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Working Document

Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC

This document has been conceived as a working document of the Commission Services which was elaborated in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives under Annex II, III and VI of Commission Directive 91/414/EEC, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

1 Introduction

Article 5 of the Directive provides that "*in the light of current scientific and technical knowledge, an active substance shall be included in Annex I for an initial period not exceeding 10 years, if it may be expected that plant protection products containing the active substance ... do not have any unacceptable influence on the environment ...*".

Annexes II and III of Directive 91/414/EEC set out the data requirements for the inclusion of an active substance into Annex I of the Directive and for the authorisation of a plant protection product at Member State level. Annex VI of the Directive includes the decision making criteria for the authorisation of plant protection products at Member State level.

It is the purpose of this document to provide guidance to Rapporteurs, peer reviewing Member States, Notifiers and Applicants on the use and interpretation of the terrestrial ecotoxicology sections of Annexes II and III and to lay down agreed procedures and criteria for decision making. The general aim is to promote consistency and transparency in decision making and to describe agreed risk assessment procedures for the assessment of plant protection products in the context of the inclusion of their active substances in Annex I to Directive 91/414/EEC.

It has to be recognised that the authorisation of plant protection products after Annex I inclusion of active substances remains the responsibility of Member States. Risk management and risk mitigation measures described in this document do not pre-empt this authority of the Member States and are meant as a non-exhaustive list of agreed options, which can be taken into consideration on the Community level for decision making concerning Annex I inclusion.

The ecotoxicology data requirements for active substances and plant protection products are set out in Annex II, section 8 and Annex III section 10 of Directive 91/414/EC, respectively. It should be noted that the introduction to these sections provides useful information on the purpose and use of data submitted. It is clearly stated that the data submitted must be sufficient to permit a scientifically valid assessment of the impact on non-target species. In order to fulfil this objective, tests additional to those outlined in Annex II and III may be needed in individual cases if there is a specific justification.

Tools and techniques in ecotoxicological risk assessment progress rapidly and it is noted that it is difficult for both notifiers or applicants as well as reviewers to take such progress fully into account in their dossiers and assessment reports during ongoing reviews. To provide a reliable framework for the review process and to avoid undue delays, the current version of this Guidance document should therefore only be used for the review of existing active substances notified in the third phase of the review programme according to Regulation 451/2000[1] and subsequent phases. For new active substances the document should be implemented for dossiers submitted from 1 August 2003. However, some flexibility may still be necessary during a transitional period of 2-3 years. Decision making should take into consideration that certain higher tier data requirements (e.g. litter bag studies) which are triggered now, may not have been obvious to applicants or notifiers at the time of their notification or dossier submission. Likewise, if this appears justified in individual cases and

[1] OJ L 55, 29.02.2000, p.25

facilitates decision making, the updated guidance may be considered also for substances in earlier phases of the review programme.

The document is to be revised regularly, in order to reflect changes of test guidelines and of scientific knowledge.

2 General issues

2.1 Introduction to the assessment of chemicals in the terrestrial environment

The assessment of the effects and risks of chemicals for the terrestrial environment is a complex matter. This complexity comes, among others, from factors such as the need for sharing of the available landscape among urban/industrial activities, agricultural production in the form of agro-systems, and supporting terrestrial ecosystems. In addition, terrestrial systems are not associated with a single compartment, but with the interface between soil and the atmosphere. Although purely soil-dwelling organisms play a clear role, basic ecosystem functioning and biodiversity is associated with organisms, such as terrestrial plants, many invertebrates, and certain terrestrial vertebrates that are simultaneously or sequentially located in the soil or above-soil compartments.

The risk assessment for terrestrial ecosystems has been reviewed by the Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE 2000). According to this document:

"General adverse effects on the terrestrial environment include:

- Effects on soil functions, and particularly on the capacity of soil to act as substrate for plants including effects on seed germination, and those on organisms (invertebrates, micro-organisms) important for proper soil function and nutrient cycle conservation.
- Effects on plant biomass production, related to contamination of soil or air including deposition on plant surfaces. Plants are the source of food for the whole system (including humans) and have additional roles in terms of land protection, nutrient cycles, equilibrium of gases in the atmosphere, etc.
- Effects on soil, above-ground and foliar invertebrates, which represent food for other organisms, and cover essential roles as pollinators, detrivores, saprophages, pest controller, etc.
- Effects on terrestrial vertebrates exposed to contaminated food, soil, air, water or surfaces, with obvious economic and/or social consequences. Poisoned birds and mammals probably constitute the highest social concern, while reproductive effects, although less evident, represent a higher ecological hazard.
- Accumulation of toxic compounds in food items and through the food chain. Is a typical exposure route for animals within the contaminated ecosystem and represents an additional concern related to the consumption of this food by humans and domestic animals.

These concerns combine human and ecological interests. Direct human interests include managed species (cultivated plants and trees, bees, domestic animals) but also wild species essential as source for supplies (e.g. forest, pasture), landscape conservation (e.g. vegetation cover), or even for leisure (from gaming to bird-watching). From an ecological point of view, any of these effects will provoke a dramatic alteration of the structure and

functioning of the ecosystem which are considered the basic protection goals in ecological risk assessment."

However, as frequently noted by the Scientific Committee on Plants, the environmental risk assessment of plant protection products requires some adjustment of the generic ecological risk assessment framework as effects on living organisms considered as pests can be both acceptable and desirable.

Directive 91/414/EC includes the need for specific assessments on certain terrestrial non-target groups, such as terrestrial vertebrates, bees, other non-target arthropods, earthworms or soil micro-organisms, as well as additional generic assessments such as on soil macro- and mesofauna when triggered by fate properties (persistence).

Targeted risk assessment, using a combination of key ecological receptors and relevant exposure routes has been recently suggested as an efficient way of solving the complexity of the terrestrial environment risk assessment (Tarazona et al. 2002). This possibility fits perfectly with a protection aim established for plant protection products, allowing the identification of target species and non-target ecological receptors.

There is a common understanding that the ecological risk assessment aims not at individuals but at the protection of populations. In general the continuance of populations of non-target organisms should be ensured. Structural and functional endpoints should be regarded of equal importance.

2.2 Animal experimentation

For reasons of animal welfare all efforts should be made to avoid unnecessary tests especially on vertebrate species.

2.3 NOEC-values as summary parameters

In several tests the aim is to determine the no-observed-effect concentration (NOEC), a concept that has been challenged on scientific grounds (Laskowski 1995, OECD 1998). The OECD, and also ISO now give preference to regression-based parameters and in newly drafted guidelines give the choice for an ECx approach. (Note: The terminology referring to concentration (NOEC, and ECx) is used for convenience; the same applies, of course, to effect levels expressed as dose, application rate, etc.). NOEC tests are still acceptable, of course, however it should be ensured that the statistical power of the individual test is satisfactory. To that end some guidelines state the maximum permissible variation coefficient for certain variables. If such validity criteria are missing the typical power of that type of test should be used as a rule. For instance, if a test usually is able to detect a 20-% difference from the control then a treatment group with a difference of 40 %, which is statistically not significant, should not be accepted as a NOEC. For background information see OECD (1998). The OECD is currently working on a guidance document on statistical analysis of ecotoxicity tests.

2.4 Test substance, formulation testing

Test substance for Annex-II data requirements

In general the studies outlined in Annex II should be conducted using the technical grade material of the active substance. However, certain study types may be conducted with a

formulated product instead of the active substance. This may be applicable to, for example, non-target arthropod studies, the earthworm reproduction test and the soil micro-flora test. The formulation used could be that covered in the corresponding Annex III dossier (the so-called lead formulation) hence the same study could fulfil the Annex II requirement as well as the Annex III requirement. As Annex II data aim at characterising the active substance it is usually not possible to use a formulation containing additional active substances. Some lead formulations contain more than one active substance; results could be acceptable when there is no effect up to the top dose level or at the limit dose; otherwise it would be difficult to attribute the toxicity to one or the other substance.

The need for standard toxicity tests on the lead formulation (Annex III)

One Annex III package for a representative formulation has to be submitted to enable Annex I listing. Annex III contains certain study types that are also part of Annex II (standard laboratory tests with birds, bees, arthropods, earthworms and soil microorganisms). Each Annex point has to be addressed; however, it is not always necessary to generate experimental data with the formulation; instead the data on the active substance could be sufficient. The decision should be based on the following considerations:

- If the risk indicators (TER, HQ) based on the active substance are well above the TER trigger or below the HQ trigger (e.g. 100-fold) then studies with the formulation could be considered dispensable. However, a decision should be made on a case-by-case analysis in agreement with the RMS and be reported.
- It might be sufficient to test the formulation with that species of a group that was most sensitive with the active substance.
- In cases where further information is considered necessary it should be examined, whether a direct step to higher-tiered-tests would be more appropriate than repeating the basic test with the formulation.

If a notifier is of the opinion that tests with a formulation are not needed, an explanation must be given.

2.5 Endocrine effects

Endocrine disruption is to be viewed as one of the many existing modes of action of chemicals and thus can be assessed in the normal conceptual framework. However, endocrine disrupting chemicals typically affect certain life stages during reproduction and development, so potential effects may remain undetected if a test covers only a part of the reproductive cycle, as is the case in the avian one-generation study. The OECD is currently engaged in reviewing the test guidelines and where necessary improving the protocols (Task Force on Endocrine Disrupter Testing and Assessment (EDTA)). As soon as amended methodology is validated and agreed on, then this should be applied in the assessment. Meanwhile it should be considered whether evidence from mammalian studies and existing ecotoxicological studies suggests on endocrine effects such as thyroid or gonadal tumors, abnormal sex differentiation and sex organ development. In such cases the available information, e.g. from a current avian reproduction test should be re-evaluated carefully (see SCP 1999).

2.6 Higher tier tests

The data requirements (Annex III) contain a suite of higher tier tests that can be submitted if the results of the basic tests are not sufficient to decide that the risk might be acceptable and to allow for a decision with regard to inclusion of an active substance into Annex I. It should be noted, however, that (semi)field tests are not the only option for refining the assessment. Before conducting such tests other possibilities to address the problem should be considered.

Higher tier tests aim at one or more of the following purposes:

- generate information on certain parameters of the risk assessment (e.g. an avian acceptance test gives information on the palatability of potential food items which is used to refine the food consumption rate and thus the exposure estimate of the exposed species)
- investigate effects under more realistic conditions (semi-field and field tests)
- produce effects data for a wider range of species and include inter-species interactions (e.g. model ecosystems or soil community tests in the field)

Higher tier tests generally provide information on exposure and effects under more realistic conditions compared with standard laboratory tests. Therefore many uncertainties are reduced, however, as some of the variables are not under the control of the experimenter, the results tend to be less reproducible.

With regard to methods some tests such as the bee field test are standardised and fairly easily conducted. Other tests have to be planned on a case-by-case basis (e.g. terrestrial vertebrate field tests). Usually the results of the basic tests together with background information are used to define clearly the objective of the study and to select the appropriate methods, endpoints and study design in order to make sure that the study focuses on the identified concerns. Thus, the following should be considered: species at risk, type of effect (e.g. mortality or sub-lethal effects), duration of effects (e.g. are acute or long-term effects expected?), whether recovery is to be studied. When planning a higher tier study the notifier might wish to discuss the protocol with the Rapporteur Member State or consult independent experts.

2.7 Persistence

Persistent active substances and metabolites are of special concern as influences on organisms can continue to act over generations, they may have multiple effects, and any recovery may take an unduly long time. Therefore, a higher degree of scrutiny is needed to assure that non-target organisms are not affected. The assessment has to ensure that all routes of exposure are adequately considered. Persistence may be accompanied by greater bioaccumulation than would be observed for a non-persistent substance and this also should be fully addressed. Aquatic bioaccumulation data cannot be transferred to terrestrial organisms; however there are models available which describe the behaviour of an active substance/metabolite in soil organisms based on simple data (e.g. Connell and Markwell 1990, Jager 1998) as well as models to describe food chains to mammals and birds (Romijn et al. 1994). It has to be observed that not all of these models are validated, and up to now they are not routinely used for regulatory purposes. Furthermore the applicability of these models is restricted to certain chemical types.

According to Annex VI 2.5.1.1 no authorisation shall be granted "*if the active substance and, where they are of significance from the toxicological,ecotoxicological or environmental point of view, metabolites and breakdown or reaction products, after use of the plant protection product under the proposed conditions of use during tests in the field, persist in soil for more than one year (i.e. DT90 > 1 year and DT50 > 3 months), or during laboratory tests, form not extractable residues in amounts exceeding 70 % of the initial dose after 100 days with a mineralisation rate of less than 5 % in 100 days, unless it is scientifically demonstrated that under field conditions there is no accumulation in soil at such levels that unacceptable residues in succeeding crops occur and/or that unacceptable phytotoxic effects on succeeding crops occur and/or that there is an unacceptable impact on the environment, ...*"

If certain persistence triggers are exceeded, further tests with soil organisms are to be conducted (see chapter 6.1). With regard to bound residues effects on soil organisms are unlikely as long as the substance is not bioavailable. However, under certain conditions bound residues may become bioavailable and therefore a risk cannot be ruled out. Therefore it is proposed that the same data requirements should apply as for those substances with a $DT90_f$ of >365 days and a $DT50_f$ of >3 months. If there is convincing evidence from the fate data package (for example release rates, release behaviour) then further data may not be necessary.

2.8 Risk assessment

Risk characterisation

For risk assessment purposes it is common to use quotients which combine exposure and effect in order to characterise the risk. However, there are numerous ways in which such indicators could be formally defined. Unfortunately terrestrial ecotoxicology within the framework of Directive 91/414/EEC is not uniform in this regard for various reasons. Currently it uses TER values (terrestrial vertebrates, earthworms) along with HQ values (for bees). In this Guidance Document it became necessary also to introduce an indicator for arthropods taken from the ESCORT II document (Candolfi et al. 2001) where it is termed HQ. This document retains the terminology and definitions laid down in Annexes II, III and VI of 91/414/EEC. Nevertheless, it is useful to give a few explanations: Risk indicators are particular with regard to the following properties:

Direction of quotient (toxicity to exposure or exposure to toxicity)

Usually indicators under 91/414/EEC relate toxicity to exposure (TER) which means that the higher the figure the greater the safety. Exceptions are the hazard quotients (HQ) for bees and other non-target arthropods where the opposite applies, exposure being divided by the toxicity (the higher the figure the greater the risk).

Unit concordance

Mostly exposure and effects are expressed in the same unit, e.g. both as concentration in soil (mg/kg), or both as dose per body weight (mg/kg bw). This is also true for arthropods (g/ha or ml/ha). The only exception is the hazard quotient for bees where application rate (g/ha) is divided by bee LD50 (µg/bee); the latter relation makes sense, of course, as the application rate is a measure for exposure and the bee LD50 is a measure for effect. However the absolute level of the resulting HQ is meaningless without calibration; (in this case calibration has been done, see next point).

Validation, rationale for critical TER and HQ

TER values are defined such that the toxicity is taken from standard tests with the most sensitive of the tested species and the exposure is an estimate of the realistic worst case. In order to account for uncertainties (e.g. tested species vs universe of species, lab to field) assessment factors are introduced which under 91/414/EEC appear as critical TER values, e.g. 10 for the acute TER for terrestrial vertebrates and earthworms. Although founded on general experience in risk assessments the critical TERs are somewhat arbitrary (Chapman et al. 1998, SCP 2002). In contrast, the critical HQ of 50 for bees as well as the critical HQ of 2 for arthropods have a different reasoning. These values have been established according to a validation procedure where the HQ was compared with (semi)field data. The predictive power of these two HQ are therefore better defined. (It should be noted that as regards the non-target arthropod trigger value of 2, there has been some criticism due to the limited nature of the data set). Two principle points have to be observed:

- The critical HQ is only applicable to situations and conditions which have been included in the validation; for example, with both, arthropods and bees, the validation included spray applications only.
- The critical HQ is only applicable if the HQ is calculated in the same way as for validation; for example with arthropods the validation has been conducted using LR50 data from glass plate tests, not for effects data from other tests (Candolfi et al. 2001).

Interpretation of TER and HQ values

TER and HQ values should be used as indicators of risk in the assessment process. In cases where the calculated values do not meet the relevant trigger the provisions in Annex VI require that no authorization shall be granted unless it is clearly established through an appropriate risk assessment that no unacceptable effects occur under field conditions. There are several options to proceed, for example:

- refined exposure estimates
- refined effects assessment
- higher tier studies
- re-evaluation of the risk in more detail, considering the magnitude, probability and ecological significance of effects
- consideration of risk reduction measures (determined at Member State level when granting authorisations); examples are given in chapters 3.4, 4.4, 5.4, 6.4
- no authorisation of certain uses of particular concern or, finally, of all uses.

Applying risk mitigation measures and refining the toxicity and exposure estimate will result in new TER values. These amended values should be compared to the appropriate Annex VI values again to indicate whether the proposed risk mitigation measure is adequate. (HQ values underlie some constraints in this regard, see above). In higher tier studies, however, exposure is usually part of the study design, so that the results are not used for a formal TER (or HQ) calculation but immediately interpreted in terms of risk. If sufficient risk reduction measures cannot be identified, non-inclusion of the substance into Annex I of Directive 91/414/EC must finally be considered.

Example 1: The basic data may show that a product is toxic to bees with a hazard quotient clearly above the trigger of 50. If higher-tier studies confirm the risk then effective risk mitigation measures are a prerequisite for the authorisation. In this case the use could be restricted to glass-houses that are inaccessible to bees (and where no pollinators are introduced), or a label phrase could be required that would exclude applications to flowering plants (if that is compatible with the intended use of the product).

Example 2: The avian acute and dietary toxicity data for a seed treatment may indicate a high risk for seed-feeding birds with TER_a- and TER_{st}-values (according to the standard calculation) below the trigger values of 10. The refined risk assessment re-examines the worst-case assumption that birds feed exclusively on treated seed. This re-assessment is reliant upon additional data, i.e. the results of palatability studies and/or field studies. These studies may demonstrate a clear avoidance of treated seed so that it is considered unlikely that birds in the field would ingest sufficient seed to cause toxic effects and the risk may be judged as acceptable.

Probabilistic risk assessment

The traditional TER-based approach uses point estimates for the input parameters (e.g. lowest available toxicity figure, highest exposure level) and involves an overall factor (= critical TER) to cover the various sources of uncertainty. Such a deterministic assessment has limitations with regard to the quantification of the risk. This problem could be overcome by newly emerging probabilistic approaches. Performing a probabilistic risk assessment (PRA) involves assigning probability density functions to the various components that affect risk, and then carrying out Monte Carlo simulations or other calculations in order to estimate the probability that a certain event takes place. At present PRA has some shortcomings:

- For many input parameters reliable information on the distribution is lacking
- There are no common standard methods for the statistical calculations

The result of the assessment appears complex in nature and thus may be difficult to communicate to non-experts. However, that should not be regarded as a drawback.

Strengths and weaknesses of PRA methods and their applicability for regulatory purposes are presented in Hart (2001). It should be noted that some weak points such as lack of information on distributions are likewise shortcomings of current deterministic approaches. Furthermore, generic data may be used where specific data are insufficient. In conclusion, PRA methods must be regarded as promising tools and already now there may be situations where their use could be envisaged.

2.9 Metabolites

Introduction

The active substance of a plant protection product may be transformed in the environment by either abiotic or biotic processes. Under Directive 91/414/EEC, the potential risks that these metabolites pose to terrestrial organisms must be assessed.

Definitions

To facilitate clear understanding the following generic definitions are used in this guidance document:

Metabolite
For the purpose of this document, the term is used for all breakdown products of an active substance of a plant protection product, which are formed in the environment by biotic or abiotic processes after the application.

Major metabolite
All metabolites that are formed in amounts of ≥10 % of the applied amount of active substance at any timepoint evaluated during the degradation studies in the appropriate compartment under consideration.

Minor metabolite
All metabolites, degradation and reaction products that are formed in amounts of <10 % of the applied amount of substance of active substance at any time during the degradation studies under consideration.

Ecotoxicologically relevant metabolite
A metabolite which poses a higher or comparable risk to terrestrial organisms as the active substance. Such a metabolite is relevant for the overall decision on Annex I inclusion or for definition of risk mitigation measures.

Definition of ecotoxicologically significant residues (Annex VI, B.2.6.2)
An active substance or – if appropriate – a metabolite for which an analytical method has to be established for monitoring purposes (see below).

Relevant compartments

When assessing risks to terrestrial organisms, metabolites in the following media and compartments have to be considered and the potential risk for the respective organisms should be addressed:

Soil
Data on metabolites in soil come from the environmental fate section, including information on time course of appearance and concentration level. These metabolites are relevant for soil organisms and ground dwelling arthropods.

Plants
Information is provided by plant metabolism studies. Metabolites may be relevant for arthropods including bees and herbivorous birds and mammals.

Vertebrates (fish, birds, mammals)
The toxicology package contains information on absorption, distribution, metabolism and excretion in mammals. Similar data on poultry are required if, according to the intended use, residues could be found in poultry feed. In the ecotoxicological assessment, residues in vertebrates, be it the active substance or metabolites, are considered in the context of potential food chain transfer. It is not considered likely that modern plant protection products magnify in vertebrate food chains, however this route should not be ignored. Should a substance be persistent and bio-accumulative in birds, mammals or fish a proper risk assessment is necessary (for details see Appendix III of the Guidance Document on Risk Assessment for Birds and Mammals (SANCO/4145/2000)).

If exposure of a certain environmental compartment is not expected (e.g. wound-healing or stored-produce uses), further assessments are not normally required (c.f. Annex VI, C2.5.1.1, and Annex II point 7).

Requirements for assessment and testing

As a general principle, it should be understood that assessments raised in this context do not always have to be addressed by experimental studies. Notifiers are invited to address the open questions by any other available information in support of a scientific and rational assessment.

As a matter of course more supporting evidence is needed for major metabolites whereas a qualitative approach can be used for minor metabolites. Valuable sources of information include, but are not limited to:

- consideration of molecular structure of the metabolite (active part intact?);
- the occurrence of metabolites in the medium in existing tests with the active substance or major metabolites;
- with regard to birds and mammals: the appearance of the metabolite in rat and poultry (Annex points II 5.1 and II 6.2);
- general knowledge on the relationship between the toxicity of the metabolite and its parent substance (e.g. from the aquatic base set (fish, daphnia, algae);
- information on pesticidal activity from biological screening data;
- available knowledge on related compounds;
- risk indicators (TER, HQ) calculated for the parent compound (clearly on the safe side of the trigger?).

If the metabolite is CO_2 or an inorganic compound, not being or containing a heavy metal; or, if it is an organic compound of aliphatic structure, with a chain length of 4 or less, which consists only of C, H, N or O atoms and has no "structures" or functional groups which are known to be of ecotoxicological concern, then no further studies are required and the metabolite is not considered to be ecotoxicologically relevant and is of low risk to the environment.

Generally a risk assessment is needed for all metabolites. However, metabolites occurring at levels lower than 10 % (minor metabolites) only have to be considered in exceptional cases, e.g. if containing the active moiety of the molecule. By definition the PEC for a minor metabolite is lower than the PEC for the parent compound by more than a factor of 10; accordingly minor metabolites even if 10 times as toxic as their parent compound can be considered as safe, provided that the parent compound is safe and also provided that no new concern with regard to persistence is brought in. It is recognised that for technical reasons it might not be possible to identify minor metabolites. If metabolites are identified in lab studies but not in field studies then field studies should be regarded more relevant unless the difference is due to the methods applied; assessments on this should be left to enviromental fate specialists.

Tests with metabolites may not be required where they are formed relatively rapidly and are short-lived, as their toxicity may be exerted in the tests on the parent compound. This conclusion should be supported by analytical measurements or other justifiable arguments (e.g. data from laboratory or field studies). If there is more than one metabolite it may be sufficient to conduct only tests with the most important metabolite (highest amount, most comparable in structure with a.s.). If higher tier studies have been conducted with the active substance, or a relevant formulation, these studies may have also encompassed the exposure to metabolites (depending on the duration of the study and the degradation behaviour af active substance and metabolites).

Information on which tests are necessary with metabolites are found in chapters 3.1, 4.1, 5.1, and 6.1 for the different groups of organisms.

The purpose of the toxicity studies is both to establish the relative toxicity of the metabolite to the parent compound, particularly for sensitive organisms, and also to provide an effect concentration for risk assessment purposes.

Risk assessment for metabolites

In principle the risk assessment process for metabolites will be similar to that for active substances, albeit recognising that risk assessment cases will not always require specific study data for certain metabolites. If the metabolite is less toxic than the parent compound, then in most cases it does not pose greater risks than those indicated for the parent compound, so that a detailed quantitative assessment is dispensable. Exceptions are metabolites which are more persistent and bio-accumulative than the parent compound so that the long-term exposure is likely to be different.

If standard risk assessments indicate potential concerns then, as for parent molecules, risk refinement is possible either by refining effect levels or by refinement of the exposure estimate.

Defining ecotoxicological relevance

If as a result of the above risk assessment, a metabolite is considered to pose a similar or even higher risk to the terrestrial environment than its parent compound, and therefore, risk mitigation measures are needed, this metabolite is considered as 'ecotoxicologically relevant'. Such a metabolite must be included in the residue definition.

Definition of ecotoxicologically significant residues (Annex VI 2.6.2)

According to Annex VI B 2.6.2 and C 2.6.2 analytical methods must be available for post-registration control and monitoring purposes among which there are methods for residue analysis of the active substance, metabolites, breakdown or reaction products. The methods must be able to determine and confirm residues of toxicological, ecotoxicological or environmental significance. With regard to foodstuff, provisions in Annex VI contain details on sensitivity etc. With regard to environmental media, however, such specifications are missing which obviously is due to the fact that there are currently only some Member States which have maximum residue levels for soil and surface water and systematic monitoring programmes for these media. Nevertheless, definition of residues for environmental compartments is requested in the Annex I procedure. With regard to soil the following definition of "ecotoxicological significance" is proposed provisionally: Apart from the parent compound the definition should include firstly metabolites which pose a higher or comparable risk to terrestrial organisms as the active substance (= ecotoxicologically relevant metabolites according to the definition given above). Secondly, also any hazardous metabolites should be included which needs establishment of a threshold for effects data. A suitable concentration level would be that which results in the classification of a substance as environmentally hazardous. Unfortunately the EU classification system according to Directive 67/548/EEC does not yet contain criteria with regard to soil organisms, but they are in preparation. As soon as these concentrations are agreed upon they should be used for the purpose here. There is often the situation that there is no separate test with a metabolite because the metabolite appears in the system during the test with the parent compound. Then it is impossible to decide whether the observed effect is to be ascribed to the parent compound or to the metabolite. This distinction could be unimportant for the risk assessment, but the question of

whether the metabolite is hazardous remains open. In such a situation the metabolite should be regarded as ecotoxicologically significant. However, additional studies can be submitted to remove the metabolite from the residue definition. Metabolites included in the residue definition need analytical methods.

It should be noted that the definition of the residues is a formal process which is different from risk assessment.

3 Terrestrial vertebrates

3.1 Data requirements and testing

Avian acute oral toxicity (Annex II 8.1.1)

Work conducted for the UK Pesticides Safety Directorate (Hart and Thompson 1995) shows that regurgitation can substantially reduce the dose absorbed by birds in acute oral toxicity tests. Therefore, during the evaluation of avian acute oral tests it should be assessed whether regurgitation or emesis has occurred. If so, it may be appropriate to repeat the study using birds which do not regurgitate, in particular if a high risk use – such as seed treatment - is being assessed.

For example, if regurgitation is observed in an acute oral toxicity test at 500, 1000 and 2000 mg a.s./kg bw but not at 200 mg a.s./kg bw, and if there is no mortality at 200 mg a.s./kg bw then the conclusion is valid that the LD50 is >200 mg/kg bw and this figure may be used in the initial risk assessment. If this assessment raises concern, i.e. TER_a less than 10, then either an acute or dietary study will be requested using a bird species which does not regurgitate. If the initial assessment does not raise concern, i.e. TER_a >10, no further data will be requested. Sometimes regurgitation may occur in all doses whilst mortality occurs only in the top doses, i.e. regurgitation is not sufficient to protect birds. Also in this situation, a further study with a non-regurgitating species will be required.

Avian short term dietary toxicity (Annex II 8.1.2)

When the test diet has been analysed the results should be reported in the monograph. According to OECD guideline 205, a deviation up to 20 % between measured feed concentrations and nominal values is considered to be acceptable. In the case of larger deviations toxicity figures should be recalculated using effective concentrations.

Avian reproduction (Annex II 8.1.3)

A reproductive toxicity study should always be conducted unless it can be demonstrated that exposure of birds (adults and young) does not occur during the breeding season. When all relevant species are considered, the breeding season could be rather long and even short exposure periods may give rise to concern with regard to potential reproductive effects. Thus, in the case of foliar applications during the breeding season, for example, the test should normally be required even if only one treatment per season is intended.

A justification for not conducting a bird reproduction study must be supported by data to indicate that no exposure will occur during the breeding season. The justification may be

based on residue data on potential feed items. Reproductive data are always required for substances which are generally persistent (see chapter 2.7) or have a bio-accumulation potential. Reproductive data are not required, for example, if plant protection products are used indoors or if a product with a short half life of <14 days on food items is applied in autumn. It should be noted that low acute and dietary avian toxicity are not sufficient to indicate a low reproductive toxicity.

Effects of secondary poisoning (Annex III 10.1.4)

Annex point III 10.1.4 mainly addresses the food chain from rodents to predators and scavengers in the case of rodenticides. For further information see Doc SANCO/4145/2000.

Metabolite testing

Metabolites in or on potential feed items have to be considered. However, apart from general considerations explained in chapter 2.9, there are some cases where experimental toxicity testing is not necessary:

- If the metabolite in question also appears in birds and mammals it can be assumed that any toxic effects would be expressed in the toxicity test with the parent compound, and that the risk from the metabolite is covered. It has to be observed that the toxicology section of the dossier/monograph always provides information on metabolism in rats, but not necessarily on metabolism in birds (poultry), and it cannot be assumed that the metabolic pathway in birds is identical to that of mammals.
- The toxicology data package may already contain mammalian toxicity tests with the metabolite. The absolute toxicity of the metabolite cannot be directly extrapolated from mammals to birds, but the relation can be used as an indication that such information might be sufficient for an assessment. For example, consider the following data and information:
  LD50 rat (parent) = 238 mg/kg,
  LD50 rat (metabolite) = 680 mg/kg,
  LD50 quail (parent) = 42 mg/kg.
  So, in rats the metabolite is 2.9 times less toxic than the parent. One should refrain from multiplying the quail LD50 (parent) by 2.9 because that would imply an undue level of accuracy. However, it would be reasonable in most cases to assume that also in birds the metabolite is not more toxic than the parent compound.

Should testing become necessary an acute oral study would be the first choice to serve as a bridging study, i.e. to compare the inherent toxicity of the metabolite with that of the parent compound.

3.2 Exposure assessment

Exposure assessment is dealt with in Doc SANCO/4145/2000

3.3 Risk assessment

Risk assessment is mainly dealt with in Doc SANCO/4145/2000. Therefore this chapter only contains some additional information.

Relevant toxicity figure for the acute assessment

Calculation of TER_a should be determined using the lowest, reliable acute oral LD50 figure. If data on the acute toxicity of both active substance and formulation are available, it should be determined whether animals are likely to be exposed to the formulation or the active substance and the more appropriate figure should be used. For instance, in the case of granules birds are clearly exposed to the formulation whereas in the case of a spray application, residues on green plant material are better considered in terms of the active substance than of the formulation.

3.4 Risk mitigation options

Risk mitigation is dealt with in Doc SANCO/4145/2000.

4 Bees

For general background information see the upcoming EPPO scheme (EPPO 2002b)

4.1 Data requirements and testing

Acute toxicity to bees (Annex II 8.3.1.1, Annex III 10.4.1)

If honeybees are likely to be exposed to the active substance both acute oral and contact toxicity tests must be conducted as the toxicity by one route of exposure cannot be predicted from the other. Where there is only one relevant route of exposure (e.g. oral exposure in the case of soil application), testing can be restricted to this exposure route. The test result should be presented as μg a.s./bee or μg formulation/bee. If there are problems with solubility of the active substance, then the test should be conducted with a representative formulation.

Toxicity tests should be conducted according to EPPO 170, or OECD 213 and OECD 214 guidelines.

Bee brood feeding test (Annex II 8.3.1.2)

The test method of Oomen et al. (1992), that is recommended in Annex II for insect growth regulators, is a worst case screening test. If no effects are found the conclusion is justified that no brood damage will occur when using the product. In the case of effects further cage/tent/tunnel or field studies are necessary to evaluate the risk under more realistic conditions. If toxicity to honeybee broods can already be predicted from the mode of action of the compound, testing may immediately start with cage/tent/tunnel or field trials.

Residue test (Annex III 10.4.2)

Aged residue tests may be valuable as an additional tool for risk assessment. However, no specific validated methods are yet available. The test should be designed to assess the duration of effects due to residual traces of plant protection products on the crop.

Higher tier tests (Annex III 10.4.3, 10.4.4 and 10.4.5)

For higher tier testing (cage/tent/tunnel or field trials), the recommendations of EPPO guideline 170 should be taken into account.

Testing of systemic plant protection products

For soil-applied systemic plant protection products (e.g. plant protection products applied as seed dressing) the acute oral toxicity of the active substance(s) have to be determined. If potential risks to honeybees are identified (i.e. very low LD50) realistic exposure conditions should be taken into account, i.e. realistic exposure concentrations as expected in nectar and pollen as indicated by residue studies. If a risk is indicated, higher tier studies (cage/tent/tunnel or field studies) with realistic exposure scenarios should be performed.

Metabolite testing

Standard lab tests are normally not required for metabolites. Exceptions may be cases where for example the metabolite is the pesticidal active molecule. Before conducting studies the general guidance given in chapter 2.9 should be observed. If higher tier studies (cage/tent/tunnel or field) are conducted with the plant protection products under realistic exposure conditions, potential risks from metabolites should be covered.

4.2 Exposure assessment

For products applied as sprays where risk as assessed according to the HQ approach exposure should be established as the maximum single application rate of the product expressed as g/ha because the HQ was validated on this measure.

For systemic plant protection products, exposure considerations and calculations should be based on the a.s. (or metabolite) present in the respective plant parts (e.g. nectar, pollen) to which honeybees could be exposed. However, it should be noted that estimates of these concentrations are rarely available.

Exposure calculations in higher tier studies are already considered within the experimental design (e.g. honeybees foraging on treated field crops).

4.3 Risk assessment

Hazard quotient for bees (Annex III 10.4)

The hazard quotient is stated to be application rate/oral LD50 or application rate/contact LD50, where the LD50 is expressed as µg a.s./bee and the application rate is in g a.s./ha. As stated above, the maximum single application rate should be used to calculate the oral and contact HQ-values. If the oral and contact HQ < 50, low risk to bees is concluded and no further testing is required. If the oral or contact HQ > 50, further higher tier testing is required to evaluate the risk to bees. The critical HQ of 50 was validated against incidents (EPPO 2002b); it is only applicable to spray products.

Higher tier risk assessment for bees

There are no clearly defined endpoints for higher tier studies, therefore, a degree of expert judgement is required to interpret both semi-field and field study results. As regards semi-field trials, where there are replicated studies, there should be a statistical comparison between key parameters, e.g. foraging density, mortality, proportion of adults, larvae and pupae in the hive. It should be noted that the parameters considered should be relevant to the compound under

consideration. For example if an insect growth regulator was being assessed then it would be more relevant to concentrate on developmental issues. As regards field trials, key parameters should be compared to either pretreatment levels or to control levels. It is important to consider any effects observed in relation to the overall survival and productivity of the hive. Key parameters which may be considered in a field trial include: mortality (assessed via the use of dead bee traps), behaviour (including foraging behaviour in the crop and around the hive), honey crop (assessed via weighing the hive at appropriate intervals) and state of colony (including an assessment of brood). Depending upon the concern highlighted in the initial risk assessment it may be appropriate to use pollen traps as well as appropriate analysis of dead bees. Analysis of honey and wax may be useful in determining exposure. The use of a toxic standard in both semi-field and field trials along with an untreated control can aid interpretation of the results. For insect growth regulators and other active substances which may cause long-term adverse effects on hive health, evidence is required confirming a lack of effects on hive health over a long time period. It should be noted that further information is available in the EPPO guideline (EPPO 2001). The design of higher tier studies is dependant upon the risks highlighted and therefore it is recommended that applicants should consult the relevant authority.

4.4 Risk mitigation options

The risk mitigation measures outlined below are options only. These measures will require consideration at a national level and implementation will depend on local agronomic practice and conditions. If predicted effects to honeybees are considered as not acceptable, the following aspects of the use pattern may be considered for modification in order to mitigate the predicted risk:

- application rate
- timing of application (e.g. apply in the evening after honeybee flight, do not apply during honeybee flight)
- GAP adaptation (e.g. do not apply during crop flowering)
- agronomic practice (e.g. mulch ground cover before application of the plant protection products)

5 Other arthropods

The risk to non-target arthropods is routinely assessed under 91/414/EEC. Annex II of 91/414/EEC states that data on two sensitive standard species as well as data on two crop relevant species are required. If effects are observed with species relevant to the proposed use then further testing may be required. Annex III of 91/414/EEC states that where significant effects have been observed the toxicity of the product to two additional species must be investigated. Both Annex II and III reference the SETAC Guidance document on regulatory testing procedures for pesticides with non-target arthropods (ESCORT, Barrett et al. 1994) as a source of guidance for testing. However, several limitations have been identified and these can be summarised as:

- The objectives of the testing scheme are not clear, e.g. it does not precisely discriminate between non-target arthropods in a general context and beneficial arthropods in an agricultural or IPM context.

- The trigger value for first tier data (30 % effects as laid down in Annex VI C point 2.5.2.4) leads to excessive higher tier testing.
- The single-dose laboratory data generated do not provide for determination of the intrinsic toxicity of the substance (except where is no effect and the test can be regarded as a limit test). In addition this kind of testing is inflexible and does not allow a satisfactory risk assessment especially for off-field habitats.
- Uncertainty about data requirements, testing methodology and evaluation, especially for multiple application products, where currently life span, spraying interval and fate are ignored and for off-crop habitats, where exposure scenarios and mitigation measures are not yet agreed.

Due to the above issues a workshop, ESCORT 2, was held in 2000 which aimed to address these shortcomings. From this workshop a guidance document resulted (Candolfi et al. 2001) which is referred to here as "ESCORT 2". This workshop was attended by all EU Member States as well as representatives from industry and academia and revised the process by which the risk to non-target arthropods should be assessed. By building on the experience gained from assessing the risk to non-target arthropods under 91/414/EEC, a new approach was proposed which offers a high level of protection, but is more focused and structured.

The process discussed and agreed on this workshop starts with glass-plate tests on the two standard sensitive species referred to in Annex II (*Aphidius rhopalosiphi* and *Typhlodromus pyri*). However, rather than a single rate study, a rate-response study is usually required. The endpoint of these studies are LR50 values (i.e. lethal rate that causes 50 % mortality) which are compared to the predicted exposure both in-field and off-field. With substances suspected to have a special mode of action (IGRs, insect feeding inhibitors) tests should include sublethal endpoints and may need other modifications. The assessment of risk for arthropods living in- and off-field is conducted separately. If the resulting 'hazard quotient' (HQ) based on the standard tests is greater than or equal to 2 then further data and/or risk management measures are required. Note: The critical trigger of 2 was proposed on the basis of the available data. It was noted at the ESCORT 2 workshop that this value should be revised when suitable data are available.

It is proposed that for active substances and their associated product(s) under consideration for inclusion on Annex I, the risk to non-target arthropods both in and off-field should be adequately addressed. The guidance given below is in line with the recommendations of ESCORT 2.

5.1 Data requirements and testing

Standard tests (Annex II 8.3.2, Annex III 10.5.1)

Testing is always required where exposure of non-target arthropods is possible.

Standard tier 1 testing comprises glass plate tests with *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Preferably these tests should be designed as rate-response studies in order to determine the LR50 as this allows for applying the data to different use scenarios and also to the risk assessment for off-crop areas. However, if the toxicity is expected to be low then limit tests can be conducted at a rate equivalent to the maximum application rate multiplied by the multiple application factor (MAF). With regard to the test substance (active substance,

lead formulation) see chapter 2.4. With substances suspected to have a special mode of action (e.g. IGRs, insect feeding inhibitors) tests should include sublethal endpoints and may need other modifications.

Details on methods are given in the ESCORT 2 document.

Higher-tier tests (Annex III 10.5.1 and 10.5.2)

Higher-tier tests are required when a risk is indicated in lower assessment tiers. There are several options for higher-tier testing or combinations of adequate tests:

- Extended laboratory tests (tests with natural substrate aiming at lethal and sublethal effects)
- Aged-residue studies
- Semi-field tests
- Field tests

ESCORT 2 provides advice regarding the choice of studies and the selection and number of species. Usually these studies are conducted with one dose rate matching the field application rate taking into account multiple applications and the use of appropriate risk mitigation measures. Advice is given in ESCORT 2 regarding the appropriate rates to use in such studies. With regard to extended laboratory tests it should be noted that due to the implementation of a correction factor[1] (default value = 5) in some cases the rules may give application rates greater than the field rate. In this case it is suggested to test at the maximum rate including the multiple application calculation. In the case of extended laboratory studies a dose response design may be more informative than a one-dose design.

Metabolite testing

Arthropods may be exposed to metabolites in/on plants and to soil metabolites.

Metabolites in vegetation: Standard lab tests are normally not required for metabolites. Exceptions may be cases where for example the metabolite is the pesticidal active molecule. Before conducting studies the general guidance given in chapter 2.9 should be observed. If higher tier studies (semifield or field) were conducted with the plant protection products under realistic exposure conditions, potential risks from metabolites should be covered.

Soil metabolites: These are assessed with regards to soil organisms, so that tests with soil-surface arthropods are not needed.

[1] In order to avoid confusion the terminology of the ESCORT document is used in this document as far as possible; actually “uncertainty factor“ or “safety factor“ would be more appropriate

5.2 Exposure assessment

Generally, exposure for non-target arthropods is expressed in terms of application rate (g/ha or ml/ha).

Tier I assessment

For the standard assessment the following scenarios are used to describe the exposure in-field and off-field. For both, the key input is the nominal field application rate supplemented by various factors:

in-field exposure = Application rate * MAF

off-field exposure = Application rate * MAF * (drift factor / vegetation distribution factor)

For calculation of MAF values, definitions and further details see ESCORT 2. With regard to the vegetation distribution factor ESCORT 2 gives a default value of 10. However, this figure is considered unreliable, therefore more appropriate data should be used as soon as they become available (a research project is currently under way). With regard to the drift factor the tables published by Rautmann et al. (2001) may be used; the standard assessment should be conducted for 1 m distance (arable crops) or 3 m (orchards and vineyards); drift factor = % drift / 100.

Basic drift values for one application Ground deposition in % of the application rate (90th percentiles)									
Distance	Field crops	Fruit crops		Grapevine		Hops	Vegetables Ornamentals Small fruit		Field crops
[m]		Early	late	Early	late		Height < 50 cm	Height > 50 cm	Water > 900 l/ha
1	2.77						2.77		4.44
3		29.20	15.73	2.70	8.02	19.33		8.02	
5	0.57	19.89	8.41	1.18	3.62	11.57	0.57	3.62	0.18
10	0.29	11.81	3.60	0.39	1.23	5.77	0.29	1.23	0.05

Higher-tier assessments

Refined assessments are based on the outcome of higher-tier studies. In such studies relevant exposure issues are considered in the study when establishing the dosing regime (be it dose-response design or single-dose design). That makes a separate exposure assessment unnecessary; it must, of course, be ensured that the study covers the use scenario under assessment.

5.3 Risk assessment

Assessing the risk 'in-field'

Step 1: Tier I assessment based on standard tests

In the first tier the risk is characterised by the 'in-field' hazard quotient (HQ):

In-field HQ = in-field exposure / LR50

where the LR50 comes from glass-plate tests with the two standard species. If the in-field HQ is less than 2 for both species, no further assessment is required (for the reasoning behind this trigger level see ESCORT 2). If the HQ is greater than or equal to 2 for one or both species then go to step 2.

Step 2: Higher tier assessment

If no appropriate risk mitigation measures can be identified, then the notifier should carry out higher tier studies on the affected species and one further species with different biology. Details of suitable species are provided in ESCORT 2. With regard to extended laboratory tests and semi-field tests lethal, and sublethal effects of less than 50 % are considered acceptable provided that the tests covered the appropriate field rate. For interpretation of aged residue studies with respect to recolonisation, and for interpretation of field studies see ESCORT 2. Generally, it has to be demonstrated that there is a potential for recolonisation / recovery at least within one year but preferably in a shorter period depending on the biology (seasonal pattern) of the species. The assessment may be based on field studies or other evidence (e.g. results of aged-residue studies, environmental fate information). In any case the data and assumptions should be fully justified.

Assessing the risk 'off-field'

Step 1: Tier I assessment based on standard tests

The risk is characterised by the 'off-field' HQ:

Off-field HQ = (off-field exposure / LR50) * correction factor

where the LR50 comes from glass-plate tests with the two standard species; the correction factor is intended to cover uncertainty with regard to species sensitivity, the default value is 10. If the off-field HQ is less than 2 for both species, no further assessment is required, if greater than or equal to 2 for one or both species then go to step 2.

Step 2: Higher tier assessment

If no appropriate risk mitigation measures can be identified, then higher-tier studies on the affected species and two additional species with different biologies should be conducted. Details regarding suitable species are provided in ESCORT 2. With regard to extended laboratory tests and semi-field tests lethal and sublethal effects of less than 50 % are considered acceptable provided that the tests covered the appropriate field rate; the default value for the correction factor is 5. Generally, it has to be demonstrated that there is an acceptable potential for recovery within an ecologically relevant period.

Basically, if the tier-1 assessment indicates a risk either risk mitigation measures or higher-tier studies are called for. It should be noted that in order to achieve Annex I listing that it is not

considered appropriate to propose unrealistic risk mitigation measures (e.g. exaggerated buffer zones) in order to avoid higher-tier testing.

Risk from solid formulations, products with a special mode of action and those of limited solubility

The standard approach is not appropriate for substances with limited solubility or for plant protection products such as granules, seed treatments and pellets. In these cases it is recommended that studies are conducted with *Hypoaspis aculeifer* or *Folsomia candida* as proposed by EPPO (2002a). If deemed appropriate, studies with *Aleochara sp.* might be conducted, e.g. at tier 2.

It is recognised that the standard approach may not be wholly appropriate for insect growth regulators or other compounds with particular modes of action. For these compounds the principles of ESCORT 2 should be followed with case-by-case modification according the specific issues for the compound in question.

5.4 Risk mitigation options

In order to reduce effects on non-target arthropods within the cropped area the following use specifications may be modified:

- application frequency and intervals
- timing of application (crop stage)
- unsprayed headlands

In order to reduce effects in off-field areas there are the following options:

- Buffer zones
- Wind breaks
- Drift-reducing application techniques

For further explanations see ESCORT 2

6 Soil organisms

6.1 Data requirements and testing

Acute effects on earthworms (Annex II 8.4, Annex III 10.6.1.1)

Testing is always required where contamination of the soil is possible. With regard to the test substance (active substance, lead formulation) see chapter 2.4.

Tests according to OECD Guideline 207 and ISO 11268-1: 1993 (which are similar to 88/302 EC) are also acceptable.

Sublethal effects on earthworms (Annex II 8.4.2, Annex III 10.6.1.2)

According to Annex II the requirement for this test depends on the exposure pattern to the active substance ('continued or repeated exposure'). The following triggers for persistence of the active substance and the number of applications are proposed:

- The test is not required when both the $DT90_f$ is less than 100 days, and the number of applications is less than 3.
- The test is always required if the $DT90_f$ is above 365 days (regardless of the number of applications).
- The test is always required if the number of applications is greater than 6 (regardless of persistence).
- If the $DT90_f$ is between 100 and 365 days and/or the number of applications is between 3 and 6, a case by case decision is made.

With regard to substances forming bound residues see chapter 2.7.

The test is also required if the assessment of the acute risk gives a TER of less than 10 (see below).

Suitable methods are ISO 11268-2:1998 and the forthcoming OECD 222. With products intended to be sprayed, surface application should be preferred (annex D of the ISO guideline) and the result given in g/ha. The test should preferably be conducted as dose-response test.

When planning the test, the upper concentration level must be chosen to be high enough in order to be able to judge whether the long-term TER meets the trigger of 5, which is provided in Annex VI of Directive 91/414/EC. It has to be taken into account that exposure under field conditions may be elevated due to repeated applications (see chapter 6.2) and that toxicity figures may be corrected for f_{oc}. If available and appropriate, data from field dissipation studies should be considered.

Earthworm field studies (Annex III 10.6.1.3)

The study is required where TER_{lt} is < 5. However, as already explained in chapter 2.6 it should be checked in such cases whether there are other options for refinement (EPPO 2002a).

The study should reflect the use of the compound, the environmental conditions and species that will be exposed. If the chemical is to be applied in the arable situation it should preferably be applied to bare soil as opposed to grassland where it may become bound to the surface thatch. Analysis of the soil would assist in confirming whether the field study is appropriate for the intended arable crop use. With regard to the dosage the test should be designed such that the highest exposure according to the intended use of the product is covered. That means that multiple applications should be made where relevant, and crop interception should be considered. If accumulation in soil is expected then a rate equivalent to the long-term (pluriannual) plateau concentration should be added. The type of application of the test substance (surface application, incorporation, etc.) should be according to the intended use.

A method is described by ISO (11268-3:1999). For further information see also Greig-Smith et al. (1992) and Sheppard et al. (1997). General remarks on higher tier tests (chapter 2.6) should be observed.

Soil nitrification and carbon mineralisation (Annex II 8.5, Annex III 10.7)

Testing is always required where contamination of the soil is possible.

With regard to methods, Annex III of Directive 91/414/EEC refers to a SETAC document (Lynch 1995). In the interim, the OECD has published its guidelines 216/217 which should be preferred when conducting new studies.

Other soil non-target macro-organisms (Annex III 10.6.2)

This Annex point requires additional data for soil organisms contributing to organic matter breakdown, depending on active substance degradation rate and on available information with regard to effects to various organisms. Principally the risk to this group of organisms, which include soil mesofauna and macrofauna, could be determined either at a species level or at a functional level. While a candidate test for the former would be a Collembola reproduction test or a test on gamasid soil mites, a candidate for the latter would be the "litter bag" test.

This Annex point particularly deals with the problem of persistent active substances or persistent metabolites ($DT90_f$ > 100 days). These are of special concern as influences on organisms can continue to act over generations and may have multiple effects, and any recovery could take an unduly long time. Therefore, a higher degree of scrutiny is needed to assure that soil organisms are not affected.

Based on the recommendations of the Lisbon Workshop (EPFES 2002) the following tiered procedure is proposed (see figure 1):

a) Collembola reproduction test or test on gamasid mites

Testing is required where contamination of soil is possible and $DT90_f$ is between 100 and 365 days and the standard HQ for arthropods (*Typhlodromus* and *Aphidius*) >2. This test is used as a potential waiver for the litter-bag-test (see next point); so, if the litter-bag test is triggered anyway by other criteria (effect on soil micro-organisms >25 % or TER_{lt} for earthworms <5) then this test could be omitted. A suitable protocol for the Collembola test is the ISO method 11267:1999; a test design with the gamasid mite *Hypoaspis aculeifer* is described by Løkke and Van Gestel (1998) and Bakker et al. (2002). As long as these methods are not validated protocols should be checked with the Rapporteur Member State.

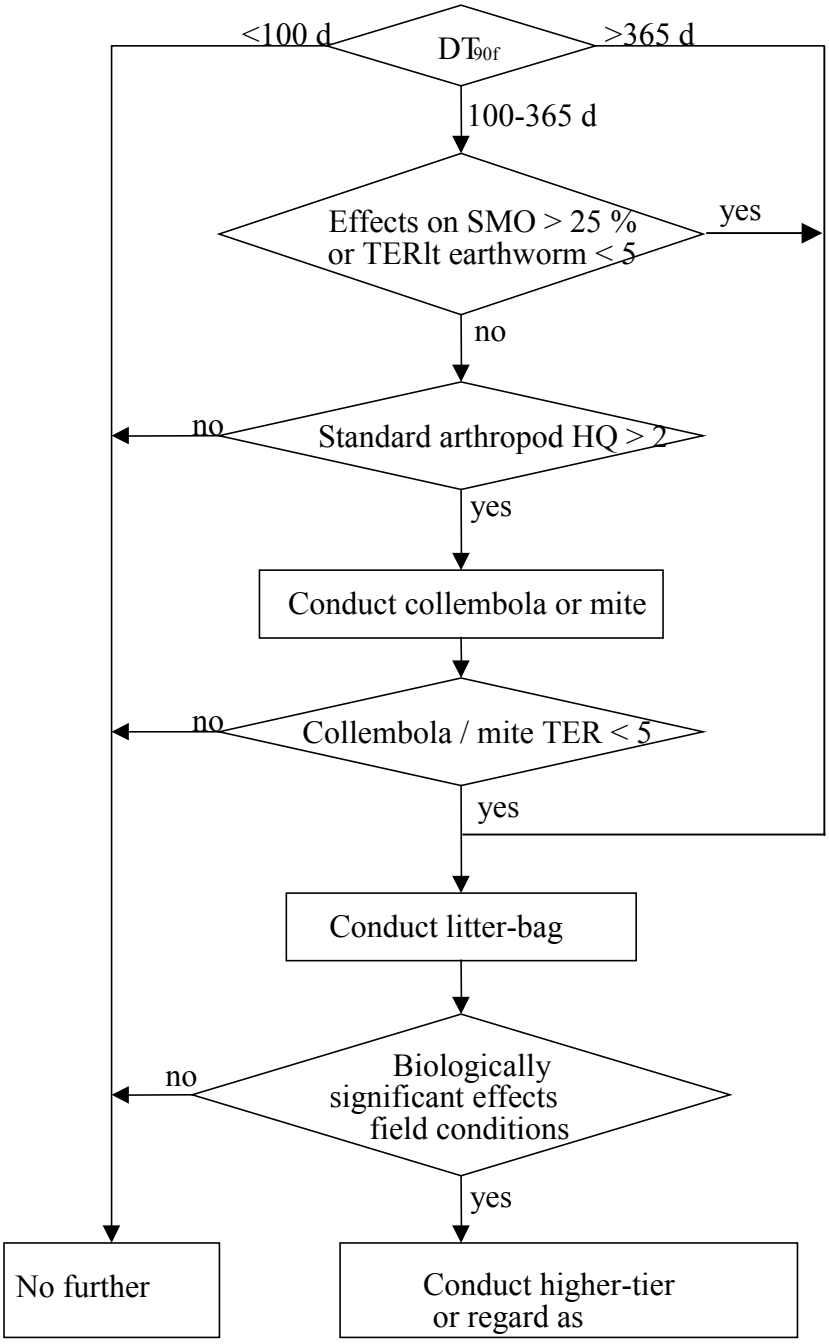
b) Litter bag test under field conditions

Testing is always required where contamination of soil is possible and DT90 is > 365 days or mineralisation is < 5% in conjunction with bound residue formation of > 70%. Testing is conditional where $DT90_f$ is between 100 and 365 days; in such cases the following auxiliary criteria are applied:
- Effects on soil microorganisms >25 % after 100 d
- or long-term TER for earthworm < 5
- or TER for Collembola or soil mites < 5

Principally this means that in the intermediate persistence range a litter bag test is not required if the above mentioned groups of organisms pass the standard tier 1 assessment.

As regards methods the test should be conducted in the field on arable sites, taking into account the intended use of the product. Concerning exposure, the plateau concentration

Figure 1: Test sequence with regard to soil organisms for persistent substances



should be applied to the soil or already be available in the soil, before the litter bags are buried. (Plateau concentration refers to the long-term pluriannual plateau over years (FOCUS 1996)). After that the annual rate is applied considering the crop interception. The degradation of fresh incorporated organic material is evaluated using at least 3 sampling dates. Minimum duration of the test should be 6 months. Special attention should be given to the method of application and the number of time points for measurement. Weight loss and the degradation rate of the organic material are the endpoints of the test. A method has been drafted at the Lisbon workshop which will appear in the workshop proceedings (EPFES 2002). As long as there are no formally harmonised protocols a certain degree of flexibility must be conceded. So, when judging the acceptability of a study it should be considered what the state of technique was when the study had been generated.

c) Higher tier tests

If the litter bag test shows biologically significant effects or there is other reason for additional concern then further testing could be an option; (there are other options such as risk mitigation; there also could be the final conclusion that there are no safe uses). If further testing is envisaged then it should be decided on a case by case basis which approach is most helpful:

- extend the on-going litter bag study or start a new litter bag study under more realistic conditions (the study may be extended for mesofauna structural endpoints; see for example Elkins and Whitford (1982), Bjørnlund et al. (2000), van Vliet et al. (2000)).
- large-scale field studies
- terrestrial model ecosystems

In any case problems and questions with the substance should be identified prior testing and tests then be targetted to these problems.

Metabolite testing

With regard to metabolite testing see general remarks in chapter 2.9. If testing of soil metabolites on soil organisms is necessary the first step should be an acute toxicity study with earthworms to compare the inherent toxicity with that of the parent compound. A particular situation may arise when the metabolite is more persistent than the parent compound. Certain tests with soil organisms are triggered by persistence (earthworm reproduction test, litter bag test, etc.), and it is possible that the persistence of the parent compound does not exceed the trigger for these studies, but the metabolite does. In such cases the additional studies should be conducted, with the metabolite, regardless of its acute toxicity.

6.2 Exposure assessment

Earthworms

The exposure is represented by the predicted in-field concentration of the substance in soil. PEC values for the various use scenarios are supplied by the environmental fate section. Initial PEC values are decisive in this context (no time-weighted averages). In the case of repeated applications, the PEC after the last application is relevant. In case of persistent substances the plateau concentration is relevant.

Soil micro-organisms and other functional tests

No separate exposure assessment is necessary for soil micro-organisms as the relevant exposure conditions (multiple application, etc.) are considered in establishing the dosing regime for the test. So the outcome of the study is immediately interpreted in terms of risk. The same is true for litter bag tests.

6.3 Risk assessment

Standard risk assessment for earthworms

The standard risk assessment is based on TER values. The acute TER is the ratio between the LC50 from the acute test and the PEC. The long-term TER is the ratio between the NOEC from the reproduction test and the PEC.

Both acute and reproductive tests are static tests where the test substance is applied to the system only once at the beginning. Therefore, the nominal dose levels in the test match initial concentrations in the field and thus it is appropriate to use initial PEC values (no time-weighted averages) for the acute as well as for the long-term TER. If it can be demonstrated that degradation in the artificial substrate and natural soils differ significantly, then it may be considered in the assessment.

The toxicity of lipophilic organic contaminants to soil organisms usually depends on the organic carbon content (f_{oc}) of the substrate as this governs adsorption and thus pore water concentration. The artificial substrate of the earthworm laboratory tests has a higher f_{oc} than many natural soils, so it could be expected that the LC50 or NOEC would be lower if the test were conducted in natural soil (Van Gestel 1992). The risk assessment should account for this difference by dividing the LC50 and the NOEC by 2 where $\log K_{ow}$ is greater than 2 (EPPO 2002a) unless it can be demonstrated by soil sorption data or other evidence that the toxicity is independent of f_{oc}. For sake of clarity the corrected toxicity figures should be denoted by a subscript (e.g. $LC50_{corr}$).

Refined risk assessment for earthworms

If the acute TER is below 10 or the long-term TER is below 5 further action is required. For general considerations see chapter 2.8. It should be decided on a case-by-case basis which option is best suited to proceed. Refinement of exposure, for example, is often quick and inexpensive and should be considered first before turning to higher tier tests.

Refined effects assessment

When the NOEC from the reproductive test is expressed in g/ha, it could be converted into mg/kg soil by a calculation assuming 100 % of substance reaching the soil, 5 cm depth and a soil density of 1.5 to give a value used in the TER_{lt} calculation. When the TER_{lt} is close to the trigger value, the calculation could be refined by considering actual test values (application rate and surface of the test unit, dry soil weight in the test unit). If there are uncertainties arising from the fact that the standard tests are conducted with artificial soil then an option might be to do the earthworm test in natural soil.

Refined exposure estimate

The exposure assessment could be improved, for example, by employing more sophisticated models, consideration of interception, or inclusion of field measurements.

Higher tier studies

Where the acute TER does not meet the trigger the earthworm reproduction test can be regarded as the next higher tier. (Note: The earthworm reproduction test fulfils two purposes. Firstly, it is a long-term test with sublethal endpoints which has its own place in the base set and is triggered by exposure considerations (continued, repeated). Secondly, it can be regarded as a higher-tier test above the acute test because it involves more realistic conditions (surface application instead of mixing into the soil).

Risk assessment for soil micro-organisms

The outcome of the soil micro-organism test is directly assessed in terms of risk. The decisive parameter is the magnitude of effect compared to the untreated control (be it increase or decrease of activity), and the time-course of recovery. According to Annex VI of 91/414/EEC the critical level is $\pm$25 % after 100 days. Larger deviations will require refinement of the assessment. As a matter of course, the concentrations used in the test must cover the maximum PEC. Generally the test concentrations are converted by calculation to equivalent doses in g/ha. Different modes of calculations are used and thus may introduce a bias in the interpretation of the risk. It is recommended to compare directly the test concentrations to the PEC values before to conclude on potential risk.

Risk assessment for non-target mesofauna

Data from a Collembola reproduction test or a soil mite test could be treated in a risk assessment in the same way as data on earthworm reproduction (TER values using PEC and NOEC)

6.4 Risk management options

Risk mitigation options for soil organisms are limited. There are possibilities to reduce the exposure (reduction of application rate and/or number of applications and/or restriction on glasshouse use only), but inevitably these measures will compromise the agricultural objectives.

7 Non-target plants

The risk of plant protection products to terrestrial plants has been until now included in a generic assessment on 'other non-target organisms (flora and fauna) believed to be at risk.' However, this aspect is considered a critical element in the evaluation of certain plant protection products, particularly herbicides and plant growth regulators, and therefore some general guidance is included.

A key element in the evaluation is the definition of non-target plants. For a generic evaluation, as required by Directive 91/414/EC, the following working definition is suggested: Non-target plants are non-crop plants located outside the treatment area.

7.1 Data requirements and testing

Annex II and III of Directive 91/414/EEC do not contain specific data requirements for non target plants. However, the introductions to these annexes generally state that there is a need to report all potentially adverse effects and to undertake additional studies where there are indications of such effects. Therefore a tiered approach is suggested starting with available data and proceeding to further steps in case of need. Data are not required, where exposure is negligible, e.g. in the case of rodenticides, substances used for wound protection or seed treatment, or in the case of substances used in stored products or in glasshouses.

Tier 1: Initial screening data

For the first tier, a preliminary assessment is conducted using available information. Preference is given to screening data; there should be at least 6 species from different taxa tested at the highest nominal application rate (1 x). These data could be supplemented by further information on efficacy, selectivity, phytotoxicity, etc. included in the biological dossier or obtained from the different field assays such as efficacy trials, residue studies, environmental fate and ecotoxicological studies, etc. The initial step is unprofitable for herbicides and plant growth regulators as these inevitably will end up in the second tier.

Tier 2: Bioassays on terrestrial plants

If a potential risk is identified (more than 50 % effect for one or more species at the maximum application rate, see chapter 7.3), then specific information on the toxicity of the substance to terrestrial plants should be requested. The second tier considers laboratory assays on a selection of plant species. It is recommended to conduct dose-response tests on 6-10 plant species representing as many taxonomic groups as possible. In order to generate data that are useful for probabilistic approaches there should not be a focus exclusively on species assumed to be the most sensitive. If, from the screening data, a specific mode of action is evident, or strong differences in the species sensitivities are identified, this evidence should be used in the selection of the appropriate test species. This may be especially true if non-herbicides reach tier-2 testing.

For foliar applications, the bioassays should be conducted by spraying the product on the plants, reproduce as far as possible the realistic exposure conditions and, in particular, spray drift. Soil application should be chosen if that is more appropriate with regard to the mode of action. The test substance should be the lead formulation (or another formulation) because formulations contain, besides the active substance, all those components and co-adjuvants required for maximising biological activity. For systemic products applied on the ground/soil, the tests should reproduce this application pattern.

Suitable test methods are the new draft OECD Guideline 208 and the OPPTS guidelines of the US EPA.

Tier 3: Field or semi-field studies

The third tier requires semi-field or field assays, to study the effects observed on non-target plants during realistic applications. Such studies are time-consuming and expensive; before undertaking them it should be checked whether there are options for the refinement of exposure and/or effects. Furthermore, as for all other non-target organisms, field or semi-field

studies are not required if the risk based on the tier 2 assessment could be managed by risk mitigation measures which could be dealt with on a Member State level.

Field or semi-field studies with non-target plants are not standardised. Therefore notifiers might wish to discuss the protocol with the Rapporteur Member State. Generally, effects on plant abundance and biomass production at different distances from the crop or at exposure levels representing different distances from the crop should be analysed. These studies are compatible with most semi-field and field studies.

7.2 Exposure assessment

Spray drift is considered the key exposure route for terrestrial plants located in the vicinity of the treated area. The drift models produced by the BBA for the exposure assessment of aquatic organisms may be used as a surrogate to cover the exposure assessment of terrestrial plants (Ganzelmeier et al. 1995, recently updated by Rautmann et al. 2001). The following table shows the drift expressed as percentage of the applied dose:

Basic drift values for one application Ground deposition in % of the application rate (90th percentiles)									
Distance	Field crops	Fruit crops		Grapevine		Hops	Vegetables Ornamentals Small fruit		Field crops
[m]		Early	late	Early	late		Height < 50 cm	Height > 50 cm	Water > 900 l/ha
1	2.77						2.77		4.44
3		29.20	15.73	2.70	8.02	19.33		8.02	
5	0.57	19.89	8.41	1.18	3.62	11.57	0.57	3.62	0.18
10	0.29	11.81	3.60	0.39	1.23	5.77	0.29	1.23	0.05

In fruit, grapevine and hops for herbicides (but not for plant growth regulators) that are applied to the ground, the column “field crops“ is applicable.

It should be noted that these drift data have been generated with regard to intake into surface waters. In particular, there is no vegetational barrier between the spray boom and the collector plates. In terrestrial scenarios, however, horizontal and vertical interception by in-crop or off-crop vegetation as well as patchy distribution is relevant (“three-dimensional-situation“); thus, when more realistic drift data become available they should be used.

The initial assessment should be conducted for a distance of 1 m from the field edge for field crops, vegetables or ground applications such as for herbicides, and 3 m for other crops. Risk mitigation measures based on buffer zones within the crop area can also be quantified using the above table. In case of aerial applications a deposition rate of 100 % is assumed as the default, however this figure may be refined by applying appropriate models (e.g. AgDrift).

7.3 Risk assessment

A tiered approach with three different steps is also recommended.

Tier 1: Initial decision on the likelihood for terrestrial plant effects

This assessment step is based on the information described above as "initial screening data". The endpoints measured in most screening studies, such as phytotoxicity, chlorosis, etc. cannot be interpreted as a NOEC value covering germination and biomass production. However, the available information usually allows the use of a conservative approach, assuming, for example, that when an untreated control has been run in parallel, any effect accounting for at least 50 % reduction in biomass production could be identified in a visual inspection. In addition, single dose experiments reported in terms of percentage of observed effects can also provide indications on the potential hazard of the substance for terrestrial plants.

The detection of potentially sensitive species in this initial assessment, or the evidence of specific mechanisms of action suggesting effects on terrestrial plants (which is evident in the case of herbicides) will trigger the need for a proper quantitative assessment. As a general rule, the risk should be considered acceptable if there are no data indicating more than 50 % phytotoxic effect at the maximum application rate. If the results show more than 50 % effect for one species or clear indications of effects on more than one species, data requirements and assessment move to the next tier.

Tier 2: Quantitative risk assessment

This tier is a quantitative risk assessment following a TER approach. Both effects and exposure are expressed in terms of application rate (g/ha). Effects data are represented by ER50 values from the studies described under tier 2 in chapter 7.1, also expressed as g/ha. There are two options, a deterministic and a probabilistic approach, from which a choice should be made with regard to the data set (the probabilistic method is not always applicable).

Deterministic approach

If the TER based on the most sensitive species is greater than 5 then effects on non-target plants are considered acceptable. This trigger of 5 presupposes that at least 6 species have been tested. The trigger may be reduced if information on more species is available.

Probabilistic approach

Probabilistic methods that make use of the species sensitivity distribution would be straightforward in this assessment step as data from 6-10 species are available. Furthermore, a probabilistic approach is considered more suitable than the deterministic one to achieve the type of environmental goal mentioned above. This approach requires that log-normal or another defined type of distribution of the data has been shown to fit the data adequately. If the ED50 for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable.

Tier 3: Higher tier risk assessment based on field studies

The third tier requires a higher tier risk characterisation and therefore, a case-by-case analysis. The ecological relevance of the observed effects, consequences on soil functions, and the potential for recovery are key elements for the assessment.

7.4 Risk mitigation options

In order to reduce exposure of non-target plants the options are similar to non-target arthropods in off-field areas:

- Buffer zones to sensitive areas
- Drift-reducing application techniques in the vicinity of sensitive areas.

As usual these measures are highly specific for Member State conditions.

8 Other non-target organisms

Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex II 8.6)

There is a requirement for a summary of available data from preliminary tests used to assess the biological activity to be submitted. It is proposed that the summary should be presented in the monograph and any areas of concern highlighted. However, as non-target plants now are dealt with separately this summary in most cases will be very brief.

9 Terms and abbreviations

ECx	Effective concentration x % (concentration causing x % effect in a dose-response test); ECx is used as an overarching term referring to any kind of dose-response-modelling; ECx values may be specified with the first letter denoting the kind of endpoint (L = lethal), the second letter denoting the kind of exposure (C = concentration, D = dose, R = rate)
ED50	Effective dose 50 %
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
DT50, DT90	Disappearance time 50 % (90 %); the time it takes in a dissipation study until 50 % (90 %) of the initial amount or concentration has disappeared; the subscript f denotes field studies
f_{oc}	fraction of organic carbon
GAP	Good Agricultural Practice
HQ	Hazard quotient
IPM	Integrated Pest Management
LD50	Lethal dose 50 %
LR50	Lethal rate 50 %
MAF	Multiple application factor
NOEC	No observed effect concentration; highest concentration in a dose-response test which is not statistically different from the control
PEC	Predicted environmental concentration
PRA	Probabilistic Risk Assessment
TER	Toxicity/exposure ratio; subscripts denote time-scales (a = acute, st = short-term, lt = long-term)

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