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EFFECTS OF PESTICIDES IN THE FIELD

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The workshop was made possible by the generous support of many organizations, including

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MATTHIAS LIESS obtained his PhD and Habilitation from the University of Braunschweig, Germany, where his studies focused on ecology and ecotoxicology.

Since 1985 he participated and lead laboratory and field studies to analyse processes in freshwater and marine ecosystems and how they are disturbed by natural and anthropogenic stressors. He is an author on more than eighty scientific publications.

Matthias Liess leads the Effect propagation group, Aquatic Ecotoxicology, UFZ-Centre for Environmental Research, Leipzig, Germany.



COLIN BROWN is Professor of Environmental Science within the Environment Department of the University of York.

He jointly leads the EcoChemistry research team that is a joint venture of the Central Science Laboratory and the University of York. Colin graduated from Leeds University with a BSc in Agricultural Chemistry and gained his PhD at Newcastle University, researching the transport of pesticides from drained land.

He worked for Cranfield University from 1993 to 2004. Colin's research focuses on fate and effects of pesticides in the environment, particularly management of aquatic exposure to pesticides and use of models within risk assessment. He recently chaired the DG-Sanco FOCUS work group on landscape and mitigation factors in ecological risk assessment.



G. PETER DOHMEN studied biology and ecology in Aachen and London and obtained his PhD in Munich working on effects of air pollutants on plants and plant/insect interactions.

Following a post-doc research project he joined BASF-AG, to work as ecotoxicologist at the Agricultural Research Center. In this position he has worked on terrestrial and aquatic ecotoxicological studies from standard laboratory tests to field studies.

He has supervised a number of mesocosm studies and performed fieldwork in paddy rice and in tropical systems. Peter Dohmen has been involved in a range of method development initiatives for ecotoxicological test systems and participated in several national and international working groups on standard and higher risk assessment methodologies and strategies.



SABINE DUQUESNE obtained her PhD in 1992 at the University of Lille, France, in marine ecotoxicology.

She then worked as a researcher at the University of Queensland until 2000 and conducted various projects in the field, in Australia and Antarctica, to study the suborganismal and organismal responses of marine species to environmental changes. Her current work focuses as well on freshwater ecosystems and on changes at the population level.

She has expertise in various research fields including bioindicators, biological markers, higher tier test systems, risk assessment. Sabine Duquesne is now based at the UFZ-Centre for Environmental Research in Germany.



ANDY HART leads the Risk Analysis Team at the Central Science Laboratory (CSL), York, UK, a research agency of the UK Department for Environment, Food and Rural Affairs.

He has a BSc in Environmental Biology and a DPhil in Behavioural Ecology, and joined the UK agriculture ministry in 1982 to research effects of pesticides on birds. He expanded his work first to other types of pesticide effects and then to the use of probabilistic methods of risk assessment.

Andy is a member of the European Food Safety Authority (EFSA) Scientific Panel on plant health, plant protection products and their residues, and co-ordinator of EUFRAM, an EU project developing a framework for probabilistic assessment of environmental impacts of pesticides (www.eufram.com).



FRED HEIMBACH is a research scientist at the Institute of Environmental Biology in the Crop Protection Division of Bayer CropScience in Monchheim, Germany.

He obtained his MSc degree and PhD in conducted research on marine insects at the Institute of Zoology, Physiological Ecology at the University of Cologne.

Since 1979 he has worked at Bayer CropScience on the side-effects of pesticides on nontarget organisms. In addition to his work, he gives lectures on ecotoxicology at the University of Cologne.

Dr. Heimbach has researched the development of single-species toxicity tests for both terrestrial and aquatic organisms and has worked with microcosms and mesocosms on development of multispecies tests for aquatic organisms. As an active member of European and international working groups, he participated in the development of suitable methods for testing pesticides and other chemicals for their potential side-effects on nontarget organisms.



JENNY KREUGER is an assistant professor at the Swedish University of Agricultural Sciences, Division of Water Quality Management.

Her research work has been focused on the environmental impact of agricultural activities on the quality of surface water and groundwater bodies. Special emphasis has been on studies of pesticide transport in soil and water at different scales, including also work on the effects of pesticide management practices on water quality at the catchment scale and studies of atmospheric deposition of pesticides.

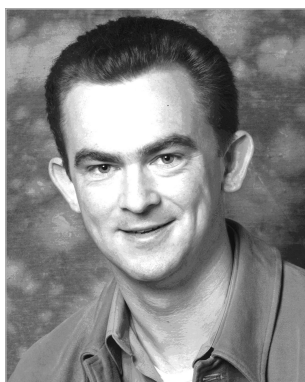
She is responsible for the Swedish national environmental monitoring program for pesticides in stream water, groundwater, and atmospheric deposition.



LAURENT LAGADIC is a Research Director at the INRA (National Institute for Agronomic Research) in Rennes, France.

He obtained his MSc Degree in Population Biology and PhD in Fundamental Toxicology from the University of Paris XI, Orsay. He has been involved for about 20 years in the study of the effects of pesticides in invertebrates, both target and non-target organisms. His work has been focused on the physiological changes (defence mechanisms, hormone and energy metabolism) induced by pesticides, and their consequences on individual reproductive performances and population dynamics. He currently leads a research group in aquatic ecotoxicology, where various approaches (biochemistry, immunology, population dynamics & genetics, community ecology) are applied to the study of multi-scale effects of pesticides in aquatic environments. Current research of the group includes endocrine disrupting effects of pesticides and interactive effects of toxicants in mixtures.

Dr. Lagadic is involved in the coordination of national research programmes in ecotoxicology, and he has participated to several international initiatives in environmental toxicology. He also provides external expertise in aquatic ecotoxicology, either for field assessment of effects of pesticides, or for the design and use of micro- and mesocosms in regulatory risk assessment of pesticides.



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effects of pesticides in the field

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a

EXECUTIVE SUMMARY

1 WORKSHOP FOCUS AND OBJECTIVES

The overall aims of the workshop were

- to consider to what extent current data demonstrate exposure and effects of pesticides under field conditions, and
- to examine whether these effects (or lack thereof) would be predicted on the basis of current risk assessment procedures.

Five specific topics were addressed to meet these aims:

- 1) review of available field studies for which the environmental effects of pesticides in agricultural landscapes, in both terrestrial and aquatic compartments;
- 2) examination of the essentials of field studies (exposure and biological effects assessment);
- 3) linking of tiers and extrapolation (comparing observed effects and predicted effects and limitations of such comparisons);
- 4) consideration of research requirements and further strategies; and
- 5) determination of the implications for regulatory risk assessment and management.

2 REASONS FOR THE WORKSHOP

Procedures for first-tier and higher-tier risk characterisation of plant protection products (PPPs, or pesticides) for the aquatic and terrestrial environment are now reasonably well defined in the European Union (EU). The EU Uniform Principles for the assessment of plant protection products require that if the preliminary risk characterisation indicates potential concerns, registration cannot be granted unless it can be demonstrated that "... under field conditions no unacceptable impact on the viability of exposed organisms..." occurs (Annex VI, Directive 91/414/EEC). To date, such assessments have been made by conducting higher-tier studies, which have included a range of laboratory, semi-field, and field experiments. In the Higher-tier Aquatic Risk Assessment for Pesticides (HARAP) workshop (Campbell et al. 1999), field studies were one of the ap-

proaches proposed to further characterise potential risks identified in a first-tier assessment. Over recent years, a number of field studies have been conducted to address exposure and effects of pesticides in the agricultural environment ("the field"). The current workshop focused on the "Effects of Pesticides in the Field". Results of field studies were discussed with respect to (1) the occurrence and magnitude of observed effects and (2) the links between observed and predicted effects (derived from the first-tier and higher-tier risk assessment schemes). This discussion was carried out to assess whether the risk assessment scheme is under- or over-protective with respect to effects observed in the environment.

3 WORKSHOP STRUCTURE AND APPROACH

The workshop comprised 75 scientists from Europe and North America, representing government, industry, and academia and experienced in risk assessment and field effects of pesticides.

The state of the art of the current knowledge and information available about field effects of pesticides was reviewed via a series of platform and poster presentations (see Appendixes A and B). These presentations were separated into 3 main topics:

- observation of effects in the field;
- ability to predict effects: linking tiers and extrapolation; and
- implications for risk assessment.

Following the platform presentations, the topics were discussed further in different breakout groups (aquatic organisms, terrestrial invertebrates and plants, and birds and mammals). Rapporteurs presented the outcomes from their breakout groups, which were further discussed in plenary sessions among all participants.

4 FIELD STUDIES

4.1 DEFINITIONS

In the context of this workshop, investigations into the effects of pesticides in the field regrouped under the name “field studies” included 2 types of studies, which were defined as follows:

Monitoring study: an investigation into the overall impact of pesticide use on a specific ecosystem through surveying or monitoring that consists of characterisation of exposure (chemical monitoring, exposure modelling) and observations of effects (biological monitoring) occurring in the field or treated area as a consequence of use and/or misuse of pesticides.

Experimental study: an experiment into the impact of a specific product or active substance applied under controlled conditions in the field. Such studies are performed in the natural environment within an agricultural context (and thus contrast with mesocosm studies).

Monitoring studies were the main focus of the workshop.

4.2 OVERVIEW

Over recent years, some studies of environmental exposure to pesticides in the agricultural environment aimed at quantifying any effects on non-target species in the different ecosystems (aquatic organisms, terrestrial invertebrates and plants, vertebrates). Effects were frequently observed and described, but a firm link to pesticide usage often was not established. Indeed, demonstrating causality between exposure and effects is difficult, especially in studies in which long-term and indirect effects are responsible for biological impairments. Reasons for this difficulty include problems in accurately characterising exposure, the presence of confounding factors, and natural variability in the system.

The workshop revealed that direct effects due to the use of pesticides have been demonstrated in some cases (e.g., aquatic insect larvae and non-target plants). Indirect

effects due to changes of predator–prey balance and competitive interactions have been observed in certain groups of organisms. However, these direct and indirect effects were often, but not always, decreased or even annulled through long-term recovery processes.

4.3 EXAMPLES OF FIELD STUDIES

The investigations summarised below considered monitoring studies a priority because this was the main focus of the workshop. However, some experimental studies were also reviewed, especially when the number of monitoring studies was too limited.

Aquatic organisms

The monitoring studies presented at the workshop dealt mainly with the effects of insecticides on macroinvertebrates. They were performed mostly in streams of intensively cultivated areas. Effects of pesticides were identified in several of the field studies (Altes Land and the Braunschweig area in Germany, the Lourens River in South Africa). When recovery was studied, it was shown that in some cases the invertebrate community composition did not recover within 1 year. In the studies that established a clear link between exposure and effects, it was often difficult to assign a specific level of exposure to the observed effects. This was due to fundamental problems and uncertainties in accurately characterising exposure.

Terrestrial invertebrates and plants

For terrestrial invertebrates, the number of available monitoring studies is limited, and those available mostly studied effects on honeybees. The UK Wildlife Incident Investigation Scheme (WIIS) is an example of such a study; one of its significant findings was that the current risk assessment scheme for bees appears to be protective.

Monitoring programmes for other species of terrestrial invertebrates (non-target arthropods and below-ground invertebrates) are not available. However, studies focused on the ecology of individual species showed

long-term effects of pesticides associated with a decline in abundance of some invertebrate families or species (e.g., Sussex study). In some cases, long-lasting effects were also shown. For example, in the SCARAB (Seeking Confirmation About Results at Boxworth) project, the Collembola was the arthropod group most affected by conventional pesticide use. Long-term effects (>4 y), reflected by a lack of recovery of some species, occurred at a location with repeated use of organophosphate insecticides.

Some field studies are available for plants, but there are no monitoring studies. In a large-scale experimental study, clear short-term effects were shown, with recovery occurring within a year after application.

Birds and mammals

The most significant monitoring scheme performed in the UK (WIIS) investigated direct effects of pesticides on wildlife, pets, and some livestock and showed evidence of pesticide poisoning.

In 1968 the Sussex study (also called the “partridge survival project”) began to investigate the reasons for the decline in the grey partridge population, and it identified indirect effects of pesticides (reduction in chick food availability) as the main cause. More recent projects have also had a similar hypothesis and findings about the relevance of indirect and/or chronic effects (e.g., the corn bunting study).

5 METHODOLOGICAL CONSIDERATIONS

One outcome of the workshop is that more guidance and research are needed to assist with the design and conducting of field studies (monitoring and experimental studies). The following requirements were identified:

- **Exposure:** Data on pesticide use in the study area (chemical use pattern) and appropriate characterisation of exposure (e.g., detection of peak concentrations, number of applications) are part of the considerations. Geographic information system (GIS) technologies may have applications in analysing landscape factors

that influence exposure, such as proximity of habitats to treated fields and connectivity of habitats.

- **Reference or control sites:** These must be established so that studies are suitably representative. They could then be extrapolated to different regions or used to determine whether risk mitigation works. Both reference and impacted sites should not be subject to major stressors other than the one tested.
- **Cause and effect:** To establish the cause of observed effects, the observations should be designed so that, wherever possible, the mechanisms causing effects can be revealed. Direct effects should be assessed, but the potential consequences of indirect effects as well as chronic (long-term) and delayed effects should also be considered, depending on the problem formulation. The use of additional measurement or experimentation (e.g., biomarkers, bioassays, mesocosms) can provide useful complementary tools to better link exposure and effects.
- **Confounding factors:** Current and/or past exposure to multiple substances and stressors (e.g., weather, water quality, species interactions) interferes with or decreases the power of detection of field effects of pesticides. The studies should be designed so that these problems are minimised.
- **Natural variability:** Field studies are conducted against a backdrop of natural variability that includes the normal operating range of the system under consideration (intersite, interreplicate, temporal, and spatial heterogeneity). This variability leads to uncertainties and/or limitations that need to be considered during evaluation of the study.
- **Biological and ecological information:** Such data are needed for many taxa to better evaluate their potential sensitivity to pesticides and also their recovery potential. Information that is lacking or incomplete can include life-history characteristics, dispersal ability, and behavioural ecology, physiology, population genetics, and ecotoxicity data.
- **Environmental parameters:** These can be typical for specific regions and can influ-

ence the fate of the pesticide, the ecological performance of organisms, and their response to toxicants (e.g., drying, variations of temperatures). Environmental parameters should be considered because they may alter the sensitivity of organisms and influence the fate of pesticides (e.g., disappearance rate, bioavailability).

- ▶ **Representativeness and regional understanding:** This will assist our ability to extrapolate results to another region, another ecosystem, and different scales of assessment. The system monitored should be characterised with respect to the agricultural landscape, pesticide usage, ecological and biological properties, and spatial and temporal scales. Influence of climatic conditions and geographic position should also be understood (i.e., the environmental parameters mentioned above). Appropriate landscape characterisation will eventually support the development of representative, region-specific scenarios.
- ▶ **Defining acceptability:** Criteria for defining the acceptability of observed effects should include both scientific recommendations and societal considerations. For example, the selection of reference sites and target images depends partly on what society considers to be acceptable. It is necessary both to establish the target image and to decide how much deviation from that target image is acceptable.

6 LINKING TIERS AND EXTRAPOLATION

Effects of pesticides were identified in several of the field studies that were presented. These include direct effects (i.e., aquatic studies: Liess, platform presentation, and Schäfers, platform presentation; soil invertebrates: Römbke, platform presentation; plant study: De Jong, platform presentation; studies on birds and mammals: WIIS from the UK and indirect effects [observed in various groups of organisms]). These studies should offer the opportunity to calibrate the relationship between biological effects observed in the field and effects predicted in

test systems based on the current risk assessment schemes. However, there are various limitations in undertaking such calibration. For example, on the basis of the knowledge usually available, it is often difficult to determine whether (1) effects are due to normal use or to misuse of pesticides and (2) exposure in the field is accurately characterised. Furthermore, effects of pesticides in the field are mostly related to multisubstance contamination, but current risk assessment does not consider mixture toxicity. Indirect effects can have important ecological consequences, but they can be difficult to quantify and they are often taken into account within current risk assessment schemes by applying safety factors to direct effects measured in mono-species tests. For these reasons, it is difficult to verify whether current risk assessment procedures provide suitable environmental protection (i.e., whether they are overprotective, underprotective, or appropriate).

7 IMPLICATIONS FOR RISK ASSESSMENT

Field studies can provide useful information and knowledge for the risk assessment that

- ▶ can be used as a “reality check” for the risk assessment process and may identify areas where the present process may be over- or underprotective; hence, they could be used to calibrate current risk assessment;
- ▶ may provide a means to evaluate the effectiveness of risk mitigation measures in the field;
- ▶ may give information on the temporal trends of environmental impacts associated with use of pesticides at a local/regional scale when performed over a few years; or
- ▶ are relatively easy to communicate to the public and other stakeholders, as real situations are represented (rather than surrogates of the reality).

However, it seems difficult to incorporate such studies into decision making with respect to registration for a single active sub-

stance because the field situation is often characterised by multiple exposure and mixture of substances and hence differs from higher-tier tests performed as part of the risk assessment. Both types of field studies (monitoring and experimental studies) can be useful in regulatory risk assessment for the post-authorisation phase or for exploring more general trends in the environment.

Regulatory risk assessment and field studies provide complementary information. Risk assessment seeks to provide a generalised, protective framework on which to base decision making. Results from field studies highlight areas that could be considered within advanced risk assessment. These include a better characterisation of chemical exposure, further developments in incorporating internal and external recovery, and characterisation of indirect effects.

Risk assessment should eventually consider landscape characteristics. Use of environmental databases containing various types of information (e.g., biological, ecological, and landscape characteristics; farming practises; pesticide usage) will help us to evaluate field studies, particularly monitoring studies, and will improve risk assessment by increasing realism.

8 MAIN OUTCOMES

Concerning the effects of pesticides in the field, the following conclusions were reached within the workshop:

- ▶ Effects of pesticides were identified in several of the field studies presented. Direct and indirect effects have been observed, as well as recovery processes that often decrease or annul these effects to a greater or lesser extent.
- ▶ The influence of natural variability, confounding factors, etc., in the establishment of cause-and-effect relationships could be reduced by strategies such as categorising species according to ecological traits, identification of suitable control sites, and more effective sampling strategies.

- ▶ An improved assessment of exposure, appropriate for each ecosystem, will enable us to establish a better correlation between the magnitude of observed effects and the level of exposure.
- ▶ The risk associated with pesticide use can be predicted in a more realistic way by including parameters into risk assessment strategies that are environmentally relevant at the landscape level (i.e., recovery through recolonisation, life-history traits, etc.).
- ▶ Monitoring studies are recognised as a valuable retrospective tool to verify the field relevance of risk assessment schemes. They can also be used to demonstrate the effectiveness of risk mitigation measures.

It was generally accepted within the workshop that it is challenging to determine pesticide exposure as the cause of effects in the field. This difficulty is due to problems of natural variability, multiple substances and multiple stressors, confounding factors, and insufficient statistical power.

Concerning the current regulatory risk assessment procedure, many participants felt that current approaches are reasonable to ensure the protection of non-target organisms and, in some cases, may be considered too conservative. However, effects of pesticides were identified in several of the field studies presented. It could not be determined whether good agricultural practise or misuse was responsible for the observed effects in many of these studies; therefore, some uncertainty remains about the actual level of protection. It was stated that further research is needed to evaluate accurately the degree of protection, so that risk assessment procedures can be adjusted if necessary.



effects of pesticides in the field

b

WORKSHOP SYNTHESIS

1 DEVELOPMENT OF WORKSHOP CONCLUSIONS

The workshop comprised 75 scientists representing government, industry, and academia and experienced in risk assessment and field effects of pesticides. Participants were from EU member states, the US, and Canada and thus a broad range of expertise, experience, and viewpoints were available.

The workshop analysed the state of the art of the current knowledge and information available about field effects of pesticides assessed by performing field or monitoring studies. This was reviewed via a series of plenary sessions, platform presentations (see abstracts in Appendix A), and poster presentations (see titles in Appendix B). These were separated into 3 main topics: (1) observations of effects in the field, (2) ability to predict effects, and (3) implications for risk assessment.

Following the platform presentations, the topics were discussed further in different breakout groups. The discussions were based on data made available at the workshop. However, additional data were considered if relevant and scientifically sound. Participants were divided according to their expertise into 4 breakout groups: (1) aquatic organisms (2 groups), (2) terrestrial invertebrates and plants, and (3) birds and mammals. Each breakout group produced a working paper that reflected the content of their discussions. Rapporteurs presented the outcomes from their breakout groups, and these were further discussed among all participants in plenary sessions. The final plenary of the workshop discussed in detail the future role of field and monitoring studies of pesticides in the EU and summarised the outcomes.

After the workshop, the steering committee decided future actions and schedules and commented on and revised the drafts of the workshop report. The report coordinator merged the working papers of the 2 aquatic breakout groups into 1 report, which was then reviewed by the rapporteurs for the 2 aquatic groups. The 3 breakout group reports (aquatic invertebrates, terrestrial invertebrates and plants, and birds and mammals)

were then distributed to the participants, who were invited to comment in 2 rounds. The first and second sets of comments were integrated by the rapporteurs and the report coordinator, respectively. The workshop steering committee decided that the 3 breakout group reports should be used as a basis to write the “workshop synthesis” and that the breakout group reports should be provided as an appendix (Appendix D) to the final report because they were very different in terms of substance, structure, and amounts of information provided. Once the workshop steering committee agreed on the content of the workshop synthesis (Part B), this was complemented by an executive summary (Part A).

The draft report was then distributed to the workshop participants, who were invited to comment. Their comments were used by the workshop report coordinator to make revisions to the report, which was then subjected to final review by the workshop steering committee.

The report (Parts A and B) was reviewed by 2 external reviewers. Appendixes A through D had to be kept as original material and therefore were not externally reviewed.

2 FIELD STUDIES

2.1 DEFINITIONS

Investigations into the effects of pesticides in the field generally fall into 2 main categories that can be defined as follows:

- 1) Monitoring study: an investigation into the overall impact of pesticide use on a specific ecosystem through surveying or monitoring that consists of characterisation of exposure (chemical monitoring, exposure modelling) and observations of effects (biological monitoring) occurring in the field or treated area as a consequence of use and/or misuse of pesticides.
- 2) Experimental study: an experiment analysing the impact of a specific product or active substance applied under controlled conditions in the field. Such studies are performed in the natural environment within an agricultural context and

thus contrast with mesocosm studies. Experimental studies are usually conducted with untreated controls and sometimes with reference treatments in replicated plots.

The main focus of the workshop was on monitoring studies. However, some experimental studies were also reviewed because they can provide valuable information to aid interpretation of monitoring data.

2.2 STUDIES ON EFFECTS OF PESTICIDES IN THE FIELD

A number of field studies were reviewed during the course of the workshop. For aquatic invertebrates, sufficient monitoring studies were available to discuss them in detail (Table 1). For terrestrial invertebrates and plants (Table 2), as well as for birds and mammals (Table 3), experimental studies were also discussed due to the limited number of monitoring studies available.

2.2.1 MONITORING STUDIES FOR AQUATIC INVERTEBRATES

Biological effects of pesticides were identified in several of the monitoring studies that were presented at the workshop. It should be noted that the studies reviewed focused mainly on aquatic macroinvertebrates and insecticides; thus, their broader applicability is limited. Furthermore, the studies were generally carried out in intensively cultivated areas. Altered communities likely due to pesticides were observed in the Altes Land (near Hamburg, Germany), a special case of intensive agriculture for apple orchards where buffer zones are not appropriate (Schäfers, platform presentation). Effects were also shown following runoff events in the Liess studies (Liess, platform presentation; (Liess 1994, 1998; Liess and Schulz 1999; Liess et al. 1999; Schulz and Liess 1999a, 1999b). These investigations took place at sites with a risk of surface runoff (Braunschweig area, Germany) ranging from

Table 1: Monitoring studies from the literature showing a clear relationship between exposure to pesticide (mostly due to surface runoff) and biological effects on field aquatic organisms (modified after Schulz, 2004; see also review of Hommen et al. 2004).

Exposure			Endpoint	Species	Reference
Substance	Quantification	Duration			
Cypermethrin	2.25 mg/kg	>150 days	Abundance, emergence	Dipteran (Chironomidae)	Kedwards et al. 1999
Parathion-ethyl	6 µg/L	1 h	Community composition	11 invertebrate taxa	Liess and Schulz 1999
Parathion-ethyl	6 µg/L	1 h	Abundance, drift, mortality	Trichoptera, other invertebrates	Schulz and Liess 1999a
Fenvalerate Parathion-ethyl	0.85–6.2 µg/L	1 h	Mortality abundance	Amphipods Trichoptera	Schulz and Liess 1999b
Endosulfan	1.3–10 µg/kg SPMD ^a	Unclear	Abundance	Ephemeroptera, Trichoptera	Leonard et al. 1999
Azinphos-methyl Chlorpyrifos Parathion-methyl	0.82 µg/L 344 mg/kg 1-550 µg/L	1–3 h	Community composition	Ephemeroptera, other insects	Schulz et al. 2002
Endosulfan	10–318 µg/kg	Few h	Abundance, drift	Various species of invertebrates	Jergentz et al. 2004
Azinphos-methyl Chlorpyrifos Endosulfan	4±2 µg/kg 29±19 µg/kg 54±19 µg/kg	Several weeks	Community composition	Various invertebrate taxa	Thiere and Schulz 2004
5 fungicides 4 herbicides 1 insecticide	Various concentrations	1 h	Community composition	Various invertebrate taxa	Berenzen et al. 2005
7 insecticides, 6 fungicides, 8 herbicides	Various concentrations	1 h	Community composition	Various invertebrate taxa	Liess and Von der Ohe, 2005

^a semipermeable device

“very low” to “high” on a scale defined for the German agricultural area (Bach et al. 2000). The Schulz studies from South Africa also took place in an intensively cultivated area exposed to pesticides via surface runoff and spray drifts (Schulz, platform presentation; Schulz et al. 2001, 2002). Only a few monitoring studies exist that focus on biological effects and include appropriate exposure monitoring to allow the establishment of cause and effect.

Indeed, only 11 studies that included the information necessary to establish causality between insecticide contamination of surface waters due to usual agricultural practises and effects on aquatic fauna (i.e., exposure quantified, effects on field populations – thus excluding in situ bioassays – measured, control situations without effects included) were identified (Table 1); most of them are reported in a recent review (Schulz 2004). It is important to note that for many of these studies, the pesticide concentrations measured in the field were not large enough to support an explanation of the observed effects based on acute toxicity data alone (Schulz 2004). Based on current knowledge, it is not known (1) whether measured concentrations in the field regularly underestimate the real exposure, (2) whether relevant long-term effects are considered in the field but not under controlled conditions, and (3) whether there are differences in sensitivity between laboratory and field organisms.

2.2.2 MONITORING AND EXPERIMENTAL STUDIES FOR TERRESTRIAL INVERTEBRATES AND PLANTS IN EUROPE

MONITORING STUDIES FOR TERRESTRIAL INVERTEBRATES

MONITORING SCHEMES

Honeybees

Monitoring of honeybees is mostly performed for food-safety purposes and to provide centralised data for incident reporting and for appraisal of wider-scale effects by regulatory bodies. The UK Wildlife Incident

Investigation Scheme (WIIS) (Barnett et al. 2002) investigates suspected incidents of honeybee poisoning by pesticides, most of which are reported by beekeepers. Previous analyses of the results showed that no pesticides classified as having “low risk” were implicated in poisoning incidents (Aldridge and Hart 1993), while, for example, pyrethroids classified as “high risk” are of low hazard in the field (Inglesfield 1989). The current risk assessment scheme for honeybees thus seems to be successful, at least in that while there are false positives (e.g., with pyrethroids), there do not seem to be false negatives. However, potential side effects of pesticides on honeybees are intensively discussed in the public domain (e.g., in France; Römbke, platform presentation). Monitoring studies have also been conducted in other countries. For example, a honeybee monitoring system is operational in Germany, but this system is more irregular than that in the UK and there are large regional differences in efficiency. The foraging activity and behaviour of the bees on the crop and at the hive entrance are recorded as part of field test guidelines (EPPO 1992).

Non-target arthropods and below-ground invertebrates

The number of monitoring studies on terrestrial invertebrates other than honeybees is very limited. Indeed, there is no system like the UK WIIS that is effective for non-target arthropods (NTAs) or below-ground organisms. The possible application of the honeybee scheme to other terrestrial invertebrates was considered difficult because developed and accepted schemes are lacking. However, the monitoring of soil invertebrates is currently facilitated by the standardisation of sampling methods by the International Organization for Standardization (e.g., tests with earthworms). When focusing on non-target arthropods and below-ground invertebrates, species from in-crop areas and off-crop areas (which are habitats for many different species) should be differentiated.

Ecological studies

Ecological studies are not usually designed to examine pesticide effects but rather to

study the ecology of individual species. However, pesticide effects can be considered and therefore detected in such investigations. It was noted that long-term data and landscape-scale studies are lacking, even though pesticides can be applied over extensive areas (Holland, platform presentation). However, several projects are in progress that aim to identify the “normal” species composition of soil invertebrates at reference sites (e.g., Ruf et al. 2003; Schouten et al. 2004).

Examples:

- ▶ The overall impact of pesticides was examined in an extensive, long-term study conducted by The Game Conservancy Trust, referred to as the “Sussex study” (or the partridge survival project). The extensive dataset includes information on the abundance of many invertebrate families or species within each field and consequently allows their range across the study area to be investigated (Aebischer 1991). In the late 1990s, the Sussex data were reanalysed to look at the impact of pesticides (Holland, platform presentation; Ewald and Aebischer 1999, 2000). Although this has yet to be fully explored, it is evident that some of the invertebrates have declined in abundance or sometimes have disappeared as a result of pesticide use (Holland 2002). Outcomes have led to management measures, including recommendations with regard to adoption of conservation headlands.
- ▶ Relatively few other studies have investigated long-term or large-scale effects. Holland (Holland, platform presentation) cited 2 examples of 2-year studies that investigated effects of insecticides and considered recolonisation (Wick and Freier 2000; Kennedy et al. 2001). The insecticide reduced numbers of invertebrates within a season, but there were no effects 1 year afterwards on open plots with no barrier to recolonisation.

Another type of ecological study covers local or regional projects that are usually supported by state or federal agencies that do not explicitly aim at monitoring effects of pesti-

cides. They try to (1) determine the biological soil quality (Germany: Ruf et al. 2003), (2) assess the habitat function of the soil (independently, whether this function is affected by pesticides, soil compaction, other factors, or a combination of all), (3) identify the reference sites, or (4) assess effects of soil pollution (e.g., heavy metals in the UK: Spurgeon et al. 1996), including an increasing intensity of (agricultural) land use (France: Ponge et al. 2003).

EXPERIMENTAL STUDIES FOR TERRESTRIAL INVERTEBRATES

As described earlier, experimental studies investigate the impacts of a specific substance introduced into the field under controlled conditions for the specific purpose of the experiment.

SMALL-SCALE FIELD STUDIES

Van Straalen and van Rijn (1998) reviewed a series of studies performed since 1964 that investigated the effects of different pesticides (lindane, dimethoate, parathion, chlorpyrifos, carbofuran, carbaryl, benomyl, and atrazine) on various species of terrestrial invertebrates. Thompson (2003) gives an overview of a wide variety of semi-field and field studies assessing bee behaviour following field applications, indicating what type of field observation is needed for predicting long-term consequences of pesticides. Guidelines are available on test methods for evaluating the side effects of pesticides on the foraging activity of honeybees and their behaviour on the crop and at the hive entrance (EPPO 1992). Some studies have also been performed by companies at agricultural sites sprayed with specific pesticides, but these results are not usually published (e.g., studies on earthworms are based on methods described in the Earthworm Field Test, ISO 1999). Vogt (1994, 2000) also compiled results from semi-field and field studies with terrestrial arthropods and compared them with results from laboratory tests based on the “worst-case assumption”. Results show that (1) using laboratory tests to screen harmless products is reliable and (2) predicting the magnitude of field effects for prod-

ucts found to be harmful in the laboratory is not possible.

FARM-SCALE STUDIES

Farm-scale studies are large-scale experiments for which large blocks of farmland comprising multiple fields are subjected to contrasting treatments, and populations or communities of terrestrial invertebrates are then monitored. Farm-scale studies are not routinely performed because of their cost, and only a few examples exist.

Examples:

► A well-known example is the Boxworth Project from the UK, which broke new ground during the 1980s by being the first long-term, farm-scale project to investigate the ecological impacts of intensive arable farming practises (Greig-Smith et al. 1992). Three treatment regimes were applied to different areas of the same farm over 5 years. Overall, 1 regime (“full insurance”) involved more than twice the number of pesticide applications and about 6 × the number of insecticide applications used in the other 2 regimes (so-called “supervised” and “integrated” treatments, similar to each other and to typical UK farm practises at the time). Over the 5 years of application, the results of full insurance treatment compared to the other 2 treatments showed that (1) ef-

fects on soil invertebrates such as springtails were not consistent, with some species showing decreases and others increases; and (2) herbivorous, predatory, and parasitic invertebrates in the crop had densities that declined by about 50%, while detritivores were little affected. The responses of individual species varied within each group, and this was attributed to complex differences in exposure, capacity for recolonisation, and changes in prey populations. Changes in some species were clear and consistent, but most showed considerable variation from year to year.

► The TALISMAN (Towards a Lower Input System Minimising Agrochemicals and Nitrogen) and SCARAB projects evolved subsequently as follow-on studies to the Boxworth Project and were designed to examine in greater detail many of the issues raised by the Boxworth Project. These 2 projects complemented each other in their aims and objectives. TALISMAN focused primarily on the economic and agronomic issues of reducing pesticide and nitrogen fertilizer use. The SCARAB project examined ecological side effects of pesticides on non-target organisms including insects, spiders, earthworms, and soil microbes and is thus of more interest in the context of this workshop. The numbers of certain species of

Table 2: Examples of monitoring and experimental studies from the literature showing effects of pesticides on non-target terrestrial invertebrates

Study	Type	Endpoint	Species	Location	Reference
WIS	Monitoring (monitoring scheme)	Lethality	Beneficial insects (bees)	UK	Barnett et al. 2002
The Game Conservancy Trust	Monitoring (ecological studies)	Abundance	Various species of invertebrates	Sussex study, UK	Holland et al. 2002
Long-term study		Long-term effects (over 2 seasons) recolonization	Nontarget arthropods (Carabidae, Staphylinidae, Linyphiidae)	Germany, UK	Wick and Freier 2000 Kennedy et al. 2001
Benomyl	Experimental (small scale study)	Long-term effects	Various species of grassland species	UK	Bembridge et al. 1998
Boxworth Project	Experimental (farm-scale studies)	Long-term effects, abundance and recolonization	Crop invertebrates, predators of pests, soil invertebrates, plants in field margin	UK	Greig-Smith et al. 1992
SCARAB		Long-term effects, abundance	Invertebrates (Collembola, earthworms, etc), soil microflora	UK	Frampton 1997a Tarrant et al. 1997

non-target insects and spiders declined after the annual application of some broad-spectrum insecticides, but recovery usually occurred within the same season. The results showed considerable inter-species variability of both initial *Collembola* vulnerability to the pesticide regimes (Frampton 1997a; Frampton 2000) and subsequent recovery rates among *Collembola* (Frampton 1997b, 1997c, 2000). Some species of Entomobryidae were eliminated from the field (but not the surrounding margins) by repeated applications of organophosphorus (OP) insecticides (Frampton 2002). Recovery of 1 species took more than 4 years after the inputs of OP insecticides had stopped, despite the presence of potential recolonists at the field margins throughout the period (Department for Environment, Food, and Rural Affairs [DEFRA] Project PNo934). A limitation of the SCARAB and Boxworth projects was the lack of spatial replication. However, this drawback was overcome in the SCARAB project by temporal manipulation of the pesticide regimes (Frampton 2000, 2001, 2002).

FIELD STUDIES ON PLANTS

The use of plants in monitoring studies can be more straightforward than for other groups of organisms because general information on life-history strategies, phenology, and habitat requirements of wild plants are readily available. Furthermore, terrestrial plants are sessile organisms, and thus their presence in samples may be clearly related to local environmental conditions. However, this area has not been considered very thoroughly so far with respect to regulation in Europe.

One study examined the effects of herbicide use in arable fields on ditchbank vegetation adjacent to sprayed fields (De Snoo and van der Poll 1999). The number of dicotyledonous species adjacent to an unsprayed buffer zone was about 50% higher compared to the number of species directly adjacent to the parcel treated with a herbicide against dicotyledonous weeds. The buffer zone reduced drift deposition from about 5% to less

than 0.1% of the field dose (De Jong, platform presentation).

In a large-scale field experiment, clear short-term effects on plant growth and phytotoxic symptoms on non-target vegetation of ditchbanks and road verges occurred at low dosages (<5% of the field rate) of a broad spectrum of herbicides at several metres from a treated plot. Effects within the same season were generally observed at higher dosages. The inclusion of unsprayed buffer zones and other drift-reducing measures could prevent these effects. Full recovery was observed in the next year for all dosages (up to 64% of the maximum field rate) and measures of effect (De Jong, platform presentation).

Studies on non-target plants in field margins during the Boxworth project suggested that they were more influenced by habitat structure than by pesticide drift or treatments with pesticides.

2.2.3 MONITORING AND EXPERIMENTAL STUDIES FOR BIRDS AND MAMMALS IN EUROPE

MONITORING STUDIES

MONITORING SCHEMES

A reactive monitoring scheme (or incident investigation scheme) is defined as one that considers whether a deleterious event (e.g., death of a bird or mammal) results from the use of a pesticide and, when evidence is gathered, whether it is attributable to the correct use, misuse, or abuse of a compound. The organisation of reactive monitoring schemes in Europe seems to be underdeveloped, as it exists in only 7 out of 18 countries (De Snoo et al. 1999). Such schemes have some limitations, including

- ▶ low probability of an incident being reported,
- ▶ use of retrospective data (measurement of lethality),
- ▶ bias towards large species or species of conservation interest, and
- ▶ no detection of long-term changes due to chronic and indirect effects.

However, they also have some advantages: they are a safety net for the regulatory systems in terms of acute direct effects, they are countrywide, and they highlight the need for appropriate risk management and issues regarding misuse and abuse.

Examples:

- ▶ The UK WIIS investigates the deaths of wildlife (i.e., birds, mammals, and beneficial insects), pets, and some livestock throughout the UK where there is evidence that pesticide poisoning may be involved. Annual reports of findings are published (e.g., Barnett et al. 2002).
- ▶ Another successful scheme, established in France, is operated by a national network (SAGIR, a sanitary surveillance network for wildlife in France) and aims at assessing wildlife health rather than just pesticide poisonings. Initially, it focused mainly on game but is now extended to other species.

ECOLOGICAL STUDIES

These long-term, species-specific monitoring studies are not usually designed to examine pesticide effects but rather to study the ecology of individual species. However, pesticide effects may be detected when there are, for example, variations with regard to pesticide use or any obvious hints of causal effects.

Examples:

- ▶ Partridge survival project: This project (simply known as the “Sussex study” and conducted by The Game Conservancy Trust) started in 1968 and was triggered by the decline of the grey partridge population. It studied general effects of farming practise and indirect effects of pesticides but excluded direct toxic effects of pesticides. The cause identified as responsible for the decline was a reduction in the availability of chick food. A chain of causal links from pesticide use to insect abundance to chick survival to population size was demonstrated (Potts 1986). Results have not been used for the assessment of individual pesticides but led to management measures (i.e., recommen-

dations with regard to the development of conservation headlands).

- ▶ Corn bunting study: This study had the same hypothesis of cause (decrease in availability of chick-food invertebrates) and chronic effects (chick mortality) as in the partridge project. This hypothesis was also demonstrated, and it was concluded that these effects may reduce breeding success (Brickle et al. 2000).
- ▶ Turtle dove study: This study compared ecological parameters in the 1990s with those in the 1960s. A change in diet, an increase in foraging distance, and a decrease in the number of nesting attempts were demonstrated, but a link to the use of pesticides was not shown (Browne and Aebischer 2004).
- ▶ Sparrowhawk study: This study demonstrated direct impacts of cyclodiene insecticides (such as dieldrin) on populations of sparrowhawk (Newton 1986; Newton and Wyllie 1992; Sibly et al. 2000).
- ▶ Yellowhammer study: This study showed evidence of some indirect effects of pesticides on breeding of yellowhammer (DEFRA Project PN0925: (Anonymous 2004).
- ▶ Studies commissioned by the Danish Environmental Protection Agency: these studies were undertaken to analyse and model the distribution of birds in Danish farmland in relation to various parameters including pesticide use (Petersen 1996; Petersen and Jacobsen 1997).

EXPERIMENTAL STUDIES

Examples of farm-scale studies are included in the following:

- ▶ The effects of pesticides on avian endpoints were studied in the Boxworth Project (Greig-Smith et al. 1992), a 5-year, government-funded investigation into sustainable methods of farming. Avian endpoints included abundance, reproductive performance, frequency of nest visits by birds, feeding of young, diet composition (from faecal samples), and cholinesterase inhibition in nestlings. The project also measured the abundance of wood mice by using capture-marking-recapture methods. The project had a num-

ber of limitations, the most important of which was the lack of replication because of cost limitations. This weakness made it difficult to assess whether differences between the blocks were due to the treatments or to confounding factors. In addition, despite the relatively large size of the blocks, the number of active bird nests available for study at the time of any particular pesticide application was small. The only vertebrate endpoint that showed a clear pesticide effect was the abundance of wood mice. Applications of methiocarb pellets in the autumn had a dramatic short-term effect on their populations. However, immigration of juvenile mice from untreated areas led to a rapid recovery in numbers. It was suggested that applications at other times of the year, or in areas without adjacent woodland to allow recolonisation, might produce longer-lasting effects.

- A large-scale field experimental study has recently been completed in the UK to assess the relative importance of food avail-

ability on the demography of farmland bird populations as part of the “Indirect Effects of Pesticides Project” (DEFRA Project PN0925; Anonymous 2004). Three treatments and a control were applied in a factorial design to 1-km² blocks of farmland at 3 sites. One treatment involved increased insecticide application in summer to decrease invertebrate abundance; another also had increased insecticide inputs in summer along with provision of extra seed in winter. Where the extra insecticides were applied, there was a reduction in the reproductive output of yellowhammers at all 3 sites. The third treatment involved providing extra seed in winter to increase the food supply: there was some evidence that this increased overwinter survival of yellowhammers, but the effect was not consistent across all sites. Results for other bird species were inconclusive.

Table 3: Examples of monitoring and experimental studies from the literature showing effects of pesticide on birds and mammals

Name	Study type	Endpoint	Species	Location	Reference
WIIS	Monitoring (monitoring schemes)	Lethality	Wildlife (birds mammals), pets, livestock	UK	Barnett et al. 2002
SAGIR		Lethality Health	Game birds, other wildlife	France	Berny et al. 1997
Partridge survival project (Sussex study)	Monitoring (ecological studies)	Lethality (through indirect effects)	Grey partridge	Sussex, UK	Potts 1986 Aebischer 1991 Ewald and Aebischer 1999; Ewald and Aebischer 2000
Corn bunting project		Lethality (through indirect effects)	Corn bunting	UK	Brickle et al. 2000
Turtle dove project		Indirect effects	Turtle dove	UK	Browne and Aebischer 2004
Sparrowhawk study		Abundance	Sparrowhawk	UK	Newton 1986; Newton and Wyllie 1992 Sibly et al. 2000.
Boxworth project		Abundance, breeding performance, behaviour, nestling growth, ChE inhibition in nestlings Abundance, Recovery	Birds Small mammals	UK	Greig-Smith et al. 1992
Indirect effects project		Abundance, Reproductive performances Overwinter survival.	Birds	UK	Boatman et al. 2004 Anonymous 2004

3 ESSENTIALS OF FIELD STUDIES

A review of monitoring and experimental studies during the workshop clearly showed that these studies should include both assessment of exposure and monitoring of biological effects in order to attempt to establish causality. Criteria or concepts that are important for these studies (especially for monitoring studies) were further discussed in breakout groups, and the main outcomes are reported in this chapter.

3.1 EXPOSURE ASSESSMENT

One issue in interpreting field data is that exposure may be poorly characterised and the validity of field studies without exposure assessment is questionable. This is particularly relevant for monitoring studies. Furthermore, studies can be used to confirm mitigation measures or to validate exposure models. Therefore, quantification of exposure concentrations should be as complete as possible.

For aquatic organisms, monitoring exposure focuses mainly on water concentrations. For terrestrial organisms, exposure data comprising residues in food items are extremely important (Anonymous 2002). For birds or mammals, monitoring exposure (expressed as dose or body burden) is extremely difficult because it not only is a function of the environmental concentration but also depends on the animal's behaviour.

The following issues are relevant for an adequate assessment of exposure of organisms to toxicants in the field, although they are mainly illustrated by examples from aquatic ecosystems.

3.1.1 CHEMICAL USE PATTERNS

Chemical use patterns include qualitative, quantitative, spatial, and temporal information on the use of pesticides within a monitored area. Such knowledge is needed because it provides valuable information for (1) evaluating potential effects of pesticides on non-target organisms and (2) designing, for

example, appropriate exposure monitoring for mixtures of chemicals.

An adequate evaluation of pesticide exposure, for example, in catchments, should include information associated with landscape characteristics (e.g., river basin structure, catchment size, soil type, rainfall, water flow) and agricultural practises (e.g., fertilizer application, land use practises, amount and type of pesticides used, as well as spraying season). However, there are often problems in obtaining such data, especially those related to pesticide use (Kreuger, platform presentation).

Studies involving field measurements should use an event-triggered sampling design. For example, it is necessary to sample at the time of application for drift inputs in aquatic systems. In contrast, it is important to sample at the start of the hydrological event for rainfall-driven inputs (runoff and drain flow) and later to capture both the peak pesticide concentration and the pattern over time. The problem is enhanced by the fact that time of pesticide application is more or less unpredictable. Problems linked to resolution in space and time could be partly reduced by using GIS. Indeed, GIS may have applications in analysing factors influencing exposure, such as landscape characteristics, proximity of habitats to treated fields, and temporal use patterns.

3.1.2 GOOD AGRICULTURAL PRACTISES

Exposure to pesticides results from field applications but may additionally result from misuse and/or point sources (e.g., failure to observe appropriate buffer strips, wastewater treatment works, or improper cleaning of equipment in the farmyard). Regulatory risk assessment of pesticides does not usually consider misuse or point sources because it is assumed that pesticides are used in accordance with the principles of Good Agricultural Practise (GAP). Predicted environmental concentrations do not currently consider exposure from poor agricultural practise. A long-term programme in Sweden that aimed at informing farmers about GAP led to a 90% reduction in pesticide contamination in

streams, whereas the amount of pesticide applied did not decrease (Kreuger, platform presentation).

The problem of point source and misuse needs to be kept in perspective because, for example, chemical monitoring data from the Netherlands indicate that chemicals that exceed maximum permissible concentrations derived from lower- and/or higher-tier tests (see www.bestrijdingsmiddenatlas.nl) may be associated with unknown sources. In addition, experience from Germany with monitoring in groundwater indicates that several cases of exceeding maximum permissible concentrations (drinking water standards) originated from point-source contamination and/or misuse. However, these cases mainly concerned water-soluble herbicides because they are easily detectable with multiresidue methods (Bach et al. 2000, 2001; Muller et al. 2002). In the case of insecticides, only a few studies used event-controlled sampling strategies in order to detect maximum short-term concentrations (Kreuger 1998). Hence, it should be pointed out that especially in the case of short-term pulses of insecticides that result from diffuse sources, measured concentrations are generally underestimated compared to the real concentrations because of the sampling and/or analytical protocols.

3.1.3 MODELLING EXPOSURE

Models are used extensively to predict pesticide exposure for regulatory risk assessment and are a possible option to reduce the problems related to field measurements. As described by Brown (platform presentation), the models are used to estimate field behaviour, mainly on the basis of laboratory measurements, and offer several advantages over direct determination of exposure in the field: modelling is faster and cheaper, it is possible to assess fate and exposure under the full range of possible use conditions, and the analysis can be repeated to account for changes in use or impact of mitigation measures. Nevertheless, it is critical to establish the extent to which model predictions for environmental concentrations of pesticides match real concentrations in the field. How-

ever, the predictive power of exposure models is questionable because extensive validation studies are missing. Problems with reliance on modelling alone include, in addition, the following: (1) pesticides and undetected, yet toxicologically important, substances may be missed either in chemical analyses or in models, (2) exposure processes are not always well understood, and (3) environmental data available to perform the calculation are not always sufficient (e.g., presence of buffer strips, pesticide usage). Nevertheless, it is possible to combine modelling with monitoring data to provide further insights into quantifying exposure. For example, catchment studies can be used to calibrate models that include product use data and landscape information, which can then be used for generic exposure assessment (e.g., the PESTSURF development in Denmark), or modelling and monitoring data can be coupled within GIS (e.g., Padovani et al., poster presentation). Currently, the validation of exposure models by comparing measured and modelled loads can be problematic because of the lack of monitoring data. For example, for avian risk assessment, the estimates of exposure have had almost no verification or validation against actual field data (Mineau, platform presentation).

According to the issues mentioned above, the pesticide concentrations measured in chemical monitoring programmes can differ from those predicted by regulatory models (e.g., Crane et al. 2003). This may be due to various reasons and may lead to the following

- Differences between predicted and measured concentrations because of
 - ▷ sampling from different types of water bodies (e.g., flowing rather than static water bodies),
 - ▷ inappropriate model or missing or inaccurate input data, or
 - ▷ differences in the dissipation of the pesticide in water, between observations in the field, and in predictions on the basis of laboratory water–sediment studies.
- Lower concentrations measured than predicted because of

- ▷ sampling during a low vulnerability situation (e.g., in relation to timing of rainfall),
- ▷ missing of peak concentrations, or
- ▷ conservatism in the initial tiers of the risk assessment process (e.g., worst-case 90th percentile modelled environmental exposure).
- ▶ Higher concentrations measured than predicted because of
 - ▷ point-source contamination (which is not included in models),
 - ▷ poor agricultural practise (e.g., buffer zone violation), or
 - ▷ addition of concentrations as a result of simultaneous uses in the area, while predicted concentrations focus on one single-use situation.

3.1.4 ROUTES OF EXPOSURE

Appropriate monitoring requires a good understanding of the routes of exposure. Routine monitoring for pesticides and other substances in surface waters is usually performed by water analyses. Indeed for aquatic organisms such as macroinvertebrates, the main route of exposure is usually via the water and only to a lesser extent through sediment or food. However, for organisms such as sediment dwellers, exposure via interstitial water associated with sediment may be of greater relevance. Routine monitoring is mostly carried out by authorities and regulatory bodies for legislative reasons that are usually unconnected with pesticide authorisation and control. Many of these programmes have poor temporal resolution (e.g., monthly or quarterly) in relation to peak pesticide concentrations. In addition, sampling locations often concentrate on larger water bodies (e.g., at the inlet of drinking water plants), and analytes are frequently biased towards water-soluble herbicides and are not related to use patterns. Routine chemical monitoring is therefore often of limited value for the prediction of ecological effects, although it is acknowledged that under the Water Framework Directive this should be augmented by monitoring for biological impacts.

The published data from case studies on exposure to agricultural insecticides in surface waters have recently been reviewed (Schulz platform presentation; Hommen et al. 2004; Schulz 2004).

For terrestrial organisms, the precise route of exposure is also a critical factor determining the risk of insecticides (Akkerhuis 1993; Wiles and Jepson 1994). Emphasis is clearly on oral exposure via food for vertebrates, for example. Therefore, exposure data comprising residues in food items are extremely important to refine the risk assessment (Anonymous 2002). There is evidence that the dermal route may also be important for certain compounds (Mineau, platform presentation; Mineau 2002), and exposure via this route is likely to be underestimated within current risk assessment schemes. Oral uptake via preening and inhalation may also need to be considered.

For birds or mammals, exposure (expressed as dose or body burden) not only is a function of the environmental concentration but also depends on the animal's behaviour – such as time spent in treated areas, feeding preferences, or feeding rates – which is highly variable and quite often unknown.

3.2 BIOLOGICAL EFFECTS

3.2.1 DEFINING REFERENCE AND CONTROL SITES

A key issue for performing field studies that are suitably representative and that can be extrapolated across different regions is to define control or reference sites. Such sites need to be selected with a view to protection goals.

Often there is no “control” site because selecting a truly uncontaminated site is unachievable in regions with historically modified ecosystems (i.e., most developed countries). Nevertheless, ecosystems modified by anthropogenic activities may have many of the properties of truly natural systems (e.g., certain types of ditches may simulate the properties of natural water bodies). The intensity of such activities (i.e., the degree of

disturbance) could be used as an indicator for selection of appropriate reference sites.

Reference sites should fulfil the following characteristics:

- ▶ ensuring that the pesticide exposure regime is such that no effect is induced;
- ▶ incorporating the natural variability characteristics of the community under consideration;
- ▶ minimising the importance of environmental parameters and confounding factors by avoiding differences other than pesticides between reference and tested sites (e.g., for aquatic systems: minimal difference in neighbouring cultures, stream or pond morphology, nutrient situation, hydrodynamics, soil and sediment type).
- ▶ excluding any major anthropogenic disturbance but including whatever disturbance induced by natural stressors (e.g., drying) is typical for the area (apart from exposure to pesticides).

To fulfil these characteristics, reference sites are often selected in smaller upstream sections, while impacted sites are often in larger water bodies located downstream. Effects in downstream sections can be detected, although not always properly quantified, when “internal” reference sites (comparisons through time at the same site) or “relative” reference sites (sites along a gradient of contamination) are used. However, in contaminated and reference sites, background contamination (current or past) should be avoided as far as possible because of the issue of adaptive processes that may have occurred in resident populations and that may lead to replacement of sensitive species or individuals by more tolerant ones and consequently to an increase in population and community tolerance (e.g., pollution-induced community tolerance [PICT]) (Blanck 2002).

Reference sites may be more useful when selected at a more integrated level (e.g., community structure according to ecological traits as opposed to species abundance).

Definition of target images would support the development of such reference conditions. There are some parallels with the definition of target images for the EU Water Framework Directive (developed for pesti-

cides and other stressors) that could prove useful in implementing the concept into regulatory risk assessment (EC 2000 cited in Römbke and Breure 2005).

In conclusion, the definition of the reference condition may be simply a pragmatic decision based upon a combination of scientific judgment, “public acceptability”, and evidence-based determination of “good quality” in agricultural landscapes. Examples of such definitions for aquatic communities are available (Nijboer 2000; Nijboer et al. 2004).

3.2.2 DETECTING EFFECTS

CONFOUNDING PARAMETERS AND NATURAL VARIABILITY

Confounding parameters and natural variability can make it difficult to identify and/or discern effects of pesticides even when a proper reference site has been selected. It is important to characterise and understand them properly in order to (1) account for them in the study design and interpretation of results and (2) reduce the uncertainties and increase the power of detection of pesticide effects in monitoring and field studies.

The importance of confounding parameters can be reduced by careful selection, but they cannot be completely excluded because they are part of the natural system. They can be site-specific, context-dependent, and ecologically relevant parameters with an important influence on the habitat. Factors can be physical (e.g., habitat structure and flow regime), chemical (e.g., water chemistry, nutrients, pesticide metabolites and degradation products with known toxicological significance, other pollutants), or biological (e.g., community composition). As an illustration, if data on long-term changes of community composition at a regional scale (e.g., species checklists) were available, they could allow us to detect changes in regional biodiversity in a particular area. However, without additional regional information on confounding factors (e.g., changes in land use and agricultural practises, water management), elucidation of causality and robust interpretation may be compromised.

The natural variability of a community is due to variations in different environmental parameters combined with the normal operating range of the biological characteristics, as determined by phenotypic plasticity and genetic variability. Some of the natural variability may be accounted for by categorising species according to ecological traits (e.g., sensitivity to pesticides, life-history characteristics, dispersal ability) and then comparing effects in these groups across different exposure concentrations, as in the concept of species at risk (SPEAR) (Liess, platform presentation; Liess and Von der Ohe 2005). Similarly, categorising habitats with reference to the organisms within them (e.g., organisms associated with oxygen-rich microhabitats) may help to elucidate the effects of other stressors or focus on specific risks and thus isolate possible effects of pesticides (e.g., the River Invertebrate Prediction and Classification System [RIVPACS] for the UK; the AQEM consortium for the EU, which has developed an assessment system for streams in 8 European countries based on benthic macroinvertebrates [www.aqem.de]; and a study on the composition of plant and invertebrate communities in field margins [Ratte, platform presentation]).

ENDPOINTS

Measuring the correct endpoints (e.g., acute, chronic, structure, function) is essential to detect effects. Endpoints commonly used and discussed in monitoring and field studies include the following:

- Individual level: survival, growth, reproductive performance, histological parameters, physiological parameters, biochemical parameters (e.g., biomarkers, enzyme assays, fat content, energy metabolism, respiratory rates, subcellular endpoints for all species, eggshell thickness for birds), behavioural responses (e.g., avoidance, feeding rates, repellency, foraging plot choice, and courtship behaviour for terrestrial vertebrates and invertebrates), and pesticide biotransformation pathways and tissue residues.
- Population level: abundance (including spatial distribution), population dynamics, population growth and extinction

rates, recolonisation and recovery, population stage and genetic structures, inbreeding coefficient, and biomass.

- Community level: species diversity and/or richness, shift in species dominance, biomass (plants, animals, potentially microbes), and functionality (community metabolism, nutrient cycles, coarse organic matter breakdown).

The most ecologically relevant and thoroughly studied effects are usually investigated at population and community levels because they are the ultimate changes of concern in the field. However, there are exceptions. For example, effects are usually studied on the individual level for endangered species and species of special public interest such as birds.

The power of monitoring schemes can be greatly increased when they take whole communities into account, because whole-community schemes maximise the likelihood of detecting effects on sensitive species and also make it possible to detect overall community changes. On the other hand, these schemes can obscure effects on some key species if these effects are compensated or hidden by other changes such as the presence of confounding parameters. In addition, the level of taxonomic identification influences the power of monitoring schemes. The ability to detect effects will be decreased when only a higher level of phylogenetic taxa is concentrated on. In such cases, the impact may not be detected because of replacement of affected species within the tested taxa.

Population- and community-level endpoints (e.g., abundance) are used to characterise populations in field studies for aquatic and terrestrial invertebrates. Those endpoints are challenging to investigate for birds and mammals because of the large home ranges of the animals and their long generation times (Kendall and Lacher 1994). Nevertheless, the best evidence for effects of pesticides on birds is at the ecosystem level, as is shown in the studies of the grey partridge survival project conducted by The Game Conservancy Trust (Potts 1986).

In addition to structural endpoints, functional endpoints (e.g., O₂ consumption, O₂ production, leaf litter decomposition, soil pa-

rameters influenced by earthworms) should also be considered for detecting effects because they are also ecologically important (e.g., herbicides often inhibit oxygen production). However, functional endpoints are usually less sensitive than structural endpoints due to possible replacement of sensitive species (i.e., functional redundancy), and they are rarely considered in field trials, which are usually performed at insufficient spatial and temporal scale to detect effects (Holland, platform presentation). Nevertheless, there are a few examples of the successful investigation of functional endpoints such as coarse organic matter breakdown (for litter-dwelling and below-ground invertebrates). Recently, the effects of pesticides on organic matter breakdown have been assessed as part of the EU pesticide registration process using the litter-bag method (EPFES 2003).

DIRECT AND INDIRECT EFFECTS

Pesticides can be deleterious to individuals, populations, and communities by exerting direct and indirect effects. Monitoring studies have the potential to detect both direct and indirect effects, such as the significant increase of Collembola abundance and the decrease of Linyphiidae abundance following use of synthetic pyrethroid insecticides (Frampton 1999). However, it can be challenging to hypothesise the causal influences behind an observed effect and to identify whether an effect is direct or indirect. The use of additional experimentation (e.g., bioassays, micro- or mesocosms) could provide useful complementary information to better distinguish between direct and indirect effects.

Direct effects of pesticides are related to the sensitivity of the species towards pesticides and are illustrated by concepts such as the species sensitivity distribution (SSD; Brock, platform presentation) and the relative sensitivity distribution (RSD; Wogram and Liess 2001; Von Der Ohe and Liess 2004). Indirect effects result mainly from changes in biological interactions (e.g., altered predator–prey relationship, competition alteration, disappearance of food or habitat resources).

Grouping species according to ecological traits (e.g., sensitivity towards toxicants, long generation time, lower ability for recolonisation) could be a promising approach to assess direct and indirect effects. For example, in the SPEAR concept (Liess, platform presentation; Liess and Von der Ohe 2005), aquatic species are grouped according to their (1) life-cycle traits to account for differences in recovery or recolonisation potential and (2) sensitivity to particular pesticide modes of action. Using this concept, it was shown that the invertebrate communities exposed to toxic pressure are characterised by a reduced number and abundance of species at risk. This leads to an increase in abundance of species not at risk due to reduced competition with or predation by species at risk, which is a typical example of an indirect effect.

Recent findings for plants suggest that crop species sensitivity to herbicides is adequate to represent the response of non-crop species regardless of chemical class or exposure (McKelvey et al. 2002). However, there are some non-crop species that have no closely related crop species. Thus, these non-crop species might not be represented sufficiently by tests with common crop species. Depending on the question underlying field tests, it might be necessary to consider non-crop species as well.

Although the respective importance of direct and indirect effects of pesticides remains unclear for birds and mammals, indirect effects (through disturbance of the food chain) can be more important than direct effects for species such as insectivorous birds (Sotherton and Holland 2002).

Further evidence concerning indirect effects of pesticides will be gained from the Indirect Effects of Pesticides Project funded by the Pesticides Safety Directorate of DEFRA in the UK (Holland et al. 2002).

TOLERANCE

It was discussed that the absence of observed acute effects is not necessarily an indication that pesticides do not exert any effects. Reasons can include monitoring with too little power to detect effects. Acute effects could also be absent due to the development of tol-

erance or resistance at different levels of biological organisation (individuals, population [common problem in pests], or community [e.g., PICT]). Thus, chronic effects need to be taken into account within the context of the development of tolerance on various levels of biological organisation.

3.2.3. RECOVERY

CHARACTERISTICS OF RECOVERY

Recovery is an integral attribute of how biological systems (individuals, populations, communities) respond to a stressor over the long term. Therefore, it should be considered in the interpretation of effects.

The term “recovery” must be properly characterised. In the context of the workshop and at the population and community levels, it includes both internal recovery (recovery from within the affected system) and external recovery (recolonisation by dispersal from external sources). Field studies should attempt to distinguish and quantify these 2 aspects. This endeavour may be very difficult because it requires incorporation of site-specific population dynamics, life-history information for affected populations, and information on metapopulation dynamics in the landscape. Evaluation of the degree of connectivity of ecosystems at local or landscape levels may also be very useful. Assessing recovery requires considerable knowledge about different landscape factors and how they interact with organism life history. Thus, time and space are important parameters to consider in the recovery process. Many questions therefore remain in this area of ecology:

- How does recovery potential differ across systems and landscapes?
- Is the possibility or probability of recovery reduced if a large part of the landscape is adversely affected by pesticide exposure (Liess, platform presentation)?
- How large should the protected part of the landscape be (e.g., what percentage of an interconnected ditch system) to facilitate recolonisation?

- How long does it take for populations in a stream or pond to recover through internal recovery versus recolonisation?

Recovery might take longer than expected in some cases. For example, removal of key-stone species could lead to alternative stable states of the community (e.g., macrophyte-dominated versus algal-dominated aquatic systems; Scheffer 1998). Also, Matthews et al. (1996) have suggested that ecosystems possess a “memory” of stressful events and that there might be a lack of return to original conditions after chemical exposure. Mechanisms of resistance are good examples of such historical changes (PICT concept, Blanck 2002).

Whether populations are r- or K-selected may influence the speed and capacity for system recovery. Adaptation to stress may lead to a change not only towards less sensitive species but also towards species that have better recovery or recolonisation potential (e.g., shorter generation time and/or aerial life stage). For example, in an aquatic field study (Liess, platform presentation), the main recolonisation process observed was by organisms drifting from the small and non-contaminated areas upstream towards the contaminated sites downstream. This result suggests that the area required as a source of recolonisation may not need to be large (depending on the species) to have a significant effect on downstream sections.

For aquatic ecosystems, it was suspected that recovery might be faster in flowing systems than in isolated ponds and lakes. It is possible that recovery in interconnected water bodies may occur within 1 year of even substantial disturbances due to pesticides, provided the stressor is removed and depending on the species under consideration (i.e., length of generation cycle; Liess and Von der Ohe 2005). It is much harder to make predictions for isolated water bodies because the recovery processes in these are poorly understood. A recent study in pond mesocosms treated with a non-persistent insecticide showed that invertebrate communities did not recover in physically isolated systems as their composition diverged from that of control (untreated) ponds, whereas full recovery was achieved after about 2 months

in ponds for which recolonisation was possible (Lagadic et al. 2004). However, because there are often gradients of pesticide concentration within larger water bodies, there are frequently refugia and thus potential for internal recovery. In addition, it is argued that when there is frequent occurrence of stressors other than pesticides, the community is r-selected and able to recover faster from a disturbance than a community that is rarely exposed to stressors. For example, a study of ephemeral ditches in northern Germany found no differences in communities between ditches within arable areas compared to those alongside meadows (Sönnichsen 2002). This result was attributed to the high recovery or recolonisation potential of the systems, possibly linked to the ability of the specific community to recover quickly because it consisted of species that recover from periodic drought. This hypothesis was further supported by the absence of species with a low recovery or recolonisation potential. It was also proposed by Lagadic et al. (2002) to explain the absence of effects of insecticides on invertebrate communities in coastal wetlands subjected to chemical mosquito control.

For terrestrial invertebrates, field data on recovery from applications of specific chemicals have been summarised in a comprehensive study (van Straalen and van Rijn 1998). Some of the outcomes, also mentioned in Römcke's platform presentation, are as follows:

- For Collembola, the potential for recovery from adjacent, untreated field margins or unsprayed areas of crop would have been overestimated (e.g., Frampton 2002). A manipulative study carried out within DEFRA Project PN0934 also suggests that limited recovery of Collembola would occur from field edges if OP insecticides were used, despite high abundance and diversity of Collembola at the field edge. However, Collembola immigration from field edges did occur in a study without pesticide use (Alvarez et al. 2000).
- For earthworms, recovery strongly depends on the persistence of the pesticides (Jones and Hart 1998), and the role of re-

colonisation is not clear (Heimbach 1997; Mather and Christensen 1998).

For terrestrial vertebrates such as birds, visible occurrence of poisoning (loss of a few individuals) may be considered unacceptable by management authorities. Regulatory action should thus be taken well below a level at which the population might be directly affected. However, indirect effects of pesticides have been demonstrated for farmland bird species, and these could reduce the chances of achieving an overall recovery of the populations. Therefore, the issue of recovery and recolonisation is relevant for small mammalian species that are sedentary in the treated area (e.g., small rodents in pastures or orchards) as well as for birds and larger mammals.

MODELLING RECOVERY

The incorporation of information about recovery in recolonisation and population models would be very useful for effects prediction and has the potential to assist in hypothesis testing. It would be desirable to use generalised information on recolonisation statistics for different species to generate hypotheses and to design experiments.

So far, recolonisation data suitable for such models are limited in availability and mainly relate to aquatic ecosystems. They deal with reproduction, drift, and dispersal. For example, data on the recovery potential of organisms are available at the family level on the PondFX website

(www.ent3.orst.edu/PondFX) and partly at the species level in Liess and Von der Ohe (2005). An extensive database at the species level is currently being collected in a cooperative project among Alterra (G. Arts), The Ponds Conservation Trust (J. Biggs), and the UFZ Centre for Environmental Research (M. Liess).

3.2.4 BIOASSAYS AND BIOMARKERS

To help establish causality between exposure and effects, a combined approach using experimentation (e.g., in situ bioassays, biomarkers, mesocosms) and field observation may be useful (Liess and Schulz 1999; Sibly

et al. 2000; Schulz et al. 2002; De Coen and Janssen 2003a, 2003b; Hanson and Lagadic 2003; Jergentz, Passacq et al. 2004). It is difficult to determine whether in situ bioassays are more or less sensitive than field trials because the sensitivity depends on the bioassay methodology (Wiles and Frampton 1996). On the one hand, it was suggested that they might be more sensitive than field trials because of increased exposure in cages and lack of escape opportunities (e.g., with *Gammarus pulex*; Schulz and Liess 1999a. On the other hand, other studies showed that in situ bioassays and field trials have similar sensitivity (Frampton 1999; Liess and Schulz 1999). Nevertheless, it was concluded that in situ bioassays could be useful systems, particularly when natural populations show variable demographics and when direct negative effects of pesticides are concerned.

It was also concluded that biomarker selection on the basis of the mode of action of pesticides is essential in order to discriminate between various stressors (Lagadic et al. 2002). Indeed, interpretation of results can be difficult because of problems in attributing a response to a single toxicant. Selection of biomarkers should also be based on their physiological role and, more specifically, on their metabolic implications in individual performance (growth, reproduction, etc.). Their biological relevance therefore depends on the possibility of extrapolating from biomarker responses to population-level endpoints (Migula 2000; Scott-Fordsmann and Weeks 2000). Nevertheless, because exposure is difficult to quantify, especially for vertebrates, the biomarker approach is very useful.

3.3 RELEVANCE OF THE STUDY SITUATION

3.3.1 RELEVANCE OF THE LANDSCAPE

The system that is monitored should be representative of the agricultural landscape, and the challenge is in defining this. For water bodies of the UK, there are examples in which classification has been systematically achieved (e.g., Anonymous 2003). In this classification, 12 agricultural landscape types

were defined using soil, hydrogeological, and cropping descriptors; then, the morphological, physicochemical, and biological properties of water bodies in the different landscapes were analysed by compiling disparate datasets, and the results were entered into a database to facilitate access to the information. A step in the same direction was made by identifying the so-called EURO-Soils that are considered representative for soils of the EU (Gawlik et al. 1996).

3.3.2 RELEVANCE OF THE POLLUTION SCENARIO

A study can be directed towards worst-case or typical scenarios or designed to represent the full range of real scenarios. The choice of an appropriate scenario will depend on the purpose and the needs of the study. However, the system investigated should have the capacity to respond to the range of contamination expected.

3.3.3 ECOLOGICAL RELEVANCE

Representatives of the main taxa and of the different life-cycle strategies (multi- to semi-voltine species, r- and K-species) should be present in sufficient numbers for adequate statistical analysis. The influence of other stressors should be minimised unless they are typical for the region investigated. For example, if drying or dredging is a typical environmental stressor in a specific ecosystem and the community is therefore dominated by r-strategist species, then this system can be considered as representative. However, excessive maintenance in non-target areas (e.g., very frequent dredging of stream beds) should not be considered representative.

3.3.4 SPATIAL AND TEMPORAL RELEVANCE

The study must be spatially (environmental variables) and temporally (climate, usage patterns) representative. At a smaller scale (e.g., small water bodies) there may be high

variability; therefore, the number of sites or times of sampling must be sufficient to reduce the uncertainties. Similarly, at a larger scale, localised impacts or peak concentrations may not be detected or captured. Spatial and temporal scales are particularly important for the understanding of the recovery from pesticide applications.

4 LINKING TIERS AND EXTRAPOLATION

The EU Uniform Principles for the assessment of PPPs require that if the preliminary risk characterisation indicates potential concerns, registration cannot be granted unless it can be demonstrated that "... under field conditions no unacceptable impact on the viability of exposed organisms..." occurs. To date, such assessments have been made by conducting higher-tier studies (e.g., mesocosms), and thus the relevance of these assessments to field situations may have certain limitations. Examples of comparisons between observed and predicted effects are presented below. Some potential problems in comparing effects observed in the field and effects occurring under controlled conditions are also listed.

4.1 EXAMPLES OF COMPARISON BETWEEN OBSERVED AND PREDICTED EFFECTS

4.1.1 EFFECTS ON AQUATIC ORGANISMS

When observed and predicted effects are compared, various endpoints can be considered. The following endpoints were discussed during the workshop.

Short-term effects

Observed effects were in line with predicted effects. Indeed, measured pesticide concentrations of around 1:10 of the acute 48-h lethal concentration (LC₅₀) of *Daphnia magna* led to a short-term reduction in the abundance and number of sensitive species. Below 1:100 of the acute 48-h LC₅₀ of *Daphnia magna*, no such effect was observed

(Liess, platform presentation; Liess and Von der Ohe 2005). Hence, an assessment factor of 100 on the acute LC₅₀ would prevent possible short-term effects. Another investigation showed a decreased effect of spray drift on the invertebrate community in the presence of a buffer zone (Schäfers, platform presentation). However, this positive effect could not be quantified because pesticide concentrations were not measured.

Long-term effects

Effects on community composition were observed at concentrations for which no effect was predicted. Measured pesticide concentrations of around 1:100 of the acute 48-h LC₅₀ of *Daphnia magna* led to a long-term reduction of a proportion of sensitive species. However, because the levels of contamination may have been quantified insufficiently, it remains uncertain at which concentration these changes occur (Liess, platform presentation; Liess and Von der Ohe 2005). In addition, it was stated that the effect of pesticides on long-term alterations of community structure depends not only on the sensitivity of the species but also on the reproduction rate of the exposed organisms. The importance of such life-history characteristics in governing recovery was shown by Sherratt (1999) and Liess and Von der Ohe (2005).

Recolonisation

The proportion of species potentially affected depends not only on the contamination at the site but also on the presence of undisturbed stream sections upstream. The levels of biological impairment observed at sites with high concentrations of pesticides and good habitat quality (indexed as undisturbed upstream sections) were similar to those at sites where pesticide concentrations were low but habitat quality was poor (Liess, platform presentation; Liess and Von der Ohe 2005). Thus, landscape and land use information are important to consider in the prediction of effects but are not included in risk assessment.

4.1.2 EFFECTS ON SOIL INVERTEBRATES

Van Straalen and van Rijn (1998) compared predicted and observed effects and targeted the study on recovery times. They showed that in 25 out of 32 comparisons, recovery in the field takes place within a time period necessary for degradation of the pesticide to a no-effect level. In some cases, recovery was slower than expected, a possible result of indirect effects rather than of direct toxicity to the organisms considered.

4.1.3 EFFECTS ON BIRDS

Mineau (2002) used regression methods to analyse the relationship between the occurrence of avian mortality in pesticide field studies (mostly conducted in North America for regulatory purposes) and a number of potential predictor variables, including acute oral and dermal toxicity, Henry's Law constant (reflecting potential for inhalation exposure), and application rate. The best model was able to classify "safe" and "lethal" applications in the study sample with better than 80% success.

In a follow-up study (Mineau, platform presentation), predictions of this model were compared with acute toxicity–exposure ratios (TER_{acute}) calculated according to the EU Guidance Document on risk assessment for mammals and birds (Anonymous 2002). They also compared the TER_{acute} results directly with the occurrence of mortality in the individual field studies. The results showed that a substantial proportion of pesticides were misclassified, therefore suggesting that the predictive power of the current Tier I risk assessment scheme is not satisfactory. Improving the parameters for the current risk assessment model (e.g., residue per unit dose [RUD]) is desirable but not sufficient because the model itself seems to have certain deficits. For example, this study showed that dermal exposure is not as negligible as the current risk assessment scheme supposes, at least for organophosphate pesticides. Improving the model structure may thus be the best way to enhance the discriminatory power of the assessment procedure.

4.2 LIMITATIONS IN COMPARING OBSERVED AND PREDICTED EFFECTS

4.2.1 LIMITATIONS OF FIELD STUDIES

CHARACTERISATION OF EXPOSURE

Characterisation of exposure can be insufficient for the following reasons:

- ▶ Sampling problems (e.g., missed peak of contamination, sample deterioration) resulting in an underestimation of pesticide concentrations. In the case of birds and mammals, exposure is not quantified in the same way as for other species because it is a function of environmental concentrations, time spent in the area, feeding preferences of the species, etc.
- ▶ Analytical problems such as matrix interference and presence of undetected contaminants or of those exerting biological effects below analytical detection levels.

ROUTES OF EXPOSURE

Whatever the study type (laboratory, microcosm, mesocosm, monitoring, or field study), the routes of exposure must be well understood so that relationships between both types of observations can be interpreted. This may be easier to achieve for short-term effects than for long-term effects because exposure to stressors (including pesticides) and the resulting effects are more difficult to characterise over longer time periods.

DETECTABILITY OF EFFECTS

The statistical power to detect effects of pesticides in monitoring and field studies is generally less than for studies performed in laboratory test systems or in indoor micro- or mesocosms because of greater natural variability and more confounding factors. However, the combination of an elevated number of investigated sites with the appropriate statistics may enable us to discriminate the impacts of individual stressors and to reveal links between exposure and effects as shown above.

SIGNIFICANCE OF ENDPOINTS

To detect field effects of pesticides, relevant endpoints have to be monitored. For exam-

ple, monitoring studies on birds and mammals are focused mostly on lethality. Thus, one should be aware that the absence of observed effects does not prove that no effect occurs. Indeed, the long-term use of pesticides is more likely to result in sublethal effects (e.g., direct effects such as eggshell thinning, indirect effects such as decline of population following removal of food-chain components) than in the death of a bird by poisoning. In light of this situation, monitoring studies revealing longer-term changes in community structure would be more appropriate.

LONG-TERM VERSUS SHORT-TERM EFFECTS, DIRECT AND INDIRECT EFFECTS

Most effects shown in monitoring and field studies of aquatic invertebrates and birds and mammals are chronic, long-term, and indirect effects. Therefore, it is insufficient to assess only acute effects (e.g., lethality), even though this is currently done, particularly in reactive monitoring schemes for birds and mammals. Indeed, if observed and predicted effects are to be compared, the same endpoints must be considered.

RELEVANCE OF THE STUDY SITUATION

The conditions selected must be representative and appropriate for the needs of the study performed. These include the characteristics in terms of landscape, pollution scenario, ecological characteristics, and spatial and temporal scales.

4.2.2 LIMITATIONS OF MESOCOSM EXPERIMENTS

Although small-scale and large-scale mesocosm studies can play an important role in establishing causality, testing hypotheses, and interpreting field data, some limitations remain.

EXPOSURE TO MULTIPLE SUBSTANCES OR STRESSORS

Single-substance tests are not necessarily predictive of field situations, where exposure is usually more complex (multiple substances or stressors). Although controlled ex-

periments may provide fairly good effects predictions for known exposures (e.g., those compounds or other stressors that are experimentally applied), these predictions are less robust when exposure is more complicated (e.g., unknown compounds). However, controlled experiments such as mesocosm studies remain the best way to approach multiple exposures (Brock, platform presentation), as compared to simplistic laboratory test systems, particularly because the former include the effects of any biologically active degradation products.

DIRECT AND INDIRECT EFFECTS

Controlled experiments are probably predictive of direct effects, but they may not be protective in all cases. Indeed, indirect effects and long-term effects may depend on factors that may not be realistically incorporated into test systems. One relevant example is the problem of communities in test systems, which may have a higher percentage of species with a short generation time compared to natural communities in permanent streams and ponds in the field. Test systems are usually established for a limited period of time, and therefore r-strategists tend to dominate the communities if colonisation of the test systems is only natural.

RECOVERY

Controlled experiments are predictive of threshold effects concentrations when compared to single-species tests. However, recovery through internal recovery and recolonisation is often context dependent (e.g., voltinism in the particular assemblage, hydrological connectivity, latitude). Recovery data from mesocosm studies should be carefully considered because they are not directly representative of natural conditions. Indeed, compared to the field situation, the recovery processes may be (1) slower for some species (e.g., those migrating inside of the water body) or (2) faster for others (e.g., those recovering through aerial stages), especially when control and exposed mesocosms are close to each other (Lagadic et al. 2004).

TIME SCALE

Consideration of time scale in relation to the generation time of the investigated species shift is essential in test systems. Effects vary according to duration of exposure, and thus the time scale with respect to persistence of effects (e.g., indirect effects, recovery) is also important in the assessment of environmental risks. Nevertheless, with the exception of some studies in outdoor mesocosms, controlled experiments are usually shorter than 1 year. There is thus a gap between what the studies can and/or set out to demonstrate and the appropriate test design to achieve this goal. The time scale in relation to the generation time of the investigated species is also of importance, especially when recovery is being studied.

5 RESEARCH REQUIREMENTS AND FURTHER STRATEGIES

The main research requirements and further strategies identified in this workshop are summarised below. Outcomes of field studies (field monitoring and field experimental studies) performed while addressing these various issues will facilitate further interpretation and characterisation of pesticide effects in the field.

5.1 RESEARCH REQUIREMENTS

5.1.1 IMPROVED EXPOSURE ASSESSMENT

Exposure assessment should be improved in the different ecosystems considered within risk assessment. There is a need to improve sampling techniques and strategies available to support field studies. Incorporation of time-varying exposure into risk assessment is an important requirement. Mathematical models for the aquatic compartment will often predict the variation in exposure concentrations with time, but this information is not used within the risk assessment procedure. Ecological aspects of exposure (e.g., timing and duration of presence of sensitive life stages) also require further development.

5.1.2 DEFINING TARGET IMAGES

Target images need to be defined to evaluate monitoring studies. Gathering extensive knowledge about the variability of ecosystems, the effects of pesticides, and the subsequent recovery of communities in the field will enable us to define principles in setting target images.

5.1.3 ASSESSMENT OF DIRECT EFFECTS

Focusing on sensitive species is very useful for detecting direct effects of pesticides, provided that the spectra of sensitive species are the same for both active substances and their metabolites or degradation products. In order to strengthen field assessments, it would be desirable to link mode of action and species-specific metabolic pathways to observed effects.

5.1.4 ASSESSMENT OF INDIRECT EFFECTS

One important outcome of the workshop is the understanding that indirect effects are of high importance when effects of pesticides in the field are studied. Nevertheless, generalities in defining ecological traits that indicate specific effects are missing (i.e., species with high recovery potential may benefit from pesticides). In addition, it was stated that indirect effects are often difficult to demonstrate. Targeted laboratory investigations, micro- or mesocosm studies, and in situ bioassays can help to elucidate underlying mechanisms and thus enable a better observation of indirect effects. Information on indirect effects (especially on their origin) could also be gained from a survey of ecological literature, especially in the field of functional ecology (e.g., food web dynamics, temporal and/or spatial competition between species).

5.1.5 ADDITIONAL BIOLOGICAL AND ECOLOGICAL KNOWLEDGE

Additional biological and ecological knowledge is needed, for example, to better assess direct and indirect effects or the potential for recovery and recolonisation. Further information is needed regarding the following:

- ▶ Species diversity (e.g., through well-educated taxonomists and better taxonomic tools such as computer-aided identification keys). This is especially relevant for terrestrial invertebrates.
- ▶ Ecological characteristics of key species in the different agro-ecosystems (e.g., food sources for terrestrial invertebrates, life history, ecophysiology, sensitivity to pesticides, life cycle, dispersal, behavioural data, interactions or competition or predation between species and trophic networks).

5.1.6 ENVIRONMENTAL PARAMETERS

The potential of environmental parameters (e.g., UV radiation, drought) to alter sensitivity of the different levels of biological organisation (individual, population, community) needs to be better defined. This issue leads to further questions such as the different sensitivity of species or communities according to their geographical origin (i.e., northern and southern Europe), population and community genetics (biodiversity, resistance and fitness, extinction risks), or stability and continuity of ecosystems (drying versus permanent water bodies) in relation to the variations of biological response to pesticides.

5.1.7 MIXTURE TOXICITY

It is necessary to reduce the uncertainty about whether or not risk assessment that considers only single compounds is also conservative for multiple compounds or stresses that may occur in the field (Brock, platform presentation). An investigation on aquatic ecosystems presented during the workshop (Liess, platform presentation) supports the

view that single-chemical risk assessment of the most toxic compound could be sufficient for the mixture of pesticides in the scenario that was presented (small agricultural streams). This will especially be the case for high-risk compounds entering small catchments. A multiple exposure with several compounds at comparable toxicity is unlikely under such scenarios. Other experiments, however, showed that even compounds at concentrations below the no-observed-effect concentration (NOEC) can contribute to the toxicity of mixtures (Rajapakse et al. 2002; Walter et al. 2002). Also, reduction in the toxicity of active ingredients by chemicals present in the mixture (i.e., antagonism) or toxicity of spray-tank adjuvants cannot be ruled out (Jumel et al. 2002; Caquet et al. 2005). This finding underlines the need for further information.

5.1.8 MAGNITUDE OF RECOVERY

Several examples during the workshop showed the great importance of recovery for population and community dynamics. More information on quantifying recovery would help us to better assess the overall effects of pesticides. Empirical information and further development of appropriate modelling approaches would be very useful for this purpose.

5.1.9 BENCHMARK CASES

The establishment of well-developed generic benchmark cases could provide references for other compounds and be used for validation of laboratory-based risk assessment schemes.

5.2 FURTHER STRATEGIES

5.2.1 DEFINING ACCEPTABILITY

Criteria for defining the acceptability of observed effects need to be further established because they should include both scientific and societal considerations. The role of sci-

ence is to identify the options and consequences so that society can make decisions. For example, the selection of reference sites and target images depends partly on what is considered acceptable by society. It is necessary to establish the target image and then to decide how much deviation from this target image is acceptable.

5.2.2 SPATIAL UNIT OF ASSESSMENT

Modelling of landscape-scale impacts helps to address the question of what magnitude of impact is significant and/or acceptable. Spatially explicit modelling using GIS combined with biogeography and dispersal data for the target or non-target species could be an effective way of achieving this goal. Reference data on the undisturbed habitat or community are necessary to provide a benchmark for assessing changes and measuring recovery. For example, recovery of impacted aquatic communities may be facilitated by recolonisation from undisturbed water bodies.

5.2.3 HARMONISATION WITHIN EUROPE

The quality and comparability of future field studies from different locations in Europe need to be improved. This improvement should be facilitated by the strengthening of a European network of expertise in this research area as a result of the workshop.

5.2.4 GOOD AGRICULTURAL PRACTISES

The studies presented at the workshop demonstrated that in many cases it is difficult to distinguish between observed effects attributable to pesticide use according to GAP and those arising from misuse. Future studies designed to resolve this problem are worthwhile applications of monitoring and experimental studies and are highly recommended.

6 IMPLICATIONS FOR REGULATORY RISK ASSESSMENT AND MANAGEMENT

6.1 CURRENT VALUE AND SCOPE OF FIELD STUDIES IN RISK ASSESSMENT

The general consensus was that it is difficult to envisage field studies (monitoring studies and experimental studies) being incorporated into decision making with respect to registration for a single active substance, because the field situation is more complex and hence differs from the higher-tier tests performed as part of the risk assessment. However, they provide useful information, as described below.

6.1.1 INFORMING AND EVALUATING THE RISK ASSESSMENT PROCESS

Field studies are useful to monitor pesticide use after approval in the most realistic way, as a check on pre-authorisation predictions. Therefore, they can be used to verify whether the current risk assessment provides suitable environmental protection by generally validating (or invalidating) the ecologically acceptable concentration derived from higher-tier test systems.

Field studies are potentially useful for reregistration purposes. They can also help in the verification and improvement of risk assessments because the information gathered can be used to formulate questions and guide the selection and design of studies at a pre-registration stage (e.g., better generic mesocosm studies).

6.1.2 VERIFYING RISK MITIGATION MEASURES

Monitoring studies provide a potential tool for checking the effectiveness of mitigation measures applied to use of a pesticide where potential risks are identified in the registration procedure. Results may help to improve risk mitigation measures where necessary. The studies may provide a possible means for identifying where further enforcement of or

training for such measures is needed (though it is acknowledged that the establishment of monitoring sites may stimulate better practise than is found more generally; see Kreuger, platform presentation). Demonstration of the efficacy of mitigation measures to farmers and other stakeholders may also be an important function.

Monitoring to evaluate mitigation measures may involve measurement of exposure and/or effects, depending upon the measure and risk under consideration.

Examples:

- ▶ Impacts can differ when spray direction is changed and can decrease significantly when a reasonable buffer zone is observed. For example, the potential benefits of effective risk mitigation were shown in the Altes Land study when the buffer zone was 3 m to 5 m (Schäfers, platform presentation).
- ▶ Vegetated areas such as constructed wetlands serve as tools to mitigate exposure and related risk (Rodgers and Dunn 1992).
- ▶ Relatively small areas in streams that do not suffer impacts (e.g., a forested area) can significantly mitigate impacts in connected water bodies (Liess and Von der Ohe 2005).

6.1.3 RISK COMMUNICATION STRATEGIES

Monitoring studies offer the potential for better communication of risk assessment decisions to policy makers, the general public, and other stakeholders because they can inform on the magnitude or absence of ecological effects occurring on a “real” scale. Coupled with GIS technology, these studies may prove a useful tool for visualising and communicating results.

6.1.4 BROADER SUSTAINABILITY ISSUES

Monitoring studies may provide a means for looking at the overlap between the various environmental protection regulations, ensuring that management practises developed for

one area do not have an impact on another. For example, there will be applications in ensuring complementarities between pesticide regulation and specific actions to support nature conservation and protection of Red List species or implementation of the Water Framework Directive. Issues in which different environmental regulations overlap may be highly complex, and it is essential to specify the appropriate questions before designing the study and to frame problem formulation depending on the regulatory application so that the different requirements are met. Ultimately, monitoring studies may assist with checking that economic and social development proceeds in a way that is environmentally sustainable.

6.2 IMPLICATIONS FOR FUTURE REGULATORY RISK ASSESSMENT

Relevant issues resulting from current knowledge and future research developments should eventually be implemented in regulatory risk assessment to enable appropriate calibration and verification of the schemes.

One important conclusion of the workshop is that exposure should be defined accurately so that critical effect concentrations detected in the field and those predicted from estimated exposure under Directive 91/414/EEC can be compared. In addition, results from monitoring studies highlighted issues that would be relevant for increasing realism in future risk assessment. For example, the following parameters or issues should be considered and/or implemented because they are or could be important to assess the occurrence of effects under field conditions.

- ▶ Chemical monitoring: This can be effectively used, for example, in surface waters for regulatory purposes to show that risk mitigation measures work properly. However, to date the prediction of effects on the basis of such data is only approximate, because many processes can influence biological effects and their recovery.
- ▶ Route of exposure: The relative importance of different routes of exposure

should be appropriately implemented in regulatory risk assessment. For example, studies on birds pointed out that the role of dermal exposure is likely to be underestimated in current regulatory risk assessment schemes.

- ▶ **Mixture toxicity:** Some results showed that risk prediction based on the most toxic product could lead to reasonable regulatory decisions. However, further research is needed to determine whether risk assessment that considers only single compounds is generally conservative for mixture toxicity.
- ▶ **Target images:** Most of the issues cited above should help to define target images that are needed to evaluate monitoring studies. Close contacts between monitoring activities under Directive 91/414/EEC and the Water Framework Directive are needed so that overlaps can be avoided.
- ▶ **Environmental parameters:** These may increase or decrease the sensitivity of organisms towards pesticides. Such parameters should therefore be included in risk assessment.
- ▶ **Biological and ecological data:** More data are needed on the different biological and ecological traits of non-target species. For example, these data could be added to the information on landscape and analysed using GIS systems to facilitate use for regulatory purposes and to improve the realism in risk assessment.
- ▶ **Indirect effects:** These have been observed for all groups of organisms, and their relevance with regard to the long-term repercussions on populations and communities should be investigated. The causal relationship to direct effects could be better investigated by, for example, the use of biomarkers.
- ▶ **Recovery and recolonisation:** These processes have important implications for assessing the occurrence of effects. But it will be challenging to include these concepts into a risk assessment in a field-relevant manner. Indeed, repeated exposure or other stressors might disturb these processes. Furthermore, species with a low recovery potential (i.e., univoltine species and/or species with low dispersal

potential) should be considered more carefully than species with high recovery potential (i.e., short generation time and/or high dispersal ability).

- ▶ **Incidence schemes:** The organisation of such schemes should be established or improved when monitoring studies are difficult to conduct (i.e., for vertebrates such as birds). The schemes are a safety net for the regulatory system and can highlight the need for appropriate risk management and issues regarding misuse and abuse.
- ▶ **Landscape analysis:** Landscape characteristics can modify the importance of field effects of pesticides and recovery (e.g., conservation headlands support a diverse community of terrestrial organisms in agricultural landscapes; forested stream sections facilitate the diversification of aquatic communities through colonisation). Classifying landscape characteristics and including habitat quality in risk assessment may put the risks of contamination into context with respect to other stressors.

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effects of pesticides in the field

EPiF

appendix *a*

**ABSTRACTS OF
PLATFORM
PRESENTATIONS**

Plenary keynote

STRESS AND DISTURBANCE IN NATURE

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In this paper, I use the terms “stressors” to mean environmental factors that decrease population growth rate when first applied (Sibly and Calow 1989; Sibly and Hone 2002) and “disturbances” to mean short-acting stresses. Examples of natural disturbances include fires, floods, avalanches, hurricanes, grazing, and trampling. Natural stressors include pH, food availability, moisture levels, and other climatic variables.

All natural populations are subject to stress and disturbance. However, documentation of the effects of stress and disturbance in nature is far from easy. The difficulties arise in part because often the occurrence of stress and disturbance cannot be predicted in advance, and thus research methods to study them cannot be planned and put in place as needed. In addition, ascription of a particular change in population size to a particular stressor is extremely difficult. In general, identification of stressors requires lengthy, replicated experiments. Even then, identification of the cause of population change in particular cases often remains controversial.

The study of stress is generally easiest at the individual level. Populations are in general a little harder because the effects of interactions between individuals also have to be taken into account. These may produce effects such as density dependence, i.e., decline in population growth rate as the population increases. Communities introduce many further complexities as well (Van Straalen 2003). In this paper, I shall deal mainly with population responses, although towards the end I shall consider how levels of disturbance affect communities. The response of communities to disturbance is dealt with in this volume in the paper by Ratte.

There are a number of examples of the effects of stress on population growth rate at low population density. Classic studies include those of Tilman et al. (1981), showing that decreasing availability of silicates reduces population growth rate in the diatom *Asterionella formosa*, and of Daniels and Allan (1981), showing similarly the stressful effects of dieldrin on the water flea *Daphnia pulex*. However, in many ways the most impressive of such studies is one of the earliest, that of Birch (1953). Birch not only demonstrated the stressful effects of temperature and moisture levels on 2 species of grain beetle but also produced a classic diagram indicating the regions of “niche space” in which each species could prosper (i.e., increase). And he related this conception of the “ecological niche” to the geographic regions of Australia in which the 2 species occur; as predicted, the warm-adapted species occurred in the north and the cold-adapted in the south. This important approach has, I think, received too little attention in recent years, though there are examples that reproduce some of its features. Ranta (1979), for instance, delineated the ecological niches of 3 species of water flea, and Ellenberg (1988) the ecological niches of the northern European trees.

How fast a population can recover from a period of temporary stress depends on the species' life history and body size; generally, small animals recover faster than large ones. Thus, for instance, the maximum population growth rate for large mammals and man is about 6% per year, whereas small rodents can increase 20-fold per year and insects more than 100-fold. The relationships between maximum population growth rate and body size and generation time are documented, for instance, in Campbell (1996) and in Caughley and Sinclair (1994). Periods of population increase are necessarily of limited duration, and in the absence of further disturbance, population abundance must eventually reach equilibrium, representing the carrying capacity of the environment.

The effect of chronic stress is generally to reduce carrying capacity. Such effects are seen particularly clearly in plant species, which cannot move to avoid stressors; thus, plant distributions may be limited by moisture levels, pH, wind, and so on (Ellenberg 1988). There are few examples of the effects of stressors on the carrying capacities of mammals and birds. Some examples in which predators (foxes) reduce the carrying capacity of the environments of several marsupial species are presented by Sinclair and Krebs (2002). An example in which dieldrin affected the carrying capacity of sparrowhawks is considered by Sibly et al. (2000).

The effect of chronic stress is, however, not always a reduction in carrying capacity. There are examples in nature and in microcosm experiments in which populations compensate for the effects of stress (Forbes et al. 2001). This may happen if the stressors reduce the competition for food so that survivors do better than they would otherwise have done. In both the sparrowhawk and the peregrine, for instance, the use of DDT in the years after 1945 had an immediate effect on eggshell thickness and a demonstrable effect on fledging success in the sparrowhawk (Newton 1986; Ratcliffe 1993). Population sizes, however, did not decline at this time. Indeed, declines were not seen until after the introduction of the cyclodiene pesticides 10 years later. Because sparrowhawk and peregrine breed at ages 1 to 2 and 2 years, respectively, and have relatively short life expectancies (about 1.3 and 3.5 years, respectively), one has to conclude that DDT did not reduce adult population sizes in the UK, even though it did reduce juvenile survival. Perhaps the smaller numbers of fledglings were individually more successful in obtaining food because they had fewer competitors. In this way, the population could compensate for the effects of DDT reducing juvenile survival.

This study of the effects of DDT shows an interaction between stress and density. At low density the effects of stress would be a reduction in population growth rate, but in larger populations, around carrying capacity the population is able to compensate for the effects of stress. A number of microcosm studies have recently shown similar effects (Forbes et al. 2001). Thus, Liess (2002) has shown that at low but not high population density, fenvalerate reduces emergence success in the caddis fly *Limnephilus lunatus*, and Forbes et al. (2003) obtained similar effects on population

growth rate exposing the larvae of the marine polychaete worm *Capitella* sp. I to fluoranthene.

In considering the effects of disturbance in nature, it is proper also to bear in mind the important idea that biodiversity may be highest at intermediate levels of disturbance (Connell 1978). Connell suggested that at low levels of disturbance, diversity is reduced because many species may be out-competed by a few dominant species; thus, without disturbance, the beech *Fagus sylvatica* would dominate the forests of much of central Europe. At the other extreme, where there is too much disturbance, forests cannot persist. These disturbances can result, for example, from fire, herbivory, trampling, or floods. In general, therefore, we expect to find highest biodiversity at intermediate levels of disturbance.

In conclusion, I would like to endorse the proposal of Nico Van Straalen (2003) that ecotoxicology is best viewed in relation to stress ecology. I see a number of advantages to this idea. In the first place, it will broaden the scope of our studies and increase scientific interest in them. There will be a consequent increase in the scientific status of the discipline. For too long, there has been a rift between “pure” and “applied” ecology, and this rift has wasted opportunities for scientific progress. In particular, ecotoxicology has an immense amount to offer in monitoring populations in the field and in linking predictions from studying microcosms and mesocosms to what happens in the field.

There is, however, much more that we should do. Using existing knowledge of ecological niches, we should develop landscape maps that will chart the distribution of key species throughout Europe. These maps will allow us to identify the risks of pesticide exposure. They will also make a huge and fundamental contribution to ecology. Second, we should initiate general ecological studies of how populations and communities recover from disturbances. The immediate effects of short-term exposure to pesticides are reductions in abundance. Subsequent recovery may often be independent of the identity of the pesticide, depending instead on (1) the species composition and life-history characteristics of the survivors, (2) the minimum population sizes to which the populations are reduced, and (3) the nearby habitats and communities from which immigration may occur.

Stress and disturbance are core processes with in ecology that merit far more attention than they have so far received. Many of the studies have

come – and will continue to come – from ecotoxicology.

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Observation of effects in the field

LINKING TERRESTRIAL VERTEBRATE RISK ASSESSMENT TO EFFECTS IN THE FIELD

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Introduction

Currently in the European Union (EU), pesticides are assessed via the EU Directive 91/414/EEC (see Anonymous 1991). This directive and the associated annexes cover the risk to the operator, consumer, and environment. Annex II outlines what data are required on the active substance, while Annex III indicates the data required for the associated product. Annex VI, or the Uniform Principles, outlines, among other issues, the decision-making criteria that need to be considered prior to an active substance being placed on Annex I and the associated product being authorised.

The risk to the environment covers both the fate and behaviour of an active substance (i.e., exposure) as well as its possible effects to non-target organisms. Non-target organisms considered under Directive 91/414/EEC include the following: birds, mammals, aquatic life (including fish, aquatic invertebrates, algae, and aquatic plants), non-target arthropods, honeybees, earthworms, soil macroinvertebrates, soil microbial processes, and terrestrial non-target plants.

The risk assessment carried out for non-target organisms currently takes a single point estimate of toxicity as well as exposure. This results in either a toxicity-exposure ratio (TER) or a hazard quotient (HQ), which is then compared to a regulatory trigger value in the Uniform Principles of Directive 91/414/EEC (Council Directive 94/43/EC). If the relevant trigger value is breached, then no authorisation can be granted “unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs after use of the

plant protection product according to the proposed conditions of use”. This “appropriate risk assessment” usually takes the form of further information on either the toxicity of the compound or the exposure of non-target organisms to the compound.

Outline of bird and mammal risk assessment

The above is a very general overview regarding ecotoxicological regulatory risk assessment. Of more relevance to this workshop is how the risk to birds and mammals is assessed. Outlined below is a brief summary of the current risk assessment procedure for birds and mammals.

When the risk to birds or mammals is assessed, the process as outlined in SANCO/4145/2000 is followed (see Anonymous 2002). For the first-tier assessment, the “estimated theoretical exposures” (ETEs) for the acute, short-term, and long-term exposure scenarios are determined. The following calculation is used to determine the ETE:

$$\text{ETE} = (\text{FIR}/\text{bw}) \times \text{C} \times \text{Av} \times \text{PT} \times \text{PD}(\text{mg}/\text{kg bw}/\text{d})$$

where:

ETE = estimated theoretical exposure,

FIR = food intake rate,

bw = bodyweight,

C = concentration on treated food,

Av = avoidance,

PT = proportion of diet obtained from treated area, and

PD = proportion of food type in diet.

Once the ETE is calculated, it is then compared with the appropriate toxicity endpoint and a TER is determined. If the resulting TER is less than the appropriate trigger value presented in the Uniform Principles, then the assessment may be refined using the various steps outlined in the document (see Anonymous 2002). For example, specific residue data can be used in place of the generic information used in the first-tier calculation. Likewise, information on the proportion of diet ob-

Table 1 Issues that need to be considered when designing a field study. (Information on protocol design as well as issues to consider are available in a number of publications including – Greaves *et al.* (1988), Somerville and Walker (1990) and Anon (1990); there also is a protocol by the U.S. EPA (OPTTS 850.2500 – Field testing of terrestrial wildlife).)

Suitability of site	The site should be climatically, biologically and geographically representative of where the product will be used.
Capture of animals	This is potentially useful in demonstrating any population level effects.
Carcase searching	This is used to indicate the level of mortality, however there is a need to demonstrate the efficiency of carcass searching. Ideally need to have an indication as to what is considered to be a lethal level in carcasses so that cause of death can be determined.
Chemical analysis of potential food items	Potentially useful in demonstrating that exposure has taken place in the field. Information can be fed back in to the above equation and hence C revised appropriately.
Faecal analysis	Potentially useful in demonstrating exposure to treated food items.
Radiotracking	This can provide an indication of the level of mortality as well as provide additional information regarding PT.

tained from the treated areas together with the proportion of food type in the diet can be used to produce more realistic estimates of exposure. In addition, it may be possible to carry out a field study to address the whole issue regarding the risk to birds and/or mammals. Outlined below is a more detailed consideration of the types of field trials that may be conducted to address concerns regarding the risk to birds and mammals from the use of pesticides.

Types of field studies that can be used in regulatory risk assessment

Field trials conducted to address a regulatory requirement may take several different designs, and hence, for the purposes of this paper, it is proposed that regulatory field trials can fit in to 3 categories: ecological, residue, and effects. These are defined below.

Ecological field trials

These are field trials that are designed to provide information on the ecology of the species under consideration. For example, these types of field studies may entail radio-tracking birds to determine how long a particular species spends in a specific type of field. Therefore, this type of information can be used to refine PT. Likewise, “ecological field trials” can also provide information on the types of food that a particular species may eat when in the treated area. This information can therefore be used to refine PD.

Residue field trials

These are trials that are carried out to provide a more realistic estimate of the concentration of

pesticide on treated food items. Therefore, this information can be used to refine C.

Effects field trials

These are trials that are designed to detect any effects of the pesticide in the field on birds and/or mammals and hence are used to refine the whole assessment.

It should be noted that 1 field trial can be designed to cover more than one of the above issues.

It is assumed that of primary interest to this workshop is the latter type of field trial, that is, the effects field trial. There is no standardised protocol for this type of study, and hence each is designed on a case-by-case basis to answer specific questions that have arisen during the risk assessment process. However, when a field study is designed, several issues need to be considered. Of primary importance is what it is that the trial is trying to address. The trial should therefore be designed to address the specific question that has arisen from the regulatory risk assessment. For example, what is the level of mortality of small mammals following the use of a certain seed treatment?

Outlined in Table 1 are some of the other issues that need to be considered prior to commencing an effects field trial. Included in Table 1 is a brief explanation of each issue. In addition to those issues highlighted in Table 1, it is assumed that the field trial will be based on the proposed use and hence be conducted on the appropriate crop and at the appropriate rate.

How are field trials used in regulatory risk assessments?

Of the 3 types of field trials outlined above, the use of residue field trials is relatively straightforward

in that residue data gathered from appropriate locations can be fed into the above equation and hence C can be revised (see Sections 5.2 and 5.3 of Anonymous 2002 for further information). Likewise, information from ecological field trials can be fed into the above equation to refine PT and PD appropriately (see Section 5.6 of Anonymous [2002] for further information). The output from both of these types of field trials is quantitative and hence can be fed into the risk assessment on a quantitative basis. An illustration of how these factors can be revised in light of more relevant data is provided in Appendix IV of Anonymous (2002).

Due to its qualitative nature, it is not possible to factor information from an effects field trial into the above equation. The following is provided as an example of how the findings from an effects field trial may be considered in a regulatory setting.

The initial risk assessment indicates that for a fictitious insecticide applied to cereals at an early growth stage, a high risk, that is, TERacute is less than 1, is predicted. A field trial has been conducted to investigate the level of mortality of geese following application of the insecticide. In trying to determine this level, a site that is frequented by geese has been chosen, and the protocol has been designed to determine likely exposure levels in treated vegetation. It has also been designed to determine residues in faeces as well as the likely level of mortality. This latter factor is assessed via casualty searches. During the field trial, no mortalities were found and no abnormal symptoms were observed. Exposure was confirmed by analysis of the cereal shoots as well as faeces analysis.

From a regulatory perspective, what does this study tell us? One thing it does not tell us is the insecticide's level of acceptability: this is not a question that any field trial can answer. Does it provide an answer to the original question, that is, what is the level of mortality of geese following application of the insecticide? Taken on face value, it does indicate that, following exposure, no mortality of geese occurred. However, it should be noted that there are concerns regarding several issues of the trial. The first issue is the representativeness of the site: Is this site representative of all sites where geese will feed? Was the trial representative in terms of the time and hence rate that the geese spent feeding on the field? The residues indicate exposure; however, is this 1 site representative of all sites that could be treated? Due to the above

questions, effects field trials provide only a qualitative indication of the potential level of impact.

In conclusion, ecological field trials and effects field trials can provide useful information that can be used on a quantitative basis in refining the risk assessment. However, in considering the overall usefulness of effects field trials in the regulatory risk assessment, the key issue is whether they are of sufficient robustness to provide either a decision maker or a policy maker (Anonymous 2000) with sufficient information to make a decision. Scientists alone cannot determine whether this is the case, and hence the usefulness of effects field trials will depend upon an interaction between scientific advisers, decision makers, and policy makers.

Monitoring schemes

There are 2 types of monitoring schemes: proactive and reactive. In a proactive monitoring scheme, the regulator requests that some form of monitoring be carried out once the product has been approved. This can take the form of "grower questionnaires". These simply seek information on what happens after a particular product is used. The information is extremely crude and hence of potentially limited usefulness in making regulatory decisions. However, this information can aid user-education schemes in ensuring the safe use of a product. Reactive monitoring is used once an incident occurs. One example of this type of monitoring is the UK Wildlife Incident Investigation Scheme (WIIS), which investigates deaths of wildlife, including beneficial insects, pets, and some livestock when there is strong evidence suggesting that pesticide poisoning may be involved.

The WIIS provides a unique means of post-registration surveillance of pesticide use, and thus registrations can be revised if necessary. In addition, it provides a measure of the success of the pesticide registration process and helps in the verification and improvement of the risk assessments made in the registration of compounds. Evidence from the WIIS may also be used to enforce legislation on the use of pesticides and the protection of food, the environment, and animals.

Animals that are suspected of being poisoned are submitted for post-mortem examination to eliminate cases of trauma, disease, and starvation and to examine tissues for signs of poisoning. A field inquiry is carried out to assess the possible cause of the poisoning, its extent, and what

species are involved. Using the post-mortem and field inquiry reports, suitable analyses are carried out to detect residues of possible compounds. If residues are detected, the significance of these are determined and reported to the regulators or enforcement authorities.

The WIIS relies on the poisoned animal being found and recognised as a possible pesticide-poisoning incident. It must then be reported to the WIIS. Annual reports of findings are published (e.g., Barnett et al. 2002).

By examining the facts of an incident, it is often possible to establish how the incident occurred. Incidents may arise from one of 3 ways:

- ▶ through approved use, where the product has been used according to the specified conditions for use;
- ▶ through misuse, which occurs from careless or accidental or wilful failure to adhere to the correct practise of using a product, or
- ▶ through abuse, which results from deliberate, illegal attempts to poison animals.

Confirmed approved-use incidents are of the greatest value to regulators because the product, being used in a correct manner, has resulted in the death of wildlife. An assessment can be made of the conditions of the incident, and remedial action can be taken to prevent incidents under the same conditions in the future. However, caution should be used in interpreting and hence using these data in the risk assessment. As part of a large research project carried out in the UK, the reporting rate of casualties was investigated via the use of telemetry, and it was concluded that when interpreting WIIS data, it should always be remembered that only a small proportion of incidents are reported and that the proportion reported is smaller for smaller species. Therefore, for large species such as the woodpigeon, a lack of reported incidents may be taken as evidence of a lack of substantial mortality, but the possibility that occasional mortalities may be occurring cannot be ruled out. While for small species of birds, a lack of reported poisoning incidents does not rule out the possibility that substantial mortality may be occurring.

But what about incidents resulting from misuse of pesticides? Are these relevant in the regulatory risk assessment? They may be relevant if the incident arose from misleading or unclear instructions.

CONCLUSIONS

Field studies and monitoring programmes can be used to aid the regulatory process. However, caution is required to ensure that the information they provide is used appropriately. In determining the value of field trials, views of decision makers and policy makers should be sought to ensure that the trials are sufficient to address their needs.

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FIELD STUDIES OF PESTICIDE EFFECTS ON TERRESTRIAL INVERTEBRATES

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Insecticides can cause toxic and sublethal effects to non-target invertebrates, leading to a decline in their abundance, diversity, and range inhabited. Most data exist on the short-term, within-season impact of insecticide applications generated from field trials conducted for registration purposes or independently to quantify the impact on species not previously tested (e.g., Moreby et al. 2001). Data generated for registration are rarely published, although they could be widely used by agronomist and conservation advisors. Arable field trials are usually conducted within single fields using a replicated block approach based upon 1-ha plots. The data from such trials have to be treated with a degree of caution, and the following reasons were discussed by Brown (1998). Whether an insecticide causes mortality, for example, is determined by the susceptibility of each organism and the level of exposure, and this is determined by the organism's activity and the spray coverage (Wiles and Jepson 1994). There may also be sublethal effects that will eventually cause a population reduction, but these may not appear during the monitoring period. The outcome is further complicated as a consequence of reinvasion by untreated invertebrates from adjacent unsprayed areas (Duffield and Aebischer 1994), natural population fluctuations in response to other unrelated factors (Holland 2002), the heterogeneous distribution of invertebrates (Holland et al. 1999), and biased sampling methodologies. In addition, invertebrate abundance is always lower in field centres of conventionally managed arable crops (Holland et al. 1999) where insecticide trials are typically conducted, and therefore only a few species will be collected in sufficient numbers for statistical analysis. Trials conducted along the edge of a cereal field are more likely to encounter a greater number and diversity of arthropods, as found by Moreby et al. (2001).

There is plenty of evidence that the impact of field-scale applications can be highly variable, with considerable variation occurring between

species and higher taxa, between trials conducted using the same methodology, and between years (Mead-Briggs 1998; Moreby et al. 2001). A typical result may find that some non-target taxa are susceptible while others suffer considerable mortality (Figure 1); moreover, there may also be indirect effects that can be equally damaging (Sotherton and Holland 2002). However, the impacts on ecosystem functioning and the food chain are most important ecologically, but these impacts are rarely considered in field trials because they are usually of insufficient spatial and temporal scale to detect such effects. The best evidence of examining pesticide effects through the food chain can be found in the studies of the grey partridge conducted by The Game Conservancy Trust (Potts 1986). Grey partridge populations have declined severely since the 1970s, and a reduction in their invertebrate food supplies was identified as one of the major constraints on chick survival. Many of their key invertebrate food items are phytophagous, but these were substantially reduced by herbicides and insecticides, resulting in poor chick survival. When more broad-leaf weeds along with their associated invertebrates were allowed to survive by selectively spraying the outer 6 m of cereal fields and insecticides were excluded from this area, the improvement in food supplies was sufficient to increase the survival rate of grey partridge chicks (Sotherton 1991). Even so, the long-term effects on the wildlife food chain remain poorly quantified, al-

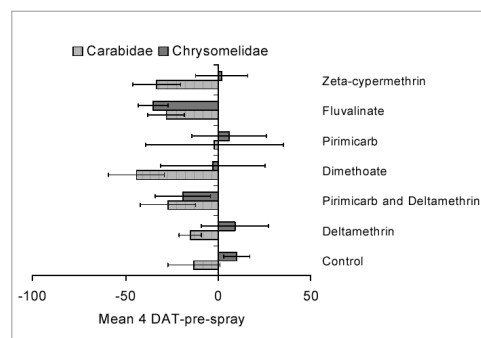


Figure 1. Variation between taxa in susceptibility to different insecticides. Reprinted with permission from Moreby et al. 2001. Copyright Society of Environmental Toxicology and Chemistry (SETAC).

though these effects may be considerably affecting many species (Campbell et al. 1997).

In addition to a shortage of long-term data, there is also an absence of landscape-scale studies, although pesticides can be applied over extensive

areas. The overall impact of pesticides was examined in an extensive, long-term study conducted by The Game Conservancy Trust. The cereal ecosystem was monitored annually over an area of 62 km² in West Sussex from 1970 until present day. Each year the abundance of beneficial insects and weeds was measured in 100 cereal fields during June, along with counts of the grey partridge in August. Pesticide usage and cropping are also known. All data were incorporated into a geographical information system (GIS). An extensive analysis of this database was conducted by Ewald and Aebischer (1999, 2000), but only some of the key relevant findings are presented here. The analyses included, among others,

- 1) comparison of pesticide inputs over the study area with the national average, and
- 2) comparison of the abundance of five groups of invertebrates eaten by farmland birds with
 - a. average number of herbicide applications;
 - b. annual proportion of area treated with fungicides and insecticides;
 - c. different groups of herbicides, fungicides, and insecticides; and
 - d. pesticide use in the previous year.

Cropping and year were taken into account in the analyses.

The trends in pesticide use were similar to the national average, with an increase in the areas treated with herbicides, fungicides, and insecticides, although these have reached a plateau in the last decade. The efficacy of herbicides has also increased, and more weed species are being classified as susceptible to the herbicide regime. The increase in autumn-sown cereals also led to a corresponding increase in autumn-applied herbicides and insecticides. As expected, the occurrence and abundance of broad-leaved weeds in fields treated with herbicide was 13% less than in untreated fields, although there was considerable variation between species. Only *Stellaria media* (chickweed) and *Myosotis arvensis* (field forget-me-not) showed a significant decline in abundance over the study period.

In the area where pesticides applications were known, the annual abundance of 3 arthropod groups (Araneae and Opiliones, Symphyta and Lepidoptera, and Chrysomelidae and Curculionidae) decreased significantly over the study period and was negatively related with the broad mea-

sure of pesticide intensity (annual number of herbicide applications, proportion of the study area treated with fungicides or insecticides). When herbicide, fungicide, and insecticide use were considered simultaneously and year of study and crop type were controlled for, the pattern was for all 5 invertebrate groups (Araneae and Opiliones, Carabidae and Elateridae, Chrysomelidae and Curculionidae, Symphyta and Lepidoptera, and non-aphid Hemiptera) to have a negative relationship with insecticide use, significantly so for the last two groups. All groups except Carabidae and Elateridae declined with fungicides, significantly so for Araneae and Opiliones. There was a consistent pattern of a negative relationship with the use insecticides in autumn and winter as well as in spring and summer. The abundance of 4 invertebrate groups declined as the application of pyrethroids increased, while all 5 groups showed a negative relationship with organophosphorus insecticides (Figure 2). None showed this relation-

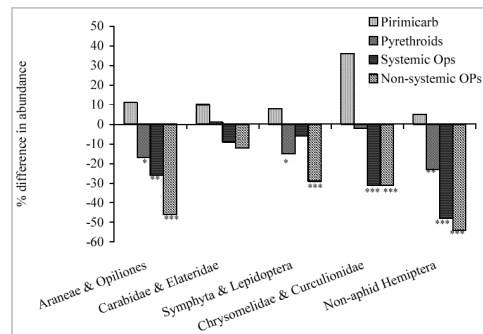


Figure 2. Comparison of the densities of invertebrate groups in insecticide-treated fields with those in untreated fields, in relation to the type of insecticide used: Sussex study 1970-1995. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; From data of Ewald and Aebischer, 1999).

ship with the selective insecticide pirimicarb. Numbers of invertebrates in all 5 groups showed a decrease after insecticide use in the previous year. In general, there was no relationship between any of the measures of herbicide usage with invertebrate numbers, but the power of this analysis was lower because herbicide inputs were widespread before the start of the study and remained consistent over the study period.

This extensive dataset includes data on the abundance of many invertebrate families or species within each field and consequently will allow their range across the study area to be investigated. Al-

though the spatial component of this dataset has yet to be fully explored, it is evident that some of the invertebrates have declined in abundance within fields or have become absent from some (Holland 2002). Relatively few other studies consider long-term effects or scale. To reduce the impact of reinvasion, 10-ha plots were located within a 100-ha field and the impact of an insecticide application was evaluated for 1 year after the application (Wick and Freier 2000). The insecticide reduced numbers of invertebrates within the year, but there were no effects 1 year afterwards. To eliminate the effect of reinvasion, Kennedy et al. (2001) used barriered plots. Insecticides reduced numbers of epigeal invertebrates, but the numbers and diversity were lower while variability was higher within the barriered plots, restricting possible analyses.

Further evidence of the direct and indirect effects of pesticides will be gained from the Indirect Effects of Pesticides Project (1999–2004) funded by the Pesticide Safety Directorate of the Department for Environment Food and Rural Affairs (DEFRA) in the UK (Holland et al. 2002; Hart et al. 2002). One of the main components was to conduct a large-scale field experiment to assess the relative importance of food availability on the demography of farmland bird populations. Two key determinants affecting survival and reproduction were considered: (1) supplies of seed food for adults in winter, which is affected by the level of herbicide inputs, and (2) abundance of invertebrate food for nestlings in summer, which is determined by insecticide and herbicide inputs. To manipulate these food supplies, 4 experimental treatments were implemented: (1) extra seed over winter, (2) high insecticide inputs during the summer, (3) extra seed over winter and high insecticide inputs during the summer, and (4) control sites, each on a 1-km² block of arable farmland. These treatments were repeated at 3 study sites. Data analysis is currently underway.

In conclusion, the ecological consequences of pesticide usage can be far-reaching, with the extent of effect being influenced by, for example, the spatial and temporal scale of the applications, the pesticides' spectrum of activity, and the species present. In addition, there may be interactions with other components of the farming system, including cropping and type of tillage. Not only short-term consequences but also those that may occur in the long term must be considered.

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FIELD STUDIES OF HERBICIDE EFFECTS ON TERRESTRIAL PLANTS

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In the recent past increasing attention has been given to the side effects of herbicides on non-target plants outside the treated area. In Europe, only recently has guidance been published concerning the inclusion of non-target terrestrial plants in the first tier of the registration procedure for pesticides. Given this background, higher-tier studies in the framework of the registration procedure are scarce, and only a few field studies aimed at effects of herbicides on terrestrial non-target plants are available.

In the present paper the results of 3 types of field studies, conducted in the Netherlands, are presented: (1) bioassay experiments, (2) field margin studies, and (3) a large-scale field experiment with natural vegetation and a dose-response design.

The bioassay studies were conducted on an experimental field, using young plants of a dicotyledonous species (*Brassica napus*) and a monocotyledonous species (*Poa annua*). The effects of wind-drift of a number of herbicides with different modes of action were studied on growth and survival of the test plants (De Jong and Udo de Haes 2001, De Jong 2001). Drift deposition was measured as well. From the results, a deposition effect correlation was derived. An example of a sensitive combination of herbicide and species is shown in Figure 1.

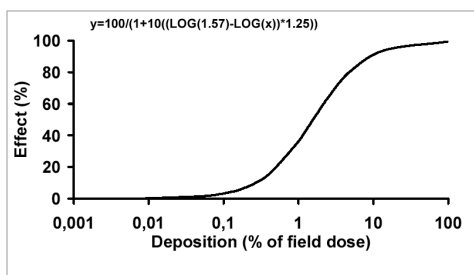


Figure 1. Modelled effect of deposition of herbicides on plant growth (De Jong 2001).

From the model in Figure 1 an EC₅ of 0.15% of the field dose and an EC₅₀ of 5% of the field dose were calculated for effects of herbicides on plant growth.

In the field margin experiment, the effects of the actual herbicide use in arable fields were studied on ditchbank vegetation adjacent to the sprayed fields (De Snoo and Van der Poll 1999). In the experiment the effects of drift adjacent to fields with and without unsprayed field margins of 3 m and 6 m wide (resulting in, respectively, low-

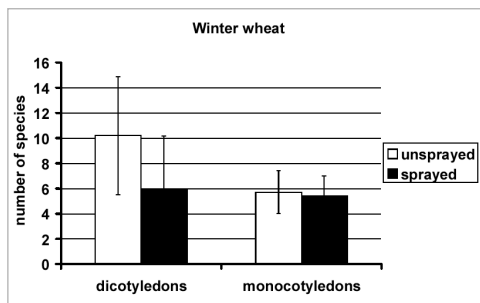


Figure 2. Number of species in a ditchbank adjacent to a winter wheat parcel with and without a 3-6 m buffer-zone.

and high-drift figures) were studied with number and cover of species as effect parameter.

Figure 2 shows that the number of dicotyledon species adjacent to an unsprayed buffer zone is higher (Wilcoxon, $P < 0.001$) than the number of species directly adjacent to the parcel (10.2 compared to 5.9 species). Drift deposition in ditchbanks adjacent to a weed parcel is 5% to 25% of the field dose. When a buffer zone is used, the deposition is diminished to less than 0.1% of the field dose.

In the large-scale field experiment, short-term (weeks), mid-term (within the season), and long-term (3 years) effects of a broad-spectrum herbicide were studied on non-target vegetation on ditchbanks and road verges. In this case the vegetation was directly sprayed with a range of dosages. Four locations were studied, with a treatment range of 0%, 2%, 4%, 16%, 32%, and 64% of

the field dosage and 5 replicates per treatment level.

For the short-term effects, phytotoxic symptoms were studied. For the mid-term effects within

Table 1. Phytotoxic effects of a broad spectrum herbicide on non-target vegetation (years and sites combined)

Dosage	After first spraying	After second spraying
2% dosage	7% (4–14%)	3% (0–5%)
4% dosage	14% (10–31%)	11% (5–14%)
16%	51% (43–57%)	48% (27–52%)
32%	65% (50–67%)	63% (54–70%)
64%	78% (69–85%)	80% (74–87%)

the season and the long-term effects between different years, the parameters biomass, number of species, and community structure were used as effect parameters.

For a discussion of the results, 3 time scales are distinguished: short-term effects on plant growth and phytotoxic symptoms, effects on the vegetation parameters within the season, and recovery in the next year.

The results (see Table 1) show that short-term effects are present at low dosages (<5%, bioassays and field experiment) of the maximum field dose. In the case of a contact herbicide, individual plants are able to recover from phytotoxic symptoms, even when exposed to the highest dosage (positive control).

Regarding effects on species number within the season, in one year a significant effect on species number was found at low dosages at two of the locations, while at the other two locations no effects were found at all, not even at the highest dose. In the second year no effects on species number were found at any of the locations at any dosage. Further analyses of these data are being carried out to determine whether the effects are a false positive or whether an ecological explanation can be found for the large differences between the years.

Concerning recovery, assessed in the spring of the next year before application, all parameters are in the range of the control at all dosages.

Table 2. Effects of a broad spectrum herbicide on non-target vegetation community.

All Sites	2000 before	2000 after	2001 before	2001 after	2002 before
P-value	> 10	0.002	> 0.10	0.008	> 0.10
NOED community	> 64%	32%	> 64%	32%	> 64%

Table 2 shows the effect on the community level, using principle response curves as the method of statistical analysis. The effects show that the NOEDcommunity within the year was 32% of the field dose. On the individual sites, however, 16% of the maximum field dose was found as a no-observed-effect dose (NOED) on several occasions. In the year after application, no effects were found.

Table 2. Effects of a broad-spectrum herbicide on non-target vegetation community.

From the results it was concluded that clear short-term effects on growth and phytotoxic symptoms can be found at low dosages of herbicides. The distance from a treated plot is several metres. Vegetation effects within the year are generally found at higher dosages, meaning that the inclusion of unsprayed buffer zones and other drift-reducing measures could prevent these kinds of effects. In the field experiment, full recovery was found in the next year for all dosages and effect parameters.

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**LANDSCAPE-LEVEL
PESTICIDE RISK ASSESSMENT
USING FRESHWATER
MACROINVERTEBRATE
COMMUNITY STRUCTURE
IN THE ORCHARD AGRICULTURE
OF ALTES LAND, GERMANY**

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Objectives:

- To develop a method to investigate landscape level effects of pesticides.
- To detect relevant differences in the macroinvertebrate community structure.
- To determine whether there is a correlation of differences to the exposure to pesticides.

Approach:

Areal approach using multivariate statistics. Investigation of 40 sites per region in spring, summer, and autumn (5 sampling in 1998 until 2000); macroinvertebrate communities and a set of potentially relevant influence factors. Pesticide exposure was not measured, but calculated. Intention: less exact for single sites, detection of changes relevant for the landscape via statistics.

Investigated regions:

Altes Land near Hamburg, apple orchards

- Ditches with nearly identical morphology
- One culture on one side of a ditch, homogeneous distance
- Nearly identical pesticide applications following recommendations of the local orchard research institute (timing, product, dosing)
 - ▷ Very homogeneous situation
- Intensive use of pesticides (20 to 40 applications per year)
- Special legal status: exempted from distance conditions
 - ▷ Very high exposure

Unique situation for an effect study: ideal for the proof of principle

Region of Braunschweig

- Heterogeneous ditches, intensive field cultures
 - ▷ "Normal" situation: application of the method

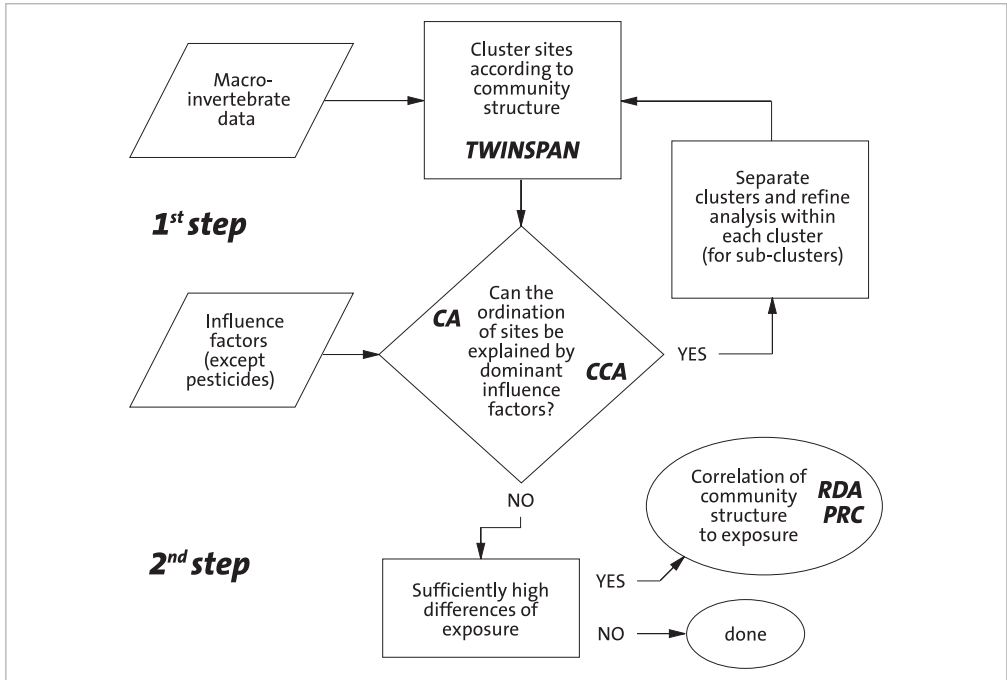


Figure 1: Decision loop to assessing causes for macroinvertebrate community structure differences. Aim: Excluding variability caused by other relevant influences than pesticides to enhance statistical power to detect pesticide effects.

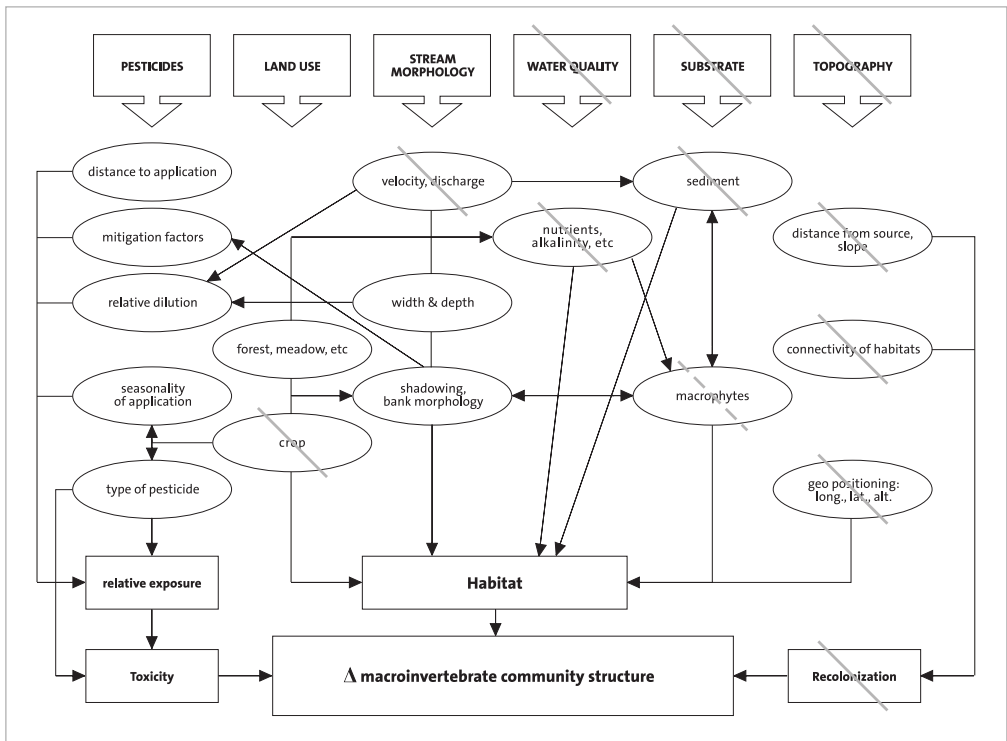


Figure 2: Factors determining differences in the macroinvertebrate community structure in waters of the agricultural landscape. Factors excluded by the selection of homogeneous sites in the "Altes Land" are struck out.

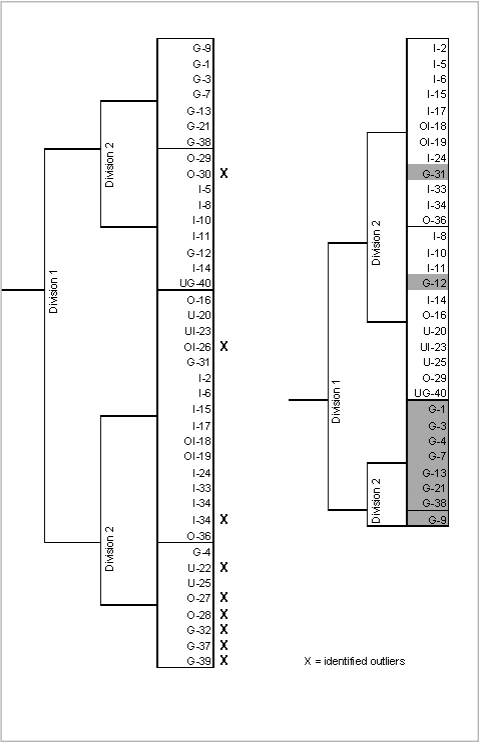


Figure 3: grouping of sites by community structure via TWINSpan

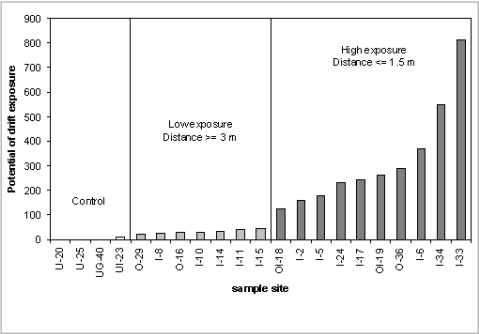


Figure 4: Calculated potential of exposure (by distance and water volume) resulted in 3 groups of sites. Meadow sites excluded as controls, organic sites excluded as exposed.

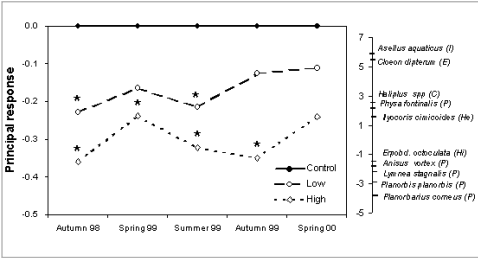


Figure 5: Principal response curve analysis of the sites (organic sites excluded): Recovery trends during winter

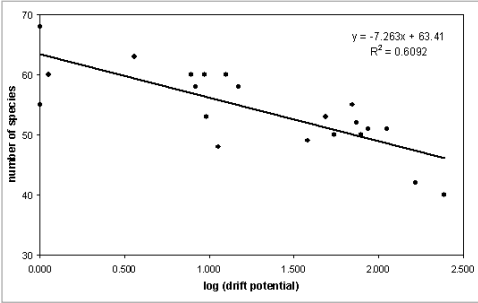


Figure 5: Species number significantly reduced at sites of high exposure potential

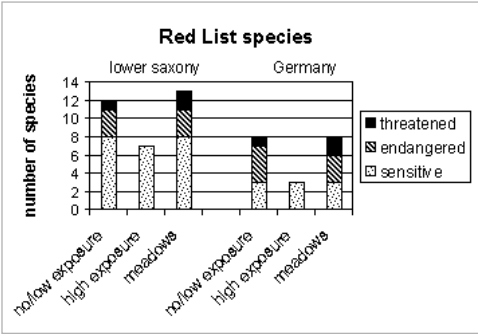


Figure 6: number of Red List species comparable at sites with no or low, but clearly reduced at sites with high exposure potential. Occurrence of these species regarded as indication of the "value" of the communities in the "Altes Land".

THE LOURENS RIVER, SOUTH AFRICA: A CASE STUDY FOR A MEDITERRANEAN AGRICULTURAL CATCHMENT

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Introduction

The assessment of pesticides in the field requires understanding of both exposure and the resulting biological effects in natural surface waters due to normal farming practises. This contribution focuses on insecticide exposure and effects in aquatic ecosystems. Besides a general description of the available literature, results from the Lourens River in the Western Cape of South Africa will be presented as a case study for a Mediterranean agricultural catchment.

Exposure

More than 60 reports of insecticide-compound detection in surface waters due to agricultural non-point-source pollution have been published in the open literature during the past 20 years, about one-third of which have been undertaken in the past 3.5 years. Recent reports tend to concentrate on specific routes of pesticide entry, such as runoff, but there are very few studies on spray drift-borne contamination. Reported insecticide concentrations are negatively correlated with the catchment size, and all concentrations $>10 \mu\text{g/L}$ (16 out of 127) were found in smaller-scale catchments, $<100 \text{ km}^2$.

The Lourens River in the Western Cape of South Africa receives non-point-source pesticide contamination from a 400-ha orchard area. Storm runoff and spray drift are the two most important routes of entry for current-use organophosphate insecticides. A variety of methods have been used to quantify transient pesticide contamination, and measured concentrations are well in accordance with predictions using simple spray-drift and runoff models. The GIS-implemented models were used to predict exposure concentrations and loads on a catchment level. Storm runoff events during the pesticide-application period leads to high short-term exposure concentrations, and runoff has been shown to be more important than spray drift as a route of entry for pesticides into surface waters.

Effects

Field studies on effects of insecticide contamination often lack appropriate exposure characterisation. About 15 of the 42 effect studies reviewed revealed a clear relationship between quantified, non-experimental exposure and observed effects in situ on abundance, drift, community structure, or dynamics. Azinphos methyl, chlorpyrifos, and endosulfan were frequently detected at levels above those reported to reveal effects in the field. However, it is important to note that for almost all of the studies that seem to establish a clear link between exposure and effect, the pesticide concentrations measured in the field were not high enough to support an explanation of the observed effects based simply on acute toxicity data.

Microcosm experiments using communities from the Lourens River were combined with surveys on the invertebrate fauna in the field, suggesting the potential to link these different approaches in order to identify causal exposure-effect relationships. Invertebrate surveys in the field suggest that transient pesticide levels affect the community composition at exposed sites. However, at this level of complexity it becomes evident that it is very difficult to clearly separate pesticides from other contributing factors such as increased turbidity during runoff events.

Conclusions and recommendations

Considerations for future studies should include but not be restricted to:

- regular and effective exposure characterisation,
- measurement and understanding of confounding factors,
- combination of endpoints (e.g., in situ, abundance, drift), and
- risk mitigation and ecosystem and landscape features.

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Ability to predict effects: linking tiers and extrapolation

EXPOSURE MONITORING FOR AQUATIC RISK ASSESSMENT

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Monitoring of pesticide exposure in the aquatic environment has been performed for various reasons, resulting in a multitude of different monitoring strategies. Commonly, monitoring is done to comply with regulatory requirements such as compliance with drinking water standards or with different conventions (e.g., HELCOM, PARCOM, the Water Framework Directive) or due to post-registration requirements laid down by the regulatory authorities as a condition for approval. Moreover, there have been research-based monitoring programmes to develop scientific understanding of pesticide fate in the environment, preferably also using the results to calibrate and validate exposure models. Monitoring can also be used as a tool to follow-up on policy decisions, (e.g., checking risk mitigation programmes). Furthermore, there are some examples of exposure monitoring for risk assessment also considering ecological endpoints. However, most studies are short term (1–3 years), thus resulting in a general lack of long-term monitoring studies.

In a report on the presence of residues and the impact of plant protection products in the EU (SSLRC 1997), data from field monitoring studies were compiled and analysed. The overall conclusion was that monitoring and data collection within Europe were uncoordinated, preventing systematic interpretation of information with respect to determining the presence and impact of pesticides. Monitoring was usually not targeted for location, timing, or a specific active ingredient with respect to effects on non-target organisms. Some general conclusions were as follows.

- ▶ Certain monitoring data were classified as confidential or presented only in a summary.

- ▶ Older, no longer registered persistent pesticides often used key resources.
- ▶ There were varying study designs, i.e., little consistency in scale of study, sampling site selection, timing of sample collection, target pesticides (other than the triazines), and detection limits.
- ▶ There was a lack of usage data.
- ▶ There was no link between chemical and biological monitoring.
- ▶ Environmental quality standards (EQS) were derived for only a limited number of pesticides and varied often by several orders of magnitude between countries.

Nevertheless, monitoring results have demonstrated a widespread presence of pesticides in streams and rivers throughout Europe. Public concern is focused on possible negative impacts of pesticides on aquatic life or on human health. Proper estimation of any hazard that pesticides may pose is dependent upon knowledge of both exposure and toxicity. For adequate exposure assessment as part of a risk evaluation, good-quality data are needed on pesticide exposure patterns and characteristics. The ecological effects of pesticides on flora and fauna in surface waters are dependent on both peak concentrations and the duration of the exposure.

Transport of pesticides from cultivated fields to surrounding surface waters generally occurs through runoff or drainage and is induced by rain or irrigation. However, wind drift from nearby spraying applications as well as incautious actions during the handling of pesticides are also sources for pesticides entering stream waters. Pesticide concentrations in streams and rivers leaving larger catchments are generally much lower than those in runoff water from edge-of-field sites. Nevertheless, transported amounts in percentage of that applied are often independent of the size of the study area. In most monitoring studies there has been a lack of site-specific data relating to pesticide occurrence in the water with ongoing activities in the catchment area.

The objective of many monitoring investigations has been to determine whether concentrations of pesticides could be detected in surface waters on single occasions. This target is insufficient to assess the ecological risks posed by pesticides in surface waters. To constitute a basis for exposure assessment, monitoring in the future should improve the sampling and analytical strategies to increase evaluation possibilities. Also, when regulatory pollution-control measurements are determined, transport calculations are useful in evaluating possible changes. Minimum background data for adequate evaluation should include the following: catchment size, land use pattern, soil type, precipitation, water flow rate, amount and type of pesticides used, and spraying season. For better recommendations to the users on how to minimise losses of pesticides to the water bodies, there is also a strong need to increase our knowledge of the different transport pathways within a catchment, including all possible processes (spills, runoff, leaching, wind drift, etc.).

Swedish monitoring experiences

A pesticide monitoring study was initiated in the spring of 1990 to examine the loss of pesticides from an agricultural catchment in southern Sweden under normal management practises (Kreuger 1998). The catchment has an area of 9 km² consisting of 95% arable land. Information on pesticide usage and handling within this area was collected annually from the farmers. About 35 different substances were used annually, and 85% to 95% (by weight) of these were included in the analyses. At the outlet of the watershed, an automatic water sampler collected, on a weekly basis, time-integrated water samples from May to November. Also, at different sites within the catchment, samples were collected on occasion to assess point sources. The overall objectives were to monitor occurrence and long-term trends of pesticide residues in stream water, to explore the reasons for pesticide contamination, and (from 1995) to minimise losses of pesticides to water by implementing risk mitigation practises.

Results have demonstrated a 90% reduction in pesticide concentrations since the onset of mitigation measurements, although applied amounts in the area have remained quite constant (Figure 1). Decreasing levels can primarily be attributed to an increased awareness among farmers on better handling and application routines. During recent

years, correct handling and application procedures have been developed further as integrated parts of programmes by authorities and in trade agreements (i.e., the industry imposing conditions on farmers in exchange for buying the sugar beet harvest), giving growers economic incentive to minimise environmental risks when pesticides are used.

Since 2002, the Swedish national monitoring programme for pesticides in the aquatic environment comprises:

- ▶ 4 small agricultural catchments (8–17 km²), including stream water with continuous sampling from May to November; shallow ground water (3–6 m deep); and information on pesticide use at a field level, and
- ▶ 2 rivers (90 km² and 500 km²).

The analysis includes ca. 70 different pesticides, including some metabolites.

Results demonstrate that even in rather small catchments (8–17 km²), a large number of pesticides were applied (26 to 54 pesticides per catchment) and that many of these also were present in stream water leaving these catchments. In addition, a number of pesticides with no registered use were detected, presumably originating from previous applications or possibly from unreported usage. The presence of a multitude of different pesticides (sometimes more than 20 pesticides in a single sample) has to be considered when potential effects of pesticides on non-target organisms are evaluated.

Future challenges

- ▶ The use of chemical vs. biological monitoring (a combination would be preferable). Considerations include how to cope with a multitude of pesticides occurring and how to select relevant control sites.
- ▶ Development of relevant, well-defined quality criteria for the aquatic environment.
- ▶ Development of guidelines on how to apply these criteria. For example, should the criteria be different for different ecosystems or regions? Are they applicable only to data from well-defined monitoring programmes? Should we use single values, a number of consecutive values, or an average over a certain time period? What should the minimum size of the catchment or edge-of-field site be?

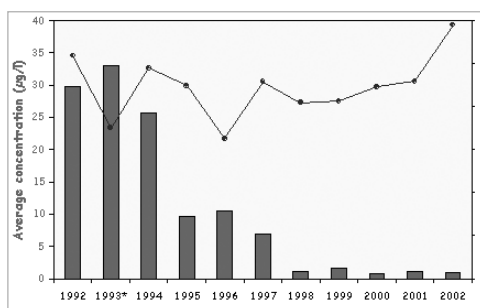


Figure 1. Time-weighted mean concentration for the sum of pesticides in stream water leaving the catchment during May–September 1992–2002 (bars) and applied amount of pesticides included in the analytical procedures during corresponding years (line).

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EXPOSURE MODELLING FOR AQUATIC RISK ASSESSMENT

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Models are used extensively to predict pesticide exposure for regulatory risk assessment. The models are used to estimate field behaviour on the basis of mainly laboratory measurements and offer several advantages over direct determination of exposure in the field. Modelling is faster and cheaper, it is possible to assess fate and exposure under the full range of possible use conditions, and the analysis can be repeated to account for changes in use or impact of mitigation measures. Nevertheless, it is critical to establish the extent to which model predictions for environmental concentrations of pesticides match measurements in the field. Several studies have addressed this question in the EU and the US. In assessing the implications for risk assessment, it is also necessary to consider a number of generic issues related to the use of models and evaluation against field data:

- **Variability:** Spatial variability associated with fate processes and exposure profiles is generally ignored in modelling for risk assessment, which has almost always been deterministic.
- **Scale:** Exposure modelling typically considers the field as the unit of assessment with time spans of days to weeks. Predictability of the system generally improves for measures that integrate across larger spatial and temporal scales (e.g., annual loadings within small catchments).
- **Data availability:** Models provide continuous output that is difficult to evaluate against monitoring information that may comprise just a few measurements in space and/or time.
- **User subjectivity:** Studies have shown that decisions made by the user result in uncertainties in the model output (Brown et al. 1996; Boesten 2000). Recent developments by FOCUS reduce but do not eliminate subjectivity in modelling.

Simulation of in-field concentrations in soil should be relatively accurate because there is a primary input to the system, although studies have shown significant variability in deposition across the treated area. Beulke et al. (2000) evaluated a model of soil degradation based on the equations

of Walker (1974) against published data from 178 studies. Laboratory half-lives were corrected on a daily basis for soil temperature and moisture content, and the simulated residue for each study was output at the time so that 50% degradation was observed in the field. Most simulated residues fell within the measured value $\pm 50\%$ and there was a marked tendency for the model to overestimate field concentrations (Figure 1). Similar work in the US using the PRZM degradation routines for a smaller dataset concluded that simulated concentrations across the whole degradation curve can be expected to be within a factor of 3 of measured values 50% of the time and within a factor of 10 80% of the time (Jones and Russell 2001).

FOCUS has recommended that the models to be used for different fate processes relevant for basic aquatic risk assessment are spray-drift tables, MACRO (drain flow), PRZM (surface runoff), and TOXSWA (fate in surface water). The timing of pesticide loading to surface water via drain flow and surface runoff is generally well predicted by the models, and most attention has focused on the ability to simulate chemical concentrations and loads. It has been shown that MACRO is better able to simulate drainage concentrations from loams and clay loams (accuracy within an order of magnitude) than from heavy clay soils, where loadings

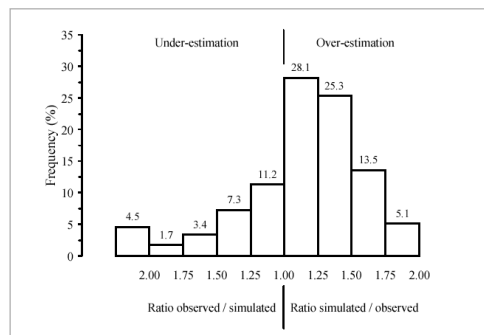


Figure 1. Simulated pesticide concentration in soil at the time of measurement of 50% loss in 178 published field studies (Beulke *et al.*, 2000).

are larger and fate is dominated by the highly heterogeneous process of preferential flow.

The most comprehensive evaluation of simulations for pesticide transport via surface runoff and erosion was undertaken by the FIFRA Environmental Model Validation Task Force (Jones and Russell 2001). The PRZM model was used to simu-

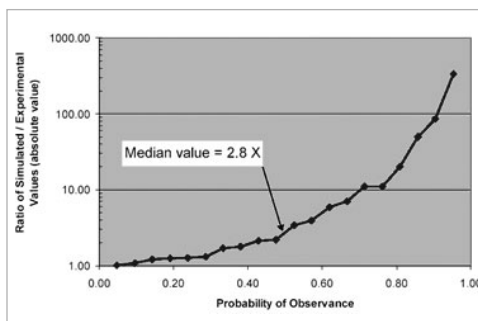


Figure 2. Rank plot of the accuracy of simulations for pesticide concentration in soil measured in 8 field studies (Jones & Russell, 2001).

late data from 8 field trials. It was found that simulations for concentrations and loads of pesticides in in-field runoff and erosion were generally within an order of magnitude of measured values. Model accuracy improved for multievent simulations.

By comparison with other exposure models, evaluation of TOXSWA is in its infancy. To date, only fitting of the model to laboratory water-sediment data or fate data from small ponds have been reported. Ongoing research in the Netherlands, France, and the UK will generate evaluations against field measurements. Evidence from regulatory simulations and catchment-level water-quality models suggests that fate in water is of lesser importance in predicting aquatic exposure than is accurate characterisation of pesticide inputs to water and of advective losses within the water body.

Regulatory modelling adopts a simplified approach to predicting exposure to pesticides that is designed to provide a protective estimation for use in risk assessment. Scenario selection considers

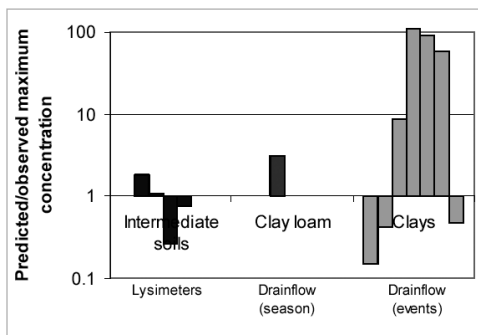


Figure 3. Relative accuracy of MACRO leaching simulations for different soil types (Brown *et al.*, 1999)

vulnerable situations and exposure estimated on the basis of maximum and time-weighted average concentrations and assumes that full bioavailability of the environmental residue will be conservative. Given the limitations outlined above and the accuracy of model simulations for site-specific conditions, the question appears to be not to what extent the models are able to match real exposure levels, but whether the exposure estimates based on deterministic modelling provide a protective input on which to base the risk assessment.

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PREDICTABILITY AND ACCEPTABILITY OF EFFECTS OF PESTICIDES IN FRESHWATER ECOSYSTEMS

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Introduction

Establishing causal relationships between pesticide exposure concentrations and effects in natural ecosystems is difficult due to the many intrinsic environmental factors that can hinder this process. In conjunction with observational field studies, controlled laboratory studies and experiments in microcosms or mesocosms can be used to investigate possible causal relationships between (combinations of) pesticides and ecological effects. The ecological realism of these controlled experiments is improved when the species and/or communities used in these experiments are representative of the species and communities at risk in the field. This also applies when the experimental exposure regime is realistic in terms of normal agricultural practise, which may imply the repeated application of a single compound or the application of a realistic combination. In addition, by incorporating the modulating factors characteristic of the natural ecosystem at risk in the experimental study design, field monitoring studies and controlled experiments complement each other.

Irrespective of whether the effect assessment is based on an observational study in the field or on a controlled semi-field experiment, it is important to realise that the properties of aquatic ecosystems vary in space and time. Consequently, an important issue in the assessment and management of ecological risks of pesticides in freshwater ecosystems is the predictive value of the current tiered risk assessment procedure. In other words, can the derived critical threshold levels and ecological responses be extrapolated in space and time? Another burning issue is whether the acceptable concentrations proposed for individual compounds sufficiently protect the aquatic ecosystem when more than one pesticide or stressor is present. In addition, the “what if” and “so what” questions may be raised when the “ecological significance” of predicted or measured pesticide concentrations and/or effects in different types of surface water are evaluated.

Rules of thumb derived from higher-tier tests with individual compounds

The first part of this paper will focus on the field relevance of effect assessments based on species sensitivity distributions (SSDs) and micro- and mesocosm experiments. The presented data for individual compounds are based on a literature review and studies performed at Alterra (Wageningen UR, The Netherlands) and the University of Sheffield (UK), with special reference to insecticides and herbicides.

Overall, the compiled single-species toxicity database reveals that SSDs can be used to predict ecological threshold levels for direct toxic effects, at least when the toxic mode of action of the compound and the exposure concentrations under field conditions are known. The studies of Maltby et al. (2002, 2005), Van den Brink et al. (2002), and Schroer et al. (2004) indicate that:

- ▶ SSDs and HC₅ values for the same compound may be comparable between different freshwater habitats and geographical regions, at least when based on the sensitive taxonomic group and taxa characteristics for these habitats and regions.
- ▶ Laboratory single-species toxicity data can be used to generate SSDs that are representative of (semi)-natural assemblages.
- ▶ In general, the HC₅ derived from short-term arthropod toxicity data, or short-term primary producer toxicity data, may be used to estimate the ecological threshold concentration for acute insecticide and herbicide exposures, respectively.
- ▶ In the absence of sufficient long-term NOEC data, SSDs based on short-term L(E)C₅₀ data may be used to derive HC₅ values for chronic exposure if an appropriate safety factor is applied (e.g., based on the acute-to-chronic ratio observed for the sensitive standard test species in the basic dataset).

Overall, the compiled micro- and mesocosm database (Brock, Lahr et al. 2000; Brock, Van Wijngaarden et al. 2000) reveals that:

- ▶ Ecological threshold levels of the same compound usually differ by less than a factor of 10 between different micro- or mesocosm experiments, at least when similar exposure regimes are tested.
- ▶ The ecological threshold levels for herbicides with a photosynthesis- or growth-inhibiting

mode of action are generally $\geq 0.1 \text{ TU}_{\text{algae}}$.
 $1 \text{ TU}_{\text{algae}}$ = geometric mean EC₅₀ of the most sensitive standard test alga (TU = toxic unit).

- ▶ In insecticide (semi-)field studies the ecological threshold level is usually higher than $(0.01-0.1) \times \text{EC}_{50}$ of the most sensitive standard test species (in most cases $0.01-0.1 \text{ TU}_{\text{Daphnia}}$).
- ▶ The recovery of affected populations depends on dynamics in exposure concentrations in the test system, on generation time and dispersal properties of the population affected, and on the ecological infrastructure of the surroundings of the locality exposed (e.g., extent of isolation).
- ▶ At exposure concentrations above the critical threshold level for direct toxic effects (usually $> 0.1-1 \text{ TU}$), the magnitude and type of indirect effects may differ considerably between different micro- and mesocosm experiments
- ▶ Insight into the types of indirect effects that can be expected in freshwater ecosystems can be obtained by combining results of ecotoxicological experiments, knowledge on the structure and functioning of the ecosystem at risk, and food web modelling.

Multistress and pesticides

To address the issue of multistress in freshwater ecosystems, the results of a complex study in experimental ditches are presented. These experimental ditches sufficiently resemble outdoor field situations in the polder landscape of the Netherlands. This study focussed on the impact of a realistic exposure to a range of pesticides commonly used in the cultivation of potatoes in the Netherlands. The main experimental aims were to provide information on the ecological impact of drift of a realistic package of pesticides into surface water and to evaluate the effectiveness of drift-reduction measures in mitigating risks. The pesticides selected and the dosage, frequency, and timing of application were based on normal agricultural practise for potato crops. During the growing season two herbicides (prosulfocarb and metribuzin), one insecticide (lambda-cyhalothrin), and two fungicides (chlorothalonil and fluazinam) were applied 1, 1, 2, 4, and 8 times, respectively. Applications were made at 0.2%, 1%, and 5% of the label-recommended rates. At the 0.2% level, no consistent treatment-related effects could be observed. At the 1% level, only short-

lived effects occurred. In the 5% treatment, clear effects on macroinvertebrates were observed, which could mainly be attributed to the insecticide lambda-cyhalothrin. The results of this complex study suggest that the current aquatic risk assessment procedure in the Netherlands (based on individual compounds, the Uniform Principles, and a drift emission of 1%) may sufficiently protect aquatic ecosystems stressed by a realistic combination of pesticides (Arts et al. personal communication).

The results of the above-mentioned study are in line with another crop-based microcosm experiment performed in Wageningen (Van Wijngaarden et al., accepted). In this microcosm experiment, the treatment regime was based on a realistic application scenario in tulip cultivation. The fungicide fluazinam, the insecticide lambda-cyhalothrin, and the herbicides asulam and metamitron were applied at concentrations equal to 0%, 0.2%, 0.5%, 2%, and 5% spray-drift emission of label-recommended rates. Again, at the 0.2% level no consistent treatment-related effects could be observed. The 0.5% treatment regime resulted in short-term effects. Pronounced effects were observed at the 2% and 5% treatment levels. In this multistress experiment, and for the end-points measured (community metabolism and species composition and abundance), the first-tier risk assessment procedure for individual compounds was adequate for protecting sensitive populations exposed to a realistic combination of pesticides. Spray drift-reducing measures seem to be efficient in protecting aquatic ecosystems in agricultural areas.

The “ecological significance” of pesticide stress

Guidance on how to deal with risks of chemicals in the environment is provided not only in a regulatory context but also by concepts based on science, ethics, and aesthetics. To sharpen the discussion, four completely different perceptions to consider ecological risks of toxicants in non-target habitats can be recognised (1) the pollution prevention principle, (2) the ecological threshold principle, (3) the recovery principle, and (4) the functional redundancy principle.

It is concluded that consensus on well-defined protection criteria, which may differ among types of ecosystems, is needed for tuning of EU policy (e.g., Uniform Principles, Water Framework).

ACKNOWLEDGMENTS

This contribution depends highly on the input of those mentioned in the references.

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PREDICTING EFFECTS OF PESTICIDES IN STREAMS

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Abstract

Our aim was to find patterns related to the effects of pesticides in aquatic invertebrate community composition in central European streams. To reduce the site-specific variation of community descriptors due to environmental factors other than pesticides, species were classified and grouped according to their vulnerability to pesticides. They were classified as species at risk (SPEAR) and species not at risk (SPE_{not}AR). Ecological traits used to define these groups were (1) sensitivity to toxicants, (2) generation time, (3) migration ability, and (4) presence of aquatic stages during time of maximum pesticide application. Results showed that measured pesticide concentrations of 1:10 of the acute 48-h median lethal concentration (LC₅₀) of *Daphnia magna* led to a short- and long-term reduction of abundance and number of SPEAR and a corresponding increase in SPE_{not}AR. Concentrations of 1:100 of the acute 48-h LC₅₀ of *D. magna* correlated with a long-term change of community composition. However, number and abundance of SPEAR in disturbed stream sections are greatly increased when undisturbed stream sections are present in upstream reaches. This positive influence compensated for the negative effect of high concentrations of pesticides through recolonisation. The results emphasize the importance of considering ecological traits and recolonisation processes on the landscape level for ecotoxicological risk assessment.

Introduction

The EU Uniform Principles for the assessment of pesticides require that if the preliminary risk characterisation indicates potential concerns, registration cannot be granted unless it can be demonstrated that "... under field conditions no unacceptable impact on the viability of exposed organisms ..." occurs. To date, such assessments have been made by conducting higher-tier studies, which have included a range of laboratory and semi-field experiments. Therefore, it is still not clear to what extent pesticides change population

dynamics and community structures in the field. Recently, some studies have quantified pesticide exposure, adverse effects on aquatic life, and recovery of these invertebrate communities in the field. Mortality of 6 mayfly species in an Australian river was linked to endosulfan contamination due to runoff (Leonard et al. 2000). Other investigations also found a link between mortality of several invertebrate species and insecticide concentrations in streams (Liess and Schulz 1999; Thiere and Schulz 2004). Several invertebrate species that declined in abundance due to pesticides were found to recover within a year (Liess and Schulz 1999). Nevertheless, most existing studies lack (1) sufficient numbers of investigations in various streams to evaluate the frequency of potentially harmful events in a specific region, (2) evaluation of long-term effects on invertebrate communities, and (3) quantification of the recovery of impacted communities due to recolonisation from undisturbed stream sections. The inclusion of habitat quality may put the risks resulting from contamination in context with other stressors.

According to these open questions, the aim of the present investigation was to find patterns in community composition that were related to the effect of pesticides. As other environmental factors may mask possible effects, a new approach that aims at reducing variability in community characterisation is presented. A detailed description of this work can be found in Liess and Von der Ohe (2005).

Results and Discussion

Environmental parameters at investigated sites

During the investigation period (1.8 years per site, 20 sites total), pesticides were detected in 125 runoff events at 18 of the 20 sites. Most of the contaminated-runoff events occurred in May, followed by June and July. The four pesticides contributing the most to the toxic units (TU_(D.magna)) were parathion-ethyl, azoxystrobin, kresoxim-methyl, and ethofumesat in decreasing contribution. Water-quality standard parameters would not be expected to have deleterious effects on the invertebrate fauna.

Correlating Environmental parameters and community descriptors

The measure for toxic stress of pesticides TU_(D.magna) best described the variance of community descriptors related to SPEAR. In general, the num-

ber and abundance of SPEAR correlated negatively with $TU_{(D. magna)}$. In contrast, the average number and abundance of SPE_{notAR} did not correlate with toxic stress. Other parameters contributing to the variability of SPEAR are length of forested stream sections, type of substrate, and coverage with submersed plants.

Temporal changes in community structure

The abundance of SPEAR decreased from April to May at sites with values of $TU_{(D. magna)}$ exceeding -2 to -1 compared with sites where $TU_{(D. magna)}$ values were below -4 . Furthermore, an increase in abundance of SPE_{notAR} occurred from April to June at sites where $TU_{(D. magna)}$ values were greater than -3 to -2 compared with the sites where $TU_{(D. magna)}$ values were below -4 .

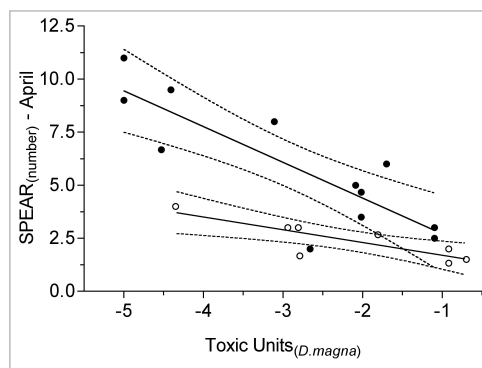


Figure 1: Relation between toxic units ($Daphnia magna$) and the number of species at risk in April ($SPEAR_{(number)} - April$). Sites are differentiated on the basis of the presence of forested stream sections closer than 4000 m upstream of the study site (filled circles; linear regression, $r^2 = 0.70$, $p < 0.01$) or absence of such sites (open circles; linear regression, $r^2 = 0.70$, $p = 0.01$). Confidence bands show the 95% confidence limit for the respective means. The slopes of the two regression lines differ (analysis of covariance, $p < 0.05$). Reprinted with permission from Liess and Von der Ohe 2005. Copyright Society of Environmental Toxicology and Chemistry (SETAC).

Contribution of uncontaminated stream sections to recovery

The presence of forested stream sections > 200 m in length and < 4000 m upstream of the investigated sites had a strong influence on the intercept and slope of the correlation between $TU_{(D. magna)}$ and SPEAR in April. When forested stream sections were present, the numbers of SPEAR tended to be greater. At the same time, the reduction of SPEAR with increasing $TU_{(D. magna)}$ was greater than at sites without forested stream sections (ANCOVA, $P < 0.05$). However, the positive influence

of forested stream sections upstream of the investigated sites compensated for the negative effect of high $TU_{(D. magna)}$ on SPEAR. Indeed, sites with $TU > -2$ and forested stream sections contained a number and abundance of SPEAR similar to those at sites with $TU < -3$ without forested stream sections (Figure 1). The described differences of sites with and without forested stream sections were apparent only in April—the time period before the highest toxic units have been measured. The differences of sites with and without forested stream sections were not detectable in June—the time period directly after the highest toxic units have been measured.

Finding patterns in community composition

In the present investigation, we reduced the problem of natural variability by grouping species according to their sensitivity to pesticides (Von der Ohe and Liess 2004) and their life-cycle traits known to influence recovery from toxicant stress. The approach of grouping SPEAR has the advantage of reducing the variability of the site-specific community characterisation and increasing the ability to detect the effect of pesticides on community composition. However, the SPEAR approach also has disadvantages: because species-level data are aggregated according to sensitivity and life-cycle traits related to recovery, the effect of a pesticide cannot be assigned to any particular species or taxon.

Temporal changes in community structure: reduction in SPEAR

Sites characterised by high TUs (between -1 and 0 based on the 48-h $LC50_{(D. magna)}$) showed a 75% reduction of SPEAR from April to May, when the highest concentrations of pesticides were measured. Other investigations of streams in agricultural areas also reported that pesticides from surface runoff can cause acute mortality of benthic invertebrates when they reach the range of the 48-h $LC50_{(D. magna)}$. No indication was found in the present investigation that parameters other than pesticides (e.g., hydrodynamic stress, water-quality parameters) might be responsible for the observed short-term reduction of sensitive species. In agricultural areas, hydrodynamic stress in streams due to increased current velocity and suspended particles during runoff events can occur frequently throughout the year. Hence, this stressor is probably not responsible for the short-term reduction of

individuals that occurred only during May. A detailed discussion of the topic can be found in Liess and Von der Ohe (2005).

Temporal changes in community structure:

alteration of SPE_{notAR} and long-term changes

Sites characterised by low TUs (below -3, based on the 48-h LC50_(D. magna)) showed a 60% reduction of SPE_{notAR} between April and June. This reduction was not observed at sites where TU values were high (above the range of -3 and -2). This pattern could result from an indirect positive effect of pesticides on SPE_{notAR} due to negative effects of pesticides on sensitive species. Such negative effects might occur even within the range of sublethal effects (TU_(D. magna)). However, previous investigations support the idea that short-term exposure to concentrations that are more than 100 times lower than concentrations causing acute mortality can cause long-term effects (Liess 2002).

Factors other than pesticides clearly can influence benthic invertebrate community structure, too, but we found no evidence that this occurred in relation to the endpoint of SPEAR at the sites we studied. However, because the levels of contamination may have been insufficiently quantified, it remains uncertain at which concentration these changes occur. A detailed discussion of these topics can be found in Liess and Von der Ohe (2005).

Contribution of uncontaminated stream sections to recovery

The length of forested stream sections upstream of the investigated site did relate significantly to the number and proportion of SPEAR in April. The positive effect of upstream forested stream sections on SPEAR at the downstream sites was not due to lower concentrations of contaminants at downstream sites; hence we suggest that the positive effect of forested stream sections on SPEAR at downstream sites can be attributed to in-stream recolonisation by invertebrates from the undisturbed stream sections where diversity is greater.

Cumulative risk

For the streams we studied, habitat quality seemed as important as toxicity for community composition. Thus, landscape information increases predictability in the assessment of risk due to pesticides. We suggest that the geographical unit of assessment should be extended to include landscape recovery potential.

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FROM LABORATORY TO FIELD: EXTRAPOLATION OF PESTICIDE RISKS TO NON-TARGET INVERTEBRATE COMMUNITIES

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Introduction

In this contribution an overview is given on the current knowledge of the relationship between the laboratory and field level when the potential effects of plant protection products (PPPs) on non-target terrestrial invertebrates are tested. In particular, the following extrapolation steps when going from laboratory tests to field studies are addressed:

- ▶ test species versus species in the field,
- ▶ artificial versus natural substrates, and
- ▶ acute versus chronic endpoints.

According to Directive 91/414/EEC, the risk of pesticides to non-target terrestrial invertebrates is routinely assessed for 3 invertebrate groups, namely, bees, non-target (above-ground) arthropods (NTAs), and the below-ground soil fauna. Recently, a functional endpoint was added, covering impacts on organic matter breakdown at a higher-tier level.

At lower tiers of risk assessment, the bees are represented by the honeybee, NTAs by two test species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*), and the soil fauna mainly by earthworms. Depending upon the lower-tier risk and the usage pattern of the pesticide, additional species may be tested at intermediate tiers (e.g., Carabidae for NTAs or Collembola for soil fauna). Standard guidelines for the highest (field) tier of risk assessment are currently available only for earthworm tests (ISO 1999), but similar guidance is given for bees and some NTAs by other organisations such as the International Organisation for Biological Control of Noxious Animals and Plants (IOBC) (Candolfi et al. 2000). The risk to soil fauna is assessed in-crop, whereas for NTAs off-crop risk assessment is also required (EPPO 2003).

Problems of extrapolation

The prediction of pesticide risks to terrestrial non-target invertebrates poses a challenge in regulatory risk assessment. Terrestrial invertebrate communities are complex, involving hundreds of inter-

acting species whose ecological roles and importance are temporally and spatially dynamic but not always well understood. Worst-case assessment of pesticide risks in the laboratory, on the other hand, necessarily focuses on a small subset of species under controlled conditions. Key difficulties are the realistic prediction of indirect effects, long-term effects, impacts of multiple stressors, and recovery.

Modelling approaches are increasingly being used to address some of these issues but are strongly limited by the availability and quality of empirical data. Risk assessment methods are available for use with small datasets (e.g., probabilistic approaches, species sensitivity distributions), but the data shortage invoking the use of such approaches could preclude their validation. Sometimes the underlying assumptions for the use of probabilistic approaches are not satisfied (e.g., species sensitivity distributions based on non-random selection of species).

Despite the fact that some tests still need to be standardised, it is no longer true that in comparison to aquatic ecotoxicology the number of terrestrial guidelines is too low. However, the availability of data (access to existing data or the production of new data) still limits any general discussion of the existing ecological risk assessment (ERA) scheme for PPPs.

Effects of PPPs on bees

- ▶ Standard guidelines for all test levels are available.
- ▶ Results of field tests are based mainly on “expert judgement”.
- ▶ Monitoring studies are not required, but are regionally performed (e.g., in the UK).

The UK monitoring shows that no pesticides classified as having “low risk” were implicated in poisoning incidents (Aldridge and Hart 1993), while, for example, pyrethroids (classified as “high risk” in the laboratory) are of low hazard in the field (Inglesfield 1989). Therefore, the current ERA scheme for bees seems to be successful. However, in the public domain potential side effects of PPPs on bees are intensively discussed (e.g., in France).

Lessons learned from bee testing

According to the Bee Assessment Scheme of EPPO (2003), field test results should be regarded as decisive when conclusions from lower-tier tests conflict with those from field tests. In addition, monitoring schemes should be used for the valida-

tion and refinement of ERA methods and results in other non-target groups. However, such an implementation will be more difficult due to higher species numbers, lack of experience, etc. (Lewis et al. 1998).

Effects of PPPs on Non-target Arthropods

- ▶ Standard guidelines for all test levels are available (but relatively few for field tests).
- ▶ Results of field tests are mainly based on “expert judgement”.
- ▶ Monitoring studies are not required but are regionally performed (e.g., in the UK).
- ▶ The results of field as well as monitoring studies are difficult to assess because in-field and off-field situations have to be distinguished, high numbers of species from various taxa with clearly diverse biological properties in very different exposure situations are potentially involved.
- ▶ Assessment of higher-tier results is still under discussion (in particular, a definition of the term “ecologically significant effects” is lacking).

However, some recent compilations have shown that it is possible to identify “standard test species” (*T. pyri* and *A. rhopalosiphi*), which are on average the most sensitive ones, and to recommend trigger values for higher tier testing if data are both sufficient and available.

The following issues need more discussion:

- ▶ Is the database, in particular from the field, sufficient (e.g., for *Aphidius* sp.)?
- ▶ How can multiple applications be considered?
- ▶ Is the sensitivity (but also biology) of standard test species comparable to the sensitivity of arthropod off-field communities? Is the ecological knowledge of off-crop communities sufficient? What about their exposure conditions?
- ▶ How can an “ecologically relevant” period and recovery be defined?
- ▶ How can local and regional variability (both in terms of exposure and effects) be included in ERA schemes?
- ▶ Is there a need for more guidance on field study design (depending on the aims and local conditions)? How can the effects of persistent PPPs plus the different recovery strategies be included?
- ▶ Assuming that the species level is the most appropriate one to address, is the taxonomic

knowledge about these species sufficient, and can it be handled in a practical way?

Effects of PPPs on soil (below-ground) invertebrates

- ▶ Standard guidelines for all test levels are available (but just one for field tests).
- ▶ Results of field tests are partly based on “expert judgement”.
- ▶ Monitoring studies are not required but are performed locally, usually as part of research projects studying the role of earthworms and other ecosystem engineers.

Due to the limited ecological knowledge (except for earthworms and, probably, Collembola) and to the problems related to field studies in general, expert judgement is clearly needed for these higher-tier studies.

Concerning species sensitivity, the following conclusions can be drawn from some reviews (e.g., on earthworms; Jones and Hart 1998) and an ongoing project sponsored by DEFRA (WEBFRAM 5):

- ▶ No general pattern is visible (e.g., the compost worm *E. fetida* is not always less sensitive than other lumbricid species).
- ▶ Sensitivity differences between taxonomic groups occur.
- ▶ Within one taxonomic group (i.e., the family level), sensitivity differs at about a factor of 10 (earthworms: 22 out of 25 pesticides), but exceptions are possible (up to a factor of 72 with PPPs; Jones and Hart 1998).
- ▶ The sensitivity ratio (the factor describing sensitivity differences for one chemical) for soil invertebrates is: $SR_{95:5} = 437$ (Elmegaard and Jagers op Akerhuis 2000).

While the influence of different species sensitivities can be substantial, different soils usually have a smaller impact on the lab-to-field extrapolation (e.g., the influence of the high organic amount in OECD artificial soil is covered by a factor of 2 for chemicals with a $\log K_{ow} > 2$; EPPO 2003). However, it must be taken into account that certain soil properties can already stress individual species; therefore, the extrapolation to, for example, acid soils might be problematic. In addition, the different behaviour of individual species can alter the exposure to PPPs considerably.

There have been several attempts to derive ratios between acute and chronic test results as well as between chronic laboratory tests and field tests

(e.g., Barber et al. 1998; Heimbach 1998), but still the amount of data is small and for the time being, “artificial” values are used.

Looking at the effects of PPPs in the field, it is good news that so far there is only one example showing that soil invertebrates were eradicated due to the impact of chemicals (species of the earthworm genus *Scherotheca* by heavy metals in parts of southern France; Abdul-Rida and Bouche 1995). However, because detailed monitoring studies are rare it is difficult to know whether such long-term and large-scale effects are genuinely infrequent or simply are not being detected (e.g., long-term effects of synthetic pyrethroids on arthropods were revealed by a unique time series of data in the UK (Ewald and Aebischer 1999).

Concerning the use of PPPs in English farmlands, some general results are listed (for details see, e.g., Frampton 1997):

- ▶ Community responses differ clearly among groups and species of arthropods.
- ▶ Short-term effects of insecticides nearly always occurred, while long-term effects were very site specific and group specific.
- ▶ Non-conventional agriculture (i.e., a reduced use of PPPs) favours soil invertebrates, but the variability in time and scale is high and effects of, for example, tillage are often higher than those of PPPs (e.g., earthworms: Tarrant et al. 1997).

In a case study with *Oligochaeta* and the fungicide carbendazim, in which laboratory, semi-field (i.e., terrestrial model ecosystems), and field studies were performed at 4 European sites, it could be shown that the increasing realism when going to higher-tier studies can lower the resulting PEC–PNEC ratios considerably (Römbke et al. 2004; Weyers et al. 2004; see also Weyers et al. this volume). However, the high variability of these complex tests is difficult to assess (would one study be sufficient to cover, e.g., different geographical localities?).

Recovery of soil invertebrates in the field after the use of PPPs has been studied with *Collembola* and earthworms (for a review, see Van Straalen and Van Rijn 1998). It seems that for the former group, the most astonishing result of these studies is that the potential for recovery from adjacent, untreated field margins or unsprayed areas of crop has been overestimated (e.g., Frampton 2002). For earthworms, some more details can be given:

- ▶ Recovery strongly depends on the persistence of the PPP (Jones and Hart 1998): DT₅₀ < 50 days = recovery within 6 to 12 months; DT₅₀ > 50 days = no reliable estimation possible.
- ▶ The role of immigration for recovery is not clear. Migration of up to 10 m/year is possible, but repellent effects are known (e.g., for benomyl: Heimbach 1997; Mather and Christensen 1998).
- ▶ The species composition is rarely considered, but for the ecosystem, anecics are usually more important than endogeics.
- ▶ Other stress factors related to agricultural practice (or even the diffuse impact of other chemicals) influence the populations as well.

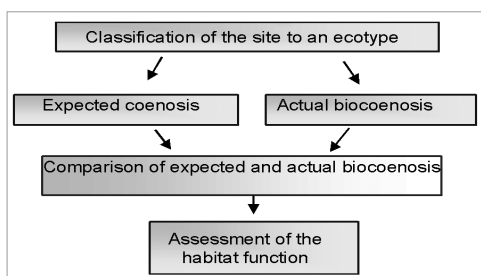
As a rule of thumb, any effect of more than 50% impact on earthworm populations or important species under farm conditions should cause concern.

Effects of PPPs on soil functions (processes)

- ▶ Standard guidelines for microbial (laboratory level) and decomposition tests (litter bag) are available (e.g., EPFES 2003).
- ▶ Trigger values are currently arbitrary.
- ▶ Monitoring (as opposed to testing) of these functional processes is not required and is hardly done at all.

The litter-bag test is triggered by the persistence of a compound and, partly, by effects in single-species laboratory tests. Since these two endpoints (mortality or reproduction of organisms and organic matter breakdown) are not directly linked, research on the structure-function relationship is needed. Two possibilities exist:

- ▶ A field test with soil invertebrates (i.e., *Collembola* and mites) in analogy to or in combination with the standard earthworm field guideline (ISO 1999).
- ▶ A monitoring approach (see also the accompanying contribution by T. Ratte): comparison of potentially impacted sites with reference values derived from “unaffected” sites in analogy to aquatic concepts like RIVPACS or BEAST (Reynoldson et al. 2000). In the following figure, the general approach is described (Ruf et al. 2003).



Recommendations

- ▶ Improvement of data availability (by either collating existing data or generating new data).
- ▶ Collation of (ecotoxicological and ecological) data of the most important (test) species in a central database.
- ▶ Use of these data in modelling (note the order!).
- ▶ Clarification of the aim of higher-tier studies (e.g., by defining what is an “ecologically relevant” recovery time).
- ▶ Provision of guidance on (regionally differentiated) field studies as well as monitoring studies (see, e.g., ISO draft guidelines on field sampling of soil invertebrates).
- ▶ Investigation of the relationship between structural and functional endpoints.
- ▶ Identification of the role and guidance on the performance of monitoring studies.

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**ABILITY TO
PREDICT EFFECTS IN BIRDS:
LINKING TIERS AND
EXTRAPOLATION***Mineau, P. and Whiteside, M.*National Wildlife Research Centre,
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Currently, most of the regulatory effort in pesticide avian risk assessment centres on the development of models incorporating laboratory-determined toxicity as well as estimates of food-item residues, consumption rates, proportion of foraging time in treated areas, and any avoidance response to treated foodstuffs. These models can be of a deterministic nature but, increasingly, natural variation and model uncertainty are incorporated into the risk assessment process through probabilistic tools such as Monte Carlo simulations. These assessments are typically carried out chemically by chemical or, in some cases, on a cluster of pesticides used for the same purpose.

Toxicity has been relatively easy to model in avian risk assessment because several approaches have been developed to process data obtained on different species in order to put any prediction of toxicity on a reasonably firm quantitative basis. Unfortunately, these techniques are often ignored by regulatory bodies in favour of arbitrary and highly stochastic single-species toxicity endpoints. Exposure, on the other hand, has proven particularly difficult to model, and such models tend to be very data intensive. Worse, estimates of exposure (hence, the very structure of existing models) have had almost no verification or validation against actual field outcomes. Fundamental assumptions inherent in the models, such as the belief that most of the exposure comes from the ingestion of contaminated food, are critical to model validity; yet the available evidence (Mineau et al. 1990; Driver et al. 1991) suggests that, for pesticide sprays at least, these assumptions are often incorrect.

A single directed field study by itself may not be sufficient to dispel a presumption of high risk that is placed on a pesticide. This deficiency results from the stochastic variability encountered in most field situations as well as from our inability to detect impacts every time they occur (in part because of the difficulty of finding evidence of an impact such as carcasses). Recognising this, the United States Environmental Protection Agency

(USEPA) proposed that a large number of fields needed to be monitored in order to increase the confidence of a finding of no effect (Fite et al. 1988). This requirement meant that substantial costs needed to be expended for each pesticide under presumption of causing avian harm (essentially mortality). This was one of the reasons field studies eventually fell out of favour with the US pesticide regulatory system. Yet, the logic was probably sound: several field studies are needed to uncover avian mortality that is either sporadic or even regular, but with a low frequency of occurrence.

A recent analysis of the avian pesticide study record (Mineau 2002) showed that it was possible to generate empirically based models to predict the likelihood of visible avian mortality. Mortality is certainly not the only endpoint of importance in avian risk assessment, but it is nevertheless the one that has attracted the most attention. The most accurate model developed by Mineau (2002) requires the following inputs: the "toxic potential" of the pesticide, a measure that incorporates HD₅ (*sensu* Aldenberg and Slob 1993; values from Mineau et al. 2001 as well as application rate); the dermal toxicity index of the pesticide (calculated from rat data as well as physicochemical constants); and Henry's law constant, a reflection of the potential inhalation exposure. This model was able to classify safe and lethal applications in the sample with better than 80% accuracy. We compared the output of this reality-based model to the generic (it stays the same regardless of the crop) small insectivore Tier 1 toxicity-exposure ratio (TER) calculation proposed in various drafts of the EU guidance document on risk assessment for birds and mammals under Council Directive 91/414/EEC (e.g., EU 2002).

For all the pesticides represented in Mineau's 2002 sample of field studies, we calculated the rate of application that is expected to lead to avian mortality 10% of the time or following 1 in 10 applications. This choice is entirely arbitrary: this frequency of wildlife incidents may not be acceptable in the case of very broad uses or where species of conservation interest are targeted. However, we think there would be general agreement that a pesticide-use pattern giving rise to mortality in 1 out of every 10 fields should warrant more scrutiny than Tier 1 can offer. For illustration purposes, we used the model developed by Mineau (2002) for field crop and pasture applications. We

then assumed that this calculated rate was the rate requested for registration and determined whether or not the registration request would be sent to Tier 2 (TER <10) or passed through without further investigation (TER >10). TERs were calculated with northern bobwhite, Japanese quail, or mallard LD₅₀ values. Based on precedence, we consider these to be the most likely species that would be tested for a new pesticide registration being submitted to the EU. We used either the maximum values available or a geometric mean of available values reflecting the chance element involved in selecting a toxicity value or a systematic bias on the part of registrants. Also, in order to remove any remaining doubts surrounding the model or the arbitrary choice of a 10% risk of mortality, we computed TERs for all available field studies with a determination of bird mortality or debilitation.

Based on the 2001 suggestion of a FIR (food intake ratio) of 1.03 and a RUD (residue level calculated for a 1 kg/ha a.i. application) of 11 mg/kg, there was a high “failure rate” in that between 29% and 44% of approximately 30 organophosphorus and carbamate insecticides would have successfully passed Tier 1, even though the field evidence suggests that, at the proposed rate of application, they kill birds 10% of the time.

In response to comments, the 2002 draft guidance document proposed abandonment of the 11-ppm RUD for “small” insects typically consumed by birds and adoption of a 52-ppm RUD, the maximum value proposed by Hoerger and Kenaga (1972) as an interim value until the issue of insect residues could be sorted out. Predictably, the “Tier 1 failure rate” for the same sample of insecticides dropped to between 0% and 16%, depending on the starting point toxicity value.

Not surprisingly, the TERs calculated for the individual field studies were also misleading for a high proportion of field studies. (Here, we combined all studies, whether on field crops, orchards, or woodlots, because the draft assessment guidelines do not specifically distinguish between these scenarios.) Using the maximum of the LD₅₀ values available for the bobwhite, the Japanese quail, or mallard, TERs calculated with an insect RUD of 11 ppm would have failed in the case of 11 separate insecticides and would not have predicted observed field mortality or severe debilitation following some applications of acephate, azinphosmethyl, chlorpyrifos, diazinon, dimethoate, feni-

trothion, fenthion, methamidophos, methomyl, mevinphos, phoxim, and propoxur. Repeat calculations with a RUD of 52 ppm still would not have predicted field studies reporting either mortality or debilitation with acephate, chlorpyrifos, dimethoate, fenitrothion, phoxim, and propoxur.

Fiddling with model constants such as the RUD can clearly be used to bring Tier 1 predictions more in line with available field evidence. However, as long as the underlying problem is not solved, this also will result in a much larger number of “false positives”. Based on a number of field studies where no mortality or debilitation was seen, we estimate that the proportion of false positives could easily be as high as 40–70% of pesticides, depending on whether a RUD of 11 or 52 was used. We believe the only scientifically defensible way of achieving the proper Tier 1 balance between false positives and false negatives is to have a good model in the first place. Based on our earlier work, this would mean considering exposure routes other than the ingestion of contaminated food. It is not a coincidence that several of the insecticides whose risk was most easily underestimated based on documented field studies (e.g., acephate, chlorpyrifos, dimethoate, and fenitrothion) are among those products with the highest dermal toxicity indices. Another (phoxim) has the highest Henry’s law constant of the products examined, suggesting, again, that the impact documented in the field resulted from exposure other than food intake. It is clearly in the interests of both regulators and industry to put more emphasis in this area and fix the problem.

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Implications for risk assessment

ECOSYSTEM DYNAMICS AND STABILITY: ARE THE EFFECTS OF PESTICIDES ECOLOGICALLY ACCEPTABLE?

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Abstract

This paper focuses on the non-target communities in agro-ecosystems that could undergo frequent perturbations by agricultural practises. Some frequently discussed concepts of theoretical community ecology and some general conclusions are briefly described. Among these conclusions are that (1) a tendency for inherent stability increases as complexity decreases; (2) relatively stable environments support complex but fragile communities, while relatively variable environments allow the persistence of only simpler, more robust communities; (3) complex, fragile communities of relatively constant environments (e.g., the tropics) are more susceptible to outside, unnatural disturbance than the simpler, more robust communities that are more accustomed to disturbance (e.g., in more temperate regions).

The main conclusion of the paper will be that in the context of an acceptability discussion, the general considerations should be followed by an applied approach, as communities exhibit different structures across the landscapes. Thus, the acceptability of effects can be discussed only on the basis of the local communities and agricultural practises. An approach is proposed on how to establish an inventory of reference habitats or communities, by means of which unacceptable effects in non-target habitats could be identified.

Introduction

The acceptability of anthropogenic effects on ecosystems largely depends on values developed by human societies rather than on conclusions de-

rived from ecological science. Valued properties of natural ecosystems undergo changes in time and are quite different among those who use nature (e.g., for agriculture, sports, game fishing, or commercial fishing) and those who want to protect nature for ethical reasons.

Plant protection products (PPPs) are applied to do their job on target sites in agricultural ecosystems¹, but non-target sites are often affected unintentionally, resulting in perturbations of terrestrial and aquatic communities or habitats surrounding the cropped areas. With respect to the target sites of PPPs, it has long been recognised that there is a need to monitor the sustainable maintenance of the soil functions necessary for growing crops and thus to protect the composition and functioning of the associated in-soil fauna.

The attractiveness of the cultivated landscape comes through a mix of various structural components in addition to the cropped areas, such as vegetation strips, hedges, ponds, small rivers, and woods, which contribute to the characteristic image of the landscape. These components form habitats for a number of biotic communities contributing to the agro-ecosystem. Although in accordance with current agricultural practises, the application of PPPs is also expected to impact non-target communities (e.g., their structure [diversity] and functioning). Consequently, the following issues arise: how these communities are affected, whether they will remain structurally stable in future, and how community composition (diversity) and functioning of the agro-ecosystem can be maintained in a sustainable manner.

Quick journey through theoretical community ecology

I shall briefly examine whether these questions (or at least some of them) can be answered theoretically by the ecological science, following the lines given in modern textbooks (e.g., Begon et al. 1990). Community ecology deals with the species composition or structure of communities and with the pathways followed by energy, nutrients, and

other chemicals as they pass through them (the functioning of communities). Unfortunately, different terminology has developed between plant and animal ecologists. The term “community” is most frequently used by zoologists in both a general and specific sense, whereas botanists (plant sociologists) use “association” as the fundamental unit representing a plant community of definite floristic composition. An association is composed of a number of stands that are concrete units of vegetation observed on a site. Zoologists have been more concerned with functional relationships such as food webs and energy flow through the community, whereas botanists have been more concerned with taxonomic and structural relationships in the community and the way these change in space and time. The often more-comprehensive studies of zoologists have dealt with plants as animal food, and botanists have tended to ignore the animals.

Important community characteristics are species diversity, growth form and structure, dominance of species, relative abundance of species, and trophic structure. Species diversity comprises all species of plants and animals living in a particular community. A species list is a simple measure of species richness or species diversity. The different growth forms determine the stratification, or vertical layering of the community (e.g., trees, shrubs, herbs, and mosses and broad-leaved and needle-leaved trees). Dominant species exert a major influence on the community by virtue of their size, numbers, or activities. Dominant species are highly successful ecologically and determine to a considerable extent the conditions under which the associated species must grow. The relative abundance is the relative proportion of species in a community. Trophic structure describes the pathways of material and energy in a community: who eats whom?

Among the methods to analyse the composition of communities in space are gradient analysis, ordination, and classification. Experience tells us that homogeneous environments hardly exist in nature. Most environments contain within them gradients of conditions or available resources. There is much evidence that the existence of one type of organism in an area immediately diversifies it for others. Changes in species and environmental conditions in time are either seasonal cycles or so-called successions. These are long-term changes and run through various stages, from the

first colonisation of new land to the stabilisation of communities. The final or mature stage is called climax, and there is a debate on whether there is one definite climax stage at a certain geographical area (mono-climax) or multiple climaxes (poly-climax). If a community develops anew on bare land, a so-called primary succession takes place, whereas a secondary succession occurs after a disturbance. With regard to agricultural activities and PPP applications, it is important that both activities prevent the natural succession or put succession back to an earlier stage at least in the target sites.

Because impacts of agricultural measures, in particular of PPPs, cannot be completely prevented, one has to study whether communities of non-target sites remain stable and sustain the associated perturbations in future times. Some major aspects of stability of ecological systems are described as follows:

- ▶ Resilience describes the speed with which a community returns to a former state.
- ▶ Resistance is the ability of the community to avoid displacement arising from a perturbation.
- ▶ Local stability is the tendency of a community to return to its original state (or something close to it) when subjected to a small perturbation.
- ▶ Global stability is achieved if this tendency is shown when the community is subjected to a large perturbation.
- ▶ Robustness describes the stability of a community in a wide range of environmental factors.
- ▶ Fragility describes the stability of a community in a narrow range of environmental factors.

The “conventional wisdom” states that “an increase in complexity leads to an increase in stability”. This statement, however, has been undermined by more recent work and mathematical modelling. No clear-cut relationship was found between the complexity of a community and its inherent stability. The stability appears to depend on the nature of the community itself, the way in which it is disturbed, and the way in which stability is assessed. Nonetheless, a tendency for inherent stability to increase as complexity decreases was obvious. Among other tendencies observed are that relatively stable environments support complex but fragile communities, whereas relatively variable environments allow the persistence

of only simpler, more robust communities. The likelihood exists that complex, fragile communities of relatively constant environments (e.g., the tropics) are more susceptible to outside, unnatural disturbance (and are more in need of protection) than the simpler, more robust communities that are more accustomed to disturbance (e.g., in more temperate regions).

Probably, there is a parallel in the properties of the community and the properties of the component populations. In stable environments populations will be subject to a high degree of K-selection; in variable environments they will be subject to a relatively high degree of r-selection. The K-selected populations (high competitive stability, high inherent survivorship, low reproductive output) will be resistant to perturbations, but once perturbed will have little capacity to recover (low resilience). The r-selected populations will have little resistance but a higher resilience.

An approach to acceptability

The rationale behind ongoing discussions on ecological stability, long carried out by risk assessors and those applying the PPPs, appears to be the reasoning that resistant or resilient communities might have developed in the agricultural areas that can sustain impacts brought about by the PPPs. However, this hypothesis cannot be derived or proven by theoretical community ecology, especially not the acceptability of impacts. In practise, we are faced with concrete habitats embedded in a landscape that is comprised of gradients of conditions as well as of fauna and flora. The species composition varies across local and global scales. Questions on the impact of PPPs and the acceptability of their effects can be answered only at a local scale. Recovery from effects also depends largely on the specific local conditions (e.g., on whether allogeneic recovery sources such as metapopulations exist). Thus, the conclusion here will be that applied ecology has to be put in place to generate the basic information on a local scale, which then can be used to discuss acceptability.

I shall now propose an approach, the central element of which is the development of target images for the major habitats and communities in non-target areas of the agro-ecosystem. The approach is similar to one performed for small rivers (e.g., in Germany) and is based on target images for rivers of the various geological areas and concrete reference rivers coming close to the target

image, though influenced by humans. A river under consideration can be compared with the reference, and measures can be taken when its quality does not come close enough to that of the reference. This approach shall be performed in fulfilment of the requirements of the EU water framework directive.

According to this directive, the terrestrial approach would be to

- ▶ generate an inventory of habitats and communities in non-target areas of the agro-ecosystem specific for the geographical regions;
- ▶ generate agreement on target images and reference sites;
- ▶ define boundaries below which deviations from the reference are seen as unacceptable;
- ▶ compare the habitats and communities in the considered region with their reference sites;
- ▶ come to conclusions on the acceptability of structural deviations from the reference; and
- ▶ come to conclusions about measures to be taken to remediate the habitats, depending on the conclusions reached above.

This approach is illustrated in Figure 1. The variety of non-target habitats appears to be small, and thus this task could be performed in relatively little time. A recurrent monitoring comparison of

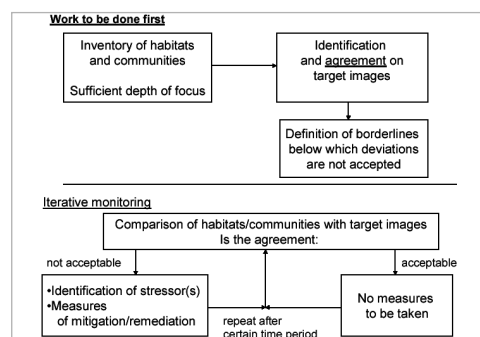


Figure 1: Proposed approach to acceptability

local habitats and communities with their references would lead to sustainable preservation of the cultivated landscape and maintenance of their typical diversity of habitats and species.

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THE ROLE OF DATA FROM MONITORING STUDIES IN THE REGULATORY FRAMEWORK

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Annexes II and III of EU Directive 91/414/EEC do not explicitly require data from monitoring studies. Consequently, in Annex VI no specific assessment schemes for monitoring data are given. This absence is not surprising, because an applicant must show that a product is safe before it is placed on the market, and current regulations are dealing with this situation. Monitoring data under realistic use conditions can only be collected later on. However, monitoring data have been required under national regulations for special purposes (e.g., to confirm that risk mitigation measures are effective). Below, examples for monitoring are presented.

Based on the state of the art in ecochemistry and ecotoxicology, the methods used to assess the risk for non-target organisms lead to conservative risk predictions, and it is very likely that unacceptable risks are precluded. However, a certain degree of uncertainty is inevitable, and therefore the predictions will never be perfect. Furthermore, the tools currently used are focused on single compounds and disregard combinations of effects. Organisms living under real-field conditions integrate all effects and all interactions. Considering that the sustainability of these populations is the ultimate protection aim, it is reasonable to conduct such monitoring studies from a scientific point of view. Additionally, these data should be used to demonstrate to the public that the use of plant protection products is safe. The interpretation of results should be conducted by an independent board of experts to ensure transparency and reliability. It is recommended that the authorities responsible for the authorisation of plant protection products identify typical vulnerable areas where continuous monitoring studies should be conducted.

Standard environmental risk assessments are based mainly on toxicity data from laboratory tests and on more-or-less sophisticated exposure estimates. During the past few years, especially in the

area of plant protection products, the risk estimates have become more realistic because, for example, data from higher-tier tests have been used. However, even when these modern tools are used, it is unclear whether the risk estimates match the actual risk prevailing in reality, that is, to which degree the risk estimate is overprotective or underprotective. Monitoring data can answer this question, although their interpretation is difficult and no precise numbers are to be expected. In a broader sense, data from monitoring studies can be used for a “reality check” of risk assessment schemes.

GEOGRAPHICAL DIFFERENCES IN THE EVALUATION AND PREDICTION OF THE EFFECTS OF PESTICIDES

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The regulatory environmental risk assessment of pesticides is conducted mostly through the extrapolation of laboratory data to natural systems. In addition, the evaluation covers generic agronomic practises. However, the biological characteristics and the pressure of natural ecosystems and communities associated with agricultural land are very different among regions.

Fortunately, direct effects of pesticides on aquatic systems and terrestrial wildlife in Europe have been significantly reduced due to regulatory measures, farmers' training, and an increase in environmental concerns.

This contribution will review the potential differences among geographical regions, particularly the specific differences of the Mediterranean region, in the assessment of effects on pesticides in the field.

The geographic dimension

Geographic differences are associated with significant agricultural differences. Four main aspects should be considered:

Regional crops: The connection of some crops to climatic and related conditions is so high that the crops are exclusively associated with a certain region and even with specific areas of a region. Rice, olive trees, and citrus trees are typical examples of Mediterranean crops.

Environmental, climatic, and agricultural differences: Environmental differences among regions include climate, geography, landscape characteristics, etc. All these factors modify the relevance of different exposure routes, as well as the type of scenario required for the assessment.

Differences in agricultural practises: Communities exposed off-crop are challenged by the whole list of pesticides used in the field and must follow the use pattern required for pest control in the area. The treatments differ among areas due to differences in the pests to be controlled and to differences in cropping characteristics, traditions, and regulatory and management practises. For example, the use of insecticides is particularly impor-

tant for Mediterranean conditions, where the climate allows rapid development of insect pests.

Ecological differences: The biological community is obviously adapted to the geographical area. The differences are related not only to differences in species but also to the role of each taxonomic group within the ecosystem. All main taxonomic groups considered in the current pesticide assessment are present in all geographical areas. The selected species for tier I assessment are generic, and there is no information to justify specific trends in the sensitivity of species among the areas. Thus, no fixed sensitivity tendencies for direct effects can be substantiated from current knowledge. However, differences in the ecological roles are evident, and therefore differences regarding indirect and long-term effects can be hypothesised. The conservation of habitats and species also presents significant differences among regions.

Several examples revealing the role of these differences when assessing the effects of pesticides in the field are discussed below.

Regional crops: Rice

Rice is a very special crop because the in-crop assessment combines aquatic and terrestrial taxa. The biodiversity of rice paddies is also very high, and most of the European area is located in the vicinity or even within areas of high ecological relevance. Wetland birds can get most of their food from rice paddies. Large bird mortalities by organophosphates have been described in rice paddies, and management plants for the use of agrochemicals in rice are assumed to be essential elements for an overall sustainable management.

Environmental differences: the role of exposure routes for aquatic systems

Spray drift is assumed to be largely responsible for effects of pesticides on aquatic systems. Spray drift is a very local and punctual event, intimately associated with the application of the pesticide by the farmer.

The effects, if any, can be observed immediately or at least shortly after application. Monitoring incidents is relatively easy. However, the relevance of spray drift in the Mediterranean region is relatively low, while runoff and soil erosion can be the largest contributors to pesticide surface-water contamination. The events are associated with rainfall, not pesticide application, and monitoring pro-

grammes require significant efforts to identify effects on aquatic communities and to quantify the role of pesticide application versus all other factors affecting the ecosystems.

Differences in agricultural practises:

Herbicide applications

Climatic conditions offer the possibility of specific uses of pesticides. The use of herbicides for facilitating harvesting is typical for several crops, including citrus and olive trees. The climatic conditions may produce different results from apparently similar practises. For example, in some areas, plant growth is associated with a very specific part

of the year, and the application of a herbicide can maintain a bare soil for several months, within the treated area and that affected by drift. Therefore, recovery of the non-target populations can be dramatically affected.

Ecological differences

Two aspects will be mentioned: the ecological relevance of indirect effects, such as the eutrophication risk associated with direct effects on zooplankton in Mediterranean systems, and the consequences on the European biodiversity, related to the effects on endangered species.



Environmental, climatic and agricultural differences.

Eutrophication processes are very common in several parts of Europe. Every year, hundreds of episodes of algal blooms and associated fish mortalities are reported in Spain. Light, temperature, reduced flow, large nutrient loads, etc., are obviously factors affecting algal growth and are not related to pesticide use. However, recent studies have confirmed that under Mediterranean conditions, the role of zooplankton in controlling algal populations is a key element for ecosystem effects. Toxic concentrations of several pesticides have been reported in areas associated with eutrophication problems, and a role of pesticides as an additional element cannot be excluded.

Effects on biodiversity are particularly relevant when endangered species are affected. In the last few years, a large number of poisoning incidents on prey birds have been reported in Spain, including the most endangered species. A total of 454 cases in black vulture (*Aegypius monachus*), 16 in bearded vulture (*Gypaetus barbatus*), and 72 in imperial eagle (*Aquila adalberti*) have been reported. Half of these poisonings have been diagnosed as pesticide poisoning, with carbamates, organophosphates, and some organochlorinated pesticides being the main responsible agents. Most of these poisonings are associated with the illegal use of pesticides for poisoning wild carnivores. However, effects associated with normal use of the pesticide by farmers have been suggested by other authors.

Discussion

Due to the particular conditions of the Mediterranean area, indirect and long-term effects associated with the use of pesticides have become a critical element for assessing the real impact of these chemicals on communities and ecosystems.

A quantitative assessment of the relevance of these effects cannot be conducted with the current level of information, but there are several indicators suggesting a significant contribution of pesticides to the overall anthropogenic hazards.



appendix *b*

LIST OF POSTER PRESENTATIONS

From laboratory to field: extrapolation of pesticide risks to non-target invertebrate communities

Amorim M¹, Soares A,¹ and Römbke J²

- 1) Department of Biology, University of Aveiro, Portugal
- 2) ECT Ökotoxikologie GmbH, Flörsheim, Germany

Aquatic risk assessment of realistic pesticide regimes in semi-field experiments

Arts GHP, van Wijngaarden RPA
and Brock ThCM

Alterra Green World Research, Wageningen University
and Research Centre, Wageningen, The Netherlands

Statistical Evaluation of the available Ecotoxicology data on plant protection products and their Metabolites (“SEEM-Project”)

Auteri D, Visentin S, Alberio P, Redolfi E,
Azimonti G, Mangiarotti M and Maroni M

International Center for Pesticides and Health Risk Prevention,
Busto Garolfo (Milano), Italy

Evaluation of methylparathion impact in freshwater open mesocosm on biocoenotic and bivalve biomarker responses

Bassères A and Guerniou A

Total, Groupement de Recherches de Lacq, Lacq, France

Chemical-biological monitoring in drainage ditches in the orcharding region “Altes Land”.

1. Residues of a.i. in surface water

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and Pestemer W¹

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- 2) Center for Research and Fruit Consultation for Fruit Growing of the Chamber of Agriculture Hannover, Jork, Germany

Entry of pesticides into surface waters: Results of the Lamspringe run-off monitoring project

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- 2) Federal Biological Research Center for Agriculture and Forestry, Institute for Plant Protection in Field Crops and Grassland, Braunschweig, Germany

Monitoring of terbutylazine in surface waters adjacent to maize fields with potential run-off to prove the efficacy of vegetated buffer zones

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Higher-tier risk assessment of fomesafen and fomesafen-nonylphenol polyethoxylate adjuvant mixture. 1. Fate and effects on planktonic communities

Caquet Th¹, Lacroix G², Deydier-Stephan L³, Le
Rouzic B³, Cravedi J-P⁴, Lescher-Moutoué F²,
Azam D⁵, Heydorff M¹ and Lagadic L¹

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- 5) Unité Expérimentale Écologie et Écotoxicologie Aquatique, INRA, Rennes, France

Higher-tier risk assessment of fomesafen and fomesafen-nonylphenol polyethoxylate adjuvant mixture. 2. Effects on macroinvertebrate communities

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Predicted versus observed effects of pesticides in a paddy rice field

Dohmen GP and Kubitz J

BASF-AG, Agricultural Center Limburgerhof, Germany

Predicting population recovery in aquatic systems using toxicity data combined with both chemical monitoring and functional determinations

Farrelly E

Braddan Scientific Ltd., Harrogate, UK

Assessing pesticide risk on birds: a case study (Parco Agricolo Sud, Milano)

Finizio A¹, Verro R¹, Sala S¹, Auteri D² and Vighi M¹

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Development of community tolerance to the antifouling agent Irgarol 1051 in marine periphyton after years of coastal water contamination

Grönvall F¹, Barceló D², Blanck H¹, Martinez K² and Nihlén P¹

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- 2) Environmental Chemistry, CID-CSIC, Barcelona, Spain

Higher-tier risk assessment of fomesafen and fomesafen-nonylphenol polyethoxylate adjuvant mixture. 3. Changes in individual performances of the snail *Lymnaea stagnalis*

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Effect of drift potential on drift exposure in terrestrial habitats

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State Agency for Agronomy and Plant Protection,
Mainz, Germany

Probabilistic risk assessment of Mancozeb fungicide in beneficial arthropods

Kramer VJ and Miles M
Dow AgroSciences, European Development Centre, Abingdon,
UK

Multi-tiered assessment of the effects of chemical mosquito control on invertebrates in coastal wetlands of Morbihan (Brittany, France)

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Linking insecticide contamination and population response in an agricultural stream

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Population response to toxicants is altered by intraspecific interaction

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Effects of pesticides in persistently contaminated areas – Detoxification enzymes as the endpoints of the pesticide effects in invertebrates inhabiting a gradient of metal pollution

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Landscape exposure assessment of chlorpyrifos in a citrus area

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Ecological impact of mixtures of antifouling agents on marine microalgal communities and the prediction of combined effects

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A critical review of pesticide related monitoring studies in Germany

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Three-year field study on effects of lambda-cyhalothrin on carabid beetles

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Modelling toxic pressure in streams – An indicator of the potential aquatic exposure caused by pesticide runoff

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A review of the analysis of morphological alterations of individuals as bioindication tools in aquatic ecosystems: examples with Chironomidae (Diptera) larvae

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Effects of short-term climatic variations on fluctuating asymmetry levels in Chironomus riparius larvae at a polluted site

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Field studies in the risk assessment of atrazine in amphibians

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Approaches for active biological monitoring of pesticides

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Chemical-biological monitoring in drainage ditches in the orcharding region “Altes Land”.

2. Ecotoxicological evaluation and survey of aquatic zoocenoses

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Functional landscape classification for the development of a GIS-based local risk assessment with realistic exposure scenarios

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Relative Sensitivity Distribution (RSD) of aquatic invertebrates to organic and metal compounds

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The effects of the herbicide metsulfuron methyl on the ecology of macrophyte-dominated experimental ecosystems

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Environmental risk assessment for plant protection products in the EU using laboratory, terrestrial model ecosystem and field data

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Magnitude and time course of residues in arthropods as potential food items for terrestrial vertebrates – obtaining data from field experiments

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Habitat use and time budgets (PT) of terrestrial vertebrates – hop yards in Bavaria: an example of obtaining data from field experiments

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effects of pesticides in the field

appendix C

REPORTS OF THE BREAKOUT GROUPS

Aquatic Ecosystems

1 GENERAL COMMENTS ABOUT EFFECTS OF PESTICIDES IN THE FIELD

The fact that agriculture per se (including land management, cattle rearing, pesticide use, fertilizers, irrigation, etc.) has an impact on water bodies in agronomic landscapes is generally acknowledged. Well-performed monitoring studies from the real world (“the field”) presented during this workshop showed that there can be effects on non-target aquatic organisms in the field from pesticide use. At the workshop the examples presented in plenary were discussed. However, several fundamental questions remain:

- ▶ There are relatively few examples of field studies and there is little information available on how widespread the described effects are.
- ▶ There is often much uncertainty over the magnitude of exposure that produces the observed effects.
- ▶ There are difficulties in attributing a particular observation of effect solely to pesticides, as the effects could often also be the result of other stressors (including natural environmental factors) having their effects upon the ecosystem. Documented effects concern mainly insecticides and arthropods. Data on other pesticides and other groups of organisms are scarce or were not presented/discussed at the workshop.

2 FIELD AND MONITORING STUDIES

2.1 DEFINITION OF FIELD/ MONITORING STUDIES

These studies generally fall into two categories:

- ▶ Field study: an investigation into the impact of specific products or active substances on a specific water body and introduced artificially for the purpose of the experiment under controlled conditions;

- ▶ Monitoring study: an investigation into the overall impact of pesticide use (or misuse) on water bodies

Within the context of this workshop, the focus is mainly on monitoring studies. However, field studies will often provide valuable information for predicting potential effects in the field and for interpretation of monitoring data.

Monitoring studies should include both chemical (exposure) and biological (effects) monitoring in order to attempt to establish causality according to widely accepted epidemiological criteria. It was noted that not all the criteria need to be met at all times (e.g., it may be difficult to link pesticide residues to indirect effects). In order to avoid spurious correlations, a combined approach using experimentation (such as mesocosm studies) and field observation is often useful to establish causality, although this may not always be feasible.

2.2 WHY DO WE NEED FIELD/ MONITORING STUDIES?

There are several reasons why field monitoring studies may be useful:

- ▶ They are a reality check for the overall risk assessment process and as such may help to identify areas of concern that are not sufficiently protected that require further investigation and also identify areas where the present process may be overprotective;
- ▶ Measurements in the field are explicitly focused on ecosystem components or processes that we wish to protect and are made under realistic conditions, rather than the surrogates for exposure and effect that are used at lower tiers in the pesticide risk assessment framework;
- ▶ They may help to demonstrate the extent to which risk mitigation measures really work in the field, in order to provide confidence to those who implement them. Conversely, they

may sometimes demonstrate that these measures are not effective enough and therefore justify the development of modified mitigation measures;

- ▶ Repeated field monitoring studies (i.e. at regular time intervals) may give information on the temporal trends of the environmental impact of agricultural use of pesticides at a local/regional scale;
- ▶ They can play a role in refinement of the risk assessment and in risk management;
- ▶ Data from monitoring studies have the advantage of being relatively easy to communicate to the public and other stakeholders.
- ▶ Currently biological monitoring is the only means to identify potential impacts from multiple stressors in the field.

Controlled field and outdoor experiments (e.g., micro and meso-cosms) can be useful predictive tools during the pre-authorization risk assessment and for post-authorization testing of hypotheses. This contrasts with monitoring studies that are useful during the post-authorization phase as a check on pre-authorization predictions, and also potentially useful for re-registration purposes. Monitoring results may help to develop more appropriate experimental designs (e.g., for common mixtures of chemicals, and to identify potential confounding factors.)

2.3 EXPOSURE MONITORING

Routine monitoring for pesticides and other substances in surface waters is usually performed by water authorities and regulatory bodies for legislative reasons unconnected with pesticide authorization and control. As a result, many of these programmes have poor temporal resolution (e.g., monthly) in relation to likely pesticide peak concentrations, with sampling locations more frequently found in larger water bodies (e.g., at the inlet of drinking water plants) and biased towards water-soluble herbicides (which are easily detectable with multi-residue methods and most likely to exceed drinking water standards). Routine chemical monitoring is often therefore of limited value for the prediction of ecological effects, although it is acknowledged that under the Water Framework Directive this should be augmented by biological monitoring for environmental impacts.

The published data from case studies on agricultural insecticide exposure in surface waters have recently been reviewed (Schulz, 2004).

On occasions the pesticide concentrations measured in chemical monitoring programmes differs from those predicted by regulatory models (e.g., (Crane *et al.*, 2003); or see www.bestrijdingsmiddelenatlas.nl). This may be due to various reasons such as,

- ▶ Sampling during a low vulnerability situation (e.g., in relation to timing of rainfall);
- ▶ Sampling from different types of water bodies (flowing rather than static water bodies);
- ▶ Peak concentrations missed when monitoring by grab sampling;
- ▶ Differences in the dissipation of the pesticide in water in the field compared to that predicted on the basis of laboratory water-sediment studies;
- ▶ Point source contamination.
- ▶ Poor agricultural practice (e.g., misuse, mainly buffer zone violation)
- ▶ Conservatism in the initial tiers of the risk assessment process (e.g., “worst-case” 90th percentile modelled environmental exposure) resulting in differences between predicted and measured pesticide concentrations.

Well-designed monitoring studies can be used to demonstrate that the risk assessment process is sufficiently precautionary in estimating exposure from single, or many, pesticide applications in the same catchment. Targeted chemical monitoring is an appropriate means of refining exposure and could be used to validate exposure models. For example, targeted catchment studies can be used to calibrate models that include product use data and landscape information, which can then be used for generic exposure assessment (e.g., the PESTSURF development in Denmark). However, chemical monitoring data on their own are of limited use without establishing a correlation between possible chemical causes and biological effects. If causality can be established, then it may be possible to examine the correlation between critical effect concentrations detected in the field and those predicted from exposure estimates under Directive 91/414.

2.4 EFFECTS MONITORING

Biological monitoring studies are useful for investigating the effects of multiple stressors, as organisms can integrate these effects. However, it is important to identify:

- ▶ What it is possible to observe and measure in the field; and
- ▶ Whether it is possible to discriminate the impacts of individual stressors.

A major problem in environmental monitoring is the variability caused by the multitude of stressors influencing the specific local and temporal ecosystem. Thus, due to the “noise” in the data the power to detect differences can be low. Despite this, studies by Leonard *et al.* in Australia (Leonard *et al.*, 1999; Leonard *et al.*, 2000), Liess (platform presentation), Liess and Schulz in Germany (Liess, 1994, 1998; Liess & Schulz, 1999; Liess *et al.*, 1999; Schulz & Liess, 1999a, 1999b), Schulz *et al.* in South Africa (Schulz *et al.*, 2001; Schulz *et al.*, 2002), and in Argentina (Jergentz *et al.*, 2004) and Schaefer *et al.* in Germany (Altes Land; Schaefer *et al.*, platform presentation) demonstrate that responses can be detected from within the variability of the data. (Schulz, 2004) has critically reviewed the available field studies on insecticide effects in surface waters (see Table 1).

It is likely that monitoring studies may not detect small and subtle effects, so it is important when designing a study to understand what one wishes to measure (and protect) and the level of difference that it is necessary to detect. Such decisions may be informed by taking species life-history strategies (e.g., r versus K-selected, short versus long generation times; Liess, platform presentation) and potential species or ecosystem sensitivity into account.

2.5 LIMITATIONS OF AVAILABLE DATA

The studies presented at the workshop concentrated on insecticides and macroinvertebrates, so generalizations to other organism types or compound groups are difficult. The number of focused, suitable monitoring data is rather low and some these studies had limitations (e.g., absence of chemical monitoring, or no clear evidence of cause and effect). There is also evidence of macrophyte loss

from the agricultural landscape (Williams *et al.*, 1998).

3 WHAT FEATURES ARE ESSENTIAL IN A SUCCESSFUL FIELD/ MONITORING STUDY AND WHY?

Monitoring studies should include both chemical (exposure) and biological (effects) monitoring in order to attempt to establish causality.

3.1 QUANTIFYING ENVIRONMENTAL VARIABLES

3.1.1 PROBLEMS IN QUANTIFYING EXPOSURE

One potential issue in interpreting monitoring data is that exposure may be poorly characterized. Field measurements should be made relative to application time to detect the peak concentrations and to aid interpretation of observed biological effects. For rainfall-driven inputs (runoff and drain-flow) it is important to sample at the start of the hydrogeological event to capture the peak pesticide concentrations. For drift inputs, it is necessary to sample at the time of application.

Effects arising from mixtures require a more complex exposure monitoring design that can detect multiple chemicals. As complete a knowledge as possible of exposure concentrations is desirable and also of other factors associated with landscape structure (e.g., river basin structure) and agricultural practice (e.g., fertilizer application, land use practices, etc.) which may confound effective interpretation. It is important to measure these potential causal factors as well as pesticide concentrations so that their role can be assessed. Establishing cause and effect may be difficult, and the use of additional experimentation (e.g., laboratory or in situ bioassays, or mesocosms) may be useful to validate monitoring data. Controlled experimental studies in combination with monitoring studies can often help to test hypotheses and to interpret field data.

In some circumstances validated/verified model estimation may be more appropriate than inadequate measurement, e.g., for rapidly dissipating

substances. However, a problem with reliance on modelling alone or on targeted chemical analyses is that legacy pesticides and undetected, yet toxicologically important, substances may be missed. Nevertheless there is the potential to combine modelling with monitoring data to provide further insights into quantifying exposure.

3.1.2 POINT VERSUS DIFFUSE SOURCES

There may be a poor correlation between point source and diffuse pesticide contamination, as some of the exposure to pesticides detected by field monitoring may arise from point sources (e.g., waste water treatment works, or improper cleaning of equipment in the farmyard) in addition to field applications. As a result, effects observed in a monitoring study may not necessarily invalidate a single-substance risk assessment.

Comparison of effects observed in field monitoring studies with the risk assessment for an active substance or product is difficult because, in general, the field exposure is insufficiently characterized (e.g., it may miss the peak contamination, potential sample deterioration during storage or matrix interference and presence of undetected contaminants or those exerting biological effects below analytical detection levels).

Risk assessment of pesticides currently does not consider point sources because under Directive 91/414 only product use according to Good Agricultural Practice (GAP) is considered. Modelled PECs therefore currently do not consider exposure from poor agricultural practice. However, this issue needs to be kept in perspective as, for example, chemical monitoring data from the Netherlands indicate that only a few chemicals exceed lower and/or higher-tier maximum permissible concentrations (see: www.bestrijdingsmiddelenatlas.nl) and these may be associated with unknown sources. In addition, experience from Germany with chemical monitoring in groundwater indicates that many cases of exceedences were from point source contamination (Bach *et al.*, 2001; Bach *et al.*, 2000; Muller *et al.*, 2002). However, the limitations of routine chemical monitoring apply (see 2.3).

3.1.3 TEMPORAL AND SPATIAL SCALES

The scale of monitoring and observation needs to be considered. At a smaller scale (e.g., small waterbodies) there may be high variability, so sufficient sites and times of sampling should be used to distinguish cause and effect relationships with adequate statistical power. Similarly, at a larger scale localized impacts may not be detected.

3.1.4 SITE-SPECIFIC BASELINE INFORMATION

Site-specific, context-dependent and ecologically important biological, physical, and chemical factors (e.g., water chemistry, habitat, and site history) that could be confounding variables should be measured in field monitoring programmes. This may include pesticide metabolites and degradation products with known toxicological significance.

The use of long-term databases on community composition at a regional scale (e.g., species checklists) may also give an opportunity to detect changes in regional biodiversity in a particular area. However, without additional regional information (e.g., changes in water management, land use, etc.) elucidation of causality and robust interpretation may be compromised.

3.2 QUANTIFYING BIOLOGICAL RESPONSE

3.2.1 DETECTING RESPONSES

Loss of species richness (or loss at another taxonomic level) was considered to provide strong evidence of an adverse biological effect in a surface waterbody. However, species should be grouped, if possible, according to their:

- life cycle traits to account for differences in recovery/recolonization potential;
- level of tolerance to particular pesticide modes of action (the percentage of species within the community that are sensitive to the particular toxicant should then be estimated);
- the species at risk (SPEAR) concept developed by Liess (see case study in this report) was considered a potentially valuable tool.

Some variability in measured ecological effects may be accounted for by categorizing species according to ecological traits (e.g., sensitivity to pesticides, reproduction potential, resilient life stages, high O₂ requirements, voltinism, microhabitat, etc.), and then comparing effects in these groups across different exposure concentrations.

Similarly, categorizing habitats with reference to the organisms within them (e.g., organisms associated with oxygen-rich microhabitats) may help to elucidate the effects of other stressors and thus isolate possible effects of pesticides. Functional endpoints should also be considered but are usually less sensitive than structural ones.

Endpoints for which tolerance or resistance has been shown to have developed should be avoided. The presence and abundance of sensitive species in natural, functioning ecosystems was considered a reliable endpoint. For example, a study in northern Germany (Sönnichsen, 2002) of ephemeral ditches found no differences in communities between ditches within arable areas compared to those alongside meadows. This was attributed to the high recolonization potential of the systems and, possibly, the ability of the specific community to withstand periodic drought. Species with a low recovery/recolonization potential were not found. Similar hypotheses were proposed by Lagadic to explain the absence of effects of insecticides on invertebrate communities in coastal wetlands subjected to chemical mosquito control (Lagadic, 1999).

3.2.2 REPRESENTATIVENESS OF THE SYSTEM

The system that is monitored should be representative of the agricultural landscape. However, the presence of extreme disturbance (e.g., very frequent maintenance, intense chemical stress) has to be avoided. The system should have the capacity to respond to stressors, and representatives of the main taxa and of the different life cycle strategies (multi- to semivoltine species, r- and K-species) should be present in sufficient numbers for adequate statistical analysis. Other important biological issues like, for example, the number of generations should also be considered. The normal range of variability in measured endpoints should be un-

derstood for both the measured system and for those that it is intended to represent.

The challenge is in defining such representativeness, but there are examples in the UK where classification of water bodies has been systematically achieved (e.g., UK aquatic landscapes project: (Anonymous, 2003).

3.2.3 CONTROL AND REFERENCE SITES

A key issue for all field monitoring studies is establishing the control or reference site. Often there is no “control” site, and water bodies can contain adapted populations due to stress from pesticides or other contaminants. It may be easier to find reference sites in flowing water systems (e.g., comparable pristine upstream parts of the contaminated waterbody), although it was acknowledged that communities may be inherently different at different sites for reasons other than pesticide use.

Establishing a “true” uncontaminated reference site is probably unachievable and therefore finding a reference site with a clearly different exposure regime from the test site is required. One possibility might be to use “relative” references, i.e. sites with a gradient of contamination, from the lowest to the highest measured levels. However pollution induced community tolerance (PICT) caused by other stressors should be avoided in the tested sites and in the control sites, respectively. Reference sites should be included in either time or space (it is acknowledged that this may be very difficult), although using an ‘internal’ reference in which comparisons at the same site are made through time may be appropriate.

To establish a control, the intensity of anthropogenic activities (i.e., the degree of disturbance) could be used as an indicator to aid site selection. Investigators should not be too concerned if water bodies have been modified by anthropogenic activities rather than being truly natural, as the former have many of the properties of truly natural systems (e.g., certain types of ditches may simulate the properties of natural water bodies). Reference sites may be better at a more integrated level (community tolerance as opposed to species abundance, ecosystem functioning, etc.).

In places where there are other recognized impacts occurring (e.g., elevated nutrient concentrations) it will usually be impossible to demonstrate conclusive correlations between observed impacts and individual stressors.

3.2.4 CONFOUNDING VARIABLES

It is important to establish what the potential multiple stressors in a system are before beginning a study so that they can be accounted for in the study design and interpretation. Confounding variables (e.g., unusual weather or fluctuating organism populations) cannot be completely avoided in field studies, as they are part of the natural systems; however, they should be monitored in order to aid the interpretation of results. Differences between reference and treatment sites (e.g., neighbouring cultures, shading, differences in upstream areas, stream or pond morphology, nutrient situation) should be avoided. Routes of exposure examined in laboratory and monitoring studies should also be understood before a study begins so that relationships between laboratory and field observations can be interpreted. This may be easier to achieve for short-term effects (e.g., lethality) than for long-term effects, because exposure to stressors (including pesticides) and the resulting effects are more difficult to characterize over longer time periods. Hence, the statistical power of field tests is generally less than for mesocosm studies because of greater variability and more confounding factors.

Biological effects observed in the field could result from multiple stresses, which may make determining pesticide exposure as the cause very difficult. On the other hand, the absence of observed effects could be due to pollution induced community tolerance (PICT) or to poor statistical power. There is a need for consensus on what kinds of effect monitoring studies are capable of detecting.

4 WHAT FEATURES ARE DESIRABLE IN A FIELD/MONITORING STUDY?

Monitoring studies should include both chemical (exposure) and biological (effects) monitoring in order to attempt to establish causality. Additionally, in order to avoid spurious correlations, a com-

bined approach using experimentation (in situ studies, biomarkers or mesocosms) and field observation is often useful to establish causality. However, a problem with field studies based on in situ bioassays is that they tend to be more sensitive because of increased exposure in cages and lack of escape opportunities. (e.g., (Schulz, 2003) for *Gammarus pulex*; (Lagadic *et al.*, 2002) for chironomids). Despite this, in situ bioassays can be useful systems for assessing potential bioavailability and exposure, particularly when natural populations show variable demographics (perhaps due to seasonality).

Sub-organism biomarkers may provide a good indicator of ecosystem stress in general (De Coen & Janssen, 2003; Hanson & Lagadic, 2003; Sibley *et al.*, 2000). However, difficulties in attributing a response to a single toxicant may make results from biomarkers difficult to interpret. Therefore, selection of biomarkers on the basis of the mode-of-action of pesticides is essential in order to attempt to discriminate between various stressors (Lagadic *et al.*, 2002). In addition, difficulties in extrapolating from biomarker responses to population level endpoints have led some to question their relevance. The selection of biomarkers should be based on their physiological role, and more specifically on their metabolic implication in individual performances (growth, reproduction, etc.) (Liess & Schulz, 1999).

5 HOW SHOULD FIELD MONITORING DATA BE INCORPORATED INTO A RISK ASSESSMENT?

The general consensus was that it is difficult to envisage monitoring studies being incorporated into decision making with respect to registration for a single active substance. However, it could be used for fine-tuning potential mitigation measures in post-registration activities.

The principal benefit of monitoring data within the current risk assessment framework is as a check on the pre-authorization assessment. The risk assessment considers single compounds in isolation, but there is uncertainty over whether or not it is also conservative for multiple stresses (including multiple pesticide exposure) that may occur in the field.

In one field study (Liess, platform presentation), it was shown that the most toxic substance (highest Toxic Units) gave the best correlation between contamination and community impact, while for all contaminants, the additive exposure concentrations gave a poorer correlation. This would support the view that the single chemical risk assessment is sufficient for the pesticides, habitat type and ecological receptors examined in this study.

This view is supported by controlled microcosm/mesocosm experiments in which the impact of a realistic exposure to pesticides used in, e.g., tulip and potato fields was simulated (Arts and Brock, presentations; Van Wijngaarden *et al.*, 2005).

The need for a clear definition of the protection aims underlying risk assessment for pesticides was identified. One approach may be to establish a reference condition (i.e., the “natural” status) that fulfils the protection aims for water bodies. However reference locations may be difficult to locate in nations with historically modified surface waters (i.e., most developed countries). Definition of the reference condition may therefore be simply a pragmatic decision based upon a combination of scientific judgement, ‘public acceptability’ and evidence-based determination of ‘good quality’ in agricultural landscapes. There are examples available of such definitions for aquatic communities in Dutch drainage ditches in the Netherlands (Nijboer, 2000; Nijboer *et al.*, 2004).

6 HOW SHOULD POPULATION RECOVERY BE INTERPRETED, ESPECIALLY FROM UNCONTAMINATED AREAS?

6.1 GENERAL

Recovery is part of the description of how natural systems respond to a stressor and should be considered within the context of both specific landscape factors and extrapolation to wider landscapes. The term recovery includes both internal system recovery and recolonization from external sources. Assessing recovery requires considerable knowledge about different landscape factors and how they interact with organism life histories,

although many questions remain in this area of ecology:

- ▶ How does recovery differ across systems and landscapes?
- ▶ There are some typical ecosystems in the agricultural landscape – is it acceptable to take these as baselines?
- ▶ Is the possibility or probability of recovery reduced if a large part of the landscape is adversely affected by pesticide exposure?
- ▶ What percentage of an interconnected ditch system should be protected to facilitate recolonization?

Measurement of “recovery” in monitoring studies may be difficult because removal of keystone species could lead to alternative stable states (e.g., macrophyte-dominated versus algal-dominated aquatic systems (Scheffer, 1998)). Also, (Matthews *et al.*, 1996) have suggested that ecosystems possess a ‘memory’ of stressful events and that there is a lack of return to original conditions after chemical exposure (i.e., irreversible effects). If an effect has been of sufficient magnitude to alter the ecosystem irreversibly, it would probably be considered an unwanted effect even if there was ecosystem stability.

Whether populations are *r* or *K*-selected will clearly influence the speed and capacity for system recovery (as shown in Liess and Brock, platform presentations). Adaptation to stress may not be a change to less sensitive species, but to those species that have better recovery/recolonization potential (e.g., shorter generation time and aerial life stage). For example, in the field study presented by Liess the main recolonization observed in the field was in-stream transport by organism drift from small, forested upstream areas. This suggests that the area required as a source of recolonization may not need to be large to have a significant positive effect.

Recovery in flowing systems might be faster than in isolated water bodies, and it is possible to make reasonable predictions for interconnected water bodies that recovery will usually occur within 1-2 years, even from substantial natural perturbations, provided the stressor is removed. It is much harder to make predictions for isolated water bodies, as the recovery processes in these are poorly understood. However, as there will often be pesticide concentration gradients within larger water bod-

ies, there will frequently be refugia and thus recovery potential from within the system.

In summary, it is important to define recovery endpoints *a priori* and, when considering recovery, monitoring studies should attempt to distinguish and quantify both internal recovery and recolonization by dispersal. This may be very difficult as it requires incorporation of site-specific population dynamics, life history information for affected populations, and metapopulation dynamics in the landscape.

6.2 MODELLING RECOVERY

Few data are available about recolonization (e.g., reproduction, drift, and dispersal) that could be used in population models. However, data on the principles of aquatic organism population recovery are available. Using very simple models it should be possible to differentiate between landscapes with isolated aquatic systems and those with interconnected and flowing water bodies. For example, data on recovery potential of organisms are available at the family level on the PondFX website (www.ent3.orst.edu/PondFX). Data at the species level are being collected in a cooperative project between The Ponds Conservation Trust, UFZ Centre for Environmental Research and Alterra. The use of relatively simple recolonization/populations models also have the potential to assist in hypothesis testing.

It is therefore possible to use generalised information on recolonization statistics for different species to generate hypotheses and design experiments. Modelling and landscape analysis can be used to identify water bodies that are most likely to be impacted and then targeted monitoring can be undertaken to investigate whether or not the impacts are observed.

6.3 RECOVERY AND RISK ASSESSMENT

Knowledge about population recovery can help us to understand how observed recovery in mesocosms should be interpreted within a wider environmental context. Despite its importance, recovery is currently not generally considered in the initial tiers of the risk assessment process, although

at higher tiers it is considered (e.g., NOEAEC; (91/414/EEC, 2002; Anonymous, 2002). There are also conceptual difficulties in incorporating recolonization into a risk assessment, as only single stressors are considered.

The FOCUS Landscapes & Mitigation working group is attempting to develop ecological information associated with the established surface water scenarios to inform risk assessment.

6.4 MITIGATION

The evidence from the Altes Land study presented in this report demonstrated differing impacts with changes in spray direction, thus indicating the potential benefits of effective risk mitigation. Furthermore, Liess's study in this report also demonstrated that relatively small areas in streams that do not suffer impacts (e.g., a forested area) can significantly mitigate impacts in connected water bodies. In addition, the workshop also heard of a German terrestrial mitigation system (using GIS information). Although this was designed for a different aim (the proportion of the landscape covered by woodland scrub was correlated with the potential for recolonization), the principle and use of GIS could be useful in assessing or refining approaches to mitigation in the aquatic environment.

7 WHAT GENERAL CONCLUSIONS CAN BE DRAWN ABOUT THE LEVEL OF AGREEMENT BETWEEN LABORATORY BASED RISK ASSESSMENT AND FIELD EFFECTS.

Specific targeted experiments do show effects of pesticides in the field. This has been shown in the Altes Land (see case study in this report) in cases where spraying was at a short distance from and in the direction of the ditch, in the Schulz studies from South Africa (where the exposure was consistent with predictions from spray drift tables as used in the EU), and in the Liess and Schulz studies following run-off events. For various reasons (demonstration of causality, or poor characterisation of exposure), it is not always possible to correlate observed biological effects with the risk assessment process (e.g., uncertainty over actual field exposure). Biological impacts observed in

monitoring could be used to indicate the need for further investigation. When the “actual” exposure is well understood, monitoring studies do not underestimate the risk.

Mesocosm studies may provide fairly good effects predictions for known exposures (e.g., those compounds or other stressors that are experimentally applied), but these predictions are less robust when exposure is more complicated (e.g., unknown compounds or stressors present). They are probably also predictive of direct effects, but as indirect effects are usually context-dependent it is difficult to be sure that generic tests such as mesocosms are protective in all cases.

Controlled experiments are predictive of threshold effects concentrations, but recovery in the field is often context-dependent (e.g., voltinism in the particular assemblage, hydrological connectivity, latitude, etc.). Insertion of species into mesocosms (e.g., univoltine species) may help us to understand potential recovery. However such data should be carefully considered since they are not fully representative of natural conditions where the time lag between contamination and recolonization may be much greater compared to the experimental test duration.

Monitoring data may help us to design better generic mesocosm studies (including inter-laboratory mesocosm ring tests) or, at least, to understand the limitations of specific mesocosm designs. For example, from monitoring we may discover important differences in the timing of algal blooms, generation time, etc., across different habitats or geographical regions, which must be taken into account in pesticide approval or re-registration.

8 WHAT FURTHER RESEARCH IS REQUIRED TO IMPROVE THE CONDUCT AND INTERPRETATION OF A FIELD/MONITORING STUDY FOR RISK ASSESSMENT?

- More well-designed field studies are required. They should include monitoring studies with a wider geographic distribution to account for differences in routes of exposure, life cycle ecology, etc. (Examples to date have been from

Northern Europe; recovery may be different in different climates, but pest/disease pressure may also be different.)

- If field studies reveal correlations between pesticide exposure and biological effects then these should be regarded as hypotheses. Subsequently mesocosm or other focused studies can then be used for hypothesis-testing.
- Additional taxonomic, life history, genetic and other ecological information (e.g., regional differences in structure and function of communities) to generate and interpret results.
- Better understanding of the mechanisms that underpin species sensitivities. For example, there was an interesting contrast in the sensitivities of molluscs between the Altes Land results (potentially quite sensitive) and forest stream results (insensitive) presented at the workshop and summarised in this report.
- Additional information on landscape and other geographical factors and development of appropriate population models. This may benefit from integration of GIS data.
- Studies intermediate between mesocosm and large scale monitoring studies: a need to resolve causality at an intermediate level and to address issues such as multiple stressors (including natural environmental factors). Methodological guidance on this (e.g., quality criteria) may be required.
- Studies of recovery: better understanding of the mechanisms associated with recovery, including generating data that can be used to parameterise landscape/metapopulation models.
- Modelling of landscape scale impacts to help address the question of ‘What magnitude of impact is significant?’
- Experiments in which stressors are deliberately added to previously minimally impaired locations, and in which stressors are selectively removed (if possible) from locations in which pesticides are used.

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Terrestrial Invertebrates and Plants

1 TO WHAT EXTENT ARE EFFECTS OF PPP SEEN UNDER FIELD CONDITIONS?

The group discussed both dedicated field trials (controlled experiments conducted with adequate controls and often reference treatments in replicated plots) and monitoring (planned or unplanned observations of effects occurring in the field or treated area as a consequence of the use or misuse of pesticides). A short discussion was held to clarify the difference between these two activities, and the essential and desirable features of studies of either kind were listed separately.

The group surveyed some of the general features that characterise good field trials and monitoring studies before going into specific aspects (Table 1). These general features were summarized by Andy Hart, as follows:

- ▶ Ecological relevance: endpoints measured should reflect the major ecological properties of the system
- ▶ Representativeness: the study system should be typical with respect to climatic conditions, for the pesticides, biological properties, geographic position and agricultural systems.
- ▶ Bias/accuracy: the study should be realistic and the measurements should accurately reflect the true structure and function of the system
- ▶ Causality: the observations should be designed so as to reveal wherever possible the mechanisms underlying their occurrence.
- ▶ Detectability: the measurements should be done with sufficient replication and power so as to maximize the likelihood of observing effects if any.

The group discussed the introductory lectures given in the morning with the aim of drawing lessons from them, which would be relevant for the issue of terrestrial invertebrates and plants.

Crucial points of the presentations:

- ▶ *Clook* paper. The UK wildlife incidence scheme seems to work adequately for birds and mammals and to a certain extent also for bees. In general they provide the most useful information for species of larger body mass and of perceived functional/economic importance. The most useful aspect of these schemes is to raise public awareness of adverse effects on non-target species.
- ▶ A honeybee monitoring system is also operational in Germany.
- ▶ No such system is effective for below-ground organisms or non-target arthropods (NTA).
- ▶ The group discussed the possible application of the honeybee scheme to other NTAs? Effects-exposure assessment for bees is possible but for other species this is not considered possible (or very difficult), because developed and accepted schemes are lacking; moreover multiple compounds are used, more than one species must be observed and the time scale when they are applied is very diverse. It is difficult to find dead individuals of many NTA's (methodological problems).
- ▶ Monitoring of bees is mainly done to provide centralized data for incident reporting and for regulatory bodies appraisal of wider scale effects.
- ▶ *Holland* paper. Difference in sensitivity to pesticides between species within the same taxonomic group is often bigger than between different taxonomic groups: so how are we going to deal with this? We need to have data on many species not only on single ones. This is collaborated by the view that the diversity of arthropod species may be greater in the field edges than in the field and concentrating on higher taxa may not give the full impact. The power of monitoring schemes is greatly increased when it takes whole communities or guilds into account, because this maximises the likelihood of detecting effects. However, according to ESCORT 2 field trials for arthropods

should focus on key/relevant NTA species or on species where issues are predicted. Need for taxonomic tools for identification. Lack of life history and generic data on species. Strength of monitoring data is the availability of long-term data (trend analyses possible). Cause-relationship is sometimes difficult to establish. Only one sample time per year could eventually be problematic as indicated by the effect of invertebrate life stage on grey partridge.

- De Jong paper. There was a large amount of variability among the plots before treatment. Random assignment of treatments to plots seems to be problematic. The group discussed that, in general, information on life-history strategies, phenology and habitat requirements

of wild plants are readily available, which is an advantage of the use of plant in monitoring schemes. How important are buffer zones? The data presented showed how important buffer zones were in reducing toxic effects of herbicides on NTP. From the results it is clear that short term effects were seen on growth and phytotoxicity symptoms at low exposure rates and that full recovery occurred. Are we underestimating effects of herbicides on plant feeders? In- or off-crop? Unsprayed field margins are important for diversity and refuge. Moreover, margins are still a very important reservoir of invertebrates.

In the context of regulatory testing protocols in respect of non- crop plant species, recent findings by

Table 1: Essential or desirable features in a successful field/monitoring study

E = essential feature; D = desirable feature; Na = not applicable

- 1) Replication within experiment
- 2) Following options are possible: a) nearby site(s) differing only in potential impact but not in site properties (e.g. climate, vegetation, soil type) – ideal but in reality difficult to find. b) “virtual” reference site(s), derived from a number of “undisturbed” sites or from time series, linking site properties with species distribution – this approach worked in limnology and plant sociology, see Ratte presentation. When an internal control is used (“before – after”-approaches), it needs to be assured that the tested/monitored community is not adapted to chemical stress and reacts sensitive to the substance of concern.
- 3) Reference item or exposure assessment needed
- 4) Guidance document available see Candolfi et al. 2000, J Pest Science 73 (6): 141-147.

Features	Field	Monitoring
Clear Objectives	E	E
Definition of endpoints	E	E
Pre-treatment observations	E	D
Replications	E 1	D
Control	E	D 2
Reference item	D 3	Na
Exposure assessment	D	E
Statistics	E	D
Duration	Shorter typically 1 crop season or one year	Longer generally several years
Area/plot size/location	E 4	D
No. of observation high	D	D
Representatives / Geography	E	E
Meteorological data	E	E
Recovery included	D	D
High taxonomic resolution	E	E
Sampling methods	E	E
Surrounding	E	E
Functional endpoints in addition to structural endpoints	D	D
GAP	E	D

McKelvey et.al. 2002 (Pest Manag Sci 58: 1161-1174) suggest that crop species sensitivity to herbicides is adequate to represent non-crop species response regardless of chemical class or exposure.

SUMMARY OF DISCUSSION:

- 1) Very limited monitoring data on invertebrates other than honeybees are available.
- 2) Limited field trial data are available in the open literature, since most of the performed studies belong to the company's assets.
- 3) More effort to collect life history data is needed for invertebrates.
- 4) Monitoring data are useful but we need to know and define the limitations.
- 5) Identification of species is an issue. For some invertebrate groups (e.g. carabids) good identification keys are available, however, for some others, (e.g. oribatids) good keys are missing. Computer aided identification keys may be helpful. At least a strong need for good educated taxonomists were identified.
- 6) Comprehensive studies needed: multiple parameter studies are needed.
- 7) Need to make more effort to determine exposure level in the environmental compartments likely to be at risk and in the target and non-target species inhabiting that compartment. Exposure data particularly of residues in food items are extremely important also to refine the risk assessment. Exposure assessment is essential in monitoring studies but less so in field studies where application is highly controlled and we have reference compounds. The validity of monitoring studies without exposure assessment is very questionable.
- 8) GIS could be used for space and time resolution; recovery and local extinction are very much dependent on spatial arrangement of treatment and dispersal of invertebrates between plots. GIS could also be very helpful in choosing appropriate sites for the field and monitoring trials.

2 LIST OF ECOLOGICALLY IMPORTANT EFFECTS OR ENDPOINTS WHICH WERE THOROUGHLY INVESTIGATED AND IDENTIFIED WHETHER EFFECTS WERE OBSERVED OR NOT

- Population dynamics
- Species composition/community structure
- Biomass (plants, animals, potentially microbes)
- Organic matter breakdown
- Behavioural studies (e.g. repellency)
- Extent, time, duration and space of effects

Lesson learned from the presentations:

- Brock paper. The author is of the opinion that NOEC and LOEC can be predicted more or less reliably from lab studies (if exposure is similar) but what will happen above the threshold depends on several factors (e.g. structure of system, species, and life cycle). This paper also indicates that current risk assessment practices for individual compounds using appropriate spray drift estimates is adequate to protect sensitive populations exposed to realistic combinations of pesticides. Limited data are available in the literature to verify this for terrestrial invertebrates (e.g. ESCORT 2). Some data are available for soil organisms (earthworms, e.g. Heimbach 1992). Chemical monitoring data are not necessarily linked to effects seen in the aquatic system: poor correlation seen with sampling results. The main determinant of recovery was the generation time especially with aquatic insect species. For the soil compartment, this is not known since no chemical monitoring was done.
- Kreuger & Brown. Effect assessment for plants would be improved if we would have chemical monitoring data. Basic terrestrial in- and off-crop exposure data and information concerning the main exposure routes of the species (e.g. where, when, how much, see Koch posters) are lacking and need absolutely to be generated. Moreover, better usage of existing data (e.g. residue data), arising at times of ecological significance rather than for determining crop residues at harvest, could improve the assessments. The bulk of contamination in the Swedish study seems to be due to point source release. Exposure assessment is an important factor when predicting effects of terrestrial invertebrates for off-crop deposits, droplet size of

spray drift is the critical factor in determining the extent of drift deposits.

- Liess. Monitoring should focus on long-term effects. Parameters other than toxicology are very important in the effects seen and measured. The species' physiological sensitivity as well as their recolonisation potential (both implemented in the species at risk concept, SAR) need to be taken into account when assessing the effects on biocenosis level. Need to define the species at risk concept!
- Römcke. Earlier expert meetings agreed that effects on earthworms and non-target arthropods in the field smaller than 50% are usually falling in the range of natural fluctuation and can often hardly be demonstrated with statistical certainty. Effects above 50% reduction are considered critical. No fundamental long-term effects on NTA were seen in the UK studies (Boxworth-Scarab) but the studies were criticised. Number of tests available for soil organisms (soil micro-organisms, earthworms, mites, Collembola) seems to be sufficient to determine potential effects on soil organisms but validation of the data generated (particularly higher-tier tests) and used for the risk assessment needs to be done. This does not apply for NTA where only data on part of the fauna potentially exposed is assessed (beneficial arthropods). For plants there are only 1st tier tests.
- Mineau: Underestimation of exposure may lead to incorrect risk assessment. Consideration of all exposure routes is essential for a correct risk assessment. We acknowledge the importance of different exposure routes for different species and for chemical substances with particular attributes.

3 HOW DO DATA FROM MONITORING STUDIES COMPARE WITH OUTPUT FROM REGULATORY STUDIES AND/OR RISK ASSESSMENT?

3.1 WHAT APPROACHES ARE APPROPRIATE FOR INCORPORATING FIELD/MONITORING DATA IN RISK ASSESSMENT?

There are appropriate test systems and risk assessment tools in place for the use of field trials for honeybees, earthworms and non-target arthro-

pods (e.g. ISO 1999, IOBC 2000, EPPO 2002). Appropriate monitoring tools are available for honeybees. Number of test available for soil organisms (earthworms, mites, Collembola, see EU Terrestrial Guidance Document 2002) seems to be sufficient to determine potential effects on soil organisms but validation of the data (especially field data) generated and their usage in the risk assessment needs to be done. For non-target plants and soil invertebrates other than earthworms, higher tier study and risk assessment tools are lacking (citation). Bee monitoring data were successfully used to validate the testing and risk assessment scheme (EPPO 2003).

The following approaches were considered to be appropriate for incorporation of monitoring data in risk assessment:

- a) Post-registration soil organisms, NTA or NTP monitoring could be used to validate the current risk assessment procedures.
- b) Such test could also be used to check the effectiveness of risk reduction (mitigation) measures imposed.
- c) Monitoring data can be used to reduce the uncertainty by enlarging the scale and "quality" of data available (e.g. spatial and taxonomic scale in different regions).
- d) Monitoring data can provide public confirmation of absence of significant ecological effects on a field scale.

3.2 HOW TO INCLUDE RECOVERY AND RE-COLONIZATION – ESPECIALLY FROM UNCONTAMINATED AREA

Recovery and re-colonisation data cannot easily be implemented as part of the risk assessment process. The spatial and temporal importance of the components of recovery was recognised. For example, recovery by immigration will be a function of the scale of the treated area. Conversely, recovery by emergence of fresh individuals from within treated areas will be unaffected by the scale of treatment. In monitoring studies these processes are likely to be included by definition. The intensity of pesticide use and spatial distribution patterns particularly for extensive cropping patterns with frequent perturbations, needs be incorporated in the risk assessment. Regional risk assessment is probably the future way to go. Spatial

explicit modelling, using geographic information systems combined with biogeography and dispersal data of the target/non-target species, could be an effective way of achieving this. Reference data on the non-disturbed habitat/community is necessary to provide a benchmark for assessing changes and measuring recovery.

3.3 WHAT GENERAL CONCLUSION CAN BE DRAWN, ON THE BASIS OF CURRENT EVIDENCE, ABOUT THE LEVEL OF AGREEMENT BETWEEN LAB-BASED RISK ASSESSMENT AND FIELD EFFECTS?

Laboratory data are considered to be worst-case and therefore appropriate to screen for harmlessness. If you get the exposure of the lab study correct (i.e. route and level of exposure) the prediction of the field effect should probably be good for the initial or threshold value. Consideration of exposure duration (appropriate test design for the species) and measuring the correct endpoints (e.g. acute, chronic, food web, function) are essential. In addition to that, indirect and long-term effects may also depend on factors, which cannot be incorporated in lab tests like landscape, climate, species composition and so on.

3.4 FURTHER RESEARCH REQUIRED TO IMPROVE THE CONDUCT AND INTERPRETATION OF A FIELD/MONITORING STUDY FOR RISK ASSESSMENT

The following research areas need further attention:

- ▶ Life-history, life-cycle, dispersal and other ecological data including food sources for soil invertebrate and NTA are needed
- ▶ More knowledge should be generated on what are the key species in the different agro-ecosystems
- ▶ Species distribution and behaviour data. Interactions between species in trophic networks.
- ▶ Taxonomy
- ▶ Exposure data especially drift deposit and distribution patterns in terrestrial off-crop habi-

tats (e.g. hedges) and probabilities of being exposed

- ▶ Relate mode of action and species-specific metabolic pathways to effects observed
- ▶ Determine maximum duration of effects that will not cause long term population decline (development of population models would be very useful for this).

Birds and Mammals

1 GENERAL COMMENTS ABOUT EFFECTS OF PESTICIDES IN THE FIELD

Agricultural fields are inhabited by a broad range of avian and mammalian species. Mammals and birds may have their nests or burrows there, or visit fields in search for food, or just pass through. Therefore they inevitably come into contact with pesticides. When trying to predict effects then the exposure assessment turns out to be a hard nut to crack. The reason is that the exposure for an individual, expressed as dose or body burden, is not only a function of the environmental concentration but also depends on the animals' behaviour which is highly variable. Therefore there is a need for monitoring studies not only to find out what happens in the field but also to improve exposure models and risk assessment schemes by supplying input data and data for calibration.

Besides direct (toxic) effects pesticides may exert indirect effects by altering the habitat and the food availability. Indirect effects may be influenced by agricultural measures other than pesticide use. Monitoring studies may be designed to determine either direct or indirect effects. Sometimes, however, it is challenging to find the causal network behind an observed effect.

Monitoring studies have clearly shown that there are direct as well as indirect effects of pesticides on birds and mammals. The extent of such effects remains unclear, however it appears that indirect effects are of more concern than direct effects.

2 FIELD MONITORING STUDIES

2.1 TYPES OF STUDIES

There are no standardized procedures for field studies and monitoring projects, instead there is a broad spectrum of approaches. For the purpose of this workshop the group considered the useful

classification which was presented in the keynote lecture by Mark Clook.

- ▶ Effects field studies
- ▶ Ecological field studies (also called 'generic' field studies')
- ▶ Residue field studies
- ▶ Proactive monitoring studies
- ▶ Reactive monitoring studies

2.2 INVENTORY OF ENDPOINTS

The following collections comprise endpoints that have been part of field/monitoring studies or that are often discussed. The aim of the workshop has not been to discuss methods and endpoints in detail, therefore a few references only are given (Greaves *et al.*, 1988; Somerville and Walker, 1990; Maltby *et al.*, 2001; Pastorok *et al.*, 2001; Munns *et al.*, in press).

a) Individual level

- ▶ Behavioural responses
 - ▷ Avoidance
 - ▷ Food collection, foraging plot choice, feeding rates
 - ▷ Time budgeting
 - ▷ Courtship behaviour
- ▶ Survival (mortality, debilitation)
- ▶ Reproductive performance
- ▶ Physiological parameters
 - ▷ Analysis of faeces for the substance and/or metabolites
 - ▷ Enzyme-assays
 - ▷ Fat content
 - ▷ Egg shell thickness
 - ▷ Sub cellular endpoints (e.g. genotoxicity)
- ▶ Tissue residues

b) Population level

- ▶ Abundance (including spatial distribution)
- ▶ Vital rates
- ▶ Population growth rate
- ▶ Return, recovery, extinction rates

- Population structure
- Inbreeding coefficient

c) Community level

- Shifts in species dominance

In the case of birds and mammals, population and community endpoints are challenging due to the large home ranges of the animals and long generation times (Kendall and Lacher, 1994). Sometimes community parameters are used to characterize the suitability of the study area rather than to interpret them in terms of effects.

3 LIMITATIONS AND DESIRABLE FEATURES OF FIELD/MONITORING STUDIES

3.1 LONG-TERM SPECIES-SPECIFIC MONITORING STUDIES

Such studies are usually not designed to examine just pesticide effects but instead look at the ecology of individual species. However, pesticide effects are sometimes detected (which depends on whether there is a variation with regard to pesticide use, or any obvious hints on causal effects).

An example of a thorough investigation is the partridge survival project (Sussex study). The study was started in 1968 as part of a suite of work to investigate the reasons for the decline of the Grey Partridge. The extensive monitoring has continued ever since. This data was used in the 1980s to identify causes behind the decline which turned out to be the reduction in availability of chickfood (Potts, 1986). This led to the Cereal and Game birds project and from this the development of Conservation Headlands. This later work included a multitude of methods, best summarised in Sotherton, 1991. In the late 1990s the Sussex data was re-analysed to look at impact of pesticides (Ewald and Aebischer, 1999; 2000; Holland, presentation). The study can briefly be described as follows:

- Triggered by observed population decline of the Grey Partridge
- Aimed at general effects of farming practice and indirect effects of pesticides, but excluded direct toxic effects of pesticides

- Multitude of methods and techniques (radio-tagging, diet composition via faecal analysis, nesting parameters)
- Length of monitoring (20 years) was important for its success
- There could be demonstrated the chain of causal links from pesticide use to insect abundance to chick survival to population size
- Results have not been used for the assessment of individual pesticides but led to management measures (recommendations with regard to conservation headlands).

Similar long-term monitoring studies:

- Corn bunting study: There was the same hypothesis of cause and effects as in the partridge project, and it was also proven (Brickle *et al.*, 2000)
- Turtle Dove study: The study compared ecological parameters in the 1990s to the 1960s; there could be demonstrated a change in diet, an increase in foraging distance and a decrease in the number of nesting attempts (Browne and Aebischer, in press)
- Sparrowhawk study (Newton, 1986; Newton and Wyllie, 1992; Sibly *et al.*, 2000)
- Studies on yellowhammer (Morris *et al.*, in press)
- Studies commissioned by the Danish Environmental Protection Agency (Petersen, 1996; Petersen and Jacobsen, 1997)

3.2 FARM SCALE EXPERIMENT

The Breakout Group identified farm-scale experiments as a distinctive type of field study, in which large blocks of farmland comprising multiple fields are subjected to contrasting treatments. Due to their cost, there are few examples of such studies. A well-known example for terrestrial vertebrates is the Boxworth Project (Greig-Smith *et al.*, 1992). Avian endpoints studied at Boxworth included abundance, reproductive performance, frequency of nest visits by birds feeding young, diet composition (from faecal samples), and cholinesterase inhibition in nestlings. The project also measured abundance of wood mice using mark-release-recapture methods. The project was affected by a number of limitations. The most important, recognised in the project report, was the lack of replication: due to cost limitations there

was only one block of farmland for each treatment. This made it difficult to assess whether differences between the blocks were due to the treatments or to confounding factors. In addition, despite the relatively large size of the blocks, the number of active bird nests available for study at the time of any particular pesticide application was small. The only vertebrate endpoint that showed a clear pesticide effect was the abundance of wood-mice, which decreased markedly and then recovered following application of methiocarb slug pellets.

Other examples of farm-scale studies involving vertebrate endpoints include:

- ▶ UK organic farming studies (Anonymous, 1995)
- ▶ Danish yellowhammer and skylark studies (Petersen *et al.*, 1995; Odderskær *et al.*, 1997)
- ▶ UK Game Conservancy study on conservation headlands (Sotherton, 1991)

3.3 REGULATORY FIELD STUDIES

Regulatory field studies are usually undertaken to determine the direct effect following the use of a specific compound. To that end they are planned as controlled field studies in contrast to monitoring studies. To be useful for pesticide regulation it must be possible to demonstrate the causal relationship of observed effects to the pesticide.

Typical study designs include one or more of the following endpoints:

- ▶ Mortality (carcass searching)
- ▶ Censuses (e.g. CMR)
- ▶ Reproductive performance (observation of nest boxes)
- ▶ Biochemical endpoints (provide a good link with the pesticide, but are not available for all kinds of pesticide chemistries)

Carcass searching is useful if it is carried out in an appropriately and the pitfalls are avoided: Efficiency and power must be demonstrated to be sufficient, observer disturbance must be avoided, and the cause of death has to be determined. Biochemical endpoints (biomarkers) may provide a good link with pesticide exposure or effects, however, are not always available. Finally, the representativeness of the study in terms of the proposed use is crucial, for example is the location and environment, climate, diversity of birds/mammals etc all appropriate to the proposed use of the compound?

3.4 INCIDENT SCHEMES

The Breakout Group discussed reactive monitoring schemes, they defined this type of scheme as one that considers whether the death of a bird or mammal has been due to the use of a pesticide and if so whether it is due to the correct use, misuse or abuse of a compound.

The scheme considered by the Breakout Group in detail was the UK Wildlife Incident Investigation Scheme (WIIS) (Barnett *et al.*, 2002). It was noted that this scheme covers other organisms apart from birds and mammals. It was agreed that WIIS was a 'gold standard' as regards reactive monitoring schemes.

The Group identified the following strengths:

- ▶ a safety net for the regulatory systems in that it highlights some incidents which had not been predicted by the regulatory system,
- ▶ it is countrywide,
- ▶ highlights the need for risk management issues,
- ▶ highlights issues regarding misuse and abuse

The Group identified the following limitations:

- ▶ Low probability: It was agreed that there was a low probability of an incident being reported; this is due to the fact that in the first place there is a low probability of an organism being found, that this carcass will then be reported to WIIS and then that the incident will be accepted by WIIS and assessed.
- ▶ Biased: The Scheme only measures lethality; it is more likely to consider large conspicuous species, species of conservation interest/value, and those that form large flocks rather than small species.

Another successful incidents scheme is established in France. It is operated by a national network (SAGIR) and generally aims at wildlife health, not only at pesticide poisonings. Its focus had been mainly on game species but the scheme is now extended also to other species. Apart from that the organisation of incident investigation schemes in Europe seems to be underdeveloped (deSnoo *et al.*, 1999).

4 HOW SHOULD FIELD/MONITORING DATA BE USED IN RISK ASSESSMENT?

4.1 INCIDENTS DATA

From the nature of these data they can only be used retrospectively. If incidents come up they flag a risk; however, in the absence of incidents no conclusions can be drawn, i.e. a lack of reported incidents does not mean a lack of incidents.

4.2 EFFECTS FIELD STUDIES

Concern was raised about the usefulness of field effect studies, particularly relating to the difficulty of proving a lack of effect. However, such studies should not be excluded and when conducted and used in risk assessments they must meet at least the following quality requirements:

- ▶ The endpoints must be relevant.
- ▶ A solid database is necessary for a quantitative evaluation. The study design and statistical power must be sufficient to detect effects.
- ▶ The trial should not be biased in any way (e.g. exposure reduced due to disturbance by study personnel)
- ▶ The link between cause and effect should be clear
- ▶ The study needs to be representative of the full range of conditions expected in normal use.

If effects are seen then this confirms risk. However, the absence of effects does not prove that the product is safe and would not immediately overturn the lab-based risk assessment. However, the results could be valuable if there are additional endpoints that help to explain why there have been no effects (e.g. biomarkers, residue analysis) and if it can be demonstrated that the study conditions are genuinely worst case. The interpretation is facilitated if the study is accompanied by other field type studies, e.g. avoidance/feeding behaviour and residue studies.

4.3 ECOLOGICAL/RESIDUE FIELD STUDIES

The main purpose of these data is to improve risk assessment procedures by feeding input parameters into exposure models (deterministic and probabilistic).

5 HOW SHOULD POPULATION EFFECTS, RECOVERY, AND RECOLONIZATION BE CONSIDERED

With birds and mammals losses of a few individuals may already be considered unacceptable by risk managers, and thus regulatory action may be taken prior regardless of any adverse effects at the population level. Nevertheless, even in those cases assessors should include a statement in the risk characterization outlining potential population effects. Experience from risk assessments has shown that the issue of recovery and recolonization mainly becomes relevant with small mammalian species which are sedentary in the treated area, for example small rodents in pastures or orchards.

6 GENERAL CONCLUSION ABOUT THE LEVEL OF AGREEMENT BETWEEN LAB-BASED RISK ASSESSMENT AND FIELD/MONITORING STUDIES

6.1 ARIOUS FIELD WORK AND MONITORING PROGRAMMES

Apart from the studies covered by the meta-analysis (see below) there are detailed data from lab and field on a range of compounds. The following list gives examples, but is not exhaustive:

- ▶ Fonofos: effects from seed treatment (Hart *et al.*, 1999)
- ▶ Dieldrin/Aldrin: effects from seed treatment use (Murton and Vizoso, 1963).
- ▶ Fenitrothion (Pauli *et al.*, 1993)
- ▶ Rodenticides: barn owl and polecat monitoring study (Newton *et al.*, 1999)
- ▶ Methiocarb: effects on wood mice from molluscicide use (Greig-Smith *et al.*, 1992)
- ▶ Azinphos-methyl: effects on small mammals (Edge *et al.*, 1996)

Without going deep into the details of individual studies the group made the following general observations:

- ▶ Field observations include evidence of effects not covered by standard risk assessment (other routes of exposure, indirect effects).
- ▶ The quality of field data is not always sufficient to draw any conclusions
- ▶ Even where data are good it is not necessarily possible to link findings with risk assessment

due to confounding factors. Often there are case-specific or compound-specific factors making it difficult to make statements with regard to the general suitability of risk assessment procedures (e.g. avoidance, persistence, secondary poisoning).

- There could be valuable material in the literature which should be searched for and evaluated.

6.2 META-ANALYSIS OF PIERRE MINEAU

The study is published (Mineau, 2002) and was presented to the workshop as a keynote lecture. The breakout group considered the study the best available and suited for this purpose. However, a few points have to be observed: Only organophosphate and carbamate pesticides are covered; with regard to location there is bias towards North American studies. Despite these issues the group felt able to draw the following conclusions:

- According to this study the current EU Tier-I-risk assessment procedure (Anonymous 2002) produces a small proportion of false negatives (i.e. TER_{acute} > 10 for pesticides which have caused mortality in field studies); when based on quail toxicity it is about 5% of the compounds that were included in the study, however, the scheme produces a high rate of false positives (70%).

Note: The figures of 5% and 70% are associated with some assumptions and uncertainties: firstly, with regard to the data which are available for running the risk assessment scheme (e.g. number and kind of species tested), secondly with regard to a potential bias in the field data set as mentioned above, and thirdly with regard to the definition of a “positive” from the field data set (critical level of mortality rate, observed vs. modelled probabilities)

- The study suggests that the predictive power of the Tier-I-risk assessment scheme is not satisfactory, because it misclassifies a substantial proportion of pesticides. Improving the parameters for the current risk assessment model (e.g. RUD = Residue per unit dose) is desirable, however, tuning the parameters to a lower rate of false negatives probably will result in an increase of false positives. It rather appears that the model itself has certain deficits (e.g. the exposure part of the model). If that were

true then working on the model structure could enhance the discriminatory power.

- There are strong indications that dermal exposure is not negligible as the current risk assessment scheme supposes, at least for organophosphate pesticides.

7 WHAT FURTHER RESEARCH IS REQUIRED TO IMPROVE THE CONDUCT AND INTERPRETATION OF A FIELD/MONITORING STUDY FOR RISK ASSESSMENT

- Proactive monitoring is currently rarely used with regard to birds and mammals, but its potential should be explored.
- Ecological data (‘generic’ field studies or literature reviews) should be generated to help interpret classical style effects studies and to produce input data for exposure modelling.
- Consideration could be given to establish “benchmark cases” that could serve as references for other compounds and that could be used for validation of laboratory-based risk assessment schemes.

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IMPRESSUM

Proceedings from the workshop:

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A SETAC publication

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The EU Uniform Principles for the assessment of plant protection products (PPP's) require that if the preliminary risk characterization indicates potential concerns, it has to be granted that "...under field conditions no unacceptable impact on the viability of exposed organisms ..." occurs. To date, such assessments have been made by conducting higher-tier studies. The aims of the workshop, were to: (i) review available field monitoring studies addressing the environmental effects of PPP's due to agriculture, (ii) compare observed effects of PPP's under field conditions with the impact predicted on the basis of the current risk assessment guidance, and (iii) identify requirements for future monitoring studies and the design for higher tier tests.

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