (bees, earthworms and predators) are taken into account. Because it is assumed that in many crops these organisms play a subordinate part, in many cases the side effects on these groups are actually barely taken into account for registration. In addition, the registration procedure does not take into account values other than agricultural ones. Nature interests etc. are not involved in the registration, or only in part. One does pay attention to the effects on water organisms and possible problems for water purification. One pays also attention to ecotoxicological aspects regarding to vertebrates. Yet other interests, like those of nature and recreation, would have to be involved more explicitly in the registration procedure.

With this, use of the field test protocol to be drafted should provide an answer to the question in how far functions other than agricultural ones are affected by the use of pesticide. Being affected means here: changes in the possibilities for use of an area, incl. impairing the nature significance; in which nature, extent, scale, duration and reversibility of these changes are being taken into consideration.

The purpose of this research can now be described as follows:

1. developing a general protocol serving as a framework for comparing usefulness and specific application possibilities of field tests
2. by doing one or more of these field tests, it should be possible to examine whether with the use of a new pesticide there is an impairment of the usage functions or nature significance of an area

3. also, it should be possible to use the protocol within the framework of the environment quality test after the registration (see: 6.2.3).

Phase 1: Compiling a draft field test protocol

In phase 1, existing and/or still to be developed tests are ranked. In this, their suitability is evaluated as related to the nature of the pesticide concerned, the crop and manner of application, and data on behaviour in the soil and in water as well as side effects on vegetation and fauna. The results of phases 1 and 2 of the side effects of pesticides project are used, but also various EPA (US Environmental Protection Agency) regulations regarding specific field tests, and other foreign expertise in the area of (field) testing. Also, a further selection takes place of the indicators of impairment of social objectives. These are then checked against the literature and the opinions of experts, and it is checked in how far already in existing programs these species and parameters are being paid attention to, c.q. the already available knowledge can be fitted in.

By asking specific questions of and obtaining reactions from a number of specialists, comments are then obtained and worked into the rough protocol, which finally, after being checked against (new) literature, is tested for its usefulness in the field (phase 2).

Phase 2: Testing parts of the field test protocol for usefulness

Insofar as the usefulness of test components still presents practical questions, or the results to be expected are not yet clear, these aspects are examined by doing empirical research. Test fields are used for this, where one can manipulate dosage, formulae and test conditions. In ditches bordering such experimental fields, bio-assays could be placed e.g., to determine the toxic side effects and if possible the ecological ones. In addition, especially method-aspects will be studied, such as minimum required size of the experimental fields, minimum duration of the tests, finding good blanks, representativity, reproducability, causality, validation and reliability margin. Also, an evaluation should be done concerning the usefulness of the field tests within the framework of the registration procedure.

In phase 2, there should be co-operation with various research institutes, to utilize existing know-how about tests and research facilities (test fields, etc.). Specifically DBW/RIZA in Lelystad (research in Flevoland i.a.), the Experimental Station for Agriculture and Market Gardening in the Open Air at Lelystad, the Laboratory for Flowerbulb Research (LBO) in Lisse and the Plant Protection Service (PD) in Wageningen have a great deal of expertise about various research techniques, test organisms and test plot situations. In the past, researchers working at these institutes have repeatedly indicated that they are actively interested and will co-operate in further research. It is also desirable to work together with other institutions in obtaining information on the physical-chemical behaviour of pesticides (e.g. RIVM, DBW/RIZA and other water quality managers and IOB). Thus it can be attained that not only indications about the causes of different effects found are given, but also correlations can be established with the pesticides present.

Phase 3: Wording the definitive protocol

On the basis of the results obtained, in phase 3 a definite proposal for a field test protocol is compiled. With that, it will be possible to determine the effects on the various social objectives by individual chemicals, viz. by measuring a number of well-described parameters.

The result of the research is embodied in the form of an elucidation of the protocol, in which the various data and existing knowledge about field tests to determine the side effects of pesticides are mutually related. The usefulness of (a combination of) these field tests to determine the environment quality and particularly in the registration procedure for pesticides is indicated as well. Ultimately the results of the study can lead to adapting the registration procedure.

6.2.3 Proposal for an integral incidents registration system

During the interviews it was repeatedly emphasized that incidents registration is required to determine nature and extent of the side effects of pesticides in the field. The proposal to start up such a registration for vertebrates was warmly received as well at the workshop during phase 1 of the side effects of pesticides project (May 1987). At that time, obtaining greater insight into the connection between incidents and illegal use of pesticides was deemed important. At the workshop in 1988 again, a structured incidents registration, linked to a fixed measures procedure, was deemed of great importance. It was, however, still unclear in how far attention could also be paid to invertebrates here. Opinions differed at the time on whether to include illegal use of pesticides and taking into account the consequences of this regarding registration.

Setting up a well managed information and documentation system for observed incidents could, after a few years, remedy the now existing lacunae in factual knowledge about the occurrence of incidents. The information so obtained is not only of great importance in doing targeted research, but also for following a targeted and justifiable environment policy concerning pesticides.

The present incidents registration is done at various institutions. A large part of the incidents in which bird mortality occurs is reported to the CDI. The cadavers often sent-in via the investigating officials are examined pathologically and chemically and are recorded. The cause of death is determined and the results are published by the "Working group Bird Mortality", a co-operation among CDI and the "Bird Protection Association" (see also: de Snoo & Canters, 1990). There is, however, hardly any time available for further interpretation of the data obtained in this manner. One disadvantage is that the material arrives fairly arbitrarily. Incidentally, representatives from other animal groups are examined. Systematic registration of incidents with mammals, reptiles and amphibians is completely lacking though. On an ad hoc basis, data are gathered by the RIN (examination of cadavers in co-operation with TNO-CIVO and NMF. A more complete incidents registration system for vertebrates and bees exists here and there abroad (Britain e.g.) (oral comm. Fletcher, MAFF-UK) or is being set up (West-Germany).

An incidents registration for invertebrates is totally lacking. This can be partly explained by the fact that these animals are too small to be observed in incidents, but also via the absence of an agency which has as its task the recording of these incidents. Worm mortality has been reported occasionally; however, mostly this happened only after targeted investigation. An exception is constituted by bee mortality; this is regularly reported by beekeepers. The incidents are reported in a scattered way, i.a. to interest organizations and AID. In the aquatic environment, incidents are reported in the first instance to the various water quality managers. For some time now though, data on fish mortality have been gathered centrally, by the Assoc. of Water Control Boards.

When an incident occurs now, it is often a matter of an ad hoc consultation among e.g. the Plant Protection Service, the manufacturer and others involved. On the basis of this, possibly measures will be taken to change the registration, For outsiders this procedure is not very transparent, however.

On behalf of an incidents registration and the measures procedure to be linked to it, the following objectives are distinguished:

1. Setting up a systematic incidents registration system concerning pesticides, with which, if possible in absolute terms, insight is obtained into the nature and extent of these incidents, also as related to illegal and improper use.
2. Drafting a measures procedure, following an incident. In the following, a clearly described and thus "user-friendly" procedure is proposed. In designing an incidents registration system in The Netherlands it is desirable to link up as much as possible with existing projects.

Setup

The procedure consists of the following parts (see also: Fig. 6.2):

- A network of people in the field, who observe the incidents; this network will have to link up as much as possible with existing organizations, such as those of police, veterinarians, hunters, environmental protection organizations, bird watchers, terrain and water managers, (sport) fishing organizations, beekeepers, farmers, etc.
- A central reporting point, to which the incidents are reported and where systematic processing and analysis of the incidents takes place; this also entails examining cadavers, carrion, and taking of samples on location of organisms or environment components. The reporting point could be located at the CDI (link with existing tasks and activities) or at the RIVM (link with Advisory Centre Toxicology).
- At the reporting centre a cause analysis is done as well. If the cause is an illegal and possibly even purposeful use of the chemicals, the AID is called in; With legal uses, the circumstances leading to the mortality will be closely investigated; also, a report is made.
- On the basis of the cause analysis, subsequently the risk analysis can take place, where the opportunity to prevent repetition is central; also, the seriousness of the incident, related to standards still to be developed, will have to be raised here.
- If desired, on the basis of objective criteria and norms, measures can be proceeded with in the form of cancelling or modifying the registration. If insufficient clarity exists about cause or seriousness of the mortality, further targeted field research can be done as well.

- Finally, the various reports must be evaluated regularly, e.g. in the form of annual reports. Thus, depending on nature, extent and regularity of the incidents, possibly more structural measures can be proceeded with.

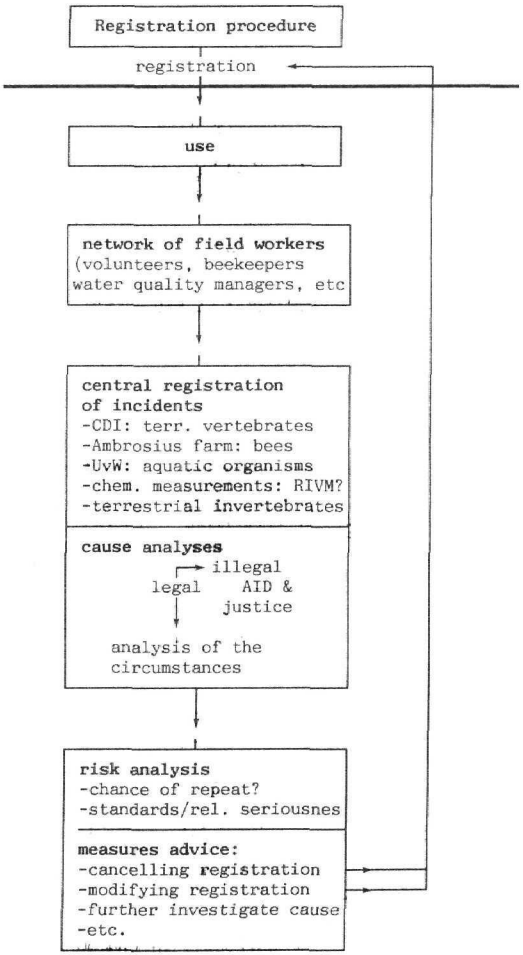


Figure 6.2 Diagram of proposed incident registration system incl. measures procedure.

Such a system really becomes valuable when it has been operational for some time (= several years). This is necessary to be able to discern trends (as a check on policy) and also to be able to adequately answer questions concerning policy.

Responsibility and financing

The workshop "Registration" (May 1988) brought out that it is felt that in the first instance, the responsibility for incidents registration lies with the government. Financing can occur by industry, consumer or government, or a combination of these. Another possibility is for financing to take place through a levy on pesticides. In the meantime it was checked whether, in analogy with the situation in Britain, possibly the NEFYTO would be willing to make a contribution to such a system. The result of this was that NEFYTO, although interested in receiving a concrete proposal, much prefers to await further developments for now (oral comm. *Jenneskens, NEFYTO chairman*).

6.2.4 Proposal for an environment quality test

Of the some 400 pesticides registered for use in The Netherlands, in agriculture a large number are used frequently and in combination with each other. In the registration procedure, so far only the side effects of the separate pesticides are examined. Additivity and synergism are hardly paid attention to. Interactions among various interventions, incl. introduction of substances alien to the environment, and interactions among organisms (ecological side effects) are left out of consideration. Consequently, it is not clear in how far organisms can maintain themselves in the field, or whether the effects of the pesticides actually affect the functioning of the ecosystem. Thus it is also difficult to extrapolate the effects toward impairment of social objectives. For these reasons, an environment quality test is proposed, the setup of which is elaborated below.

The purpose of the environment quality test is described as follows: a test to check in an integrated manner in how far the environment quality c.q. the multifunctionality in the agrarian area is affected by the use of pesticides and other interventions in the rural area. The proposal to further elaborate such a test was received positively by a large majority of participants in the workshop. To distinguish it from the field test described in 6.2.2 it is noted that the environment quality test is aimed at evaluating the effects of already registered pesticides in a certain region, and the field test is aimed at tracing the effects of pesticides to be newly registered. The field test, in contrast with the environment quality test, is directly linked to the registration procedure. The latter test should be seen as an indicative measure for reading out the environment quality in a certain area. On the basis of this, though, consequences may result for the registration of pesticides in a certain area.

Setup

By means of field research, in a certain region and/or certain crop, it is examined by which interventions and in what manner the ecosystem and its components are affected. To this end, at various locations in a region, during one season i) vegetation and fauna (and possibly fungi) are sampled, ii) concentration measurements are done in the various environment compartments and biota, and iii) by means of bio-assays it is investigated whether abnormal behaviour or mortality is occurring among specific test organisms (see: 6.2.2). This field research and the procedure to be followed afterwards are shown in Fig. 6.3. In the following the process is explained further, insofar as it concerns terrestrial invertebrates and aquatic fauna at least.

Occurrence of species

By means of various sampling methods, at a number of points in time spread out over the entire season the occurrence of terrestrial invertebrates and aquatic fauna is examined, in the field itself as well as in the ditches surrounding it. Possible sampling methods are: various kinds of nets, pitfalls, exhausters, litterbags, soil and water samples, etc. The various interventions are subsequently correlated with occurrence of the species, with attention also being paid to developments of numbers in time. It is a condition for a good study that not all interventions (restricted for the time being to the use of pesticides) occur simultaneously everywhere in the study. A possibility to cope with this problem is the use of local reference and background levels of the occurrence of terrestrial invertebrates and aquatic fauna.

Bio-assays

Because in the agrarian area not nearly the same (a)biotic conditions obtain everywhere, it is necessary, supplementary to the research on the occurrence of species, to use test organisms, the behaviour of which is examined under standard conditions. We are thinking here of the following setup: lab research for which water and soil are collected from the field and in which survival and behaviour of raised species or species gathered elsewhere are determined. As an example here we can use the so-called "bio-alarm" of water fleas and Leuziscus idus, which have been in use for some time by water utilities and the Department of Public Works, i.e. 'Rijkswaterstaat', to monitor the quality of extensive waters. Experiments with algae, periphyton, tubifex, mussels, snails, bait shrimp, mosquito larvae, etc. are also possible. For the terrestrial environment, in this connection, experiments with nematodes, earthworms, isopods, spiders and springtails are very well possible. It is also possible to keep certain species in small cages in the field (bio-assays in the field) and keep track of mortality. We are thinking here of placing epigeal invertebrates in enclosures, of setting up beehives, and of placing fish inside cages in ditches.

Concentration measurements

It is necessary to be able to relate the effects found on the fauna in the field and in the bio-assays to the intervention. Determinations of concentrations in time, specifically in the soil and the water, and determinations of residue(s) on the plant(s) are suitable for this and are required.

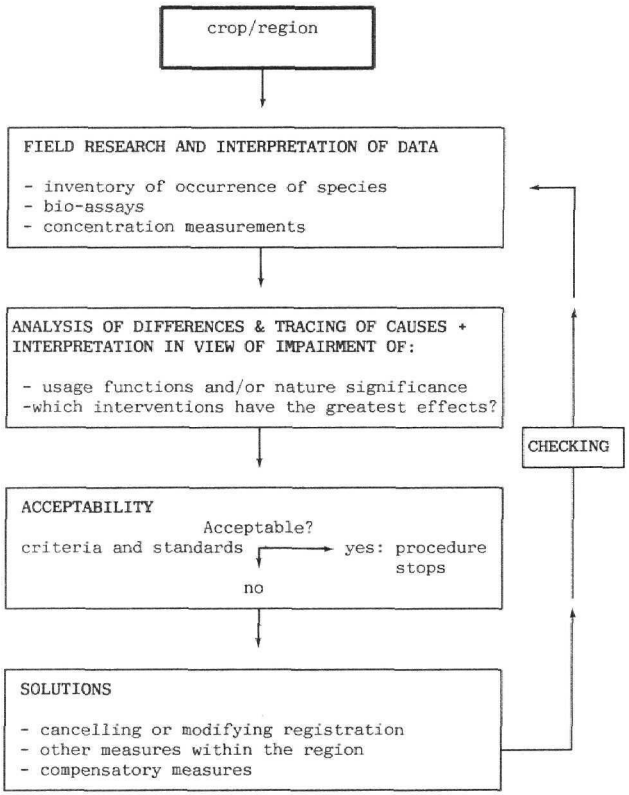


Figure 6.3 *Diagram of environment quality test, worked out for the side effects of pesticides.*

Interpretation

On the basis of the research on occurrence of species, bio-assays and concentration measurements, the relationship between intervention and effect must be established. From these, a picture originates of the toxic side effects of pesticides in correlation with other interventions in the agrarian area. By also, in addition to the occurrence of invertebrates, taking inventory of plant species present, and combining with the data about the abundance of vertebrates, a statement can be made as well about possibly occurring ecological side effects. The result of this is, that statements can be made about the quality of the environment and about the

question whether the objective-variables in the area under study have been effected.

Use of the test results

After the field research a cause analysis takes place, in which it is examined what the relative contribution is by the (possible) interventions to the effects indicated. The extent of the damage is then checked against the reference framework of the environment quality. If the damage is unacceptably great, one proceeds to look for alternatives, e.g. other means, modifying or cancelling the registration or taking compensatory measures. Finally, the measures taken will have to be checked for effectiveness, in which field research too is among the possibilities.

In the first instance, the environment quality test, insofar at least as aimed at the side effects of pesticides should be done for large crops in certain characteristic areas, such as potato crop in East-Groningen, as critical points have already been found here and consequently an environment quality test can best be examined for its usefulness in such an area.

6.3 Policy recommendations

In the preceding sections, a number of more or less specific recommendations were made about the registration (procedure), research, nature and extent of the side effects of the large-scale use of pesticides in agriculture. Below some more general points are formulated, which must have or should get attention from the policy side.

Pesticides Act
For what regards the registration of pesticides one could think of rewording art. 3 of the Pesticides Act concerning the side effects on pesticides on organisms. A wider interpretation should be given to the organisms, the maintenance of which is desirable. Explicit extension to non-target organisms must be considered. Further, the Act as a whole would have to be attuned to the (draft version of) the Flora and Fauna Act, the Pollution of Surface Waters Act ('WVO') and the General Environmental Hygiene Stipulations Act ('WABM') (now revised).

Research
In view of the extent of the use of pesticides in The Netherlands and of the extent to which these substances end up in the environment, it is important to (continue to) make extra research efforts in this direction. Point of attention here should be first of all research on reduction of use and emissions of pesticides into the environment. There is, however, also a great need for research aimed at effects, especially in the field, and for research on evaluation aspects.

Crops and regions
The field research has shown that for the aquatic fauna in any case there are danger points in the potato crop and in those grown in greenhouses. Other studies give indications of critical points in the growing of fruit, flowers, flower bulbs and mushrooms, For many other crops no research has been done yet, so that data are lacking.

In concentration areas of the crops concerned these problems come to the fore most clearly, as is evident e.g. in the Westland. Consequently, research and cleanup efforts should be directed mainly at these areas. It should be seen to though that no "end of pipe" measures are chosen, as is proposed for the Westland (central drain into the New Waterway), but that a source-directed approach merits preference (e.g. a closed loop system with re-use of the water in the greenhouses).

Emissions
The research shows that in most agrarian areas, for cholinesterase inhibitors and chlorinated hydrocarbons, the standard for the basic water quality is exceeded. This is a very worrisome fact, and definitely justifies great(er) attention from policy, e.g. in the form of suitable measures to limit emissions. One should think here not only of improvements in spraying equipment, but also e.g. of alternative field-margins management in agriculture, limiting ditch shoulder treatment and more general measures in the area of reducing the use of chemical means.

Chemical agents
The research shows that the use of a number of (groups of) chemicals has led to mortality in the past among terrestrial invertebrates and aquatic fauna, or shown to be (very) toxic for these organisms. Registration of these (groups of) chemical merits special attention from policy.

Within the fungicides group, it is known about the benzamidazols (benomyl e.g.) that in the field there are effects on earthworms, while they are also very toxic for predators. Carbendazim has been found in the surface water and is very toxic for the aquatic fauna. Organic phosphor fungicides, such as pyrazophos, are very toxic for bees and fish and have also been shown in surface water. Tolclofos-methyl too has led to fish mortality. Finally, the dithiocarbamates (i.a. thiram and mancozeb) are toxic for predators. Other representatives of this group (maneb e.g.) have been found in the surface water in high concentrations, and are (very) toxic to the aquatic fauna.

Most insecticides are very toxic to all non-target organisms (also including insects). Incidents are known with bees and organic phosphor compounds and earthworms and carbamates (carbaryl and carbofuran); aldicarb too is toxic for earthworms under field conditions. In the aquatic environment, high concentrations of cholinesterase-inhibiting substances are measured; among these are very toxic ones for the aquatic fauna. In The Netherlands endosulfan causes by far the greatest number of fish deaths in the aquatic environment, and for that reason alone merits policy attention.

On many herbicides there are no data. When there are, however, herbicides appear to be not very toxic for terrestrial invertebrates. Toxic, however, for bees and predators i.a. are the dinitroalkylphenols and -phenylesters (such as dinoseb). Dinoseb was also found a number of times in high concentrations in the water and has led to fish mortality. Other toxic herbicides are bromefenoxim and fenmedifam. MCPP, paraquat and diquat have led to bee mortality in the field.

Soil disinfectants are very toxic to earthworms and predators. This is known especially of fumigants such as D-D, metam-natrium and methyl bromide. Granulates like aldicarb are also toxic though. D-D has been found

in the water in high concentrations. Metam-natrium has led to fish mortality. In view of the fact that soil disinfectants are used on such a large scale, extra attention for this group appears desirable.

Still, it should not be concluded from the foregoing that there is a complete overview of the toxicity of pesticides for terrestrial invertebrates and aquatic fauna. This is obvious when the 20 pesticides most used in The Netherlands are compared with the toxicity data available. Data on only 3 pesticides were found for aquatic invertebrates, on 10 for fish, on 3 for earthworms, on 5 for predators, and on 6 for bees. Augmenting these data is desirable.[18]

Species
The following species came to the fore as possibly good and usable indicators of impairment of usage functions and nature significance (in view of the impairment of environment quality, the side effects of pesticides occurring in these species merit more and explicit attention from policy):

General environment quality

* terrestrial environment:
  - microbivore species, such as orbatid mites, nematodes and springtails
  - saprophagous species, such as earthworms, isopods, land snails and myriopods
  - predators, such as ground spiders, rove beetles and ground beetles

* aquatic environment:
  - detritus-eaters, such as isopods
  - predators within the mesofauna, such as water mites.

Specific environment quality

* agriculture
  - useful organisms, such as bees and earthworms
  - explicit expansion with other species which can be considered useful for agriculture, viz. predators and parasites such as ichneumon wasps and predatory mites, but other species as well qualify for this.
  - more attention for species with a long generation time (in connection with long-term effects as related to long recovery time): overwintering isopods, predatory mites, butterflies and spiders
  - more attention for species with a long range (in connection with effects occurring at some distance): flying species such as ichneumon wasps and ladybirds, but also certain ground beetle species and possibly wolf spiders.

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[18] Among these 20 pesticides, specifically captan for fish, and dinoseb for fish, bees, as well as predators, turn out to be very toxic.

* drinking-water:
  - freshwater mussels?

* forestry:
  - phytotoxicity for higher plants
  - ditto for fungi (specif. mycorrhiza)

* commercial fisheries:
  - eel and/or pike or pickerel
  - three-spined stickleback

* sports fishing:
  - diversity parameter related to average number of fish species per water type

* recreation:
  - diversity parameter related e.g. to average number of prominent species
  - (see also: sports fishing and nature)

* nature:
  - protected species:
  . terrestrial invertebrates with main distribution in agrarian area, specifically the rare to very rare species
  . aquatic fauna with main distribution in agrarian area, specifically the rare to very rare species

  - condition-creating species:
  . more attention for condition-creating species; also important for vertebrates with main distribution in the agrarian area: insectivorous mammals (six species + all bat species) + badger and otter, some 25 summer breeding bird species with a downward population trend, and practically all indigenous amphibians and reptiles (17 species)
  . more attention for flying insects, of importance in flower pollination e.g.

This report shows that invertebrates and aquatic fauna are in potential useful as indicators for the impairment of several functions for the society; yet further research will be needed to realize this potential.

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A N N E X E S

Annex 1.1 List of persons interviewed

Dr. A.J. Baars*	CDI
Dr. L. Brussaard*	IB
C.F. van de Bund	former employee RIN/PD
Ing. L.J.M. Butot*	former employee RIN
Drs. J. van der Does*	Water Control Board of Rijnland
P. van der Eijk*	Ag. rep. Haarlemmermeer Polder
Ir. J.W. Everts	LUW - Toxicology
Ing. R. Faasen	DBW/RIZA
E.W. Flentge*	Municipal Water Utility Groningen
Dr.ir. M. van der Gaag	DBW/RIZA
Ir. C.A.M. van Gestel	RIVM
Dr. P.A. Greve*	RIVM
Drs. H. van der Hammen	PWS, North-Holland
Dr.ir. C. van Heemert*	Experimental Beekeeping Station "Ambrosiushoeve"
Ir. J.A. Jobsen	PD
Ir. C. Jol*	Water Purification Board West-Overijssel
Ing. H. Jonker*	Market Gardening rep. Midden-Holland
Dr. K. Kersting	RIN
Dr. S.P. Klapwijk*	Water Control Board of Rijnland
Dr.ir. T. Kooistra	PD
Dr. P. Leeuwangh	IOB
Dr. J. G. van der Made*	LUW-Nature Management
Mw. J.L. Maas*	DBW/RIZA
Drs. A. Minderhoud*	RIVM
Dr.ir. P.A. Oomen	PD
Mw. J.M.A. Pos*	Ass. to further Beekeeping in The Neth.
Ing. K. Roggeveen*	Ag. rep. Haarlemmermeer Polder
Dr. N.M. van Straalen	VU - Ecotoxicology
E.P. Valent*	AID
Dr. P.J.R. de Vries	UvW
Drs. B. van der Wal	Water Control Board of Delfland
Ing. F.H. Wagemaker*	DBW/RIZA

* = short conversation of phone contact

Annex 1.2 Participants in Workshop "Registration of Pesticides"

Mw. D.M.M. Adema	TNO
Dr. A.J. Baars	CDI
D.A. Bekooy	ZWO
Dr. G. van den Berg	Duphar
Dr. L.H.M. Blommers	Exp. Orchard St. "De Schuilenburg"
Drs. K.J. Canters	CML
Drs. J.H. Canton	RIVM
Mw. ir. I. van Dijk	ICI
Drs. J. van der Does	Water Control Board of Rijnland
Ir. J.W. Everts	LUW - Toxicology
Ing. R. Faasen	DBW/RIZA
Dr. M.R. Fletcher	MAFF (UK)
Dr.ir. M. van der Gaag	DBW/RIZA
Prof.dr. H. van Genderen	-
Dr. J.H. van Haasteren	DGM
Dr. Ch.W. Heckman	Inst. Hydr. & Fisch, Hamburg
Dr. J. den Hollander	L&V - NMF
Dr.ir. C. van Heemert	Exp. Beekeeping St. Ambrosiushoeve
Dr. Ch. Inglesfield	Shell
Ir. G. Jagers op Akkerhuis	LUW - Toxicology
Ir. J.A. Jobsen	PD
Mw. drs. P.N. de Jong	L&V - VKA
Drs. F.M.W. de Jong	CML
Mw. prof.dr. E.N.G. Joosse-van Damme	VU - Ecotoxicology
Dr. K. Kersting	RIN
Dr. P. Leeuwangh	IOB
Dr. B.H. van Leeuwen	L&V - NMF
Dr. C.J. van Leeuwen	DGM
Ir. J.B.H.J. Linders	RIVM
Ir. T.G. Lössbroek	L&V - VKA
Dr.ir. W.C. Ma	RIN
Mw. drs. H. Meyer	RMNO
Mw. drs. A.J. Murk	Health Coucil
Dr.ir. P.A. Oomen	PD
Mw. ir. C.J. Roghair	DGM
Mw. ir. J.M. de Ruiter	DGM
Drs. G.R. de Snoo	CML
Ir. H.J.M. Straathof	PD
Drs. T. Traas	VU - Ecotoxicology
Prof.dr. H.A. Udo de Haes	CML
Dr. J. Vegter	TCB
Dr. R. van Venetië	L&V - VKA
Drs. L.H.H. van Vliet	L&V - NMF
Dr. P.J.R. de Vries	UvW
Drs. B. van der Wal	Water Control Board of Delfland

Annex 3.1 Existing tests of the IOBC (IOBC, 1988)

	lab test	semi-field test	field test
Phytoseiulus persimilis (predator mite)	X	X	X
Typhlodromus pyri (predator mite)	X		X
Amblyseius potentillae (predator mite)	X		
Amblyseius finlandicus (predator mite)			X
Anthocorus nemoralis (predator bug)	X		
Chrysoperla carnea (gauze fly)	X	X	
Syrphus corollae (hover fly)	X		
Trichogramma cacoeciae (ichneumon wasp)	X	X	
Encarsia formosa (ichneumon wasp)	X		X
Aphidius matracariae (ichneumon wasp)	X	X	
Phygadeuon trichops (ichneumon wasp)	X	X	
Coccygomimus turionellae (ichneumon wasp)	X		
Bembidion lampros (ground beetle)	X		
Aleochara bilineata (short shield beetle)	X	X	
Semiadalia 11 notata (ladybird)	X		

Annex 3.2a Occurrence of protected species: terrestrial invertebrates

Occurrence of terrestrial invertebrates - to be protected under the draft bill of the Flora and Fauna Act (1987) - in The Netherlands in various biotope types; * - species now protected under Nature Protection Act; rarity classes (according to our own interpretation): zzz = extremely rare or extinct; zz = very rare; z = rare; vz = fairly rare; mz = less rare; ma = less general and a = general; 19861 (X) = occurs sometimes in biotope concerned; bold: species within the agrarian area wich are at least rare; sources: Geijskens & van Tol, 1983; RIN, 1983 and Geraedts, 1986.

species	grass-land	arable land	bush/ heather	Details, where occurs	Status	Agra.
INSECTS						
butterflies:						
Thymelicus sylvestris	X			Limb.lime	zzz	Yes
Erynnis tages	X		X	Limb.lime	a	Yes
Spalia sertorius	X				zzz	Yes
Aporia crataegi			X		zzz	No
Strymonidia w-album				mainly Limb.	zzz	?
Lycaena dispar*				Nw. Ov. reeds	z	No
Cupido minimus				Limburg	zzz	?
Plebejus argus	X		X	high moor	zz	Yes
Lycaeides idas	X		X	poor grassl.	zzz	Yes
Vacciniina optilete			(X)	high moor	zz	No
Limenitis populi			X	mainly Limb.	zzz	No
Nymphalis polychloros			X		z	No
Nymphalis antiopa			X		zzz	No
Boloria aquilonaris				high moor	zzz	No
Argynnis paphia			X		zz	No
Euphydryas aurinia	X			poor grassl.	zzz	Yes
Melitaea cinxia	X			Limburg	zzz	Yes
Coenonympha arcania			X	Veluwe	zzz	No
Hipparchia statilinus				sand drifts	zz	No
ants:						
Formica rufa	(X)		X		?	No
Formica polyctena			X		?	No
Formica pratensis	(X)		X		?	No
Formica fusca	(X)		X		?	No
Formica exsecta	(X)		X	North.Neth.	?	No
Formica pressilabris	(X)		X	North.Neth.	?	No
Formica piniphila					?	No
beetles:						
Lucanus cervus*			X		zz	No
Omophron limbatum				sand banks	vz	No
moluscs:						
Helix pomatia	(X)		X		?	Yes
Vitrinobrachiom breve				Gelderland		?
Vertigo moulinsiana				South Limb.	zzz	Yes?
Catinella arenaria				dunes	z	No

Annex 3.2b **Occurrence of protected species: aquatic fauna**

Occurrence of aquatic fauna, to be protected under draft bill Flora and Fauna Act (1987) in The Netherlands in various water types; for further elucidation see: annex 3.2a. Sources: Nijssen & De Groot, 1975; Kelle & Sturm, 1979; RIN, 1983; Anon., 1986; oral comm. Van Tol, RMHN.

species	ditch	large water	brook	river and pools	salt and/or brack.	details where occurs	status	agra.
INSECTS								
mayflies:								
Ephemera danica			X	X			z	Yes
Ephemera vulgata			X	X			zz	Yes
dragon flies:								
Calopteryx virgo			X			Limb. & N.-B.	vz	Yes
Nehalennia speciosa						sedge swamp	zzz	No
Aeshna viridis	X	X				Stratiotes al	z	Yes
Cordulegaster boltonii			X			Limb. & N.-B.	zz	Yes
Orthetrum coerulescens			X			L&NB. swamp	z	Yes
lace-wing flies:								
Osmylus fulvicephalus						well-areas	z	No
bugs:								
Gerris *najas*			X			*east&south Nl*	zz	Yes
beetles:								
Deronectus latus			X			Geul/Hierden	zz	Yes
Dytiscus lapponicus						fens	zz	No
Cybister lateralimargin.	(X)	X					z	Yes
Orectochilus villosus			X	X			z	Yes
Hydrochara caraboides	X	X					zz	Yes
CRUSTACEANS								
Astacus astacus*			X	X			zz	Yes
MOLLUSCS								
Hydrobia stagnorum					X		zz	No
Avenionia brevis roberti						Limb.undergr.	zzz	No
Valvata pulchella	X						zz	Yes
Cerastoderma glaucum					X		?	No
VERTEBRATES								
fish:								
Lampetra fluviatilis*			(X)	X	X		a	No
Lampreta planeri*			X				z	Yes
Phoxinus phoxinus*			X			Geul	zz	Yes
Rhodeus sericeus amarus*		X	X?	X?			z	Yes
Misgurnus fossilis*	X	X	X?	X?			ma	Yes
Cobitis taenia*	X	X	X?	X?			va	Yes
Noemacheilus barbatulus*			X				va	Yes
Silurus glanis*				X			z	No
Cottus gobio*		X	X	X			va	Yes

Annex 3.3a **Food spectrums of indigenous mammal species insofar as occurring in the agrarian area (selection according to de Snoo & Canters, 1990).**

Herbivorous: veg. = "vegetables", gr = grass, br = branches, bark, wood(fibres), st. = "starch", se = seeds, fr = fruits, ss = subsoil parts; carnivorous: invertebrates, wo = (rain)-worms, mo = molluscs, cp = caterpillars, ar = arthropods (a.o. insects); vertebrates, fi = fish, eg = eggs, tv = terrestrial vertebrates (i.e. mice, small birds and amphibians & reptiles); x = part from diet, x = essential part from diet; in addition, the occurrence and the trend in the occurrence (own estimations) is given of those species whose food largely consists of invertebrates or aquatic fauna: I = occurring in < 5% of the grid cells (measuring 5x5 km); II = in 5-10%; III = in 10-25%; IV = in 25-50% and V = in > 50%; 0 = stable population; - = decresing; + = increasing and ? = uncertain or unknown (sources: de Snoo & Canters (1990) and own interpretation.

food	herbivorous							carnivorous							occur-	trend
	vegetables				starch			invertebrates			vertebraten					
species	gr	hb	lf	ta	se	fr	ss	wo	mo	cp	ar	vi	ei	tv	ing	
Erinaceau europaeus						x	x	x	x	x	x			x	V	0
Sorex cf. araneus					x			x	x		x				V	0
Sorex *minutus*					x			x	x		x				V	*0*
Neomys fodiens									x		x	x		x	IV?	-?
Crocidura leucodon								x	x		x			x	I	-?
Talpa europaea								x	x	x	x			x	V	0
Bats											x				?	?
Lepus capensis	x	x		x	x	x	x									
Oryctolagus cuniculi.	x	x		x												
Sciurus vulgaris				x	x	x			x		x		x	x		
Cricetus cricetus		x			x	x	x		x		x			x		
Clethrionomys glareolus	x	x	x	x	x	x	x				x			x		
Arvicola terrestris	x	x	x	x	x	x	x	x	x		x			x		
Ondatra zibethicus	x	x					x									
Pitymus subterraneus	x	x			x	x	x				x					
Microtus arvalus	x	x	x	x	x	x	x									
Microtus agrestis	x	x	x		x	x	x				x					
Microtus oeconomus	x	x		x	x		x									
Mycrotus minutus		x		x	x						x					
Apodemus sylvaticus				x	x	x		x			x			x		
Apodemus flavicollis				x	x	x		x			x			x		
Muscardinus avellanarius				x	x	x										
Myocastor coypus	x	x				x	x									
Vulpes vulpes				x				x			x	x	x	x		
Mustela erminea														x		
Martes foina						x					x			x		
Meles meles					x	x	x	x	x	x			x	x	III	-
Lutra lutra									x			x	x	x	<I?	-
Mustela nivalis									x		x			x		
Putorius putorius								x	x		x	x	x	x		
Sus scrofa	x	x			x		x	x			x			x		
Capreolus capreolus	x	x	x			x										

Annex 3.2b **Occurrence of protected species: aquatic fauna**

Occurrence of aquatic fauna, to be protected under draft bill Flora and Fauna Act (1987) in The Netherlands in various water types; for further elucidation see: annex 3.2a. Sources: Nijssen & De Groot, 1975; Kelle & Sturm, 1979; RIN, 1983; Anon., 1986; oral comm. Van Tol, RMHN.

species	ditch	large water	brook	river and pools	salt and/or brack.	details where occurs	status	agra.
INSECTS								
mayflies:								
Ephemera danica			X	X			z	Yes
Ephemera vulgata			X	X			zz	Yes
dragon flies:								
Calopteryx virgo			X			Limb. & N.-B.	vz	Yes
Nehalennia speciosa						sedge swamp	zzz	No
Aeshna viridis	X	X				Stratiotes al	z	Yes
Cordulegaster boltonii			X			Limb. & N.-B.	zz	Yes
Orthetrum coerulescens			X			L&NB. swamp	z	Yes
lace-wing flies:								
Osmylus fulvicephalus						well-areas	z	No
bugs:								
Gerris najas			X			east&south Nl	zz	Yes
beetles:								
Deronectus latus			X			Geul/Hierden	zz	Yes
Dytiscus lapponicus						fens	zz	No
Cybister lateralimargin.	(X)	X					z	Yes
Orectochilus villosus			X	X			z	Yes
Hydrochara caraboides	X	X					zz	Yes
CRUSTACEANS								
Astacus astacus*			X	X			zz	Yes
MOLLUSCS								
Hydrobia stagnorum					X		zz	No
Avenionia brevis roberti						Limb.undergr.	zzz	No
Valvata pulchella	X						zz	Yes
Cerastoderma glaucum					X		?	No
VERTEBRATES								
fish:								
*Lampetra fluviatilis**			(X)	X	X		a	No
Lampreta planeri*			X				z	Yes
Phoxinus phoxinus*			X			Geul	zz	Yes
Rhodeus sericeus amarus*		X	X?	X?			z	Yes
Misgurnus fossilis*	X	X	X?	X?			ma	Yes
Cobitis taenia*	X	X	X?	X?			va	Yes
Noemacheilus barbatulus*			X				va	Yes
Silurus glanis*				X			z	No
Cottus gobio*		X	X	X			va	Yes

Annex 3.3a **Food spectrums of indigenous mammal species insofar as occurring in the agrarian area (selection according to de Snoo & Canters, 1990).**

Herbivorous: veg. = "vegetables", gr = grass, br = branches, bark, wood(fibres), st. = "starch", se = seeds, fr = fruits, ss = subsoil parts; carnivorous: invertebrates, wo = (rain)-worms, mo = molluscs, cp = caterpillars, ar = arthropods (a.o. insects); vertebrates, fi = fish, eg = eggs, tv = terrestrial vertebrates (i.e. mice, small birds and amphibians & reptiles); x = part from diet, x = essential part from diet; *in addition*, the occurrence and the trend in the occurrence (own estimations) is given of those species whose food largely consists of invertebrates or aquatic fauna: I = occurring in < 5% of the grid cells (measuring 5x5 km); II = in 5-10%; III = in 10-25%; IV = in 25-50% and V = in > 50%; 0 = stable population; - = decresing; + = increasing and ? = uncertain or unknown (sources: de Snoo & Canters (1990) and own interpretation.

food	herbivorous							carnivorous							occur-ing	trend
	vegetables				starch			invertebrates			vertebraten					
species	gr	hb	lf	ta	se	fr	ss	wo	mo	cp	ar	vi	ei	tv		
Erinaceau europaeus						x	x	x	x	x	x			x	V	0
Sorex cf. araneus					x			x	x		x				V	0
Sorex minutus					x			x	x		x				V	0
Neomys fodiens									x		x	x		x	IV?	-?
Crocidura leucodon								x	x		x			x	I	-?
Talpa europaea								x	x	x	x			x	V	0
Bats											x				?	?
Lepus capensis	x	x		x	x	x	x									
Oryctolagus cunicul.	x	x		x												
Sciurus vulgaris				x	x	x			x		x		x	x		
Cricetus cricetus		x			x	x	x		x		x			x		
Clethrionomys glareolus	x	x	x	x	x	x	x				x			x		
Arvicola terrestris	x	x	x	x	x	x	x	x	x		x			x		
Ondatra zibethicus	x	x					x									
Pitymus subterraneus	x	x			x	x	x				x					
Microtus arvalus	x	x	x	x	x	x	x									
Microtus agrestis	x	x	x		x	x	x				x					
Microtus oeconomus	x	x		x	x		x									
Mycrotus minutus		x		x	x						x					
Apodemus sylvaticus				x	x	x		x			x			x		
Apodemus flavicollis				x	x	x		x			x			x		
Muscardinus avellanarius				x	x	x										
Myocastor coypus	x	x				x	x									
Vulpes vulpes				x				x			x	x	x	x		
Mustela erminea														x		
Martes foina						x					x			x		
Meles meles					x	x	x	x	x	x			x	x	III	-
Lutra lutra									x			x	x	x	<I?	-
Mustela nivalis									x		x			x		
Putorius putorius								x	x		x	x	x	x		
Sus scrofa	x	x			x		x	x			x			x		
Capreolus capreolus	x	x	x			x										

Annex 3.3b **Food spectrums of indigenous summer breeding birds insofar as occurring in the agrarian area or tied to aquatic environments; population trends are also given.**

For an explanation of the letters used see: 3.3a; further: kr = herbs (for some species incl. waterplants and/or algae); bl = leaves; gg = large arthropods; kr = small crusaceans; aa = carrion; al = omnivores; j = mainly a source of food for juveniles; 1 = decrease of more than 25% when comparing period 1973-79 with 1979-85; 2 = decrease 10-25%; 3 = remained about the same; 4 = increase 10-25%; 5 = increase of more than 25%; A = agrarian area; W = aquatic environment; sources: De Snoo & Canters, 1990; SOVON, 1987, 1988; Vogelwerkgroep Avifauna West-Nederland, 1981 and written communication Bird Protection (somewhat modified).

food	biotope	herbivorous							carnivorous									trend
		'vegetable'				'starch'			invertebrates						vertebrates			
species		gr	kr	bl	ta	za	vr	on	wo	sl	la	gp	gg	kr	vi	ei	tv	aa	al	
Tachybaptus ruficol.	W									x	x	x		x	x					1
Podiceps cristatus	W										x	x			x					5
Podiceps nigricollis	W									x	x	x		x	x					5
Phalacrocorax carbo	W														x					5
Botaurus stellaris	W											x			x					1
Ixobrychus minutus	W										x	x			x					1
Nycticorax nycticorax	W											x			x		x			1
Ardea cinerea	W											x			x		x			3
Ardea purpurea	W											x			x		x			1
Ciconia ciconia	A								x	x			x				x			3
Platalea leucorodia	W													x	x					5
Cygnus olor	A	x	x					x												5
Anser anser	W	x	x			x		x												5
Anas strepera	W		x			x		x				x							x	5
Anas creccag	W					x			x			x					x			3
Anas platyrhynchos	A	herbivorous							carnivorous										x	3
Anas acuta	W		x			x						x								3
Anas querquedula	A	herbivorous							carnivorous										x	3
Spatula clypeata	A	herbivorous							carnivorous										x	3
Netta rufina	W		x	x	x		x	x	carnivorous										x	3
Aythya ferina	W		x				x			x		x								4
Aythya fuligula	A									x		x		x						4
Circus aeruginosus	W														x	x	x	x		3
Buteo buteo	A								x				x				x	x		5
Falco tinnunculus	A											x					x			2
Perdix perdix	A			x		x	x			x	x	x								1
Coturnix coturnix	A		x			x	x		x	x		x								3
Phasianus colchicus	A		x			x	x					x					x			2
Rallus aquaticus	W	herbivorous									x	x							x	3
Porzana porzana	W					x	x				x	x								3
Crex crex	A								x	x		x								3
Gallinula chloropus	W	herbivorous								x	x	x								3
Fulica atra	W	herbivorous								x	x	x								3
Haematopus ostralegus	A								x	x		x		x						5
Vanellus vanellus	A								x			x								5
Philomachus pugnax	A								x	x		x		x						1
Gallinago gallinago	A								x		x	x								1
Limosa limosa	A								x			x								1
Tringa totanus	A								x			x		x						3
Larus ridibundus	A								x	x	x		x							3
Larus canus	A								x	x	x	x		x	x		x			5
Chlidonias niger	W										x	x		x	x					1
Streptopelia decaocto	A					x	x													5
Cuculus canorus	W											x	x							2
Tyto alba	A																x			3
Athene noctua	A								x			x					x			3
Alcedo atthis	W														x					1
Alauda arvensis	A		x	x		x			x		x	x								1
Riparia riparia	A											x								1
Hirundo rustica	A											x								3
Delichon urbica	W											x								1
Anthus pratensis	A										x	x								3
Motacilla flava flava	A										x	x								3
Motacilla cinerea	W										x	x								5
Motacilla alba alba	A										x	x								3
Luscinia svecica	W										x	x								5
Saxicola torquata	A										x	x								1
Cettia cetti	W											x								1
Cisticola juncidis	W											x								1
Locustella naevia	W											x								3
Locustella luscinioi.	W												x							2
Acrocephalus schoeno.	W											x								1
Acrocephalus palustr.	W											x								3
Acrocephalus scirpac.	W											x								3
Acrocephalus arundin.	W											x								1
Hippolais icterina	A						x				x	x								3
Panurus biarmicus	W					x						x								1
Lanius collurio	A												x			x	x			1
Pica pica	A					x	x		x			x				x	x	x	x	5
Corvus monedula	A					x			x			x				x	x	x	x	3
Corvus frugilegus	A					x	x		x	x	x	x					x	x	x	5
Corvus corone corone	A					x	x		x	x		x				x	x	x	x	5
Sturnus vulgaris	A						x		x		x	x								3
Passer montanus	A					x						x								1
Carduelis carduelis	A					x						x								4
Carduelis cannabina	A					x														2
Emberiza citrinella	A					x						x								3
Emberiza hortulana	A					x					x									1
Emberiza schoeniclus	W					x				x	x	x								3
Millaria calandra	A					x						x								1

Annex 4.1 Toxicity data of aquatic invertebrates, fishes, earthworms, predators & parasites and bees

Elucidation
This annex gives toxicity data about the above. To arrive at the table, use was made of toxicity data from: Edwards & Brown, 1982; Hague & Ebing, 1983; Verschueren, 1983; Heimbach, 1985; VROM, 1985; Mansour et al., 1984; Mayer & Ellersieck, 1986; ONC, 1984; Oomen, 1986; Hassan et al., 1987; Worthing & Walker, 1987. Further, warnings about the toxicity of agents were taken from Van Rijn, 1987.

The toxicity data are divided into classes: the limits are based on: van Gestel, 1985 and VROM, 1985. The classes toxic and very toxic were combined (see: Table B4.1).

organisms / toxicity classes	aq. fauna (LC50 in mg/l)	earthworms (LC50 in mg/kg ground	bees (LC50 in g/bee)
I toxic to very toxic	<1	<1 - 10	<0,1 - 1
II moderately toxic	1 - 10	10 - 100	1 - 10
III little toxic	10 - 100	100 - 1000	10 - 100
IV not toxic	>100	>1000	>100

Table B4.1 Class limits as used for the description of the toxicity of pesticides

For predators and parasites we used the toxicity classification according to SPV, 1987; the class limits are based on the following mortality percentages: 61-100% = class I; 60-41% = class II; 11-40% = class III and 0-10% = class IV. In addition, for the species and chemicals for which no data were included by the SPV, use was made of Hassan et al. (1987) in which dangerousness is indicated (class limits: not dangerous [under 25% mortality]; little dangerous (25-50%); moderately dangerous (51-75%) and dangerous (over 75%).

The following species (groups) come under the group predators and parasites:

predator Heteroptera:	Anthocoridae (Anthocoris spp., Orius spp.); Miridae; Nabidae.
Neuroptera:	Chrysopa spp.
Syrphida:	Syrphus vitripenis.
Ichneumon wasps:	Aphelinidae (Encarsia formosa); Chalcidae: Trichogramma spp.; Drino inconspicua; Coccygomius turionella, Phygadenon trichops.
ladybugs:	Coccinellidae (Stethorus spp., Coccinella septempunctata).
predator mites:	Phytoseiidae (Phytoseius persimilis, Typhlodromus pyri, Amblyseius spp.).
ground beetles:	Carabidae (Bembidion spp., Pterostichus spp.).
short-shield beetles:	Aleochara bilineata.

Toxicity data aquatic invertebrates, fish, earthworms, predators and parasites & bees; shown is the number of times that the chemical concerned, classified by toxicity classes I to IV, is mentioned for the species groups in the sources consulted; the chemicals are listed by application groups, viz. bactericides and fungicides, insecticides and acaricides, herbicides, and soil disinfectants. Classification: I = very toxic to toxic; II = moderately toxic; III = little toxic; IV = not toxic. Example: carbomix: this is moderately toxic for two fish species (N.B. "X" stands for the group "earthworms" or "bees"; it was not possible to distinguish the individual species here). * = highest concentration tested. (For discussion of table, see: 4.1 and 5.1).

toxicity classes	aq. invertebrate				fishes				earthworms				pred. & par.				bees			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
BACTERICIDES & FUNGICIDES																				
Anilides																				
carboxin						2														
cyprofuram						1	1													
fenfuram																				
furalaxyl							2													
metalaxyl								3								2			X	
Antibiotics																				
streptomycine																				
validamycine																				
Benzamidazoles																				
benomyl	1		1	1	3	2			X							1			X*	
carbendazim	1				1	1			X				1			1			X*	
fuberidazool																				
thiabendazool									X										X*	
Captan, related compounds																				
captan					11						X			1		8			X*	
dichlofluanid	1				1										3	1			X*	
folpet		I			8						X					1			X*	
tolylfluanid					3														X*	
Chlornitrobenzene comp.																				
quintozene																				
Dicarboximids																				
iprodion															2	1			X	
procymidon																				
vinchlozolin							3					X			3	2			X	
Dithiocarbamates																				
ferbam																2			X*	
manam																				
mancozeb													2		1	7			X*	
maneb					1														X*	
metiram																			X*	
sodium-dimethyl-dicarb.																				
thiram	1				1								3	1	2	2			X*	
zineb	1					1		1								1			X	
ziram	1				1														X*	
Phosphor compounds																				
fosethyl-aluminium								1			X							X		
pyrazofos					2	2							6	2				X		
tolclofos-methyl						1														
Chlorinated phenols																				
pentachlorophenol					7															
Copper compounds																				
copperhydroxide																				
coppernaftenate	1					1														
copperoxychinolate					1															X
copperoxychloride									X											
coppersulfate					4	1														
Mercury compounds																				
ethylmercurybromide																				
Morpholine compounds																				
dodemorf							2													X
fenpropimorf																				
tridemorf																			X*	

toxicity classes	aq. invertebrate I	II	III	IV	fishes I	II	III	IV	earthworms I	II	III	IV	pred. & par. I	II	III	IV	bees I	II	III	IV
Pyrimidine-compounds																				
bupirimate	1					2									1	4			X*	
fenarimol					1						X								X*	
Tin-compounds																				
fentin-acetate																				X
fentin-hydroxide					1															
Triazole-compounds																				
bitertanol	1				1	2										1				
penconazole						2													X*	
propiconazole								1											X*	
triadimefon							1								2	2				
triadimenol							3				X*									
Diverse																				
cymoxanil							2													
chloorthalonil							2									3				X
dichloran																				
dithianon					3															
dodine		1			1														X*	
etridiazole																				
fenaminosulf		1					2	1												
formaline				1			1	5												
guazatine																				
hymexazole																				
imazalil		1				1														
nitrothal-isopropyl																				
pencycuron						2														
prochloraz					1									2		4				
propamocarb								3					1		2					
thiofanaat-methyl									X						3	1				
triforine								3												X
trioxymethyleen																				
sulphur													1	1	3	1				X
INSECTICIDES & ACARICIDES																				
Acyl-ureum compounds																				
diflubenzuron	1						5	5							1	4				X
teflubenzuron								1												
Bacterie compounds																				
Bacillus thuringiensis		1					2								4	4				X
Carbamates																				
bendiocarb						1											X			
carbaryl	9	1			7	10	3		X				8		2	1	X			
carbofuran					8				X				1		1		X			
ethiofencarb						1	2						3	1	2	1		X		
fenoxycarb														1			X			
mercaptodimethur	1				3												X			
pirimicarb							2							2	10			X		
propoxur	3					2	1		X				5	2	1		X			
Carbamoyl-oximen																				
aldicarb					2				X											
butocarboxim																				
butoxycarboxim								1									X			
methomyl	3				4	4				X			10		1		X			
oxamyl						3	1										X			
thiofanox					2															
Cyanides																				
calciumcyanide									X								X			
sodiumcyanide																	X			
Dinitroalkylfenolen																				
DNOC		2			2						X						X			
Phosphor compounds																				
acefaat		1	1			1	2	5					9	2	2		X			
azamethifos					1	2											X			
azinfos-methyl	8				18	6							3		1		X			
bromofos					1								1					X		

toxicity classes	aq.invertebrate				fishes				earthworms				pred. & par.				bees			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
bromofos-ethyl	1	1			2	1											X			
carbofenothion	3				2	1											X			
chloorfenvinfos	2				4											1		X		
chloorpyrifos	6				6	1							2		2		X			
cumafos	2				7		4													
demeton-S-methyl-sulfon	1				5	2							1	1			X			
diazinon	4				4	4							1			1	X			
dichlofenthion	3				4	3														
dichloorvos	2				3	2											X			
dimethoaat	2					3							8		1		X			
ethoprofos						1	2													
etrimfos							1						10	1			X			
fenitrothion						3							4				X			
fenthion	7	1			4	20											X			
fonofos					2															
formothion							1										X			
fosalone					4								5	1	6			X		
fosfamidon	3	1				3	3	2					2		1		X			
fosmet	2				8	2							1	1	3		X			
foxim	1				7	2											X			
heptenofos						1							6	1	2	4	X			
isofenfos						1												X		
joodfenfos					3													X		
malathion	20	1			26	7	2					X	5		1		X			
methamidofos							2			X							X			
methidathion					2				X				9				X			
mevinfos	10				11		1						2				X			
omethoaat																	X			
oxy-demeton-methyl	2	2				7							3	1	1		X			
parathion	16				8	7				X			3				X			
pirimifos-methyl					2								2				X			
profenofos	1				4												X			
propetamfos																				
sulfotep					3												X			
temefos	4				2	7	2											X		
terbufos	1				3	1			X									X		
tetrachloorvinfos					3							X	5				X			
thiometon						1	1						1				X			
triazofos						2				X							X			
trichloorfon	7	1			9	4	2	1		X			7				X			
trichloronaat	1				2															
vamidothion													4		3	1	X			
Chlorinated hydrocarbons and related compounds																				
dicofol	1				5								3	1	3	2			X*	
dienochlor																			X*	
endosulfan	5				10				X				3	1	2			X		
endrin	14				28				X								X			
lindane	6				32							X	1		1	1	X			
methoxychlor	9				29															X
Vegetable extracts																				
pyrethrum	3				12								3				X			
Sulfides en sulfones																				
tetradifon	1				1	2									3	2				X
Synthetical pyrethroids																				
alfacypermethrin					1												X			
bio-allethrin	3				6												X			
cyfluthrin					4								1				X			
cypermethrin					1								9				X			
deltamethrin					1								8	2	1	1	X			
fenothrin					2															
fenpropathrin													3				X			
fenvalerate	1				4								8				X			
flucythrinate					2								1		1					
permethrin	1				7								8		1		X			
resmethrin					10															
tetramethrin					1												X			
Tin-compounds																				
azocyclotin					2									2					X	
cyhexatin	1				3								1	1	1	1		X		
fenbutatinoxide					3															X

toxicity classes	aq.invertebrate I	II	III	IV	fishes I	II	III	IV	earthworms I	II	III	IV	pred. & par. I	II	III	IV	bees I	II	III	IV
Divers																				
amitraz						1							2	2	3			X		
broompropylate					3								1			1				X
chlofentezine																				
minerale oil															1					
naftaleen																				
plifenate																				
polybutenes																				
coaltardistillate																				
synthetical adhesive																				
thiocyclam						1												X*		
trichlofenidine																				
HERBICIDES																				
Alkaancarbonacids																				
dalapon				1				6				X								
TCA								1												
Anilides																				
benzolyprop-ethyl							1	2				X								
di-ethathyl-ethyl																				
dimethachlor						2	1													
mefluidide								2												
metazachlor						2	1													
metolachlor																				
propachlor														1	1	1				
propyzamide														1		2				
quinonamide																				
Anorganic compounds																				
ammoniumsulfamate																				
ferrosulfate																				
sulphuracid																				
Benzo acids																				
dicamba		1		2			2													
Benzonitrilles																				
bromoxynil																				
dichlobenil		3	5			6														
ioxynil																				
Carbamates																				
asulam								5												
carbeetamid								1												
chloorbufam (BiPC)																				
chloorprofam (chloor-IPC)																				
fenmedifam													1		1	2				
profam (IPC)		1	1				2													
Difenylethers																				
aclonifen						2														
bifenox					1		1													
Dinitroanilines																				
pendimethalin					1															
trifluralin		1			1	1														
Dinitroalkylfenoles en dinitroalkylfenylesters																				
dinoseb (DNBP)		1			3								2				X			
dinoseb-acetate																	X			
dinoterb																	X			
DNOC		2			2					X							X			
Dipyridilium-compounds																				
diquat			2	3		2	6	1												
paraquat			2	1		2	2				X									
Fenoxyacetic acids																				
2,4-D							8	4												
MCPA							1													
Fenoxypropionacids																				
dichloorprop (2,4-DP)																				
mecoprop (MCPP)																				

toxicity classes	aq. invertebrate I	II	III	IV	fishes I	II	III	IV	earthworms I	II	III	IV	pred. & par. I	II	III	IV	bees I	II	III	IV
bromofos-ethyl	1	1			2	1											X			
carbofenothion	3				2	1											X			
chloorfenvinfos	2				4											1		X		
chloorpyrifos	6				6	1							2		2		X			
cumafos	2				7		4													
demeton-S-methyl-sulfon	1				5	2							1	1			X			
diazinon	4				4	4							1			1	X			
dichlofenthion	3				4	3														
dichloorvos	2				3	2											X			
dimethoast	2					3							8		1		X			
ethoprofos						1	2													
etrimfos							1						10	1			X			
fenitrothion						3							4				X			
fenthion	7	1			4	20											X			
fonofos					2															
formothion							1										X			
fosalone					4								5	1	6			X		
fosfamidon	3	1				3	3	2					2		1		X			
fosmet	2				8	2							1	1	3		X			
foxim	1				7	2											X			
heptenofos						1							6	1	2	4	X			
isofenfos						1												X		
joodfenfos					3													X		
malathion	20	1			26	7	2					X	5		1		X			
methamidofos							2			X							X			
methidathion					2				X				9				X			
mevinfos	10				11		1						2				X			
omethoast																	X			
oxy-demeton-methyl	2	2				7							3	1	1		X			
parathion	16				8	7				X			3				X			
pirimifos-methyl					2								2				X			
profenofos	1				4												X			
propetamfos																				
sulfotep					3												X			
temefos	4				2	7	2											X		
terbufos	1				3	1			X									X		
tetrachloorvinfos					3							X	5				X			
thiometon						1	1						1				X			
triazofos						2				X							X			
trichloorfon	7	1			9	4	2	1		X			7				X			
trichloronaat	1				2															
vamidothion													4		3	1	X			
Chlorinated hydrocarbons and related compounds																				
dicofol	1				5								3	1	3	2			X*	
dienochlor																			X*	
endosulfan	5				10				X				3	1	2			X		
endrin	14				28				X								X			
lindane	6				32							X	1		1	1	X			
methoxychlor	9				29															X
Vegetable extracts																				
pyrethrum	3				12								3				X			
Sulfides en sulfones																				
tetradifon	1				1	2									3	2				X
Synthetical pyrethroids																				
alfacypermethrin					1												X			
bio-allethrin	3				6												X			
cyfluthrin					4								1				X			
cypermethrin					1								9				X			
deltamethrin					1								8	2	1	1	X			
fenothrin					2															
fenpropathrin													3				X			
fenvalerate	1				4								8				X			
flucythrinate					2								1		1					
permethrin	1				7								8		1		X			
resmethrin					10															
tetramethrin					1												X			
Tin-compounds																				
azocyclotin					2									2					X	
cyhexatin	1				3								1	1	1	1		X		
fenbutatinoxide					3															X

toxicity classes	aq. invertebrate I	II	III	IV	fishes I	II	III	IV	earthworms I	II	III	IV	pred. & par. I	II	III	IV	bees I	II	III	IV
Divers																				
amitraz						1							2	2	3			X		
broompropylate					3								1			1				X
chlofentezine																				
minerale oil															1					
naftaleen																				
plifenate																				
polybutenes																				
coaltardistillate																				
synthetical adhesive																				
thiocyclam						1												X*		
trichlofenidine																				
HERBICIDES																				
Alkaancarbonacids																				
dalapon				1				6				X								
TCA								1												
Anilides																				
benzolyprop-ethyl							1	2				X								
di-ethathyl-ethyl																				
dimethachlor						2	1													
mefluidide								2												
metazachlor						2	1													
metolachlor																				
propachlor														1	1	1				
propyzamide														1		2				
quinonamide																				
Anorganic compounds																				
ammoniumsulfamate																				
ferrosulfate																				
sulphuracid																				
Benzo acids																				
dicamba		1		2			2													
Benzonitrilles																				
bromoxynil																				
dichlobenil		3	5			6														
ioxynil																				
Carbamates																				
asulam								5												
carbeetamid								1												
chloorbufam (BiPC)																				
chloorprofam (chloor-IPC)																				
fenmedifam													1		1	2				
profam (IPC)		1	1				2													
Difenylethers																				
aclonifen						2														
bifenox					1		1													
Dinitroanilines																				
pendimethalin					1															
trifluralin		1			1	1														
Dinitroalkylfenoles en dinitroalkylfenylesters																				
dinoseb (DNBP)		1			3								2				X			
dinoseb-acetate																	X			
dinoterb																	X			
DNOC		2			2					X							X			
Dipyridilium-compounds																				
diquat			2	3		2	6	1												
paraquat			2	1		2	2				X									
Fenoxyacetic acids																				
2,4-D							8	4												
MCPA							1													
Fenoxypropionacids																				
dichloorprop (2,4-DP)																				
mecoprop (MCPP)																				

toxicity classes	aq.invertebrate I	II	III	IV	fishes I	II	III	IV	earthworms I	II	III	IV	pred. & par. I	II	III	IV	bees I	II	III	IV
Pyridazone-compounds																				
chloridazone																				
Pyridyloxaceticacid comp.																				
fluroxypyr					1			1												
trichlopyr								2												
Thiocarbamates																				
butylate			1			2														
cycloate						1														
di-allate						2														
EPTC			2				4													
tri-allate						3														
metham-sodium												X								
Triazines																				
atrazine						1	2													
cyanazine		1				2	4													
desmetryn											X				1	2				
prometryn						2														
propazine							1	1												
simazine		1	1	1		1	1	2			X				1	9				
terbutryn					1	2														
terbutylazine						1	2													
Triazinones																				
hexazinone								4												
metamitrone								1												
metribuzine							2	2												
Uracil-compounds																				
bromacil																				
lenacil						1														
Ureum-compounds																				
chloorbromuron						1	1													
chloortoluron							2	1												
chloroxuron					2		1	1												
difenoxuron																				
diuron	2	1	1		1	4														
isoproturon						1	1	3												
linuron						1	2					X								
methabenzhiazuron							2													
metobromuron							3													
metoxuron																				
monolinuron									X					1	2					
Diverse																				
alloxdim-sodium																				
amitrol				1				2												
benazolin																				
bentazone																				
broomfenoxim													1		2	4				
chlorthal-methyl																				
chlorthiamid																				
chloralhydrate												X								
difenzoquat								2						1		1				
endothal-sodium							1	4												
ethofumesate																				
fluazifop-P-butyl					1	2														
flurenol							2													
glufosinate-ammonium								1												
glyphosate		1	1			2	1	1												
pyridate							1	2												
trichlopyr																				
selective working oil																				
sethoxydim																				
SOIL DISINFECTANTS																				
aldicarb					2				X											
dazomet										X										
dichloropropene						3			X											
dichl./etridiazole																				
dichl./methylisothiocyan.					3															
ethoprofos						1	2													
metam-sodium									X											
oxamyl						3	1										X			

Annex 5.1 Use of *chemicals in Haarlemmermeer* Polder and on crops in greenhouses

. = chemical is used, but no indications about quantities; x = chemical is used; xx = used on a large scale. Sources: Berends, 1988; LAC (written comm.); oral comm. Ag. Rep. Haarlemmermeer Polder; oral comm. de Vries, Union of Water Control Boards; Working Group "Effects.....", 1989.

	crops in greenhous.		Haarlemmermeerpolder		
agents	vegetable	flowers	potatoes	wheat	sugar bts
aldicarb		xx			
amitraz	x				
azinfos-methyl	.	.			
benomyl	x	xx			
bentazon/mecoprop				x	
bitertanol	x	x			
bupirimate	x				
captan	x	x	x		
carbendazim				x	
carbofuran		x			
chlorbromuron	.	.			
chlorfenvinfos	.	.			
chlormequat	.	.			
chlorprofam	x				
chlorthalonil	x	x			
chloridazon					x
cypermethrin	x	x			
deltamethrin	x	x			
diazinon	x	x			
dicarboximid	x				
dichlofluanid	.	.			
dichloorvos	x	xx			
dicofol		x			
di nochlor		xx			
diflubenzuron	x	x			
dinoseb			x		
dinoterb				x	
dinoterb/mecoprop				x	
DMPP			x		
DNOC			x	x	
dodemorf		x			
endrin	.	.			
endosulfan	.	.			
ethofumesaat					x
etridiazole	x	x			
fenaminosulf		x			
fenarimol	x				
fenbutatin-oxide	xx	x			
fenmedifam					x
fenpropathrin		x			
fenpropimorf				x	
fluroxypyr					x
formaline	x				
fosethyl-aluminium	x				
fosfamidon			x		x
furalaxyl		x			
heptenofos	x	x			
imazalil	x				
iprodion	xx	xx			
lindane	.	.			
malathion	x	x			
mancozeb		x			
maneb/fentinhydrox.			xx		
MCPA/MCPP				x	
mercaptodimethur	x	x			
metalaxyl	x				
metamitron					x
metamsodium	x	x			
methamidofos		x			
methomyl	xx	x			
methylbromide	xx	xx			
metobromuron			x		
metribuzin			x		
mevinfos	x	x			
oxamyl	xx	x			
oxy-demeton-methyl					x
paraquat	.	.			
parathion	x	x	x		x
permethrin	.	.			
pirimicarb	xx	xx	x	x	x
propamocarb	x	x			
propiconazol				x	
propoxur		x			
pyrazofos	x	xx			
quintozeen		x			
silverthiosulfate		x			
simazin	.	.			
thiofanate-methyl	x	xx			
thiram	x	x			
tolchlofos-methyl	x	x			
tolyfluanide	xx	x			
triadimencl				x	
triazofos		x			
trichloorfon	.	.			
triforine	x	x			
vinchlozolin	xx	xx			
zineb	x	xx			

Annex 5.2 **Physical-chemical measurements August**

pH, chloride content and cholinesterase-inhibitor contents (in parathion equivalents) in the August water samples; measurements of cholinesterase inhibitors done by the RIVM.

sample no. & location	pH	Cl^- (mg/l)	Chol.(g/l)
blank			
10.IJsselmeer	8,49	144	1,52
control			
2. Aalsmeer	8,24	158	2,36
potatoes			
28.Haarl.meerpolder	8,32	387	1,32
29.Haarl.meerpolder	8,17	838	0,60
sugar beet			
3. Aalsmeer	8,27	134	2,84
30.Haarl.meerpolder	8,05	849	0,96
wheat, narrow (ditch)			
4. Aalsmeer	8,62	126	1,24
16.North-East polder	8,11	67	1,16
greenhouses, narrow			
7. Aalsmeer	7,97	40	2,56
17.Westland	8,32	159	3,52
20.Westland	7,97	212	2,60
21.Westland	8,19	193	22,4
22.Westland	8,08	110	6,40
23.North-East polder	7,91	89	0,80

Annex 5.3 Physical-chemical measurements September

pH, chloride and phosphate contents and cholinesterase-inhibitor content (in parathion equivalents) of the water samples taken in September (3rd. sampling round); measurement of cholinesterase inhibitors done by RIVM.

sample no. & location	pH	Cl^- (mg/l)	PO_4^- (mg/l)	Chol.(g/l)
blanks				
1. Leiden	8,04	119	2,14	0,72
31.Markermeer	8,12	167	0,03	0,64
controls narrow				
32.Aalsmeer	7,95	493	0,69	1,24
33.Aalsmeer	7,93	159	0,59	0,92
34.Westland	7,63	124	0,63	0,40
35.Westland	8,39	63	0,44	0,80
36.Haarl.meerpolder	7,62	1367	4,58	0,48
37.Haarl.meerpolder	7,80	50	0,24	0,96
38.North-East polder	8,32	62	0,17	0,80
39.North-East polder	7,88	51	0,08	0,96
controls wide				
2. Aalsmeer	7,90	122	0,07	5,52
40.Aalsmeer	8,11	127	2,43	0,88
41.Westland	8,09	228	2,93	2,80
42.Westland	7,88	92	0,57	5,44
43.Haarl.meerpolder	8,18	261	0,18	0,80
12.North-East polder	8,18	64	0,07	2,08
13.North-East polder	7,70	499	<0,01	0,72
potatoes narrow				
44.Aalsmeer	7,76	88	0,41	2,28
45.Aalsmeer	7,97	125	0,87	1,16
46.Haarl.meerpolder	7,60	644	0,93	1,16
47.Haarl.meerpolder	8,27	55	0,83	0,88
48.North-East polder	7,31	57	0,08	0,84
49.North-East polder	7,96	114	<0,01	1,28
sugar beet narrow				
52.Aalsmeer	8,20	39	0,04	0,76
53.Aalsmeer	7,93	73	0,11	0,80
54.Haarl.meerpolder	8,12	68	2,32	1,44
55.Haarl.meerpolder	7,94	99	0,33	0,88
56.North-East polder	7,99	62	0,05	0,56
57.North-East polder	8,18	235	<0,01	0,52
wheat narrow				
4. Aalsmeer	8,24	70	0,03	0,64
5. Aalsmeer	7,79	110	1,05	<0,30
59.Haarl.meerpolder	7,82	83	2,56	1,72
60.Haarl.meerpolder	7,88	629	1,88	0,96
16.North-East polder	7,77	89	0,06	0,80
61.North-East polder	8,05	98	<0,01	0,92
fields wide				
3. Aalsmeer	8,00	116	0,25	3,60
50.Aalsmeer	8,00	98	0,47	<0,30
62.Aalsmeer	7,61	154	0,72	1,52
28.Haarl.meerpolder	8,14	318	0,78	0,60
30.Haarl.meerpolder	7,86	1087	0,59	0,48
64.North-East polder	8,16	81	0,05	1,04
51.North-East polder	8,06	389	0,03	1,48
58.North-East polder	8,03	219	0,03	1,32
63.Haarl.meerpolder	7,87	420	0,36	1,80
greenhouses narrow				
65.Aalsmeer	7,64	32	0,19	0,68
66.Aalsmeer	8,15	125	0,95	2,28
7. Aalsmeer	7,59	38	0,83	1,76
17.Westland	7,94	71	3,17	21,6
21.Westland	7,47	107	1,69	18,4
22.Westland	7,67	23	0,77	1,44
23.North-East polder	8,14	62	0,10	0,64
greenhouses wide				
67.Aalsmeer	7,77	134	1,35	0,96
68.Aalsmeer	7,86	123	0,30	0,88
20.Westland	7,75	226	3,02	1,92
69.Westland	8,00	43	1,26	1,40

Annex 5.4 EOCl-contents

Extractable chlorinated pesticide contents (in ng/liter) in some water samples taken in August (3rd. sampling round); the analyses were done by the Rhineland (Rijnland) Water Control Board; listed are the substances for which standards were formulated in IMP-W, 1985-1989.

N.B. In a number of samples from the North-East Polder as well, chlorinated hydrocarbons were measured (by the West-Overijssel Water Purification Board); in none of these samples was a concentration measured of more than 10 ng/liter.

	Aalsmeer				Westland				Haarlemmermeer-polder			Mark.-meer
	2	3	4	7	17	20	21	22	28	47	30	31
IMP-substanc												
aldrin	1	<1	2	<1	10	<1	4	7	<1	<1	<1	<1
dieldrin	<1	<1	<1	2	25	8	1	15	<1	30	1	<1
endrin	1	15	<1	10	<1	6	6	<1	<1	<1	<1	<1
heptachloor	25	25	30	15	25	65	35	10	15	7	15	10
HEPO	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
DDT	<1	<1	<1	4	8	2	3	15	<1	<1	<1	<1
DDD	<1	<1	<1	4	8	15	5	15	<1	<1	<1	<1
DDE	<1	<1	<1	<1	3	<1	7	<1	<1	7	8	<1
HCB	<1	<1	<1	<1	4	10	2	10	<1	<1	<1	<1
HCH-A	5	5	9	6	<1	15	7	7	5	3	5	<1
HCH-G	10	8	6	6	4	9	10	20	7	3	4	10
endosulfan-A	<1	<1	<1	4	<1	1	70	<1	<1	<1	<1	<1
subtotal	42	53	47	51	93	131	150	99	27	50	33	20
other pesticides												
captan	<10	10	<10	<10	<10	<10	10	20	<10	10	<10	<10
dichlobenil	35	15	25	20	10	<1	10	6	25	15	10	10
endosulfan-B	<1	<1	<1	3	<1	<1	35	<1	<1	<1	<1	<1
HCH-B	3	2	<1	4	3	4	3	2	<1	<1	<1	4
HCH-D	20	15	20	10	15	10	15	15	20	10	15	10
PCNB	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
vinchlozolin	<1	<1	<1	<1	<1	<1	2	4	<1	<1	<1	<1
total pesticides	100	95	92	88	121	145	215	146	72	85	58	44

Annex 5.5 Determining the dithiocarbamate content

In co-operation with the biopharmacy group of the University of Leiden and some people working in the Environmental Hygiene Section of the Centre for Environmental Studies, it was attempted to determine the dithiocarbamate content of a number of samples. The CS_2-method used by the RIVM (Wegman & Hofstee, 1981) was used for this purpose.

With this method, tin chloride and acid are added to a water sample, which in a closed bottle at a temperature of 70 C react with dithiocarbamates. One of the breakdown products is CS_2, a gas which escapes from the water and gathers in the air above the liquid (the headspace). By means of injecting this gas into a gas chromatograph, the quantity of CS_2 in the gas can be determined, and thus also the amount of dithiocarbamates in the sample of water. It is true though the yield in the conversion to CS_2 may differ from substance to substance.

An electron capture detection gas chromatograph was used for the measurements. With this, one can measure many times more accurately than with a flame photometric detector. A standard solution of maneb was used as control. As it turned out later, this substance has a relatively low, and also variable yield (85 plus or minus 14%, written comm. Haarman, Water Utility Co. 'Overijssel').

It was also found, after convering the values measured to CS_2-contents, (in which an efficiency of 85% was assumed), that the values measured lay in the range of 0-10 mg/l of CS_2. This is a factor 100 to 1000 times higher than the values found by others in surface water, e.g. 30 g/l near Anna Palowna (Wegman & Hofstee, 1981), 13.7 g/l in Woudse Land reclamation, and 3.8, 4.9 and 6.9 g/l near Staelduinen (Wammes et al., 1986), 14 g/l in the Enser- and Herten canal and 57 g/l near Marknesse pumping station (ZWO, 1988). Only in waste water from cleaning soil-disinfecting machines was a concentration of 50 g/l found once (Ens unloading pier [ZWO, 1988]).

In view of these results, it has become clear that the method needs to be considerably improved to arrive a reliable results.

Annex 5.4 EOCl-contents

Extractable chlorinated pesticide contents (in ng/liter) in some water samples taken in August (3rd. sampling round); the analyses were done by the Rhineland (Rijnland) Water Control Board; listed are the substances for which standards were formulated in IMP-W, 1985-1989.

N.B. In a number of samples from the North-East Polder as well, chlorinated hydrocarbons were measured (by the West-Overijssel Water Purification Board); in none of these samples was a concentration measured of more than 10 ng/liter.

	Aalsmeer				Westland				Haarlemmermeer-polder			Mark.-meer
	2	3	4	7	17	20	21	22	28	47	30	31
IMP-substanc												
aldrin	1	<1	2	<1	10	<1	4	7	<1	<1	<1	<1
dieldrin	<1	<1	<1	2	25	8	1	15	<1	30	1	<1
endrin	1	15	<1	10	<1	6	6	<1	<1	<1	<1	<1
heptachloor	25	25	30	15	25	65	35	10	15	7	15	10
HEPO	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
DDT	<1	<1	<1	4	8	2	3	15	<1	<1	<1	<1
DDD	<1	<1	<1	4	8	15	5	15	<1	<1	<1	<1
DDE	<1	<1	<1	<1	3	<1	7	<1	<1	7	8	<1
HCB	<1	<1	<1	<1	4	10	2	10	<1	<1	<1	<1
HCH-A	5	5	9	6	<1	15	7	7	5	3	5	<1
HCH-G	10	8	6	6	4	9	10	20	7	3	4	10
endosulfan-A	<1	<1	<1	4	<1	1	70	<1	<1	<1	<1	<1
subtotal	42	53	47	51	93	131	150	99	27	50	33	20
other pesticides												
captan	<10	10	<10	<10	<10	<10	10	20	<10	10	<10	<10
dichlobenil	35	15	25	20	10	<1	10	6	25	15	10	10
endosulfan-B	<1	<1	<1	3	<1	<1	35	<1	<1	<1	<1	<1
HCH-B	3	2	<1	4	3	4	3	2	<1	<1	<1	4
HCH-D	20	15	20	10	15	10	15	15	20	10	15	10
PCNB	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
vinchlozolin	<1	<1	<1	<1	<1	<1	2	4	<1	<1	<1	<1
total pesticides	100	95	92	88	121	145	215	146	72	85	58	44

Annex 5.5 Determining the dithiocarbamate content

In co-operation with the biopharmacy group of the University of Leiden and some people working in the Environmental Hygiene Section of the Centre for Environmental Studies, it was attempted to determine the dithiocarbamate content of a number of samples. The CS_2-method used by the RIVM (Wegman & Hofstee, 1981) was used for this purpose.

With this method, tin chloride and acid are added to a water sample, which in a closed bottle at a temperature of 70 C react with dithiocarbamates. One of the breakdown products is CS_2, a gas which escapes from the water and gathers in the air above the liquid (the headspace). By means of injecting this gas into a gas chromatograph, the quantity of CS_2 in the gas can be determined, and thus also the amount of dithiocarbamates in the sample of water. It is true though the yield in the conversion to CS_2 may differ from substance to substance.

An electron capture detection gas chromatograph was used for the measurements. With this, one can measure many times more accurately than with a flame photometric detector. A standard solution of maneb was used as control. As it turned out later, this substance has a relatively low, and also variable yield (85 plus or minus 14%, written comm. Haarman, Water Utility Co. 'Overijssel').

It was also found, after convering the values measured to CS_2-contents, (in which an efficiency of 85% was assumed), that the values measured lay in the range of 0-10 mg/l of CS_2. This is a factor 100 to 1000 times higher than the values found by others in surface water, e.g. 30 g/l near Anna Palowna (Wegman & Hofstee, 1981), 13.7 g/l in Woudse Land reclamation, and 3.8, 4.9 and 6.9 g/l near Staelduinen (Wammes et al., 1986), 14 g/l in the Enser- and Herten canal and 57 g/l near Marknesse pumping station (ZWO, 1988). Only in waste water from cleaning soil-disinfecting machines was a concentration of 50 g/l found once (Ens unloading pier [ZWO, 1988]).

In view of these results, it has become clear that the method needs to be considerably improved to arrive a reliable results.

Annex 5.4 EOCl-contents

Extractable chlorinated pesticide contents (in ng/liter) in some water samples taken in August (3rd. sampling round); the analyses were done by the Rhineland (Rijnland) Water Control Board; listed are the substances for which standards were formulated in IMP-W, 1985-1989.

N.B. In a number of samples from the North-East Polder as well, chlorinated hydrocarbons were measured (by the West-Overijssel Water Purification Board); in none of these samples was a concentration measured of more than 10 ng/liter.

	Aalsmeer				Westland				Haarlemmermeer-polder			Mark.-meer
	2	3	4	7	17	20	21	22	28	47	30	31
IMP-substanc												
aldrin	1	<1	2	<1	10	<1	4	7	<1	<1	<1	<1
dieldrin	<1	<1	<1	2	25	8	1	15	<1	30	1	<1
endrin	1	15	<1	10	<1	6	6	<1	<1	<1	<1	<1
heptachloor	25	25	30	15	25	65	35	10	15	7	15	10
HEPO	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
DDT	<1	<1	<1	4	8	2	3	15	<1	<1	<1	<1
DDD	<1	<1	<1	4	8	15	5	15	<1	<1	<1	<1
DDE	<1	<1	<1	<1	3	<1	7	<1	<1	7	8	<1
HCB	<1	<1	<1	<1	4	10	2	10	<1	<1	<1	<1
HCH-A	5	5	9	6	<1	15	7	7	5	3	5	<1
HCH-G	10	8	6	6	4	9	10	20	7	3	4	10
endosulfan-A	<1	<1	<1	4	<1	1	70	<1	<1	<1	<1	<1
subtotal	42	53	47	51	93	131	150	99	27	50	33	20
other pesticides												
captan	<10	10	<10	<10	<10	<10	10	20	<10	10	<10	<10
dichlobenil	35	15	25	20	10	<1	10	6	25	15	10	10
endosulfan-B	<1	<1	<1	3	<1	<1	35	<1	<1	<1	<1	<1
HCH-B	3	2	<1	4	3	4	3	2	<1	<1	<1	4
HCH-D	20	15	20	10	15	10	15	15	20	10	15	10
PCNB	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
vinchlozolin	<1	<1	<1	<1	<1	<1	2	4	<1	<1	<1	<1
total pesticides	100	95	92	88	121	145	215	146	72	85	58	44

Annex 5.5 Determining the dithiocarbamate content

In co-operation with the biopharmacy group of the University of Leiden and some people working in the Environmental Hygiene Section of the Centre for Environmental Studies, it was attempted to determine the dithiocarbamate content of a number of samples. The CS_2-method used by the RIVM (Wegman & Hofstee, 1981) was used for this purpose.

With this method, tin chloride and acid are added to a water sample, which in a closed bottle at a temperature of 70 C react with dithiocarbamates. One of the breakdown products is CS_2, a gas which escapes from the water and gathers in the air above the liquid (the headspace). By means of injecting this gas into a gas chromatograph, the quantity of CS_2 in the gas can be determined, and thus also the amount of dithiocarbamates in the sample of water. It is true though the yield in the conversion to CS_2 may differ from substance to substance.

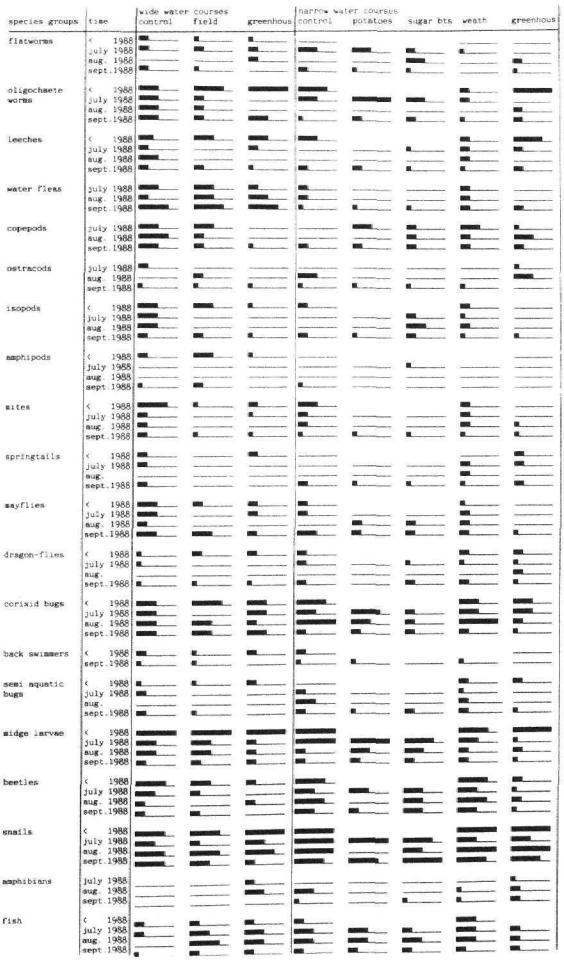
An electron capture detection gas chromatograph was used for the measurements. With this, one can measure many times more accurately than with a flame photometric detector. A standard solution of maneb was used as control. As it turned out later, this substance has a relatively low, and also variable yield (85 plus or minus 14%, written comm. Haarman, Water Utility Co. 'Overijssel').

It was also found, after convering the values measured to CS_2-contents, (in which an efficiency of 85% was assumed), that the values measured lay in the range of 0-10 mg/l of CS_2. This is a factor 100 to 1000 times higher than the values found by others in surface water, e.g. 30 g/l near Anna Palowna (Wegman & Hofstee, 1981), 13.7 g/l in Woudse Land reclamation, and 3.8, 4.9 and 6.9 g/l near Staelduinen (Wammes et al., 1986), 14 g/l in the Enser- and Herten canal and 57 g/l near Marknesse pumping station (ZWO, 1988). Only in waste water from cleaning soil-disinfecting machines was a concentration of 50 g/l found once (Ens unloading pier [ZWO, 1988]).

In view of these results, it has become clear that the method needs to be considerably improved to arrive a reliable results.

Annex 5.6 Comparison of occurrence of species groups of the various sampling rounds

■ = 1 specimen; ▬ = one class; to arrive at this table, per animal group and per crop the average of classes per ditch was taken; this yardstick gives an idea of the occurrence of species groups with an indication concerning abundance.



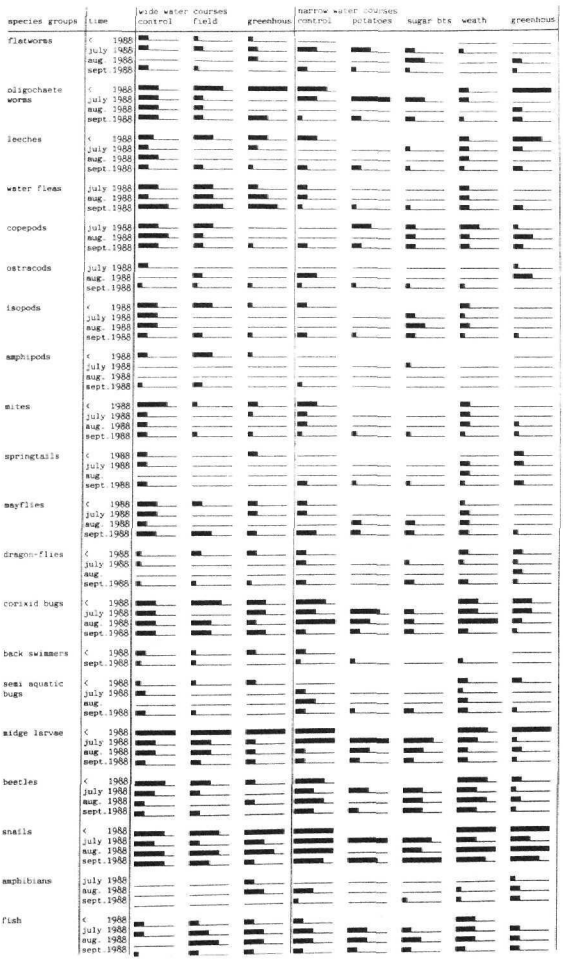
Annex 5.7 **Mortality percentages of water fleas in bio-assays of June and July**

Shown is the mortality in two non-filtered and two filtered samples after different lengths of time. Oostv. pl. = Oostvaarders Ponds; Zw. Meer = Black Lake; IJsselmeer = IJssel Lake; N.O.P. = North-East Polder; samples 1 to 7 incl.: June 25, 1988; samples 8 to 27 incl.: July 5, 1988.

sample no. and location	24 hours		48 hours	
	non f.	filt.	non f.	filt.
blank				
1. Leiden	0-20	0-30	0-30	0-30
control				
2. Aalsmeer	20-30	30	70-90	50-60
sugar beet				
3. Aalsmeer	50-60	50-60	90	70-80
wheat				
4. Aalsmeer	0-10	0-20	10-20	10-20
5. Aalsmeer	0	0-20	0-20	20
greenhouse				
6. Aalsmeer	0-30	0-20	10-30	10-20
7. Aalsmeer	90-100	100	100	100
blank				
1. Leiden	70-90	70-80	70-90	80
8. Oostv.pl.	30-100	30-80	40-100	50-90
9. Zw. Meer	40-60	50-60	70	50-70
10.IJsselm.	30-50	40-50	50-60	50-90
11.Leiden	70-90	30-60	90	50-60
control				
12.N.E.P.	20-80	40-50	50-80	80
13.N.E.P.	60-70	20-60	60-80	40-70
wheat				
14.Westland	60-80	20-70	60-90	20-70
15.N.E.P.	0-10	0-10	10-60	20-40
16.N.E.P.	40-50	60-90	40-50	70-90
16a.N.E.P	80-90	40-90	80-90	40-90
greenhouse				
17.Westland	70-80	70-90	90-100	90-100
18.Westland	60-70	60-90	70	60-90
19.Westland	30-60	60	60-70	60
20.Westland	30-60	10-80	40-80	30-80
21.Westland	60-90	40-90	70-90	50-90
22.Westland	70-80	90-100	70-80	90-100
23.N.E.P.	20-50	20-80	60-70	30-80
fruit				
24.N.E.P.	10-20	10-30	20	10-40
25.N.E.P.	50-70	10-30	50-70	30-40
flowers				
26.N.E.P.	90-100	70	90-100	80
market gard				
27.N.E.P.	70-90	70	80-100	70

Annex 5.6 **Comparison of occurrence of species groups of the various sampling rounds**

■ = 1 specimen; ▬ = one class; to arrive at this table, per animal group and per crop the average of classes per ditch was taken; this yardstick gives an idea of the occurrence of species groups with an indication concerning abundance.



Annex 5.7 **Mortality percentages of water fleas in bio-assays of June and July**

Shown is the mortality in two non-filtered and two filtered samples after different lengths of time. Oostv. pl. = Oostvaarders Ponds; Zw. Meer = Black Lake; IJsselmeer = IJssel Lake; N.O.P. = North-East Polder; samples 1 to 7 incl.: June 25, 1988; samples 8 to 27 incl.: July 5, 1988.

sample no. and location	24 hours		48 hours	
	non f.	filt.	non f.	filt.
blank				
1. Leiden	0-20	0-30	0-30	0-30
control				
2. Aalsmeer	20-30	30	70-90	50-60
sugar beet				
3. Aalsmeer	50-60	50-60	90	70-80
wheat				
4. Aalsmeer	0-10	0-20	10-20	10-20
5. Aalsmeer	0	0-20	0-20	20
greenhouse				
6. Aalsmeer	0-30	0-20	10-30	10-20
7. Aalsmeer	90-100	100	100	100
blank				
1. Leiden	70-90	70-80	70-90	80
8. Oostv.pl.	30-100	30-80	40-100	50-90
9. Zw. Meer	40-60	50-60	70	50-70
10.IJsselm.	30-50	40-50	50-60	50-90
11.Leiden	70-90	30-60	90	50-60
control				
12.N.E.P.	20-80	40-50	50-80	80
13.N.E.P.	60-70	20-60	60-80	40-70
wheat				
14.Westland	60-80	20-70	60-90	20-70
15.N.E.P.	0-10	0-10	10-60	20-40
16.N.E.P.	40-50	60-90	40-50	70-90
16a.N.E.P	80-90	40-90	80-90	40-90
greenhouse				
17.Westland	70-80	70-90	90-100	90-100
18.Westland	60-70	60-90	70	60-90
19.Westland	30-60	60	60-70	60
20.Westland	30-60	10-80	40-80	30-80
21.Westland	60-90	40-90	70-90	50-90
22.Westland	70-80	90-100	70-80	90-100
23.N.E.P.	20-50	20-80	60-70	30-80
fruit				
24.N.E.P.	10-20	10-30	20	10-40
25.N.E.P.	50-70	10-30	50-70	30-40
flowers				
26.N.E.P.	90-100	70	90-100	80
market gard				
27.N.E.P.	70-90	70	80-100	70

Annex 5.8 **Mortality of water fleas in bio-assays in August 1988**

Mortality percentages of water fleas raised at the DBW/RIZA (in duplicate) and of water fleas bought at aquarium shop (single) in the August bio-assays.

	24 hours		48 hours	
sample no. & location	RIZA	shop	RIZA	shop
blank				
10.IJsselmeer	0	20	0	30
control				
2. Aalsmeer	0-40	10	0-60	40
potatoes				
28.Haarl.meerpolder	0-10	0	0-20	0
29.Haarl.meerpolder	0	20	0	30
sugar beets				
3. Aalsmeer	90-100	100	100	100
30.Haarl.meerpolder	0	10	0	10
wheat				
4. Aalsmeer	0	20	0-10	20
16.North-East polder	0	0	0	0
greenhouses				
7. Aalsmeer	0	30	0	30
17.Westland	0-20	10	100	20
20.Westland	0	20	0	20
21.Westland	0	10	60-80	10
22.Westland	70-100	80	100	100
23.North-East polder	0	10	0	10
fruit				
24.North-East polder	0	20	0	30
flowers				
26.North East polder	0	0	0	0
market gardening				
27.North-East polder	0	20	0	40

Annex 5.9 Mortality percentages of water fleas Daphnia magna in the September bio-assays

When duplicated differ, both percentages are listed.

sample no. & location	24 hours	48 hours
blanks		
1. Leiden	0	0
31.Markermeer	0	0
controls narrow		
32.Aalsmeer	0	0
33.Aalsmeer	0	0
34.Westland	0*	0*
35.Westland	0-10	0-10
36.Haarl.meerpolder	0	10-20
37.Haarl.meerpolder	0	0
38.North-East polder	0	0
39.North-East polder	0	0
controls wide		
2. Aalsmeer	0	0
40.Aalsmeer	0	0
41.Westland	0	0
42.Westland	0	0
43.Haarl.meerpolder	0	0
12.North-East polder	0	0
13.North-East polder	0	0
potatoes narrow		
44.Aalsmeer	0	0
45.Aalsmeer	0	0
46.Haarl.meerpolder	0	0
47.Haarl.meerpolder	0	0
48.North-East polder	0	0
49.North-East polder	0	0-10
sugar beets narrow		
52.Aalsmeer	0	0
53.Aalsmeer	0	0
54.Haarl.meerpolder	0	0
55.Haarl.meerpolder	0-10	10-20
56.North-East polder	0	0-10
57.North-East polder	0	10-20
wheat narrow		
4. Aalsmeer	0	0
5. Aalsmeer	0	0
59.Haarl.meerpolder	0	0
60.Haarl.meerpolder	0	0
16.North-East polder	0	0
61.North-East polder	0-10	0-10
fields wide		
3. Aalsmeer	0	10-20
50.Aalsmeer	0	0
62.Aalsmeer	10-20	10-50
28.Haarl.meerpolder	0	0
30.Haarl.meerpolder	0	0
63.Haarl.meerpolder	0	0
51.North-East polder	0	0
58.North-East polder	0-20	0-20
64.North-East polder	0	0-40
greenhouses narrow		
65.Aalsmeer	0	0
66.Aalsmeer	0	0
7. Aalsmeer	0	0
17.Westland	90-100	100
21.Westland	100	100
22.Westland	0	0
23.North-East polder	0	0
greenhouses wide		
67.Aalsmeer	0	0
68.Aalsmeer	0	0
20.Westland	0	0
69.Westland	0	0

* = tested without duplicate.

Annex 5.10 **Mortality percentage of water mites Unionicola minor in September bio-assays**

When duplicated differ, both percentages are listed.

sample no. & location	24/48 hours	120 hours	192 hours
blanks			
1. Leiden	0-10	0-20	0-30
31.Markermeer	0	0	0
controls narrow			
32.Aalsmeer	0	0	0-20
33.Aalsmeer	0	0	0
34.Westland	0	0	0
35.Westland	0	0	0
36.Haarl.meerpolder	0	0-10	0-40
37.Haarl.meerpolder	0	0-20	0-20
38.North-East polder	0	0	0
39.North-East polder	0	0	0
controls wide			
2. Aalsmeer	0	0	0
40.Aalsmeer	0	0-10	0-10
41.Westland	0	0-20	0-40
42.Westland	0	0	0-20
43.Haarl.meerpolder	0	0	0
12.North-East polder	0	0-20	0-40
13.North-East polder	0	0	0
potatoes narrow			
44.Aalsmeer	0	0	0
45.Aalsmeer	0	0	0
46.Haarl.meerpolder	0	0	0
47.Haarl.meerpolder	0	0	20-40
48.North-East polder	0	0	0
49 North-East polder	0	0	0
sugar beets narrow			
52.Aalsmeer	0	0	0-20
53.Aalsmeer	0	0	0
54.Haarl.meerpolder	0-40	0-40	0-40
55.Haarl.meerpolder	0	0	0
56.North-East polder	0	0	0
57.North-East polder	0	0	0
wheat narrow			
4. Aalsmeer	0	0	0-10
5. Aalsmeer	0	0	0
59.Haarl.meerpolder	0	0	0-10
60.Haarl.meerpolder	0	0	0
16.North-East polder	0	0	0-20
61.North-East polder	0	0	0
fields wide			
3. Aalsmeer	0	0-10	0-10
50.Aalsmeer	0	0-20	0-20
62.Aalsmeer	0	0	0
28.Haarl.meerpolder	0	0	0
30.Haarl.meerpolder	0	0	0-30
63.Haarl.meerpolder	0	0	0
52.North-East polder	0	0	0-10
58.North-East polder	0	0	0
64.North-East polder	0	0	0
greenhouses narrow			
65.Aalsmeer	0	0	0
66.Aalsmeer	0	0	0
7. Aalsmeer	80-100	100	100
17.Westland	0	0-20	0-20
21.Westland	0	20	20-50
22.Westland	0	0	0
23.North-East polder	0	0	0-10
greenhouses wide			
67.Aalsmeer	0	0	0
68.Aalsmeer	0	0	0
20.Westland	0	0	0-10
69.Westland	0	0	0

Annex 5.11 Mortality percentages of water isopods Asellus aquaticus in September bio-assays

When duplicated differ, both percentages are listed.

sample no. & location	24 hours	48 hours	120 hours	192 hours
blanks				
1. Leiden	0	0	0	0
31.Markermeer	0-20	0-20	40	40-60
controls narrow				
32.Aalsmeer	0	0	0	0
33.Aalsmeer	0	0	0	0
34.Westland	0	0	0	0
35.Westland	0	0	0	0
36.Haarl.meerpolder	0	0	0	0
37.Haarl.meerpolder	0	0	0	0
38.North-East polder	0	0	0	0
39.North-East polder	0	0	0	0-20
controls wide				
2. Aalsmeer	0	0	0-20	0-40
40.Aalsmeer	0	0	0	0
41.Westland	0	0	0	0-20
42.Westland	0	0	0-20	0-20
43.Haarl.meerpolder	0-20	0-20	0-20	0-20
12.North-East polder	0	0	0-20	0-20
13.North-East polder	0	0	0	0
potatoes narrow				
44.Aalsmeer	0-20	0-20	0-40	0-100
45.Aalsmeer	0	0	0-20	0-20
46.Haarl.meerpolder	0-20	0-40	0-40	0-40
47.Haarl.meerpolder	0	0	0	0
48.North-East polder	0	0-20	0-20	0-20
49.North-East polder	0	0	0	0
sugar beets narrow				
52.Aalsmeer	0	0	0-10	0-20
53.Aalsmeer	0	0	0	0
54.Haarl.meerpolder	0-80	20-100	20-100	40-100
55.Haarl.meerpolder	0	20	20	20-40
56.North-East polder	0-20	0-20	0-20	0-20
57.North-East polder	0-20	0-20	0-20	0-20
wheat narrow				
4. Aalsmeer	0	0	0	0-20
5. Aalsmeer	0	0	0	0
59.Haarl.meerpolder	0-20	0-20	0-20	0-20
60.Haarl.meerpolder	0	0	0-20	0-40
16.North-East polder	0	0	0	0
61.North-East polder	0	0-20	0-20	20-40
fields wide				
3. Aalsmeer	0-20	0-20	20	20-40
50.Aalsmeer	0	0-20	0-20	0-20
62.Aalsmeer	20	20	20-40	30-40
28.Haarl.meerpolder	0	0	0-40	20-40
30.Haarl.meerpolder	0	0	0	0
63.Haarl.meerpolder	0	0-20	0-20	0-20
51.North-East polder	0	0	0	0-20
58.North-East polder	0	0	0	0-20
64.North-East polder	0	0-20	0-20	0-20
greenhouses narrow				
65.Aalsmeer	0	0	0-20	40
66.Aalsmeer	0	0	0-20	0-20
7. Aalsmeer	0-20	20-60	100	100
17.Westland	0	0	0-20	20-40
21.Westland	0	0	0-20	0-40
22.Westland	0	0-20	0-20	0-20
23.North-East polder	0	0-20	0-20	0-20
greenhouses wide				
67.Aalsmeer	0	0-20	0-40	0-40
68.Aalsmeer	0	0	0	0-20
20.Westland	0	0-20	0-20	0-20
69.Westland	0	0	0	0

Annex 5.12 LC-50 experiment with parathion

Mortality percentages after 24, 48 and 96 hours at various parathion contents. No mortality at all occurred in water mite Mideopsis orbicularis. When two figures are shown, mortality differed in the duplicates.

water flea Daphnia magna

hour	blank	0,18 g/l	0,37 g/l	0,74 g/l
24	0-60	100	100	100
48	0-90	100	100	100
96	30-100	100	100	100

water mite Unionicola minor

hour	blank	0,18 g/l	0,37 g/l	0,74 g/l
24	0	0-10	0-20	10-20
48	0	10-20	10-20	20-40
96	0	20-30	20-40	40-60

isopod Asellus aquaticus

hour	blank	0,37 g/l	200 g/l	600 g/l
24	0	100*	100	100
48	0	100*	100	100
96	0	50	100	100

* = still alive, but immobile.

Annex 6. List of abbreviations

AID	Algemene Inspectie Dienst = General Inspection and Investigation Service
CABO	Centrum voor Agro Biologisch Onderzoek = Centre for Agro Biological Research
CCRX	Coördinatie Commissie voor de meting van Radioactiviteit & *Xenobiotische Stoffen = Co-ordination Commission* for the Measurement of Radioactivity & Xenobiotic Substances
CDI	Centraal Diergeneeskundig Instituut = Central Veterinary Institute
CML	Centrum voor Milieukunde RU-Leiden = Centre for Environmental Studies Leiden University
CNB	Commissie voor onderzoek inzake Nevenwerkingen van Bestrijdingsmiddelen en aanverwante stoffen = Commission for Research on Side Effects of Pesticides and related compounds
CTB	Commissie Toelating Bestrijdingsmiddelen = Commission for the Registration of Pesticides
CUVWO	Coördinatie-commissie Uitvoering Wet Verontreiniging Oppervlaktewater = Co-ordination commission Implementation of Pollution of Surface Waters Act
DBW/RIZA	Dienst Binnenwateren van Rijkswaterstaat/Rijksinstituut voor Zuivering van Afvalwater = Institute for Inland Water Management and Waste Water Treatment
DGM	Directoraat-Generaal Milieubeheer = Directorate-General for Environmental Protection
DGM-S&R	DGM - Directie Stoffen en Risicobeheersing = Substances & Risk Control Directorate
EIS	European Invertebrate Survey
EPA	US Environmental Protection Agency
IB	Instituut voor Bodemvruchtbaarheid = Institute for Soil Fertility
IMP-M	Indicatief Meerjarenplan Milieubeheer = Indicative Multi-Year Program Environmental Management
IMP-W	Indicatief Meerjarenplan Water = Indicative Multi-Year Program Water Management
IOB	Instituut voor Onderzoek Bestrijdingsmiddelen = Institute for Research on Pesticides (at present, after fusion with some other institutes a.o. Stiboka: "Staring Centrum" [SC] = Wynand Staring Centre for integrated land, soil and water research)
IOBC	International Organization for Biological Control of Noxious Animals and Plants
KEMA	NV. tot Keuring van Electrotechnische Materialen = Joint Laboratory and other Services of the Dutch Electricity Supply Companies
LBO	Laboratorium voor Bloembollenonderzoek = Bulb Research Centre
LUW	Landbouw Universiteit Wageningen = Wageningen Agricultural University
L&V	Ministerie voor Landbouw & Visserij = Ministry of Agriculture and Fisheries (at present: "Ministerie voor Landbouw, Natuurbeheer & Visserij" [LNV] = Ministry of Agriculture, Nature Manegement and Fisheries)
MAFF-UK	UK Ministry of Agriculture, Fisheries & Food

NEFYTO	Nederlandse Stichting voor Fytofarmacie = Dutch Association of Fytopharmacal Products
NEN	Nederlands Normalisatie-insituut = Netherlands Standardization Institute
NMF	Directie Natuur, Milieu en Faunabeheer = Nature and Management Directorate (from L&V)
NRLO	Nationale Raad voor Landbouwkundig Onderzoek = National Council for Agricultural Research
OBS	Ontwikkeling Bedrijfssytemen = Developing farm Management Systems
OECD	Organization for Economic Co-operation and Development
ONC	Office National de la Chasse = National Hunting Office
OVB	Organisatie ter Verbetering van de Binnenvisserij = Organization to Improve Inland Fisheries
O&W	Ministerie van Onderwijs en Wetenschappen = Ministry of Education and Science
PAGV	Proefbedrijf voor de akkerbouw en de groenteteelt in de volle grond = Research Station for Arable Farming and Field Production of Vegetables
PCBB	Pragramma-Commissie Basiskennis Bodemonderzoek = Programming Commission Basic knowledge Soil Research
PCTB	Programma-Commissie Techniekontwikkeling Bodemonderzoek = Programming Commission Technique Development Soil Research
PD	Plantenziektenkundige Dienst = Plant Protection Service
RAWB	Raad van Advies voor het Wetenschapsbeleid = Advisory council for Scientific Policy
RIN	Rijksinstituut voor Natuurbeheer = Research Institute for Nature Management
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiëne = Netherlands Institute for Public Health and Environmental Protection
RMNO	Raad voor het Milieu- en Natuuronderzoek = Council for Environment and Nature Research
RUL	Rijksuniversiteit Leiden = Leiden University
RUU	Rijksuniversiteit Utrecht = University of Utrecht
SB	Speerpuntprogramma Bodemonderzoek = Spearhead Program Soil Research
SC	Staring Centrum = Winand Staring Centre for integrated land, soil and water research
SCMO-TNO	Studie Centrum Milieukundig Onderzoek - TNO = Study Centre for Environmental Research (from TNO)
SDU	Staats Drukkerij & Uitgeverij = Governemental Printing & Publishing Office
SOVON	Samenwerkende Organisaties Vogelonderzoek Nederland = Cooperating Organisations on Bird Census Work in The Netherlands
SPV	Service de la Protection des Vegetaux = Plant Protection Service
STIBOKA	Stichting voor Bodemkartering = Foundation for Soil Surveillance (at present, after fusion with some other institutes a.o. IOB: "Staring Centrum" [SC]
SZW	Ministerie van Sociale Zaken en Werkgelegenheid = Ministry of Social Affairs
TCB	Technische Commissie Bodembescherming = Technical Commission Soil Protection (from VROM)

TNO	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek = Organisation for Applied Physical Research
UvW	Unie van Waterschappen = Union of Water Control Boards
VBBN	Vereniging tot Bevordering van het Bijenhouden in Nederland = Association for the Promotion of Beekeeping in The Netherlands
VKA	Voedsel en Kwaliteitsaangelegenheden = Food and Quality Affairs (from L&V)
VROM	Ministerie voor Volkshuisvesting, Ruimtelijke Ordening and Milieubeheer = Ministry of Housing, Physical Planning and Environment
VTCB	Voorlopige Technische Commissie Bodembescherming = Provisional Technical Commission Soil Protection
VU	Vrije Universiteit = Free University Amsterdam
V&W	Ministerie van Verkeer en Waterstaat = Ministry of Transport and Public Works
WABM	Wet Algemene Bepalingen Milieuhygiëne = General Environmental Hygiëne Stipulations Act
WVC	Ministerie voor Welzijn, Volksgezondheid en Cultuur = Ministry of Welfare, Public Health and Culture
WVO	Wet Verontreiniging Oppervlaktewater = Pollution of Surface Waters Act
ZWO	Zuiveringschap West-Overijssel = West-Overijssel Water Purification Board