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SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on

THE AVAILABLE SCIENTIFIC APPROACHES TO ASSESS THE POTENTIAL EFFECTS AND RISK OF CHEMICALS ON TERRESTRIAL ECOSYSTEMS

Opinion expressed at the 19th CSTEE plenary meeting

Brussels, 9 November 2000

CSTEE OPINION ON THE AVAILABLE SCIENTIFIC APPROACHES TO ASSESS THE POTENTIAL EFFECTS AND RISK OF CHEMICALS ON TERRESTRIAL ECOSYSTEMS

FOREWORD AND SCOPE OF THIS DOCUMENT

The concept "terrestrial environment" cannot be easily defined. It is characterised as the part of the biosphere that is not covered by water, less than one third of the total surface. From a geological viewpoint it just represents a thin line (a few meters wide) of the interface between both the solid (soil) and the gaseous (atmosphere) phases of the Earth, several orders of magnitude wider than this line. However, from the biological point of view, this thin line concentrates all non-aquatic living organisms, including human beings.

Humans use the terrestrial environment for living and developing most of their activities, which include the commercial production of other species by agriculture and farming. Human activities deeply modify the terrestrial environment. Particularly in developed areas such as Europe, the landscape has been intensively modified by agricultural, mining, industrial and urban activities and only in a small proportion (mostly in extreme conditions such as high mountains, Northern latitudes, wetlands or semi-desert areas) of the European surface the landscape still resembles naive conditions. Wildlife has been forced to adapt to the new conditions or to disappear. Nature shows examples of adaptation and species extinction. Nevertheless, from the Polar Regions of Scandinavia to the arid zones of the Mediterranean countries terrestrial ecosystems more or less adapted to human activities, in particular agriculture, can be found.

Chemical pollution represents an additional threat for living organisms. In the particular case of the terrestrial environment it can potentially affect human populations, human economy by acting on crop and livestock production and quality, and wildlife.

Ecotoxicologists normally consider as the ultimate end-point the assessment of effects on the structure and function of the ecosystem. This also implies that this level of protection will also guarantee the anthropogenic uses of the environment. The protection of soil functions also protects the capability of the soil to be used for agricultural purposes; protection of populations include domestic as well as wild species. The protection goal in both cases is at the population or community level Hence, it is not necessary to protect each single rabbit or each single plant of wheat, but the rabbit population and the wheat yield. Domestic species represent an infinitesimal percentage of the total number of species, and it is expected that in most cases, the levels of chemicals in the environment required to protect ecosystems should be lower than those required for the protection of these human activities. In other words, a proper ecological risk assessment is sufficient for the evaluation of adverse effects on real ecosystems and associated agro-systems. Other concerns, indirectly related to the loss of living organisms (soil erosion associated to the loss of vegetation cover, climate change associated to deforestation) are also covered by the ecosystem evaluation; obviously, any significant change to the vegetation cover, including trees will provoke dramatic changes on the structure and function of the ecosystem.

If human beings are explicitly included in the evaluation, there are both similarities and differences. Humans are part of the terrestrial environment and as such will be exposed to chemical pollutants in similar ways to other vertebrates. Environmental exposures to contaminated soil, air and food can be evaluated at least in parallel ways for humans and for wild vertebrates. However, the required level of protection is different. As for environmental concerns, the population as a whole must be protected (e.g., in terms of growth rate). However for human populations, a higher level of protection is also needed where it is necessary to protect each individual human being.

Therefore risk assessments are often divided into Human Health risk assessment (which include the exposure of humans through the environment as well as direct exposure during the life cycle of the chemical) and Ecological risk assessment (which by protecting ecosystems is also expected to protect the "use" of the environmental resources by humans)

The CSTEE is in favour of the on-going approaches regarding the integration of both Human Health and Ecosystem risk assessment. However, it is necessary to have an adequate understanding of each part before any integration. Therefore, this CSTEE opinion focuses exclusively on the hazard and risk assessment of the effects of chemicals on **terrestrial ecosystems**, recognising that a holistic assessment of the **terrestrial environment** requires additional considerations and in particular, the integration of human beings and their activities as part of the terrestrial compartment.

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CHAPTER 1

INTRODUCTION

1.1 REGULATORY USE OF HAZARD IDENTIFICATION AND RISK ASSESSMENT

Hazard and risk assessment are key elements in the regulation of chemical substances. Their role within the European Union system has been recently reviewed by Hard et al., (1998).

In principle, four main regulatory uses can be identified:

- Identification and comparison of dangerous chemicals
- Setting quality standards
- Development of environmental indicators
- Decision-making at the local (contaminated sites) and generic (activities, life-cycle assessment of substances, regulation of chemicals including registration/authorisation, etc.) levels.

The current status of each aspect will be discussed below.

Hazard assessment constitutes the essential tool for the evaluation of the potential effects of chemicals on organisms and ecosystems. It includes a first step, hazard identification, which must detect the potential dangers of the substance (i.e. the kind of effects that the substance may produce), and a second step to quantify each danger and to set the expected dose/response relationships. At the regulatory level, hazard identification/quantification can be used as independent tools or, alternatively, as part of the risk assessment.

Risk Assessment aims to estimate the probability for adverse effects to occur, in doing this assessment the risk manager applies specific protocols to compare the potential hazard with the expected level of exposure. It can be considered, nowadays, the “best available methodology” to give scientific support to decision-makers regarding the management of chemical substances.

RISK ASSESSMENT

The development of protocols and guidance documents on the use of Ecological Risk Assessment allowed the expansion of risk-based methods from decision-making in the Human Health arena to management decisions regarding the protection of the environment, and in particular, the protection of the structure and functioning of ecosystems.

Ecological risk assessment has been defined as a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (US EPA; 1998). The aim can be expanded to cover other environmental aspects not particularly related to effects on ecosystems but to other concerns such as global warming, contamination of groundwater and other resources, etc. The term Environmental Risk Assessment then defines more correctly the intended evaluation.

The aims of an Ecological/Environmental risk assessment can be very different, and therefore the protocols are also adapted to the specific conditions of each study. Just within the European Union system, we can distinguish several types of risk assessment procedures, all of them adapted to specific pieces of the legislation.

Two main groups can be observed, depending on the type of risk assessment that is going to be conducted. The first group is conducted on chemicals already on the market, allowing an evaluation of real exposure levels and environmental problems. The second group represents risks assessments conducted previous to the authorisation of the product to be put on the market, which obviously will depend exclusively on modelled predictions and default values. Several examples are included below:

A- RISK ASSESSMENT FOR SUBSTANCES ALREADY ON THE MARKET.

- Comprehensive risk assessment for High Production Volume Chemicals. Related to Commission Regulation (EC) 1488/94 it includes a full risk assessment of the whole life-cycle of certain priority chemicals. The assessment covers local, regional and continental scenarios and tries, whenever possible, to use real emission data and to establish comparisons between predicted and observed level. The CSTEE has produced opinions on several comprehensive risk assessment reports.
- Targeted risk assessment for problematic substances/uses. Represent a shortened version of the previous type concentrated on certain specific uses of dangerous chemicals, trying to support decisions on specific bans or restrictions. They do not cover the whole life-cycle of the chemical but certain aspects, and mostly focuses on local or regional scenarios. The use of real emission/exposure data is crucial for a proper decision and in most cases includes a comparative study with those other substances/technologies considered as proper alternatives for the studied chemical. The CSTEE has produced several opinions on targeted risk assessment reports.
- Risk Assessment for Existing Pesticides. In principle this is a type of targeted risk assessment to address the risks associated with the specific use of plant protection products by farmers under Directive 91/414/EEC. The outcome of this risk assessment is the inclusion of the active substance in a positive list (Annex I) of substances that can be used in plant protection products, with or without restrictions, or the total-preventive ban of the substance. Monitoring data are essential for a proper identification of the properties of the substance. The Scientific Committee on Plants is responsible for producing opinions on these assessments.

- Risk assessments as part of other regulatory decisions. In addition to specific risk assessment studies such as those presented above, risk assessments also constitute the basis for several decision-makings in the related areas. To give an example relevant to the CSTEE work, risk assessment decisions are incorporated, for example, in the Water Framework Directive to give guidance on prioritisation of pollutants and to set Environmental Quality Standards.

B- RISK ASSESSMENT AS A PREREQUISITE FOR THE COMMERCIALISATION OF THE SUBSTANCE

- Predictive risk assessment of new notified substances. Related to Commission Directive 93/67/EEC constitutes a pre-requisite for the production-import-commercialisation of substances that are not currently on the EU market. Represents a predictive approach for a holistic risk assessment of all potential risks associated with the life-cycle of the substance.
- Risk Assessment for New Pesticides. In principle is a type of targeted risk assessment to address the risks associated with the specific use of plant protection products by farmers under Directive 91/414/EEC. The outcome of this risk assessment is the inclusion of the active substance in a positive list (Annex I) of substances that can be used in plant protection products when a “safe use” is identified under the Uniform Principles. It mostly focuses on local scenarios
- Risk Assessment for Veterinary Medicines. Under the adaptation of Directive 81/852/ECC the environmental risk of new veterinary medicines must be assessed previous to their commercialisation. Is also a type of targeted risk assessment for inclusion in a positive list. It mostly focuses on local scenarios
- Risk Assessment for Biocides. Regulated by Directive 98/8/EC, also represents a targeted risk assessment for inclusion of biologically active chemicals in a positive list. Nevertheless, this regulations presents a much larger variability on intended uses than those related to pesticides or veterinary medicine and therefore, a larger diversity of scenarios both local and regional should be required. The Directive is currently under implementation and the opinion on the CSTEE on the first technical guidance document has been produced.

Risk assessment also plays a key role in the regulation of chemical substances, including Pollution Control and Sustainable Development. For example risk assessment methods can be perfectly incorporated in Environmental Impact Assessment and Environmental Audits of those activities that include the management of toxic chemicals and wastes.

HAZARD IDENTIFICATION AND QUANTIFICATION

A proper Hazard Assessment constitutes the essential element for the evaluation of the potential effects of chemical substances on organisms and ecosystems; and obviously, Hazard Assessment is considered one of the main steps in the Risk Assessment process of chemical substances (EC, 1996; USEPA, 1998), being considered as an independent initial step or as part of the effect assessment structure.

Nevertheless, Hazard Identification schemes also have a specific role in regulatory decisions, independent of the risk assessment protocols, being related to environmental management in some regulations. The use of hazard approaches without connection to a whole risk assessment is considered in all those regulatory decisions that do not include the exposure part within their aim.

The main topic is related to the "identification" of substances of high concern. Within the European Union, hazard identification constitutes the scientific basis for the classification and labelling of chemical substances according to Directive 67/548/EEC and its amendments (EC, 1997), while Hazard quantification is used for setting environmental standards and criteria, such as the Water Quality Objectives under Directive 76/464/EEC (Bro-Rasmussen et al., 1994). Similarly, hazard identification is being incorporated in regulations related to the control of environmental emissions, toxic waste management, restoration of contaminated areas, etc.

Most efforts related to the development of hazard identification systems are concentrated in the aquatic compartment. The EU regulation offers a good example. In 1991 the EU formalised criteria for hazard identification-classification of substances dangerous to the environment (EC, 1991) and in 1993 updated and published the present classification and labelling protocol (EC, 1993). In theory, the approach covers both, the aquatic and the terrestrial compartment, but in practice, only the criteria for the aquatic compartment were developed and therefore the whole EU environmental classification covers, exclusively, the toxicity for aquatic organisms and the ozone layer. (The hazards for the ozone layer were not specifically developed but directly referred to in the Montreal Protocol).

A similar situation is observed for hazard quantification. Formalised proposals to estimate "safe concentrations" for the aquatic environment were established by the CSTEE and used to set European Water Quality Objectives for several priority aquatic pollutants, while no European criteria for the soil are available.

The exponential growth of terrestrial ecotoxicology during the last decade allows an optimistic view on our capability for the extrapolation of hazard identification systems for the terrestrial compartment. Most efforts have focused on the soil compartment, and even specific proposals for soil hazard identification-classification schemes have been presented by regulatory and industrial organisations (e.g., Nordic Council of Ministries, ECETOC). However, during the international Workshop on Hazard Identification Systems and the Development of Classification Criteria for the Terrestrial Environment, held in Madrid in November 1998, there was a general consensus on the need to cover the whole terrestrial environment (i.e. soil and above ground compartments).

1.2 MANDATE

This opinion tries to present an overview of the current scientific basis of the hazard and risk assessment approaches included in the EU regulation and at the same time to discuss the current state of the art of terrestrial ecotoxicology regarding their capacity to give scientifically sound advice on the prediction and assessment of the potential dangers and effects of chemical substances on the structure and function of terrestrial ecosystems.

Although this is in fact an initiative of the CSTEE, it must be recognised that several opinions requested by the Commission to the CSTEE included aspects related to the scientific evaluation of the effects on terrestrial ecosystems and the assessment of the potential risk (see section 8.5).

Recognising the comparatively low level of attention received in the past by terrestrial ecosystems from both, regulatory bodies and the scientific community, and at the same time considering the exponential growth of the concern on this environmental compartment and of terrestrial ecotoxicology, the CSTEE considered it appropriate to give its opinion on the basic rules for a proper regulatory use of terrestrial hazard and risk assessment.

1.3 HAZARD AND RISK ASSESSMENT FOR THE TERRESTRIAL COMPARTMENT IN THE EU REGULATION

As previously explained, hazard and risk assessments are expected to support a significant number of regulatory decisions such as those included below:

- Classification and Labelling
- Quality criteria (water, soil and air)
- Environmental indicators
- Notification of new chemicals
- Registration of pesticides
- Registration of biocides
- Registration of veterinary products
- Comprehensive risk assessment for HPVC
- Targeted risk assessment for specific problems

For decades, the concern and the research activities regarding the environmental effects of pollution were dominated by the aquatic compartment. Obviously, this situation was extrapolated to the regulatory arena, and the terrestrial ecosystems are considered of secondary importance or even not considered at all in legislative initiatives.

The revision of the current situation of the European regulation on industrial chemicals detects several “Burdens of the Past”, regarding low relevance of the terrestrial environment in environmental hazard and risk assessment when compared with the aquatic system. There are different reasons to explain this fact, including the lack of appropriate scientific support on this issue. However, terrestrial ecotoxicology has achieved a substantial level of development in recent years, and therefore it is time to considere if the current state of the art allows a scientifically sound approach for the use of terrestrial hazard and risk assessment in a holistic regulatory frame.

To present the state of the art of terrestrial assessment, using the aquatic environment as a reference, three main aspects have been selected: hazard identification, hazard quantification and risk assessment.

HAZARD IDENTIFICATION-CLASSIFICATION FOR THE TERRESTRIAL ENVIRONMENT

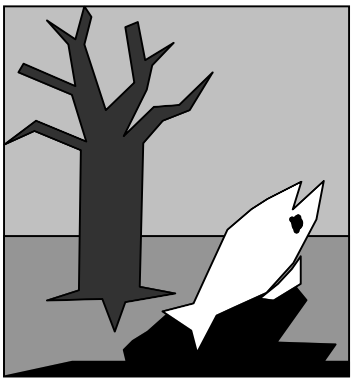
Hazard assessment constitutes, within the EU regulation, the basis for the classification of chemical substances according to Directive 67/548/EEC. The Directive establishes a classification and labelling system integrated by:

a: A symbol of hazard.
b: A set of risk (“R”) phrases.
c: A set of safety (“S”) phrases.

In principle, this system should be expected to cover “all relevant concerns” regarding the potential environmental hazards related to the intrinsic properties of chemical substances.

The symbol and the phrases indicate different environmental hazard, trying to cover the main issues for the aquatic, terrestrial and atmospheric compartment.

The symbol includes a tree and the bare soil as a representation of the terrestrial environment:



The classification categories can either express a single concern, and therefore are represented by a single R-phrase, or a combination of properties that express a combined hazard, i.e toxicity and persistence can be combined to express the potential for long-term effects.

These categories are established according to the following ten phrases which cover the toxicity for aquatic organisms and several terrestrial key groups, as well as the hazards for the ozone layer

R50 Very toxic to aquatic organisms
R51 Toxic to aquatic organisms
R52 Harmful to aquatic organisms
R53 May cause long term effects in the aquatic environment
R54 Toxic to flora
R55 Toxic to fauna
R56 Toxic to soil organisms
R57 Toxic to bees
R58 May cause long term effects in the environment
R59 Dangerous for the ozone layer

The safety phrases are general phases related to environmental release and controlled handling, which does not regard on any specific environmental compartment, for example S60 and S61:

S60 This material and its container must be disposed of as hazardous waste

S61 Avoid release to the environment. Refer to special instructions/Safety data sheet

In fact, one of the basic prerequisites considered during the development of this system was to cover all relevant compartments, and, therefore the system is expected to cover the aquatic and terrestrial compartments. However, in reality this prerequisite has not been fulfilled.

Until now, only the criteria for the "R" phrases related to the aquatic environment (R50-R53) have been developed, as well as the reference to the Montreal protocol for the effects on the ozone layer (R59). Therefore, substances are only classified as dangerous for the environment when they are toxic for aquatic organisms and/or dangerous for the ozone layer. In our opinion, this situation provokes a lack of coherence between the hazard communication (which indicates that it is dangerous for the environment as a whole) and the intrinsic properties used for the classification (which only cover toxicity for fish, daphnia and algae and fate properties also related to the aquatic compartment).

An additional problem is that certain substances that are known to be environmental hazards cannot be classified as dangerous for the environment, even when a large set of validated information clearly indicates their high potential to produce effects on certain key organisms. Certain substances such as hydrogen fluoride or acrolein which are specifically toxic for the vegetation constitute a perfect example of this lack of consistency. Even although the environmental hazards have been identified in the risk assessment programme, the substances still remain as not classified for the environment because the criteria for the R-phrase “Toxic to Flora” have not yet been developed. A similar situation can be observed for substances which are highly toxic for mammals but which are not classified as toxic to fauna, or even for insecticides which can be classified as toxic to aquatic organisms but not toxic to bees.

Currently, the European Chemicals Bureau is working on the development of classification criteria for the terrestrial environment, and this opinion is expected to give some general recommendations on the best available methods to identify the hazard of chemical substances on the basis of their intrinsic properties, recommendations which should facilitate the work of decision-makers.

HAZARD QUANTIFICATION FOR THE TERRESTRIAL ENVIRONMENT

Setting environmental quality standards or criteria can be considered as a key element regarding hazard quantification. The basic concept is to set “ecological thresholds” or the highest concentrations for which no unacceptable effects are expected.

Several proposals to estimate “safe concentrations” are available for the aquatic environment; summaries of the application factors employed by the different methods can be found in ECETOC, 1992; OSPAR, 1994; Tarazona, 1998. Taking into account these review papers, it is simple to conclude that, when deterministic approaches are used to set these threshold values, a significant level of consistency can be found among the different proposals. Even more, as pointed out in the risk assessment part, there is also a consistency between the margins of safety used here and those employed in the effect assessment part of the risk analysis.

This level of agreement is not observed for the terrestrial environment.

The current regulation also reflects these differences between the hazard quantification for the aquatic versus terrestrial compartment. In fact European Water Quality Objectives, corresponding to thresholds for the protection of the structure and functioning of aquatic ecosystems, are established for several Water Priority Pollutants. Specific criteria for the development of these objectives were established by the former CSTE (see Bro-Rasmussen et al., 1994). No EU harmonised soil quality criteria have been developed.

RISK ASSESSMENT FOR THE TERRESTRIAL ENVIRONMENT

Risk assessment protocols usually try to cover all environmental compartments, including water, soil and air in their exposure analysis although later on the effects do not always get the same relevance for all types of ecosystems.

The aquatic system is treated in a more or less homogeneous way in the technical recommendations developed in relation to the different effect and risk assessment programmes currently on-going in the EU, while large differences can be observed for the terrestrial environment.

The comparison of the methodologies recommended to set the Water Quality Objectives (Bro-Rasmussen et al., 1994), the PNEC for industrial chemicals according to Regulation 793/93 (EC, 1997); the trigger values for pesticides according to Directive 91/414/EEC; or the PNEC for veterinary products according to directive 81/852/EEC (EMEA, 1997), all follow a set of rules that can be summarised as follows:

1.- The effect assessment focuses on the toxicity data for three taxonomic groups: Fish, invertebrates (represented by daphnia) and algae.

2.- The standard EC, OECD test guidelines are the recommended protocols. The exposure is via water column in all cases.

3.- All taxonomic groups have the same weight (only for pesticides algae data are used differently than fish and daphnia data).

4.- Two procedures are used for the risk characterisation –the PEC/PNEC ratio or trigger values for the TER. However, in both cases the thresholds for acceptability are obtained by dividing the toxicity results by an application (uncertainty) factor which represents the margin of safety between toxicity for the standard species and the environmentally relevant effects.

5.- The margins of safety are in most cases equivalents. The values are mostly 100(-1000) for the acute tests and 10 for the chronic tests.

Therefore, it can be concluded that the recommendations for the risk assessment for the aquatic environment follow a similar scientific approach in all cases, obviously adapted to certain specific requirements of each assessment/use.

By contrast, the situation is not equivalent for the terrestrial environment. The methodology and/or recommendations are in most cases not as developed as those relating to the aquatic system, maybe with the only exception of pesticides.

The effect assessment for the terrestrial compartment is considered in very different ways depending on the specific technical recommendation developed for each risk assessment procedure.

The terrestrial environment is in reality an interface located around the ground level. It has been traditionally divided in the soil and the above soil compartments. This distribution can be followed for the exposure assessment, but creates a lot of problems when applied to the effect assessment. In fact, although some organisms can be clearly defined as soil dwelling or above ground dwelling organisms, there is a large percentage of organisms, including plants, different invertebrates and even some vertebrates, which are distributed simultaneously or alternatively between both compartments.

The TGD for industrial chemicals includes a specific chapter on the risk assessment for the terrestrial environment. However, this assessment only includes the soil compartment. The recommendation for the assessment, regarded as provisional, is the use of toxicity data on micro-organisms, earthworms and plants (exposed through the soil). The risk characterisation follows the PEC/PNEC approach and the same safety factors are suggested for the aquatic and for the terrestrial (soil) compartment (1000 for acute effects, 10 for chronic effects when the full data set is available). The risk can also be assessed by extrapolation from the aquatic toxicity data considering partitioning equilibrium between soil particles and soil pore water to quantify the exposure.

In addition this TGD for industrial chemicals also includes chapters for the assessment of the effects on the atmosphere and through secondary poisoning. The second approach covers the risk for terrestrial vertebrates (mostly mammals) while the first one is a mixture of abiotic effects and biotic effects on “organisms exposed through the air” which is not currently developed.

The recommendations for the risk assessment of pesticides included a full set of taxonomic groups which represent the terrestrial environment. The effects on mammals, birds, bees, non-target arthropods, earthworms and micro-organisms are always assessed, and additional possibilities for soil-macro-organisms and other non-target flora and fauna are also included. The risk characterisation follows absolutely different approaches for each group: TER for mammals, birds and earthworms; Hazard Quotients for bees, and non dose-response related limit values (percentage of effect observed at the highest application rate) for non-target arthropods and micro-organisms. The safety factors estimated for these4 proposals are highly variable and not in agreement with those proposed for industrial chemicals (i.e 10 for acute effects and 5 for chronic effects on vertebrates and earthworms).

The recommendation for the risk assessment of veterinary products (EMEA, 1997) also considers, at different tier levels, potential effects on terrestrial organisms. For plants, earthworms and micro-organisms, the exposure is expected to be via soil, and the recommended assessment factor for acute effects is 10 (with some variations depending on the persistence). For mammals and birds the same assessment factor, 10, is recommended for oral exposures. For arthropods the approach also considers a pre-established level of effect although this level is much higher than that proposed for pesticides and is different for different tier levels.

The recommendations for the risk assessment of biocides mostly consider the terrestrial environment in a similar way to that described for industrial chemicals (effects on soil organisms and secondary poisoning) while some groups of biocides are expected to reach the environment in a similar way to pesticides. In fact, the effects on soil organisms are not included in the core data set, and the first assessment must be done using the equilibrium partitioning method developed for industrial chemicals. The CSTEE has recently pointed out that the equilibrium partitioning method is not appropriate for chemicals which, like biocides, have specific modes of action.

Clearly, the effect assessment for the above soil compartment is less developed than the other “terrestrial” exposure routes. The guidance for the assessment of the effects of ozone on vegetation (WHO, 1996) is a good example. The only possibility to set recommendations on acceptable values was to look directly to the effects observed under field situations, using data for which the margin of safety must be established under case-by-case approaches.

For low-tier assessment, in most cases deterministic methods are employed, and therefore it is possible, although not always easy, to estimate the margins of safety employed in each case. Table 1 summarised the margin of safety employed to protect different terrestrial taxonomic groups in the recommendation agreed for industrial chemicals versus those recommended for veterinary medicines and for pesticides.

Table 1. Comparison of the margins of safety for the protection of terrestrial organisms employed in the environmental risk assessment of veterinary medicines, industrial chemicals and pesticides.

Group	Exposure route	Timing	Margin of safety		
			Veterinary Medicines	Industrial chemicals	Pesticides
Vertebrates (birds and mammals)	Direct	Acute Chronic	10 -	Not considered Not considered	10 5
	Secondary poisoning	Acute Chronic	10 -	1000 100-**10**	10 5
Plants	Soil	Acute Chronic	10 -	1000 100-**10**	Not considered
Earthworms	Soil	Acute Chronic	10 or 100 depending on persistence in soil	1000 100-**10**	10 5
Bees	Oral Contact	Acute Acute	Not considered	Not considered Not considered	5-17 5-1500
Other arthropods	Contact	Acute	<1-1	Not considered	1-5
Soil micro-organisms	Soil	Acute Chronic	10 -	1000 100-**10**	1-5

Similarly, Table 2 presents the same information for aquatic organisms.

Table 2. Comparison of the margins of safety for the protection of aquatic organisms employed in the environmental risk assessment of veterinary medicines, industrial chemicals and pesticides.

Group	Exposure route	Timing	Margin of safety		
			Veterinary Medicines	Industrial chemicals	Pesticides
Fish	Water column	Acute Chronic	100 -	1000 100-**10**	100 10
Invertebrates (Daphnia)	Water column	Acute Chronic	100 -	1000 100-**10**	100 10
Algae	Water column	Acute Chronic	100 -	1000 100-**10**	10 10
Aquatic plants	Water column	Acute	Not considered	1000 100-**10**	10 10

For industrial chemicals, numbers in bold represent those that are employed when a whole data set is available. Obviously, for pesticides the data set must always be fulfilled and additional information is requested when no valid information is available for any key taxonomic group. For veterinary medicines, the evaluations are only required if a set of previous requisites is fulfilled.

The objective of these tables is just to compare the level of agreement among different proposals. It can be clearly observed that the variability observed for the terrestrial environment is much higher than for the aquatic compartment. For the same chemical the differences in the estimation of its ecotoxicological threshold or maximum acceptable concentration/dose for terrestrial organisms could be as high as three orders of magnitude depending on if it is considered as an industrial chemical or as a pesticide. The differences are not restricted to the numbers, they also cover the epistemiology of the assessment, i.e. for industrial chemicals similar margins of safety are applied to all taxonomic groups (terrestrial and aquatic), while specific values for each group are applied for pesticides and veterinary medicines. This variability can be explained by several reasons, including a lower development of the scientific basis on which these safety factors are constructed.

All these reasons justify the initiative of the CSTEE to produce this document.

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CHAPTER 2

PRINCIPLES FOR ECOLOGICAL RISK ASESSMENT AND THEIR APPLICABILITY TO TERRESTRIAL ECOSYSTEMS

2.1 PROBLEM DEFINITION AND SELECTION OF SCENARIOS

The starting point of any risk assessment is the clarification of the issue that is going to be evaluated. Adopting the conventional definition of risk assessment as the quantification of the likelihood of an event to occur, this problem definition must clarify this event as well as the specific hazards covered by the evaluation, including the sources for these hazards.

These general needs are obviously also valid for ecological risk assessment.

A clear distinction between the assessment rules for the terrestrial environment and those for terrestrial ecosystems is required. This opinion focuses on evaluation of the ecosystem risk, defined as the likelihood for the occurrence of adverse effects on the structure and functioning of the ecosystems. Other aspects, such as those reflected in effects on human health, socio-economic aspects (crop production, livestock, etc.) or landscape conservation, although obviously relevant for the terrestrial environment, are not covered by ecological opinion. Nevertheless, it is normally assumed that in the case of chemical pollution, the levels required for the protection of the ecosystem should in almost all cases also be protective for those populations of economic interest such as crops or farm animals, as well as for the relevant landscape issues such as avoiding erosion problems due to reduction of vegetation cover.

Human Health risk, however, is not adequately covered by an ecological risk assessment, mostly because the level of ecological protection (populations, communities) does not cover the protection of individuals which is obviously required for humans. The CSTEE is aware of the efforts to develop methodologies for Integrated Risk Assessment and recognises the need to cover human health aspects in the assessment of the terrestrial environment as well as the possibilities for an integrated assessment in the future. However, as already mentioned, this opinion only covers issues directly related to the evaluation of the terrestrial ecosystem.

A short review of published ecological risk assessment reports clearly indicates a large variability of potential "types" of ecological risk assessments. These include holistic and predictive assessments such as those conducted prior to the registration of a new pesticide or under the notification procedure of a new chemical. They also include quite specific evaluations such as those conducted to associate environmental problems to contamination sources, i.e. searching for cause-effect relationships between decline of fish populations and upstream effluent dicharges. These differences reflect the wide range of problems for which risk analysis of terrestrial ecosystems is required.

The US EPA guideline on ecological risk assessment (US EPA, 1998) considers that the problem formulation phase provides the foundations for the entire assessment. It is the first step of the process, in which the purpose for the assessment is articulated, the problem is defined, and a plan for analysing and characterising risk is determined. In this phase the integration of the available information must be able to produce three different outcomes:

- The list of assessment endpoints or environmental values that are to be protected
- The conceptual model, which describes the relationships between the ecological entities and the stressors
- The analysis plan, including the assessment design, data needs, measures, and methods for conducting the assessment.

When risk assessment results are used for regulatory purposes, this phase is modulated, at a certain level, by the legal considerations established by decision-makers. Usually, the problem is legally defined, incorporating scientific basis but also socio-economic and other related issues in the definition of the regulatory goal.

Differences in the problem definition according to the regulatory use can obviously be expected. The kind of risk assessments mostly included as relevant tools for the regulation of chemicals can be grouped as:

- Holistic risk assessments. These are intended to offer a general view on the likelihood of occurrence of environmental problems. Usually include conditions for acceptability and/or triggers for additional (more specific risk and higher tier) assessments. The problem definition can be formulated in several ways, i.e. probability for occurrence of adverse effects on: ecosystems in general, on compartments (aquatic, terrestrial), on specific groups of organisms, etc.

- Targeted risk assessment. Focused on a quite specific problem, are restricted to a clear problem in terms of both source and type of hazard to be assessed and the environmental problem considered in the evaluation. Can be independent, i.e. conducted to identify the benefits of certain restrictions and conditions for use, or a higher tier risk assessment conducted for a specific problem identified during a generic risk assessment.

Problem definition for holistic risk assessment: the terrestrial environment.

The assessments covering a holistic evaluation of a chemical (including all or a significant part of its life cycle) are usually based on a fixed problem definition described in the legal instrument to which the risk assessment is expected to be useful. This is a common situation in the EU. Commission Regulations 1488/94 and 142/97 define the environmental spheres and the principles for the environmental risk assessment of existing chemicals. Directives 91/4141/EC and 94/43/EC define the conditions and decision-making criteria for pesticides. A similar situation can be found outside Europe. The ECOFRAME (1999) has proposed a generic conceptual model for the registration of pesticides in the USA, and the US EPA guidance document (USEPA 1998) recognises that in certain cases (new chemical assessment) the analysis plan is already part of the established protocol and a new plan is generally unnecessary.

Due to the complexity of both the exposure routes and the organisms potentially affected, the definition of the study goal to cover terrestrial ecosystems will normally require further elaboration.

There are a number of possible ways of making assumptions about the terrestrial environment in order to make a holistic assessment more manageable. One assumption frequently made is to evaluate the terrestrial environment exclusively with regard to soil exposures. Then, for holistic risk assessment, other relevant hazards for terrestrial ecosystems must be considered as independent items. The TGD on existing and new chemicals offers the best example for this situation (EC, 1996). Terrestrial ecosystems are covered under three different topics: "terrestrial risk assessment" which deal with soil dwelling organisms, the "atmospheric risk assessment", which deals with exposures through the air as well as those related to atmospheric deposition, and the "risk for secondary poisoning", which is expected to cover exposures via food.

This approach is a simplification that can be perfectly valid if it is able to cover all relevant assessment endpoints. The main concern is that it can in some cases produce an under-estimation of the real risk for the terrestrial environment. For example, in the TGD (EC, 1996) approach, the risk for terrestrial vertebrates is only covered as secondary poisoning related to bioaccumulable substances in a simplified approach, which can be summarised as follows:

Table 1. TGD approach to cover bioaccumulation potential (EC, 1996).

ENVIRONMENTAL COMPARTMENT	BIOCONCENTRATION IN INTERMEDIATE ORGANISMS	RISK FOR TERRESTRIAL VERTEBRATES
PECwater	PECfish=PECwater x BCFfish	PECfish/PNECoral
PECsoil	PECearthworms= PECsoil x BAFearthworms PECplants=PECsoil x BAFplants	PECearthworms/PNEC oral PECplants/PNECoral

The main problem of this approach is that it does not consider other relevant exposure routes, such as the deposition of the chemical "on" the surface of food items due to atmospheric deposition (industrial chemicals) or overspray (pesticides, biocides). Nor is biomagnification through the food chain included, which is considered an essential element for a proper risk assessment of persistent and bioaccumulable substances.

Figures 1 and 2 present a rapid comparison on the potential exposure routes that should be addressed in a generic environmental risk assessment for aquatic and terrestrial ecosystems.

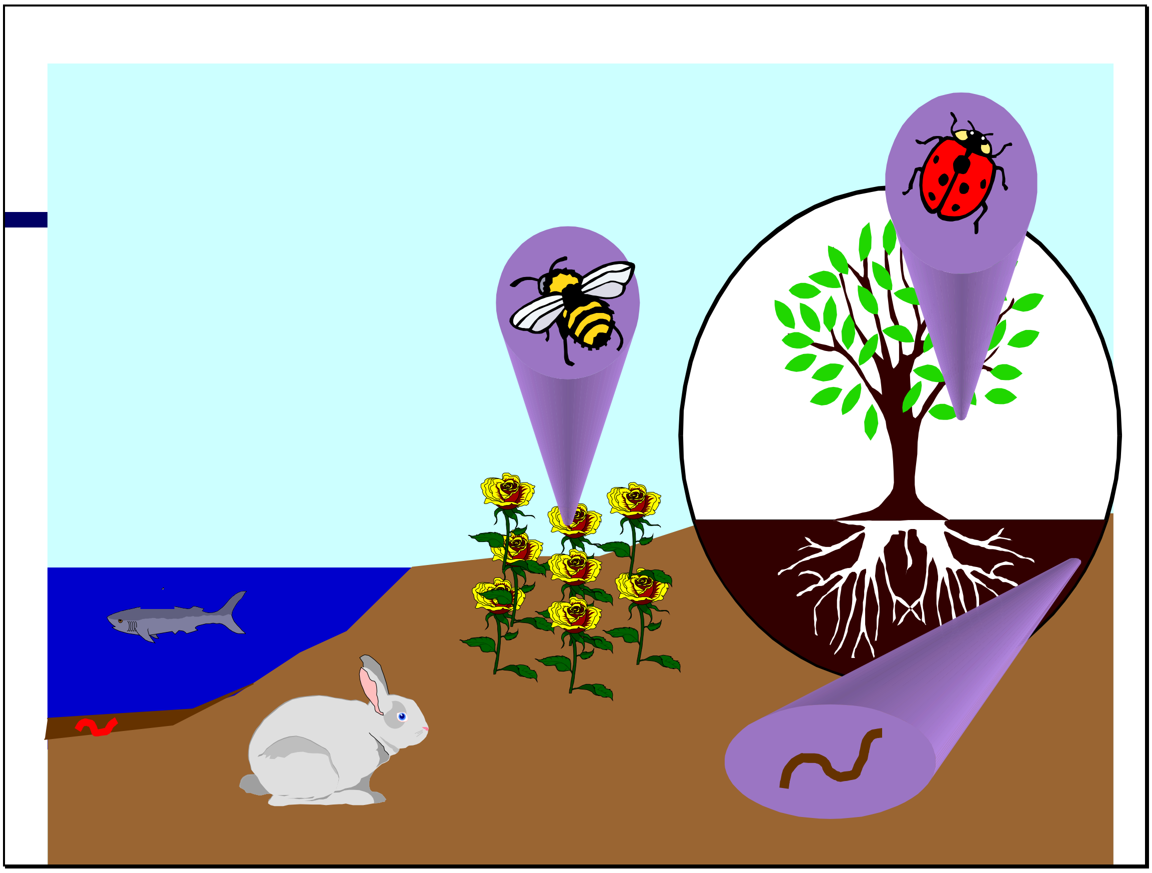

Figure 1. Key elements for an environmental exposure assessment of the terrestrial and aquatic ecosystems.

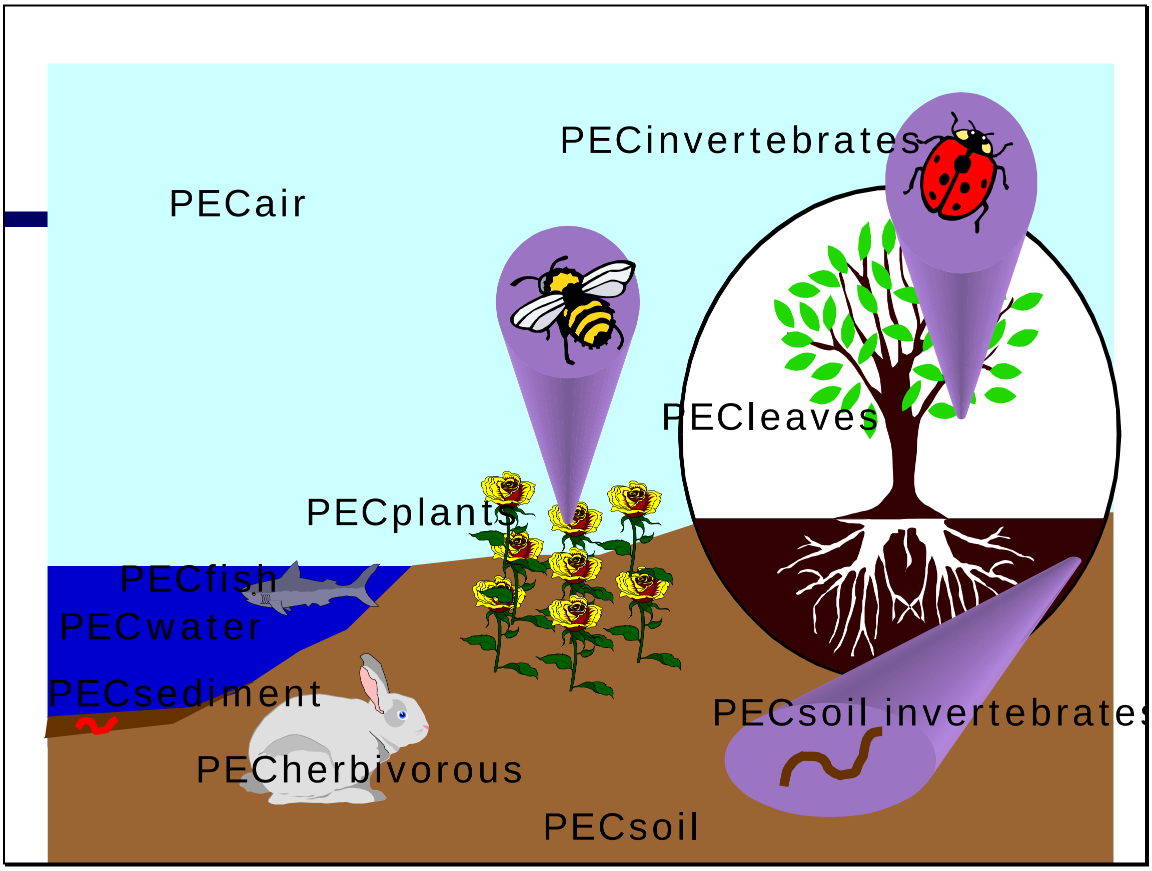

Figure 2. Examples of Predicted Environmental Concentrations which must be estimated for an assessment of the aquatic and terrestrial ecosystems.

The assessment of the terrestrial environment requires the estimation of many more PEC values than required for aquatic systems. The assessment is also much more complex as the number of processes involved in the assessment is also higher and with different time frames.

Therefore a proper hazard identification scheme is recommended. This scheme should be able to establish which components of the terrestrial ecosystem are expected to be at potential risk and which exposure routes are expected to be the most relevant.

This approach, when applied to generic risk assessments, requires a wide frame capable to cover all relevant hazards, and a tiered decision-making protocol to establish those representing the essential concern on a case-by-case basis.

Problem definition for targeted risk assessment: the terrestrial environment.

By definition, a targeted risk assessment requires a specific problem definition. The scheme on problem formulation proposed by the US EPA (US EPA, 1998) is recognised as an excellent proposal. Looking at targeted risk assessments published in the open literature, this specificity can solve the problems of complexity associated with the terrestrial environment. A good example is the risk assessment for bird populations conducted with granular pesticide formulations, when both the exposure route with consumption of granules remaining on the soil surface after treatment by the farmer, and the potentially endangered ecological receptors, are well defined.

However, the situation is not so clear when the targeted risk focuses on a certain use of the chemical, but trying to cover all relevant hazards. The situation in this case is similar to the generic risk assessment and a proper identification of the receptors of the terrestrial environment expected to be at risk is therefore required. A particular problem appears in the "comparative risk assessments" (evaluations intended to establish the relative risk of several chemicals), which include targeted risk assessment where the evaluated substance is compared versus potential alternatives. If the properties of the different chemical are different, the relevant receptors and hazards can also be different. For example, the alternative to a chemical that represents an unacceptable risk for soil dwelling organisms can be perfectly acceptable for soil dwelling organisms although it represents an unacceptable risk for top predators due to biomagnification through the food chain. If the comparative risk assessment is restricted to the soil compartment, the risk for biomagnification will not be considered, and therefore the outcome can be to recommend the most dangerous chemical. These issues need to be addressed directly when choosing the specific approach for the risk assessment.

2.2 IDENTIFICATION AND QUANTIFICATION OF RELEVANT EXPOSURE ROUTES

Exposure characterisation describes potential or actual contact or co-occurrence of a substance with receptors. It is based on measures of exposure and ecosystem and receptor characteristics that are used to analyse sources of chemicals, their distribution in the environment, and the extent and pattern of contact and co-occurrence. The objective of the exposure assessment is to predict the concentration profile or dose of a substance to which the receptor will be exposed. The exposure profile should identify the receptor (i.e. the exposed ecological entity), describe the exposure pathway from the source to the receptor and describe the intensity and spatial and temporal extent of co-occurrence or contact. Thus, for ecological exposure, the assessment should, in principle, consider all stages of the life cycle of a substance, from production, through use, to disposal and recovery. The profile should also describe the impact of variability and uncertainty on exposure estimates and reach a conclusion about the likelihood that exposure will occur.

A complete picture of how, when and where exposure occurs or has occurred is developed by evaluating sources of releases, the distribution, fate and behaviour of the chemical substance (stressor[1]) in the environment and the extent and pattern of contact or co-occurrence (EPA/630/R-95/002F, April 1998):

([1]The term “stressor” in the EPA document is defined as any physical, chemical or biological entity that can induce an adverse response. The term is used as synonymous with “agent”. In this document the term is restricted to chemical agents only.)

Description of the source(s)

The objective of this step is to identify the sources, evaluate what stressors are generated and identify other potential sources. In Guidelines for Ecological Risk Assessment, EPA has provided some useful questions to ask when describing sources (US EPA 1998):

- Where does the stressor originate?

- What environmental media first receive stressors?
- Does the source generate other constituents that will influence a stressor's eventual distribution in the environment?
- Are there other sources of the same stressor?
- Are there background sources?
- Is the source still active?
- Does the source produce a distinctive signature that can be seen in the environment, organisms or communities?

Description of the distribution of the chemical(s) (stressor) or the disturbed environment
The objective of this step is to describe the spatial and temporal distribution of stressors in the environment. This characterisation is prerequisite for estimating exposure since exposure occurs when receptors co-occur with or interact with stressors.

For evaluation of distribution of the chemical(s) (stressor) the following questions may be asked (US EPA, 1998):

- What are the important transport pathways?
- What characteristics of the chemical(s) influence transport?
- What characteristics of the ecosystem will influence transport?
- What secondary stressors will be formed?
- Where will they be transported?

Evaluating transport pathways

Chemical(s) (stressors) can be transported via many pathways, by air currents, in surface waters, over and/or through the soil surface and through ground water and through the food web. A careful evaluation can help ensure that measurements are taken in the appropriate media and locations, and that models include the most important processes. To evaluate transport pathways physicochemical properties of substances must be taken into consideration. The main physicochemical properties that affect the fate and behaviour of a chemical in the environment are molecular weight, melting point/boiling point, vapour pressure, solubility in water and partition coefficient between water/sediment, water/soil and water/natural lipids. (For reference with regard to the role of physico-chemical properties see e.g. Mackay 1991). Constituents of chemical mixtures may have different properties, and how the composition may change over time as the mixture moves through the environment, should also be considered.

To more accurately predict the fate and behaviour of a chemical in the environment, information on the transformation and behaviour of the substance should be linked to the simple physicochemical properties of the substance. Simple tests that will add to predicting the eventual fate and behaviour of chemicals are experiments on hydrolysis as a function of pH, adsorption/desorption tests, photolysis studies and studies on degradation in natural water/sediment.

Persistence along with mobility can be considered the two most important factors that dictate the fate of a chemical in the environment. Like mobility, persistent chemicals are characterised by specific properties, which can be predicted from physicochemical data and fate and behaviour studies.

When a chemical enters the environment it is subject to two degradative forces: physical (e.g. UV light) and biological (e.g. microbiological and zenobiotic metabolising enzyme systems of an organism). In considering the biological degradation of environmental pollutants it is important to consider the enormous array of species that might be involved in degradation in a single ecosystem and to bear in mind that there are many metabolic pathways by which a xenobiotic might be metabolised. Bacteria are without doubt the most important members of an ecosystem in terms of biodegradation. .

Adsorption/desorption is another important process, which affects the fate and behaviour of a chemical in soil, water and sediment. Chemicals which are strongly bound to soil or sediment will not be mobile except by physical transportation.

In assessing the environmental impact of a chemical it is important to consider the ability of the chemical to vaporise.

Predictions of fate and behaviour of chemicals in the environment based on knowledge of physicochemical properties, persistence and mobility data and partition coefficients are still at best tenuous. Considerations of biotransformation of the chemical as well as the enormous physical variations (e.g. climatic conditions) which occur in real ecosystems should also be included. Ecosystem characteristics influence the transport of all stressors. The challenge is to determine the particular aspects of the ecosystem that are most important.

Chemical distribution in the environment may be obtained by direct measurement, from modelling or a combination of both. If chemicals already have been released, direct measurement of environmental media or a combination of modelling and measurement is preferred. Models enhance the ability to investigate the consequences of different management scenarios and may be necessary if measurements are not practicable or possible. The models may be simple or complex and may include models that predict quantitative relationship of sources and stressors. For more details in fate decription of chemicals see chapter 6.

Evaluating secondary stressors (e.g. metabolites, biodegradation products or chemicals formed through abiotic processes)

Secondary stressors can greatly alter conclusions about risk; they may be of greater or lesser concern than the primary stressor. Secondary stressor evaluation is usually part of exposure characterization, however it should be coordinated with the ecological effects characterization to ensure that all potentially important secondary stressors are considered. Secondary stressors may be formed by microbial action and biotransformation. They can also be formed through ecosystem processes, for instance can nutrient input into an estuary decrease dissolved oxygen concentrations because they increase primary production and subsequent decomposition? Physical disturbances in the environment can also generate secondary stressors.

Description of contact or co-occurrence

The objective of this step is to describe the extent and pattern of co-occurrence or interaction between stressors and receptors (i.e. exposure). If there is no exposure there will be no risk. Exposure can be described in terms of stressor and receptor co-occurrence, actual stressor contact with receptors or stressor uptake by receptor.

For chemicals, exposure is quantified as the amount of a chemical ingested, inhaled or topically applied (potential dose). It can be quantified as an environmental concentration, with the assumption that the chemical is well mixed or that the organism moves randomly through the medium. This approach is commonly used for respired media (water for aquatic organisms, air for terrestrial organisms). For ingested media (food, soil) another approach combines modelled and measured contaminant concentrations with assumptions or parameters describing the contact rate (US EPA, 1993). Finally, some stressors must be internally absorbed. The absorption over biological membranes is dependent on the physicochemical properties of the chemical, the medium, the biological membrane (integrity, permeability) and the organism (nutritive status, health status). Absorption is usually assessed by modifying an estimate of exposure with a factor indicating the amount of the stressor that is available for uptake. Pharmacokinetic models can also be used to estimate internal dose. Free concentration of the chemical in the target tissue would be the most appropriate measure to evaluate risk of a chemical exposure to an organism. However, most stressor-response relationships express the amount of stressor in terms of media concentration (monitoring data) or potential dose rather than internal dose. However, to confirm that exposure has occurred determination of tissue concentrations and the use of biomarkers can be valuable and stressor concentrations in prey may be used to indicate exposure of predators.

The various ways of assessing exposure have different merits and degrees of uncertainty. Whether exposure is measured directly or modelled, predictions are made about the exposure of the environment in general or of specific subgroups/populations, either as “average” or “worst case” exposures.

For risk assessment of pesticides in the terrestrial environment, earthworms, bees and birds are used as risk indicators. With regard to relevant exposure routes, it has been assumed that earthworms be primarily exposed via soil (EU´s WG FOCUS (Forum for the coordination of pesticide fate models), for bees oral exposure and contact exposure are both considered relevant (EU´s Uniform Principle (EC, 1997)), while birds are assumed to be exposed mainly through the intake of residues in their food (for pesticides; treated plants, seeds or insects). ECPA (European crop protection association) have proposed to ignore exposure via water, drifting spray, other prey, inhalation etc for birds; furthermore, they also propose a method by which one can calculate residues on various food-stuffs immediately after spraying of the pesticides.

2.3 EFFECT ASSESSMENT: HAZARD IDENTIFICATION AND DOSE-RESPONSE ASSESSMENT

The effect assessment part must describe the more relevant effects produced by the pollutant and evaluate the expected level of damage associated to the different levels of exposure. The US EPA (1998) guidance document establishes two phases, identification of the effects of interest, and evaluation of the magnitude of the effects according to the stressor levels. In the EU, these phases, identification and quantification, are even more clearly established. For example, in the risk assessment of new (EC, 1993b) and existing chemicals (EC, 1994) it clearly states that the effect assessment comprises two different steps:

- Hazard identification: "the identification of the adverse effects which a substance has an inherent capacity to cause"
- Dose(concentration) – response (effect) assessment: "the estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect". The result of this estimate is in many cases the predicted no effect concentration (PNEC)

The hazard identification is particularly relevant for the terrestrial environment, considering the complexity of the interaction between the receptors and the exposure routes.

Hazard identification is also the basis of the EU legislation for the classification of dangerous chemicals, which is based on the hazard, and which is related to the intrinsic properties of the substance. The classification is not based on the risk of the substance, since a risk classification would require knowledge of all relevant uses of the substance, and this information is rarely available. Therefore, this hazard identification exercise must identify the relevant ecological adverse effects that can be caused by the substance. In addition, the classification exercise must also include consideration of the numbers of categories, (e.g., highly toxic, toxic, harmful, not-dangerous) that are needed to describe the adverse effect appropriately.

The quantification or evaluation of the magnitude of the hazard should in principle produce a full range of predictions on the expected effects at different levels of exposure. However, in most cases this aspect is simplified in a quite dramatic way, and the whole approach is restricted to the estimation of the level of exposure (dose or environmental concentration) which is considered "safe enough". To follow with the approach selected by the TGD (EC, 1996), the PNEC is defined as the concentration below which an unacceptable effect will most likely not occur.

Other European risk assessment guidelines, for example those recommended for the registration of pesticides, also include the identification and quantification of the effects, but do not try to establish an "acceptable" level or PNEC, and the effect assessment concludes with the production of the relevant list of endpoints ($L(E)C_{50}$s, LD_{50}s, NOECs, LOECs, NOAELs, LOAELs, etc.)

The lack of harmonisation among the different EU guidelines for ecological risk assessment can be clearly observed in the recommendations for veterinary products (EMEA, 1997). This document follows the TGD approach for aquatic organisms, and therefore the effect assessment includes the estimation of a PNEC for aquatic organisms, and the alternative ECCO (EC Co-Ordination programme for pesticides) approach for terrestrial organisms, and therefore instead of a PNEC for soil-dwelling organisms, just a list of validated data on the toxicity (L(E)C_{50}s) for terrestrial plants, earthworms and micro-organisms is produced.

As extended under the next point, the use of one approach or the other produces clear differences in the approach selected for the risk characterisation, which must be scheduled according to the information provided by the risk analysis and in particular, by its effect assessment part.

When considering relevant adverse effects, the primary need is to identify the effects that are of concern, and the second step is to select appropriate methods to assess and quantify these effects. This process may identify effects on certain ecological receptors which cannot readily be either assessed or quantified by existing test methodology. Although the efforts on the terrestrial side have been clearly significant in recent years (see chapter 4) the need for relevant and standardised assays, at different tier levels, is still evident.

The assessment of relevant effects is mostly done according to the information supplied by laboratory toxicity tests. Therefore, for the terrestrial compartment, the effect assessment should consider two different needs regarding hazard identification and quantification. Firstly, the way in which the organisms have been exposed to the chemical and secondly, the effects observed in the organisms. For each assay, the exposure routes are restricted to those covered by the experimental approach, and must be clearly identified. There are two major possibilities, the chemical can be incorporated in the system, i.e., added to the soil, or the organisms can be dosed directly, i.e., incorporating the chemical into their food. In addition, there are several possibilities for each approach. For example, for soil exposures the chemical can be mixed with the soil or sprayed on the soil surface. The recent OECD guidelines on acute toxicity on bees offer two possibilities for direct dosing: oral and contact exposures.

Significant efforts on the terrestrial side of the effect assessment have been made in the arena of pesticide risk assessment. However, several test designs reproduce the typical exposure conditions expected for pesticide applications (over-spray) and are not conducted as dose/response assays but only as limit tests measuring the effects at the intended application rate (expressed as kg pesticide/ha). Modifications in the test design are required if this assay is to provide information that is also relevant if these systems are to be used for testing chemicals in general.

Therefore, the effect assessment analysis should produce not only a list of validated toxicity information on terrestrial (soil and ground dwelling) organisms, but also clear indications on the relevance of each type of toxicity data for each of the main potential exposure routes identified by the exposure assessment analysis.

2.4 RISK CHARACTERISATION AND RISK REFINEMENT

There is a clear agreement in all risk assessment protocols on the definition of the risk characterisation as the combination of exposure and effect assessment. However, some differences can be observed in the way in which this comparison is formally conducted, and on how the results obtained for this comparison are considered with regard to the acceptability of the obtained level of risk.

Both the EU approach for industrial chemicals (TGD, EC, 1996) and the US EPA guidelines (US EPA, 1998) consider risk characterisation as an independent concept in the risk assessment process which takes place after the characterisation of exposure and ecological effects. However, the EU guidelines for pesticides and veterinary products incorporate the risk characterisation in the "ecotoxicological evaluation" (effect assessment and risk characterisation).

As stated before, the way in which the effect assessment is conducted also affects the procedure for the risk characterisation.

This is particularly relevant for assessments based on the extrapolation of single species toxicity data (lower tier assessments). When the effect assessment has included the establishment of a PNEC or any other estimation for an "acceptable concentration" or ecotoxicological threshold, then the initial risk characterisation is reduced to the comparison of the predicted environmental concentration, PEC, with this acceptable value. The condition for acceptability is obviously related to an expected concentration lower than (or equal to) the acceptable concentration, i.e.:

The risk is considered acceptable for PEC/PNEC ratios lower than (equal to) 1[1]

When the effect assessment has been restricted to the production of a validated list of toxicity data, then the risk characterisation guidelines must include the acceptability conditions. The usual approach is to estimate the ratio between the toxicity endpoint and the predicted exposure (PEC). The guidelines for pesticides use directly the terminology of toxicity/exposure ratio (TER).

$$TER = Toxicity/PEC$$

The TER corresponds to the margin of safety (MOS), which is also used in other evaluations, including the assessment of effects on human health. As discussed in Chapter 1, there is no general agreement on the level of an acceptable MOS in the different EU guidelines for the assessment of effects on terrestrial organisms.

In addition to the "pure mechanical" issue (produce a ratio with the PEC in the upper or lower part of the equation), the use of PEC/PNEC versus TER-MOS approach has a major conceptual difference:

By definition, the PNEC or equivalent value integrates all the available information for the relevant receptors in the compartment considered in each risk characterisation. Thus there is a single PNEC for soil dwelling organisms which incorporate acute and

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[1] For new substances, if the PEC/PNEC ratio is greater than one, the Competent Authority should evaluate whether further information is needed immediately or at the next tonnage threshold. The TGD suggests that immediate testing is required if the PEC/PNEC ratio is greater than 10. Thus values between 1 and 10 are normally considered acceptable until the next tonnage threshold.

chronic effects on all soil dwelling organisms (mostly terrestrial plants, soil invertebrates and soil micro-organisms). However, in the TER or MOS approach each toxicity endpoint is considered in a separate way, and therefore for the same compartment this results in several TERs or MOSs, i.e., in the risk assessment for pesticides there are up to thirteen different TERs, five food items (grass, insects, grains, fish, earthworms) and six TERs for each food item, three for birds (acute, short-term and long-term) and three for mammals (acute, short-term and long-term). In comparison, for industrial chemicals following the TGD approach, there is a single comparison between the PNEC for terrestrial vertebrates and the PEC in food (normally assuming the worst case).

Each approach has advantages and disadvantages. While the TER comparisons allow the identification of those aspects of potential risk (i.e., acute but not long-term risk; risk for herbivorous but not for insectivorous organisms, etc.) allowing expert judgement for the ecological relevance of the identified potential risk, a simplified comparison such as the PEC/PNEC ratio can usually produce more transparent decisions by decision-makers and allows a simple risk communication which reflects a basic ecological principle: a risk for a key element of the ecosystems means a risk for the ecosystem as a whole.

The way in which the output of the scientifically-based risk characterisation is incorporated into the decision-making process is obviously related to the specific legislation. Nevertheless, in all cases when a potential risk is identified in a lower tier risk assessment, this triggers a refined (higher tier) assessment: if the potential risk is confirmed in higher tier assessment then risk reduction measures, use restrictions, and/or cost/benefit analyses are required.

A particular problem for the risk characterisation for the terrestrial environment is the need to co-ordinate the results from the exposure and the effect assessment. The system is relatively simple for soil and food exposures, as PEC_{soil} and PEC_{food} can be easily compared to toxicity data for soil and food exposures respectively. However, difficulties appear for all other exposure routes, those usually regarded as above ground exposure routes, such as atmospheric deposition (including intended spray on ground and vegetation), direct and indirect contact, etc.

For industrial chemicals the TGD includes local and reginal exposure estimations, but no guidance on effect assessment nor risk characterisation. For pesticides, pragmatic approaches for bees and non-target arthropods have been adopted.

The effect assessment for bees, as previously mentioned, provides information on the LC_{50} by oral and contact exposures, which are expressed as ug/bee. The exposure part provides the application rate, expressed as g/ha. Currently, a proper scenario to combine toxicity (weight of pesticide/animal) with exposure (weight of pesticide/surface) is not available. The pragmatic decision has been to use, for the initial risk assessment, the Hazard Quotient (HQ) or ratio between the application rate and the toxicity, selecting a cut-off value for acceptability of this value (currently 50 for the application rate expressed as g/ha and the LC_{50} expressed as ug/bee).

Finally, the risk characterisation can only be a very preliminary assessment when the information provided by the effect assessment has not adequately addressed the magnitude of the hazard. This aspect is particularly relevant when the effect assessment is based on a limit test instead of on a full dose/response assay. The best example in the EU pesticide legislation comes from the risk assessment for non target arthropods other than bees (the so-called beneficial arthropods) and for soil micro-organisms in the risk assessment for pesticides. Both are based on tests conducted at the intended application rate, and the maximum intended rate must not produce more than a pre-selected toxic response (30% of effect for arthropods, 25% of effect for micro-organisms).

Regarding the risk refinement, the possibilities obviously depend on the kind of risk characterisation conducted in each case. Both aspects of the risk analysis, exposure and effect, can be refined.

For the exposure assessment, the refinement can incorporate aspects such as using real emission data instead of default values, moving from worst case to realistic scenarios, or presenting the exposure as a probability function for the PEC.

For the effect assessment several possibilities can also be mentioned. In the PNEC approach the clearest refinement is by incorporating chronic toxicity data (the safety factor can then be reduced by two orders of magnitude). In all cases, when the basic data set (acute and chronic toxicity data on key species for all relevant groups), the main options are: producing more information to incorporate species-distribution curves in the assessment, to consider more realistic bioassays, i.e., incorporating changes in the bioavailability; or to enhance the ecological relevance of the effect assessment using multi-species and field studies.

2.5 DEFINITIONS AND ABREVIATIONS

Agent: Any physical, chemical, or biological entity that can induce an adverse response. (Synonymous with stressor). (US EPA, 1998).

Assessment factor: An expression of the degree of uncertainty in extrapolation from test data on a limited number of species to the real environment. Application factor; uncertainty factor and safety factor are synonymous with assessment factor.

Bioacccumulation: The total uptake in the living organism through all routes of exposure (bioconcentration through food and environmental exposure via air (pore), water, soil, sediment etc.). Bioaccumulation factors (BAF) describe the steady-state concentrations and may be referenced to any exposure medium.

Bioavailability: That fraction of the total amount of a chemical that can be taken up by a (specific) organism in a (specified) time period. Bioavailability thus depends on the properties of the soil as well as properties of the organism. (Or more elaborately: The amount/percentage of a compound that is actually taken up by an organism as the outcome of a dynamic equilibration of organism-bound uptake processes, and soil particle related exchange processes, all in relation to a dynamic set of environmental conditions (Eicsackers et al. 1997))

Bioconcentration: The direct uptake of a chemical from the external environmental compartment (air, water) through gas exchange surfaces (leafs in plants, respiratory systems and, to a minor extent, skin in animals). Bioconcentration is a simple physico-chemical process based on equilibrium partitioning among different phases. Bioconcentration factors describe the steady-state concentration.

Biomagnification: The accumulation and transfer of chemicals via the food web due to ingestion, resulting in an increase of the internal concentrations in organisms at succeeding trophic levels. Biomagnification factors (BMF) describe the steady-state concentrations.

CA: Concentration addition in a mixture of toxic chemicals

Comparative risk assessment: Evaluations intended to establish the relative risk of several chemicals

Degradation time: The time from the end of the lag time till the time that 90% of maximum level of degradation has been reached.

Deterministic risk assessment: A risk assessment that expresses risk as a point estimate for an endpoint, usually as the ratio of exposure and effects (e.g. PEC/PNEC or TER).

Dose (concentration) - response (effect) assessment: The estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect (Article 2 of Existing Substances Regulation, EC, 1993a). This is the second of four steps in risk assessment consisting in the analysis of the relationship between the total amount of an agent absorbed by a group of organisms and the changes developed in it in reaction to the agent, and inferences derived from such an analysis with respect to the whole population. (Lawelle, 1999).

DT_{50}: Express the persistence in soil as the time needed for degrading 50% of an organic compound.

EC_{50}: Median effective concentration (the estimated exposure at which 50% effect is observed).

EC_x: Concentration causing x % effect.

Exposure assessment: Exposure assessment is the determination of the emissions, pathways and rates of movement of a substance and its transformation or degradation, in order to estimate the concentrations/doses to which human populations or environmental spheres (water, soil and air) are or may be exposed (Article 2 of Existing Substances Regulation (EC, 1993a)). The objective is to predict a PEC. A step in the process of risk assessment consisting of a quantitative and a qualitative analysis of the presence of an agent (including its derivatives) which may be present in a given environment and the inference of the possible consequences it may have for a given population of particular concern. (Lawelle, 1999).

GIS: Geographical Information System

Hazard assessment: Hazard assessment is the identification of the adverse effects which a substance has an inherent capacity to cause (Article 2 of Existing Substances Regulation, EC, 1993a). This is a process designed to determine factors contributing to the possible adverse effects of a substance to which a human population or an environmental compartment could be exposed. (The factors may include mechanisms of toxicity, dose-effect and dose-response relationships, variations in target susceptibility etc.) The process includes three steps: hazard identification, hazard characterisation and hazard evaluation (Lawelle, 1999).

Hazard: Inherent property of an agent or situation capable of having adverse effects on something. Hence, the substance, agent, source of energy or situation having that property (Lawelle, 1999).

Henry's law constant (H or K_h): The ratio between vapour pressure and water solubility; it is usually expressed with the dimensions of Pa m^3 mol^{-1}

HQ: Hazard Quotient. Ratio between the application rate (in grams/hectare) and the toxicity, LD_{50}, to evaluate risk of pesticides to bees (in ug/bee).

IA: Independent action in a mixture of toxic chemicals

IC_{50}:Concentration causing 50% inhibition of a given parameter, e.g. growth).

Inherently biodegradable: A classification of chemicals for which there is unequivocal evidence of biodegradation (primary or ultimate) in any recognised test of biodegradability.

K_{aw}: air water partition coefficient; it is a dimensionless expression of the Henry's constant (H) and can be calculated as H/RT (R gas constant, T temperature in °K)

K_{oa}: n-octanol air partition coefficient

K_{oc}: Organic carbon sorption coefficient

K_{ow}: n-octanol water partition coefficient

Lag time: The time, in a biodegradation test, from inoculation until the degradation percentage has increased to at least 10%.

LC_{50}: Median lethal concentration (the estimated exposure at which 50% effect is observed).

LD_{50}: Median lethal dose (the estimated exposure at which 50% effect is observed).

LOAEL(C): Lowest Observed Adverse Effect Level (Concentration)

LOEL(C): Lowest Observed Effect Level (Concentration)

MOS: Margin of Safety. For human health expressed as a ratio between a measure of toxicity (e.g. NOAEL) and the exposure (calculated or measured exposure) (EC, 1996). Corresponds to TER=Toxicity/PEC.

NOAEL(C): No Observed Adverse Effect Level (Concentration)

NOEL(C): No Observed Effect Level (Concentration)

PEC: Predicted Environmental Concentration. The predicted concentration of the substance which is likely to be found in the environment. It is defined for each environmental compartment (air, water, soil, biota, etc.)

PNEC: Predicted No Effect Concentration. The predicted concentration of a substance below which adverse effects in the environmental sphere of concern are not expected to occur. The PNEC may be calculated by applying an assessment factor to the values resulting from tests on organisms (LD50, LC50, EC50, IC50, NOEL(C), LOEL(C)or other appropriate methods.

POP: Persistent Organic Pollutant

Primary Biodegradation: The alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of specific properties of that substance.

Probabilistic risk assessment: A risk assessment that results in a quantitative statement about the probability or likelihood of adverse effects (e.g. in the form of a probability distribution).

QO: Environmental Quality Objective

QSAR: Quantitative structure-activity relationship: QSARs represent mathematical models relating the observed properties (activities) of chemicals to descriptors of their structure. The molecular structure may be quantified by various descriptors such as molecular surface or physico-chemical properties like 1-octanol/water partition coefficient.

Readily Biodegradable: An arbitrary classification of chemicals which have passed certain specified screening tests for ultimate biodegradability; these tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environment under aerobic conditions.

Risk assessment: “The risk assessment shall entail hazard identification and, as appropriate, dose (concentration) response (effect) assessment, exposure assessment and risk characterization.”(EC, 1993a, EC 1994). A process intended to calculate or estimate the risk for a given target system following exposure to a particular substance, taking into account the inherent characteristics of a substance of concern as well as the characteristics of the specific target system. The process includes four steps: hazard identification, dose-response assessment, exposure assessment, and risk characerisation. It is also the first step in *risk analysis* (Lawelle, 1999).

Risk characterization: The estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental sphere due to actual or predicted exposure to a substance, and may include 'risk estimation', i.e. the quantification of that likelihood (Article 2 of Existing Substances Regulation (EC, 1993a)). For any given environmental sphere, the objective, is to entail comparison of the PEC with the PNEC or toxicology with exposure (TER). This is the last step of risk assessment comprising the qualitative and/or quantitative estimation, including attendant uncertainties, of the severity and probability of occurrence of known and potential adverse effects of a substance in a given population (Lawelle, 1999).

Risk quotient: The ratio of an exposure level and a (no-) effect level (or vice versa) as in a PEC/PNEC ratio or a TER. In the strict sense, these quotients do not quantify risk as the likelihood of adverse effects is not quantified.

Risk: The probability of adverse effects caused under specific circumstances by an agent in an organism, a population or an ecological system (Lewalle, 1999)

Stressor: Any physical, chemical, or biological entity that can induce an adverse response. (Synonymous with agent). (US EPA, 1998).

TDI: Total Daily Intake

TER: Toxicology/Exposure Ratio. The ratio between effects on living organisms and environmental exposure as PEC. It can be calculated using different parameters to evaluate the effects:

- a specific toxicological end point (LC_{50}, LD_{50}, NOEL); in this case it quantifies the hazard in terms of possibility of occurrence of a given acute (LC_{50}/PEC) or chronic (NOEC/PEC) effect on a given tested organism;
- a PNEC, estimated with suitable procedures on the basis of the ecotoxicological information; in this case (PNEC/PEC) it quantifies the hazard for a given ecosystem (terrestrial, aquatic).

TTC: Trophic Transfer Coefficient

TU: Toxic Unit; it is defined as the ratio between the actual concentration of a chemical (C) and a given toxicological end point (e.g. C/EC50)

TWA: Time Weighted Average

Ultimate Biodegradation: The level of degradation achieved when the test compound is totally utilised by micro-organisms resulting in the production of carbon dioxide (in aerobic conditions), water, mineral salts and new microbial cellular constituents (biomass).

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CHAPTER 3.
HAZARD IDENTIFICATION

3.1 INTRODUCTION

Hazard assessment constitutes the essential tool for the evaluation of the potential effects of chemical substances on organisms and ecosystems. It includes a first step, hazard identification, which must detect the potential dangers of the substance (i.e. the kind of effects that the substance may produce), and a second step to quantify each danger and to set the expected dose/response relationships or stressor response profile (EC, 1996; USEPA 1998; Tarazona et al., 2000) which will be compared to the exposure assessment during the risk characterisation.

The Hazard and Risk assessments are considered administrative tools supported by a scientific basis, and as previously quoted "*Hazard Assessment and Risk Assessment of Chemical Substances in the EU may differ from Hazard Assessment and Risk Assessment of Chemical Substances in other parts of the world, simply because the administrative context in the EU is not the same as in other parts of the world*" (Hart et al, 1998). Nevertheless, all these tools are expected to be supported by a common scientific background, and the capability of environmental sciences to support these tools will depend on the level of development achieved in each particular context.

Similarly, under a common scientific basis, the specific support for developing hazard identification schemes should be related to the final goal of the assessment, and different strategies will be required depending on the intended uses for the hazard identification results.

Typical "uses" of hazard identification strategies include:

- Sorting chemicals according to their hazard
- Selection of relevant targets for establishing quality criteria.
- Selection of the assessment endpoints which must be analysed in the risk assessment.

Each example can also cover a large list of potential applications. In terms of environmental hazards, a main distinction can be established between local approaches, which focus on the specific environmental and ecological conditions of a certain area, and holistic approaches, which deal with very generic environmental and ecological conditions.

The hazard identification should obviously detect all potential effects on human health and the environment. As previously pointed out, this opinion only deals with the effects at the ecosystem level, which should be able, in most cases, to cover the effects on populations including those of economic or social value. Effects requiring an assessment at the individual level, such as human health effects, require a different approach and are not considered.

Under this context, the identification of ecological hazards in local approaches means the detection of relevant endpoints for the assessment of this particular activity on this particular ecosystem/s. As pointed out in the US EPA guideline document (USEPA, 1998) the whole process requires a proper problem formulation, with the selection of the assessment endpoint and the integration of the available information to establish a conceptual model and a specific analysis plan.

By contrast, the hazard identification in holistic approaches cannot go into a detailed description of particular environmental/ecological conditions. It is based on the identification of effects on a "generic" ecosystem or more properly on a set of generic environmental concerns represented by a selected group of receptors (key functions or taxonomic groups) and exposure routes.

3.2 ESTABLISHING ENVIRONMENTAL CONCERNS

Concerns are necessarily seen from a human perspective, since it is humans that are involved in the process: contamination is the result of human development, risk analysis is a human activity, and the results will be implemented according to human laws.

The concerns related to chemical pollution can be grouped in three main aspects:

Adverse effects on human health
Adverse effects on human economy and the use of natural resources
Adverse effects on other living beings

The distinction between the second and third points is mostly philosophical. Humans use the environment, and the second group expresses the concerns on the effects of environmental contamination on fisheries, forest, agricultural areas, etc. which are sources of food and other items as well as leisure for humans. The third point represents an ethical concern: humans have duties to non-human beings and are not legitimate to destroy other living beings (Vesilind et al., 1990).

The reality is not so clear, because it is not so clear what can or cannot be useful for humans in the future. In fact a typical argument for the protection of biodiversity is the conservation of genes which could be used in the future to treat human diseases or to combat pests.

Even more, nowadays it is clear that both points require a common assessment: the absence of effects on the structure and functioning of the ecosystem is the only way to guarantee the conservation of all potential "uses" of the natural resources. The philosophy of the Water Framework Directive is a perfect example: achieving a good ecological quality (i.e., avoiding unacceptable effects on the ecosystem) is the best guarantee to protect the resource and its anthropogenic uses. In fact, the whole concept of Environmental Sustainable Development is supported by this duality.

Regarding the terrestrial environment, humans live in it, use it producing massive changes and try to keep certain areas as naive as possible in terms of landscape and wildlife; all at the same time.

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, ground and foliar invertebrates, which represents food for other organisms, and covers essential roles as pollinators, detrivores, saprophages, pest controllers, etc.
- Effects on terrestrial vertebrates, domestic and wild species, 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 a 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 whole system.

Therefore, it is also in our own interest that concerns on chemical pollution reflect ecosystem effects.

3.3. SELECTION OF ASSESSMENT ENDPOINTS

Due to the complexity and diversity of terrestrial ecosystems, the assessment of potential hazards can only be achieved through a set of assessment endpoints. Operationally an assessment endpoint is an ecological entity and its attributes which should be selected on the basis of two scientific bases, ecological relevance and susceptibility to the stressor, plus their relevance to management goals (USEPA, 1998).

Terrestrial ecosystem is the habitat and the biotic web of animals, plants and microorganisms subsurface, on, in and above the soil. (Fig. 1) and offer great possibilities for the selection of assessment factors. Two main concerns, effects on soil functions and on ecosystem structure, can be identified.

3.3.1. Assessment endpoints related to soil functions

Chemicals can produce adverse effects on soil functioning which can be measured using assessment endpoints related directly to certain measurable functions or indirectly through the soil structure. Three major topics are discussed below.

Detritus, consisting of litter and animal debris, is the largest source of organic material in soil. The amount of litter fall per annum and area varies greatly with soil quality and climatic conditions (refs.). Litter consists of surface litter and root litter below ground. Depending on the biotope, root litter may equal the surface litter in quantity.

Mineralization of detritus. Grazing animals, soil invertebrates and microorganisms are responsible for the mineralization of the detritus. Mineralization is necessary for reverting the nutrients into inorganic form and thus renewing their availability to the vegetation. Chemicals adversely affecting the mineralization process must be considered ecotoxic. To assess whether inhibition of mineralization has occurred or not in the environment, is not simple, because different biopolymers are not similarly available to the mineralizing organisms. Hence, the composition of soil detritus changes with age (Fig.2).

The rate of soil microbial processes, such as the mineralization, depends on the nutrient status of soil, pH, moisture and on climatic factors. The biota responsible for the mineralization will be different between different soil ecosystems. Microbes differ widely in their tolerance to environmental toxicants. Examples on microbial taxa involved in degradation of cellulose, the most abundant biopolymer in the terrestrial detritus, are listed in Tables 1 & 2. Soils where the species diversity of degraders is large, may be expected to be more robust towards toxicity by chemicals than soils with low diversity. Deep (1…10 m) subsurface is expectedly lower in biotal diversity than humus, litter layer or surface soil. Soil organic matter content is important for attentuating the toxicity of both organic and inorganic chemicals. Soil toxicity may therefore be higher in deep subsurface that in surface soil.

Tests to assess the potential of a chemical to adversely affect mineralization should be done with soil relevant to the ecosystem studied, at pH, salinity and moisture content of relevance to the ecosystem assessed, under conditions mimicking those in the environment close to. Using a “standard soil” or pure or mixed microbial cultures may not correctly indicate the hazard.

Effects on soil microbe - plant interactions. The subsurface contains microorganisms down to a depth of 10 m or more. Plant roots can penetrate equal depths. Plant roots are associated with, and often live in symbiosis with, dense populations of bacteria (rhizoplane and rhizosphere, root nodule bacteria, Fig. 3) and fungi (mycorrhiza, Fig. 4).

The microbial metabolic activity of the soil associated to plant roots is up to 10^6 times higher than that in adjacent non-associated soil. This reflects the mutual feeding between the plant and the microorganisms. Examples of this are the conversion of atmospheric dinitrogen (N_2) into ammonium (NH_4^+), that only can occur in prokaryotic organisms (figs.3, 5). Some of the beneficial root microflora grow inside plant root tissue, as endosymbionts, e.g. nitrogen fixing bacteria of legumes, alder and many other tree species (Fig. 4), endomycorrhiza of coniferous trees (Fig. 5).

Root associated microorganisms generate organic acids by the microorganisms to facilitate dissolution of mineral nutrients from the litosphere to serve as plant nutrients. The plant root exudates that feed the root associated fungal and bacterial populations with carbohydrate and amino acids. In many plants, a proper development of root hairs only occurs in the presence of suitable root-associated microbial flora (Fig. 6). Most, if not all, trees depend on their root associated bacteria and fungi to be able to grow normally under environmental conditions.

The different species of the root associated microbial community may be antagonistic to one another (Fig. 7). The presence of beneficial rhizosphere organisms can protect the plant against pathogenic microorganisms (plant growth promoting bacteria, PGP). Damage to or elimination of the root associated microbes may result in increased vulnerability of the plant to diseases.

Plant roots, soil animals and vegetation cover attenuate the effects of extreme temperatures in soil and the habitat above it. Trees and other large plants (macrophytes) protect from sunlight and attenuate heat from solar radiation by evaporation of water. Microbial degradation of plant litter on soil surfaces generates heat that protects surface soil against frost. Roots and soil animals maintain soil porosity guaranteeing access of oxygen to subsurface microorganisms. Damage to vegetation cover, such as defoliation, changes the seasonal changes of soil temperature if the defoliated area is more than minor. In extreme climates, warm or cold, desertification or permanent loss of forest may occur because of draught or extension of frost in soil - even after the toxicant has dissapeared.

Soil animals. Earthworms, nematodes, insects and other soil invertebrates habitat usually in the topmost meter of soil. Their activity is important for the dynamic soil ecosystem. The topmost meter of earth surface is also the shelter and breeding ground of many soil animals, reptiles, mammals and birds. Earthworms and small, predating soil animals have mostly been chosen as the topics of toxicity studies, because of the complexity of the interactions between the larger animals, relatively low number of individuals in a given area and the difficulty of maintaining them in the laboratory.

Earthworms are thought to be vital in maintaining soil porosity, and transport of water and oxygen in soil. However, earthworms are mostly not found in natural, noncultivated soil ecosystems, such as nonmanaged forest soil. They appear to be an antropogenic addition to soil biota (Huhta, 2000).

3.3.2. Assessment endpoints on ecosystem structure.

Terrestrial ecosystems are usually constituted by four main taxonomic groups: micro-organisms, plants, invertebrates and vertebrates. Micro-organisms are mostly related to the soil and assessed through their contribution to soil functions. Terrestrial plants have the roots in the soil and the vegetative part in the atmosphere, while animals have complex habitats where soil, ground, vegetation and atmosphere play different roles depending on the species and even the developmental status within the species.

Assessment endpoints should be related to direct effects on each group (plants, invertebrates and vertebrates) using key species or population parameters, and to indirect effects related to the role of each group in the community. In addition to the described effects on soil functions, plant-animal interactions are evident.

Plants. Terrestrial animals, the vertebrates mammals, birds, reptiles, as well as invertebrates, insects, worms, small grazing animals in soil, all depend directly (herbivores) or indirectly (predators) on the vegetation, i.e. productivity of plants. For many of these, live plants or plant litter also offer a habitat for nesting, breeding and hibernation. Therefore animal welfare is ultimately dependent on the welfare of vegetation (ref).

Animals. Many plants depend on animals for the spread of their pollen and seeds. Bees and many other flying insects transport pollen. Birds and animals grazing on the soil surface feed themselves on fruits and berries, transporting the seeds to distant locations and thereby assisting the plant in finding alternate breeding grounds. They maintain the local diversity of terrestrial plants playing a significant role in the interspecies competition being pest and pest-controllers.

3.4. SELECTION OF MEASURABLE ECOLOGICALLY RELEVANT PROPERTIES.

In some cases direct effect measures can be done on the attribute of concern. Then, the assessment endpoints are equal to the measurable property. When direct measurements are not possible surrogate measurements must be selected.

A selection of biochemical, physiological and ecological measurable attributes are listed below.

Potential measurable biochemical attributes

Damage to the soil machinery of mineralization of organic matter can be measured at different levels:
Enzymic activities of soil and soil organisms hydrolysing important constituents of the detritus:
- cellulose and hemicellulose hydrolysing enzymes (β-glucosidases, -xylosidases)
- storage carbohydrate hydrolysing enzymes (α-glucosidases)
- proteases, peptidases
- specific and unspecific esterases (lipid hydrolysing)

These enzymes can be conveniently measured making use of surrogate substrates (chromogenic or fluorogenic). Depending on the logKow of the surrogate substrate used, the measured activity represents cellular plus exocellular activities (substrate logKow >2) or only exocellular enzyme.

Nonhydrolyzable organic matter mineralization by soil or soil organisms can be measured by

- methane oxidation
- oxygen uptake or ^{14}C labelled substrate oxidation into $^{14}CO_2$ (lignin, phenols, resins)
- dehydrogenase activity can be measured using surrogate substrate;
- CO_2 evolution or O_2 consumption can be measured using endogenous (soil respiration)or added substrate (substrate induced respiration, SIR)

Xenobiotic compound mineralizing activity.

$^{14}CO_2$ evolution from ^{14}C-labeled genuine xenobiotic compounds (pesticides, industrial pollutants..)

Damage to microbes / biocatalytes involved in the biogeochemical cycles of nutrients

- organic phosphate hydrolysing enzymes (phosphomono- phosphodiesterases)
- organic sulphate hydrolyzing enzymes
- proteases, peptidases
- denitrifying actvity
- ammonium oxidizing activity (nitrification)
- nitrite oxidizing activity (nitrification)
- nitrogenase (N_2-assimilation, nitrogen fixation)

Damage on terrestrial plants can be measured as

- diminished rates of photosynthesis
- changes in chlorophyl(or other photosynthetic pigments) patterns.
- dismissed evapotranspiration (forest ecosystem)
- enzymatic alterations
- effects on biochemical plant protection mechanisms

Damage to terrestrial animals can be measured as

- enzymatic alterations
- endocrine disruption
- effects on the immune response
- production of stress-response proteins (i.e., metallothioneins, heat-sock proteins)

Potential measurable physiological attributes

Damage to the soil biotic machinery (microbial & small soil animals) (“biomass”)

Quantify changes of soil biomass in response to chemicals by:

- measure respiration with added substrate (SIR)
- measure respiration on endogenous, killed biomass (fumigated soil, inoculated with non-fumigated)
- ATP content
- methane production from endogenous or added substrate (quantification of methanogenic activity)

Damage to terrestrial plants can be measured as:

- mortality

- ineffective pollination
- reduction of gain of biomass (forestry, cultivated plants)
- loss of leaves or needles (forest trees)
- reduced needle lifetime (evergreen)
- loss of mycorrhiza of trees (endomycorhizza or ectomycorhizza)
- loss of rhizoplane / root microflora
- diminished nodulation (leguminous plants and actinorrhizal trees)
- increased susceptibility to pathogens (viruses, bacteria, fungi) or pests

Damage by chemicals to terrestrial animals can be measured as:
- mortality
- effects on reproduction rates
- change of natural sex ratio
- increased susceptibility to infectious diseases

Potential measurable ecological attributes

Autoecological and synecological measures including:
- changes on population dynamics
- effects on species diversity
- yield reduction (cultivated plants, forest)
- energy transfer
- food chain structure

The selection of relevant assessment endpoints and measurable atttibutes is the aim of hazard identification. For specific risk assessment, this selection should be related to the properties of each particular ecosystem and the management goals, and a specific analysis plan should be elaborated. For generic assessment a set of relevant tools to cover all major hazards of a generic terrestrial ecosystem is required. The selection will depend on properties of the chemical in classification schemes and on the use and emission patterns in the case of risk assessment.

3.5. ALTERNATIVES TO INCORPORATE SCIENTIFICALLY SOUND CRITERIA WHEN SETTING HAZARD CATEGORIES.

The toxicity of a chemical substance is in reality the result of a complex interaction between a live being and the chemical under certain environmental conditions. However, several legislative decisions require a drastic simplification of the whole issue in order to rank chemicals according to their potential dangers.

Ranking chemicals as a result of a legislative measure is primarily an administrative process which should agree with the management goal determined by the legislative body. However, hazard identification principles can contribute to the technical application of these legislative objectives incorporating transparency into the process.

Several techniques have been used for setting the “level of concern” of a chemical. These options include aspects related to the potential for exposure, such as the production or storage volume, related to the toxicity of the chemical and/or related to the environmental fate of the chemical.

At the EU level, the Classification and Labelling system can be considered as the main regulatory tool. The primary purpose of the Directive (EC, 1967) is to establish a formal basis to classify chemicals in a number of categories of danger, and hence, to identify chemicals which must be considered as “dangerous” in a legal sense. The Directive also sets the labelling requirements for dangerous chemicals. In addition, the concepts and definitions of “danger” in this Directive are also used as a basis for several other directives.

The general aim is to establish categories, each reflecting a particular potential concern. These categories of danger reflect societal rather than scientific concerns and will normally be related to management decisions. As such, they are included in the main body of the Directive, and can only be modified by a full amendment of the Directive, under a procedure that involves both the Council and the European Parliament. The Directive includes a number of definitions of various categories of danger in its Article 2. Five categories describe physical chemical hazards, and nine categories describe hazards to human health. A final category (first included in 1979, EC 1979) defines danger to the environment: “substances and preparations which, were they to enter the environment, would present or may present an immediate or delayed danger for one or more components of the environment”. The application of these definitions requires a further technical development which must be operative and scientifically sound. The verbal definitions in the Directive, which reflect the social/political concerns reflected in the Directive are supplemented by detailed scientific criteria in an Annex. (EC 1993, 1996, 1997, 1998, 2000).

Some of the categories of danger defined in the Directive listed above reflect societal/political concerns for different degrees of the same effect. For flammability, three levels of concern are defined: “extremely flammable”, “highly flammable” and “flammable”. For toxicity, three levels of concern are defined which cause damage to (human) health: “very toxic”, “highly toxic” and “harmful”. For other physical chemical and health related concerns (e.g. explosivity, cancer) as well as for dangers for the environment, only a single category of danger is defined in the Directive. This single category can then be split into several levels during the technical development (carcinogen category, toxicity level for aquatic organisms).

These categories of danger can then be used as a common definition for the specific effects that can be addressed in other legislative measures intended to limit or remove the potential dangers and environmental concerns related to the category. (These legislative measures are sometimes described as “down-stream” legislation).

The EU legislation includes measures related to hazard communication as a first step (labelling), followed by precautionary recommendations, special instructions for transport and storing, waste management conditions, priority control, etc.

Whilst establishment of the concerns to be addressed by legislation is a task for society as a whole (including scientific concerns as well as many other issues), there is a particular role for scientific advice in the elaboration of the detailed criteria. Identification of specific assessment endpoints and the associated test methodology is an area where scientific concerns play a more central role.

When hazard identification is applied to sort chemicals in a generic way, i.e. to establish if one chemical is more or less dangerous than another, the assessment is based on the "intrinsic properties" of the chemical. The dangers for human health and the environment are mostly related to the so called "inherent toxicity" represented by a set of measurable parameters (LD_{50}, LC_{50}, NOEC, NOAEL, ...) determined for each substance on a set of species under standardised conditions. Similar approaches have been suggested outside Europe (Davis et al., 1994; Swanson et al., 1997).

> The outcome of the hazard quantification process can be either a distribution function (a hazard value is applied to each chemical using a set of algorithms) or a set of ranges representing hazard categories (each chemical is ascribed to a specific category). The first approach is usually employed in setting Environmental Indices and Environmental Indicators, while the second is used for classification.

When the second approach is employed, the number of categories depends on the objective of the classification exercise. For a legal classification scheme, it it is not useful to create more categories than are needed to reflect different legal consequences. Secondly the number of possible categories are limited by the capacity to produce sound criteria to distinguish between categories. A system with more categories than those realistically identifiable with the current state of the art will produce arbitrary results. The combination of both factors will recommend to use either a bi-compartment (YES/NO) or a multi-compartment (several levels of concern from very high to very low or no concern) approach for each type of hazard.
The criteria to discriminate between categories can be set on the pure basis of the social concerns, e.g. applying political decisions or the judgement of expert panels. In addition, there are a number of possible theoretical approaches that can be considered to select the different cut-off points. Some of the possible options are described below:

1. Each category represents a fixed percentage of the total number of chemicals; e.g. the hazardous category (or the most hazardous category) includes the 10% most toxic chemicals.
2. Comparative distributions: Each category represents a certain rate versus a fixed statistical parameter such as the average or the upper/lower limit; e.g. hazardous category (or the most hazardous category) includes those chemicals which are at least 100 times more toxic than the average.
3. The value is set independently of the distribution curve, on a non-statistical basis, but representing a certain level of concern. As an example, a fixed amount of a chemical showing a particular level of toxicity is able to pollute a fixed amount of the relevant environmental compartment (i.e. 1 tonne of the substance is able to pollute 1 million tonnes of water or soil).

The selection of the method must be related to the aim of the classification scheme and the available information. Alternatives 1 and 2 require enough statistical information on the toxicity distribution curve to produce reliable parameters (percentiles, average, etc.). Alternative 3 does not require information on this distribution, but needs a proper description of the environmental concerns associated with the potential emission conditions of the substance.

Each alternative can contribute to give scientifically sound guidance to the technical development of the regulatory needs. Ideally, the scientific contribution should be based on a combination of the different alternatives (the categories have environmental meaning and at the same time are related to the distribution curve). However, it must be recognised that in general, the first alternative is more acceptable for setting priorities while the others are preferred for emission-control and reduction measures.

The information reviewed on the current EU classification criteria for the aquatic environment (i.e Lundgren, 1992) suggests that the third alternative was the basis for their development in the eighties. However, recent studies (Tarazona et al., 1999) indicate that these criteria are also consistent with the second alternative.

To keep coherence among the procedures for covering different hazards within the same legislation is considered essential, and therefore alternatives 3 and 2 should be recommended as the starting points for developing the methodology for setting the criteria for the terrestrial environment. A recent review produced by the ECB (Allanou, et al., 1999) suggests that the current data base for terrestrial toxicology although limited (toxicity data available for about 30% of the chemicals) should be enough to consider the incorporation of some statistical bases. Similar conclusions can also be reached looking at the papers published by Riepert et al., (1999), Claussen (1999), and Vega et al., (1999). As previously stated, the number of categories should be compatible with the application of the classification framework (avoiding setting categories of no management goal) and the capability of the hazard identification tools to discriminate among levels of hazard.

As clearly expressed in the first part of this chapter, the hazard of chemicals on terrestrial ecosystems can arise from a set of different ways, which include different taxonomic groups exposed through several routes. Additionally, chapter 4 shows the current tools for assessing the effects of chemicals on terrestrial organisms. Particularly for lower tier effect assessment, the reader can recognise two items with a certain level of standardisation (toxicity via soil for plants, soil dwelling invertebrates and micro-organisms, and oral toxicity in vertebrates) plus a miscellaneous group of tools, mostly developed for very specific purposes and with different levels of standardisation, to cover other relevant hazards, particularly those associated with exposure via air and air-ground transfer (atmospheric deposition, volatilization, and spraying).

This level of complexity requires covering different hazards. The US EPA guideline (USEPA, 1998) recommends, as the first step of the problem formulation, the analysis of the available information before selecting the assessment endpoints. The list of available information on terrestrial toxicity for a full data set will include:

- Toxicity on soil dwelling organisms exposed through soil
- Oral toxicity on vertebrates, invertebrates.
- Inhalation toxicity on vertebrates, toxicity via air to plants, invertebrates.

- Contact toxicity on plants, invertebrates, vertebrates

The information provided by each group of tools can be handled separately, producing independent classification categories, or can be combined in a single category, with two or more levels of hazard. The final decision should be administrative (obviously related to the management goals). In both cases, from a scientific viewpoint, the level of hazard represented by each equivalent category or sub-category should represent a similar degree of ecosystem hazard (a chemical considered "toxic to soil organisms" should represent the same level of hazard for the structure and functioning of the ecosystem as a chemical considered "toxic to vertebrates").

Technically, it is possible to group those values expressed as doses (mg/kg b.w.) even those obtained for different exposure routes, but this is not feasible when the toxicity end-point is expressed in terms of concentration (mg/kg soil; mg/kg food; $\mu g/m^3$ air). Therefore, different criteria for the different tools are required, and coherence among these criteria is needed.

The approach should be:

1. To establish a set of fixed levels for each type of hazard (most classification schemes establish between 2 and 5 levels),
2. To develop a common rationale for establishing the cut-off values among categories,
3. To adapt this rationale to the characteristic of the effect assessment tools in order to establish appropriate criteria identifying equivalent levels of ecological danger for each hazard type (or group of tools),
4. Finally, when the management goal requires the combination of the different hazards in a single category, a potential solution from a scientific point of view is to follow the methodology applied for complex environmental indices and indicators. Each specific hazard is handled individually and in parallel, but using a common structure to establish sub-indices for each hazard and, then, the sub-indices are combined in a single index using an aggregation algorithm. Then, criteria for moving from each specific hazard to the final category of hazardous for terrestrial ecosystems are required.

A proper review of the available information is required in order to establish the level of scientific basis achievable in this process.

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CHAPTER 4.
TOOLS FOR EFFECT ASSESSMENT

4.1. INTRODUCTION

Since the early 90s, as a result of the European and national (e.g., Germany) legislative output concerning normative regulation for environmental chemicals and plant protection products, the number of ecotoxicological effects assessment tests in the terrestrial environment has been increasing. Starting from less than 5 tests in the late 80s to more than 50 in the middle 90s, developed protocols include almost all functional groups, at least in the soil compartment (ranging from microorganisms to arthropod predators). The outbreak of these protocols was originated mainly by several National research programmes, like the Dutch NIRSP (Netherlands Integrated Soil Research Programme; Eijsackers, 1989) or the Swedish MATS (Mark Test System; Torstensson, 1993a), by EU funded projects (e.g., SECOFASE; Løkke & Van Gestel, 1998), and by activities of several working groups (e.g., Pesticides and Beneficial Organisms from the IOBC/WPRS; Hassan, 1992). However, despite the number of existing tests, only some of them have been standardized and inter-calibrated and are currently in use for normative purposes.

This chapter is divided into two parts. In Part I methods of different complexity, starting with laboratory tests and ending with field tests, will be presented. Besides tests using structural endpoints (e.g. mortality), functional methods (e.g. litter decomposition) will be included. In Part II, besides an outlook of the methods presented, some recommendations will be made regarding the implementation of a test strategy for the terrestrial environment and further development needed on specific tests.

4.2. LABORATORY "SINGLE-SPECIES" ECOTOXICOLOGICAL EFFECT TESTS

According to Römbke & Moltmann (1996) the existing effect tests can be divided into several groups: (1) tests with soil microorganisms; (2) tests with plants; (3) tests with soil invertebrates (mainly saprophagous organisms); (4) tests with beneficial arthropods; (5) tests with higher vertebrates and (6) bioaccumulation tests with animals and plants.

Tests with microorganisms

Microorganisms are the keystone players in biological processes in the terrestrial environment (e.g. decomposition of organic matter and nutrient cycling) and soil pollution may exert direct or indirect effects on microbial populations affecting these microbial mediated processes (Rossel *et al*., 1997). Therefore a cost-effective battery of tests aiming to evaluate effects of chemical substances on microbial populations is needed. Due to the taxonomic obstacles of a qualitative analysis of microbial communities in the soil, most of the available tests incorporate function parameters measuring the end products of metabolic activity (CO_2 production and enzymes) or methods for estimating microbial biomass (Table 1).

Despite following several of the requirements on ecotoxicological testing (e.g., low cost, easy standardization, ecologically relevant), the use of microorganisms has some drawbacks. First, microbial activity is very sensitive to changes in soil parameters (especially moisture content and pH) and special care should be taken when performing the analysis and interpreting the results. Second, different tests have different degrees of sensitivity (Domsch *et al*., 1983). In the case of enzyme activity, for instance, soil exo-enzymes may be active even after the death of microbial cells, meaning that short-term effects may only be observed later on.

Due to these problems, more important than the selection of a single representative test indicator of toxic effects, a battery of tests, using different tests with different sensitivities, should be adopted. Depending on the resolution wanted (more general metabolic activity or a specific segment of community activity) different enzymes (or enzyme groups) should be selected (Sinsabaugh, 1994). To overcome the problem of the interpretation of exo-enzyme data, the use of indicators of the potential activity of physiological active microorganisms (e.g., DHA and respiration) is advisable (BBA, 1990; ISO, 1997a) and are currently in use with satisfactory results both as a measure of short-term exposure and resilience on different time scales (Rossel & Tarradellas, 1991). Moreover, the measurement of enzyme activity related to nitrogen transformations (nitrification and denitrification) is being highly advised as sensitive parameters to detect effects of pesticide application or metal pollution (e.g., BBA, 1990; NEN, 1988; Torstensson, 1993b; ISO, 1997b; Pell *et al*., 1998; Fairbrother *et al*., 1999). Currently, methods on nitrogen transformation as well as soil respiration are being internationally standardised by the OECD.

Bioluminescent bacteria tests using aqueous soil extracts (using *Pseudomonas fluorescens* or *Vibrio fischeri*) are also advised as highly sensitive screening tests for metal toxicity in soils (Fairbrother et al., 1999). These tests assume that a reduction in metabolic activity, caused by the chemical, induces a reduction in light emission. However, this phenomenon is a physiological indicator, and may not always be correlated with the metabolic activity (e.g., a decrease in the bioluminescent output may be related to the presence of easily degradable organic substances in a sample and not to the presence of the chemical). Moreover, the color and/or turbidity of the extracts may cause a decrease in light emission, which enhances the care that should be taken when interpreting the outcome of these tests. Another important aspect concerning these assays is the extrapolation of results. Despite their high relevance and sensitivity, due to the use of soil extracts, these tests should only be used to evaluate effects on bacterial populations (or at least microbial populations) and data extrapolation to higher levels (e.g., soil fauna) is not recommended (unless reliable toxicokinetic data confirming the relation between chemical residues on the extracts and on the animal's body).

Dighton (1997) suggests the use of integrating parameters (measurement of litter decomposition and nutrient mineralization) allied to methods that can describe the physiological status of the microbial community, thus possible changes in the microbial structure (e.g., the BIOLOG plate; Zak *et al*. 1994; Garland 1997).

According to this author, only with this integrated approach is it possible to have a sound interpretation of the pollution-induced effects on the structure and function of soil microbial communities.

Tests with plants

There are several protocols dealing with the evaluation of effects of pollutants (mainly heavy metals) on plant species. However, only some of these are standardized and useful for normative purposes (Table 2). One of the major drawbacks in plant testing is the great variability in sensitivity between species (Fletcher *et al*., 1990). Moreover, the use of nutritive solutions as substrates in several tests leads to an over-estimation of sensitivity to the chemicals and makes it more difficult to interpret the obtained results, especially during extrapolation for a real situation (contaminated soil).

Despite those constraints, simple tests based on parameters like seed germination and root elongation are used for evaluating pollution effects (e.g., EPA, 1992a&b; ISO, 1993, 1995b). Others, based on physiological parameters like photosynthesis, were proposed but they are not yet standardized (Römbke & Moltmann, 1996). The well-known OECD 208 guideline (OECD, 1984b) and its relative BBA test (BBA, 1984) are still required for pesticide registration, but their use should be carefully revised; in their actual form, Römbke *et al*. (1996) only recommend their use for range-finding purposes, stressing the need for a main-test afterwards (currently, the guideline is under review by the OECD). This test is also criticized by some authors (Price & Hikino, 1984) due to the reduced number of species used per test (mainly due to the specific effect of chemicals to plants) and by others (e.g., Marschner, 1992) because it does not use wild plant species. However, the latter author also states that despite the higher sensitivity of wild species, a plant test should rely on a genetically more homogeneous cultured species. Moreover, a possible way to move on in plant testing, and getting relevant information on sub-lethal effects, is to develop chronic and life-cycle tests (Kapustka & Reporter 1993). For the moment only two standardised long-term tests are available as a test guideline (both using *Brassica* species): one called life-cycle test (ASTM, 1999) and one chronic test according to ISO format (Kalsch & Römbke 2000).

Tests with soil invertebrates (including beneficial arthropods)

The number of existing protocols with terrestrial invertebrates is, without any doubt, much higher than the protocols from the groups considered above. This figure embraces tests ranging from soil dwelling animals (like nematodes or mites) to soil predators (like Carabid or Staphylinid beetles) or parasitoid species (like some Hymenoptera). However, only a few of them exist as international guidelines and are actually required for chemical registration (Earthworms, Collembola and some beneficial arthropods). From these, the acute earthworm test (OECD, 1984a) is the most used, although it has been heavily criticized mainly due to its lack of ecological relevance, especially due to the use of a typical compost species (*Eisenia fetida/Eisenia andrei*) to assess soil contamination and the use of artificial soil medium (Römbke *et al*., 1992). When using earthworms, effects can be assessed more accurately using the chronic test (ISO, 1996), since reproduction is incorporated as the main test effect parameter, although the motives for criticism are still present. To overcome this representativity problem a test with other type of oligochaetes, the enchytraeids, has been recently inter-calibrated and proposed to be an official international guideline for chemical testing (Römbke *et al*, 1999).

The selection of species for a battery of tests with soil invertebrates should take into account not only the most important trophic levels in the soil compartment (decomposers, parasites and predators), but also the different routes of exposure to the chemical substances and the different sensitivity to the toxicants (Laskowski *et al*., 1998). So, besides tests with earthworms, collembola and enchytraeids, some other protocols were developed in the past few years, mainly due to the outcome of the SECOFASE project and the activities of the IOBC and EPPO organizations (Table 3).

As referred to above, some of these tests are already published as guidelines, but others need further development and/or standardization. From these, special attention should be given to the saprophagous organisms, especially the isopods, since although widely used in ecotoxicological research, no official guideline is available for the trophic level represented by them. Protocols have been proposed by Hornung *et al*. (1998) to evaluate effects on growth and reproduction, but further standardization and more data (especially with organic chemicals and pesticides) is needed. Further progress passes by improving culturing conditions (Caseiro *et al*., in press), considering other exposure routes (Vink *et al*, 1995; Sousa *et al*., in press) and by re-evaluating the use of measured effect parameters, especially on the growth test, by incorporating them in physiological models (Ribeiro *et al*., in press).

Moreover, other types of parameters should be considered in invertebrate testing. Studies on effects at cellular, biochemical, physiological and behavioral levels have been increasing in the past few years. From these, biochemical responses, like the expression of heat shock proteins, the induction of monooxygenases and those enzymes revealing neurotoxic effects have been receiving most of the attention with promising results (Kammenga & Simonsen, 1997). However, more studies are needed to link biomarker responses with toxic effects at higher levels of biological organization.

Tests with vertebrates

Tests with vertebrates are usually reduced to mammals and birds. Laboratory toxicological tests with mammals are often regarded as models for toxic effects of a defined substance to humans. Existing guidelines (e.g. from OECD) comprehend many different acute and long-term tests where the test chemical is given orally, injected or applied in or on to the animal skin (Table 4; note that only examples are given). Required for chemical and pesticide registration, these tests are done with mice, rabbits or guinea pigs, and, rarely, with wild mammals (in the U.S. tests with wild mammals are required if previous results showed a significant effect). The toxicity tests on mammals developed for the evaluation of human health effects are currently used in the ecological assessment for both pesticides and industrial chemicals. This use is highly recommended because of ethical (reduction in the use of toxicity tests on vertebrates) and economic issues. In general, the guidelines recognise the need for the re-evaluation of the test results from an ecotoxicological perspective. Therefore, the NOAELs selected for the protection of ecosystems are not necessarily

<u>the same used for the protection of Human Health. Some recommendations on the selection of ecologically relevant endpoints from the standard mammalian toxicity tests are under preparation at the EU level for both pesticides and industrial chemicals. Effects on reproduction rate, survival and growth rate are normally considered as ecologically relevant, while other effects such as bioachemical or physiological alterations are normally considered as not relevant if the results clearly demonstrate that these effects do not affect the reproduction and growth rates. The CSTEE considers that, in addition to general guidance, specific issues such as the relevance of effects on the endocrine system, reproduction and genotoxicity should be covered. However , basic scientific knowledge is still required for a proper ecological evaluation of all these issues and, therefore, this issue should be considered as a research need. Specific recommendations for the assessment of endocrine disrupting chemicals have already been produced by the CSTEE.</u>

Avian tests are also required for pesticide registration and are mainly performed with quail and duck species. OECD and EPA guidelines exist for both acute and chronic assessments (Table 4). Although more relevant in ecotoxicological terms than mammal tests, most of the bird tests performed are being used not so much for assessing effects at ecosystem level, but more for protective reasons, e.g., for assessing the risk to endangered species or the possible accumulation of chemicals in animals used or consumed by humans (Walker, 1993; Römbke & Moltmann, 1996).

Due to the growing concern of using live animals, especially in acute tests, the use of non-destructive techniques is gaining more relevance in vertebrate testing. Here biochemical biomarkers also have the leadership, but more studies are needed to link biomarker responses to effects at individual and population levels (Scott-Fordsmand & Weeks, 1998).

<u>Bioaccumulation tests</u>

Bioaccumulation and bioavailability are terms closely associated with soil contamination issues. Their study is important not only in terms of contaminants transfer along food chains, but also for a robust effect assessment of chemicals in the environment. It is recognised that the detectable concentration of contaminants in the soil can not fully predict a biological or ecosystem effect. Under this context it is essential to understand the pathways and the mechanisms of how the chemical enters the organism and also to evaluate the bioaccumulation potential of a certain chemical under defined circumstances (e.g., analyzing bioavailable fractions).

Bioavailability is, in fact, the key property here. Perhaps more important than finding the most efficient way to extract the available fraction of the contaminants from a defined soil, researchers should focus on the adaptation of the existing extraction methods to more realistic conditions, assessing which chemicals (or chemical fractions) were really available for soil organisms (measuring real bioavailability).

There are some underlying hypotheses that can explain the use of different methods to measure the bioavailability in soil and sediment. The most relevant hypotheses that have been discussed is the Equilibrium Partitioning Theory (DiToro *et al.*, 1991) which states that organic compounds that are sorbed to the soil and sediment are in equilibrium with the aqueous phase or pore water, the same aqueous phase to which benthic and terrestrial organisms are exposed. But this theory has some deviations and to complement it some other studies are made such as bioaccumulation kinetics (Sijm *et al.*, 2000).

Under this line of research, several studies analysing the different exposure scenarios on the uptake pathways and bioaccumulation of chemicals using soil and sediment organisms have been performed. In these studies, the use of kinetic models has shown that the route of uptake (food, soil and pore water) has a major importance on the bioaccumulation potential of a defined chemical. Some studies were made evaluating the bioaccumulation of Lindane and hexachlorobenzene by tubificid sludgeworms (Oligochaeta) (Egeler *et al.*, 1997) and by earthworms (Connell and Markwell, 1990; Van Gestel and Ma, 1988; Belfroid *et al.*, 1996); also some studies with terrestrial isopods were made comparing different uptake routes of lindane (Sousa *et al.*, in press) and evaluating the uptake and elimination kinetics of benzo(a)pyrene in the isopod *Porcellio scaber* (Brummelen & Van Staalen, 1996). With heavy metals, several studies were also performed where the kinetics of Cadmium and Zinc were evaluated using several organisms (e.g., Janssen & Bergema, 1991; Janssen *et al.*, 1991; Crommentuijn et al., 1997; Van Gestel & Hensbergen, 1997).

Although there is a considerable amount of individual data in the terrestrial ecosystems, most of it present a high variability in test conditions and cannot be used as a comparison, especially data on pesticides. This enhances the need for the development of bioaccumulation guidelines using soil organisms. Under this context, several studies (using mainly earthworms, enchytraeids and isopods) are currently being performed aiming at guideline development (e.g. Amorim et al., 2000a; 2000b; Sousa et al., 2000; Bruns et al., 2000 personal information). Some of these studies (especially those using enchytraeids and isopods) also include the measurement of the bioavailable fractions of the test substances and the assessment of bioaccumulation and bioavaliability over time, that is, evaluating the ageing effect. This last aspect is gaining more and more importance in the assessment of contaminated sites.

4.3. EXPERIMENTAL APPROACHES: MULTISPECIES TESTS

Organisms can be affected by direct as well as by indirect substance effects. In single species tests, direct effects of the test substance on an organism are determined, whereas multispecies tests additionally include interactions between organisms. (e.g. changes of food supply). Therefore, the test result is significantly influenced by the composition of the biotic community and the position of the single organisms within the food chain.

So far, only very few internationally standardised tests or guidelines for terrestrial multispecies tests exist. Investigations can be performed in the laboratory as well as in the field. While laboratory testing allows a standardisation of the test conditions, field tests are characterised by a higher variability due to climatic influences. Semi-field tests, a very heterogenous group, are somewhere in-between. Multispecies tests require considerably higher testing efforts than single species tests, therefore in general, fewer concentration gradients are investigated. In addition, the number of replicates is usually lower. The calculation of an ECx frequently is either not possible or it is based on a smaller set of data. With respect to single species tests reliability/precision and reproducibility is lower however the ecological relevance is higher.

Since there is hardly a clear borderline between tests on the three investigation levels, in this chapter it will be distinguished between multispecies tests performed under more or less controlled conditions (laboratory and semi-field tests) and field studies. In addition, some remarks are made on functional test methods which can be used at each investigation level.

4.3.1 Laboratory and semi-field tests

Gnotobiotic laboratory tests

These tests, relatively similar to single-species tests, are run under very controlled conditions. Usually a few species (2 – 5), either from laboratory cultures or caught in the field, are exposed together in an artificial or (often sieved) field soil. One example is a two-species chronic test (Schlosser & Riepert, 1992), in which the predatory gamasid mite *Hypoaspis aculeifer* is exposed via their food (collembolans or enchytraeids). This test is quite complicated since the prey organisms have to take up the test chemical via their food firstly, making the test in its most elaborated version very time-consuming (up to 24 weeks).

Several proposals have been made where various invertebrates or plants have been added to an artificial assemblage of sieved soil. For example, Mothes–Wagner *et al.* (1992) used nematods, enchytraeids, gamasids (all laboratory-reared) and beans to test the effects of pesticides. Recently, much work has been done with a gnotobiotic system called the Ohio type microcosm (Edwards *et al.*, 1998), which can be classified as an intermediate method, ranging in complexity between laboratory tests and terrestrial model ecosystems (see next paragraph). So far none of these methods has been regularly performed or was required by governmental agencies.

Terrestrial microcosms/mesocosms

Terrestrial microcosms/mesocosms can be used as integrative test methods in which fate and effect parameters (partly including bioaccumulation) are investigated at the same time and under conditions that are „closer to nature“ than in the laboratory. A huge number of different approaches has been proposed so far (Morgan & Knacker, 1994; Sheppard, 1997). Some methods are very complex: for example, the NATEC „plant metabolism box“ and related systems are closed ecosystem segments that were developed to investigate the environmental fate of radio-labelled chemicals and consist of an intact soil core and planted crop species (Figge *et al.*, 1983). However, due to their size and complexity they are not suitable for routine application as part of the registration or notification of chemicals.

In contrast, „Terrestrial Model Ecosystems” or TMEs are small enough to be replicated but large enough to sustain soil organisms for a long period of time (Römbke *et al.*, 1994). The TME was developed in the early 1980s as an open model ecosystem consisting of undisturbed soil cores extracted in the field. It is the only multispecies test method that is fixed as a guideline for the registration of chemicals in the USA (EPA, 1987; ASTM, 1993). Whereas during the development of the tests the emphasis was on the environmental fate of the test chemical and its effects on the nutrient cycle, the inclusion of effect parameters (e.g. on microflora, plants, mesofauna (enchytraeids) and macrofauna (earthworms)) has enhanced the relevance of the method considerably (Knacker & Römbke, 1997). Recently, the applicability of this method was investigated by studying the effects of the fungicide Carbendazim on many endpoints using four European soil types (Förster *et al.*, 1999). Like the TME, other relatively small model ecosystems were able to simulate filed conditions. For example, earthworms have been tested successfully in such systems (Förster *et al.*, 1996; Svendsen & Weeks, 1997). The influence of animals on ecosystem functions like the mineralization of organic matter can also be examined in comparatively small units (e.g. isopods feeding on poplar leaves; Van Wensem *et al*. 1991). These studies have repeatedly confirmed that microcosm tests can react more sensitively than pure laboratory tests (e.g. Teuben & Verhoef, 1992; Vink & Van Straalen 1999).

Field enclosures

Tests classified as field enclosures consisted of small (usually less than 1 m^2) parts of, e.g., a meadow, which is separated from its surroundings in such a way that there is no exchange of organisms but which is exposed to the normal climatic conditions. Most of these methods were developed in order to detect side-effects of pesticides on beneficial arthropods (Hassan, 1992). The most widely used example is the carabid semi-field test which has been standardised recently (Heimbach *et al*., 2000). Ground-beetles, usually the species *Poecilus cupreus*, are exposed for two weeks after application of a pesticide in a cage consisting of steel frames put into the soil. Comparable methods are available for staphylinid beetles and spiders. The results of these tests have been successfully used as part of the registration process of pesticides, especially since they very closely resemble real field conditions.

4.3.2 Field studies

Field tests

Up to now, nearly no standardised methods for evaluating the ecotoxicological hazard potential of chemicals in terrestrial field ecosystems are available. In the European Union testing of the side-effects of pesticides on earthworms, beneficial arthropods or even on litter degradation can be required as part of the registration process (EU Guideline 9/414/EC). In the USA, terrestrial field studies performed under actual pesticide use conditions can be required by the EPA (Fite *et al*., 1988). The latter address the potential acute, subacute and/or chronic adverse effects of pesticide residues to non-target mammals and birds. Field studies generally serve to determine a current state, which is influenced by climatic conditions and chemical-physical soil parameters as well as by anthropogenic actions, as soil use or chemical input. Dose-response relationships normally are not established.

The best-known example is an earthworm test in which the long-term effect of a pesticide on the natural lumbricid community of an arable or meadow ecosystem is examined (BBA, 1994; ISO 1999b). There is also an English guideline that is comparable in design, albeit extremely general, for the effects of insecticides on beneficial arthropods (various groups of beetles and spiders) in summer grain fields (MAFF, 1993). According to a newer proposal Collembola are used as test organisms (Wiles and Frampton, 1996). Otherwise, several methods for evaluating the effect of pesticides on beneficial arthropods in the vegetation layer are available, e.g. of vineyards (BBA, 1991). All of these tests are done by applying one or more concentrations of a pesticide under conditions comparable to normal agricultural practice. After a few weeks of up to one year abundance, biomass and dominance spectrums of the test organisms are used as measurement endpoints. The methods used are well known from soil ecology (Dunger and Fiedler, 1997).

Community approach

Due to space limitations it is not possible to present here a compilation of all bio-indication methods useful for the assessment of the effects of chemical contamination (or soil quality in general). Instead the question of the selection of test species is discussed in more detail. First of all, the community of species (i.e. the biocoenosis) should be used when evaluating the ecosystem but not an individual species as is often the case for other environmental compartments (e.g. protection of endangered birds or mammals). Therefore, with one important exception (see next paragraph on “ecosystem engineers”), it is necessary to select several organisms from various size classes, and functional and trophic groups since there is usually not one „perfect“ indicator. It must be decided whether the investigation effort aims to detect effects of potential contaminants on the organisms (reaction indicators) or whether the accumulation of a chemical in soil organisms and possible secondary poisoning effects (e.g. for birds of prey) should be identified (accumulation indicators). Finally, functional methods should be used in addition to the structural endpoints mentioned so far (cf. section 3).

Organisms to be selected as test species in field studies (including monitoring programs) should fulfil the following criteria:

- clearly identifiable (especially the active life stages);
- sensitive to a wide range of stress factors;
- quick reproduction (i.e. 1 – 4 generations per year);
- preferably a good correlation with microbiological activities;
- easy sampling; e.g. high density;
- not migratory (so changes can be attributed to a certain site).

In addition, it is recommended to select “ecosystem engineers” (often less clearly named “key species”). These are those organisms which directly or indirectly affect the availability of resources to other organisms through modifications of the physical environment (Jones *et al*., 1994). In the soil ecosystem, earthworms are the organisms most often identified as the principal engineers (Lavelle *et al*., 1997), although in some case other groups might also be important in this context (e.g. millipedes, ants or, more rarely, mesofauna groups like springtails or enchytraeids). Since ecosystems engineers have sufficient numerical and biomass densities to exert a predominant influence in the formation and maintenance of soil structure and to regulate processes to an extent that overrides organisms in other functional categories, they are primary targets in any assessment concept. By testing or monitoring ecosystem engineers one can be sure that any changes in their densities or species structure will also have clear influences on soil structure and ecosystem processes. Such studies have been performed with several organism groups (e.g. springtails (Hopkin, 1997); nematodes (Bongers, 1990); mites (Ruf, 1998).

Accumulation studies

The use of earthworms as accumulation indicators in the field (often combined with other endpoints) to monitor soil quality is best exemplified by a study performed at Superfund Site (Holbrook, USA; Callahan *et al*. 1991). In this field test, containers were placed on transects located across the site in the impacted as well as in reference sites located nearby. These plastic chambers were filled with the excavated soil from the same sites, and five *Lumbricus terrestris* were placed on the soil surface. Mortality and morbidity endpoints were evaluated at the end of 7-day field exposures. Soil samples as well as earthworm tissue samples from survivors exposed to those soil samples were randomly selected for chemical analysis. This on-site method appeared to be a sensitive test for assessing the potential impact of soil contaminants at field sites, especially when combined with laboratory tests and/or the investigation of native populations. Recently, the same approach was used to assess the effects of soil contamination on the fertility and population dynamics of springtails (Kopeszki, 1999). However, up to now no terrestrial bioaccumulation guideline has been standardised.

4.4. MEASUREMENT OF SOIL PROCESSES

In this chapter three methods related to two processes (litter decomposition and feeding rate) will be presented in detail since they are relatively easily measurable (Kula & Römbke, 1998). Despite the fact that some of these methods can also be used in semi-field tests, they are usually performed under field conditions. In the European Union, data on litter decomposition using the litter-bag method can be required as part of the registration process of persistent pesticides according to Guideline 91/414/EC.

The cotton strip assay (Harrison et al., 1988; Kratz, 1996)

The cotton strip assay uses only a physical parameter (loss of tensile strength). An advantage of this method is the availability of a well-standardised cotton material. One problem is the need for special equipment, which can only be used to measure tensile strength loss. Another disadvantage is that the assessment of degradation of pure cotton compared with degradation of natural litter will result in a simplification of this complex soil function.

The litter bag method (Crossley and Hoglund, 1962; Paulus et al., 1999)

The litter bag method allows one to determine the effects of chemicals on both mass loss and patterns of nutrient dynamics in decomposing litter. Although it requires more effort than the method described above, this technique provides ecologically-relevant information and is therefore preferable. The disadvantages of this method are: (i) substrate packed in a litter bag may create a microclimate condition different from bulk soil, (ii) substrate in a litter bag does not come into contact with contaminated soil, and (iii) litter in the bag may attract soil organisms which in turn may lead to increased biological activity and faster decomposition.

The bait-lamina test (Von Törne, 1990; Paulus et al., 1999)

In addition to these highly integrating methods the feeding rate of soil invertebrates can be measured by using the Bait-Lamina-Test. Their production is well standardised. It is the simplest and most practical test with just a „yes“ or „no“ answer. A disadvantage is that the contribution of different groups of soil biota to the decomposition process is not known.

4.5. CONCLUSIONS AND OUTLOOK

From this brief overview of effect assessment tests for the terrestrial environment it is possible to verify that there is no shortage of test ideas. According to Römbke *et al*. (1996) what is missing is reliable test data. We believe that there is a general agreement that the effect assessment as part of the registration of chemicals (especially those known to be biologically active like pesticides or certain pharmaceuticals) should not be restricted to the established official guidelines. In this context more protocols, using different test species, embracing more trophic levels and different life-history strategies, need to be required in order to improve the effect assessment of chemicals in the soil compartment. This implies both the need for further improvements / adjustments and standardization of some of these protocols, and the agreement on a tiered test strategy with increasing ecological relevance and complexity. Such a test strategy would be more demanding, in terms of ecosystem safety, than the one requested so far by the EU (EEC, 1992). Several strategies have already been proposed, each focussing on a special group of chemicals, which makes comparisons difficult (e.g. Leon & Van Gestel, 1994; Pedersen & Samsoe-Petersen, 1995; Römbke *et al*., 1996, Cortet et al., 1999). However, all of them need refinement, especially concerning the following issues:

- the incorporation of all relevant organism groups (e.g., soil invertebrates as well as microorganisms and plants);
- measurement of structural (i.e effects on populations) <u>and</u> functional (i.e. soil processes –like decomposition of organic matter);
- definition of criteria (like trigger values) in order to decide when tests of the next higher tier are necessary or not..

Virtually all of these proposals for a tiered test strategy use the same criteria for general considerations and especially for the selection of test guidelines. For example, usually not more than three tiers are proposed in order to perform the testing in a practical way. Tier I often consists of short-term, acute tests whereas on the second tier, long-term, chronic tests are required. The third tier is reserved for semi-field or field tests selected on a case-by-case basis. However, the usefulness of acute mortality tests is more and more doubted due to their relatively low sensitivity and ecological relevance (e.g. for invertebrates: Fairbrother et al., 1999).

The test methods proposed for the individual tiers should be identified according to the following criteria. The (ideal) test strategy should:

- include only standardised and (preferably) validated test methods published by (international) organisations
- be useful for different stress factors (e.g. pesticides, chemicals)
- cover various trophic and taxonomic groups as well as size classes (i.e. different exposure situations)
- include structural and functional endpoints as well as bioaccumulation studies
- be flexible in selecting the most appropriate test with increasing tiers
- be efficient in using limited resources (e.g. avoiding unnecessary tests)
- produce test results which are useful for an overall risk assessment for the “living” compartment soil.

Taking into consideration the proposals published so far, the following tests could be included in an “ideal” tiered test strategy for the soil compartment. Despite the fact that an in-depth review of the various test strategy proposals is beyond the scope of this contribution, some general recommendations can be given. It should be noted that special requirements (e.g. testing the side-effefts of pesticides on beneficial organisms) are not covered here.

On Tier 1, at least two microbial tests (representing carbon and nitrogen mineralisation), one (acute) plant test, two saprophagous invertebrate tests like an (acute) earthworm and an (acute) collembolan test and one test with a predatory organism like a gamasid mite or a staphylinid beetle should be performed.

If effects are observed, the risk for the sensitive group(s) has to be examined further on Tier 2, e.g. by performing reproduction (or better: life cycle) tests and by expanding the species list (e.g. including nematods, enchytraeids, carabid beetles and isopods). Depending on the properties of the test chemical (especially the log Pow) bioaccumulation tests with either plants, earthworms, enchytraeids or isopods have to be included.

On Tier 3, either a TME soil microcosm test or a field test is needed. In any case on this tier functional endpoints like litter decomposition are required; especially if the test substance is persistent.

In spite of all the difficulties involved, multispecies tests on the various investigation levels have played an important role in ecotoxicology in the last three decades and will continue to do so in the foreseeable future. In general, multispecies tests should be required on higher levels of a tiered test strategy when chemicals are registered or notified (as referred to above). The most promising approaches on each level (one microcosm test method and two field approaches (one test and one community-orientated method)) have to be standardised, probably as a guidance paper, since the variability of test conditions does not allow the formulation of a strict and detailed guideline. Currently an ongoing EU funded project is addressing these aspects, by improving and validating a TME test system (Knacker, 1998). In relation to field testing, besides assessing effects at population or community level of selected organisms, it is advisable and necessary to integrate functional tests at the ecosystem level (e.g., litter decomposition and bait-lamina tests (Kula & Römbke, 1998)).

Finally, all the tests we have mentioned above were developed to assess the effects of a certain chemical on a specific test organism. However, the major goal of soil ecotoxicological research is to be able to predict the effects of harmful substances in real situations. This means in addition to evaluating the effects of emissions of chemical substances, soil ecotoxicologists must also be able to predict the hazard potential of contaminated soils. Therefore, besides further improvement on the existing extrapolation methods, more research is needed not only focusing on multi-exposure scenarios (by developing multi-exposure and multi-species tests), but also on the development of new test guidelines using more realistic conditions (e.g., adapting the existing protocols to be used with a variety of natural soils; e.g. Dott & Hund 1995, Crane & Byrns 2000) and/or embracing other types of test procedures (e.g., *in situ* testing). Currently, the strategic background of such an approach is under discussion for contaminated soils in general (ISO 1999c). Figures 1 and 2 attempt to picture the strategies and differences underlying the “single chemical” testing and the assessment of soil quality.

One concept aiming in the same direction is the comparison between the biocoenosis actually found at a – potentially contaminated - site (characterised by using mainly qualitative parameters like dominance spectrum but also the abundance) with the community which should live there if the soil had not been contaminated or otherwise (anthropogenically) affected (e.g. SOILPACS (England): Spurgeon *et al*. 1996; BBSK (Germany): Römbke *et al*. 1997) (see Fig. 2). This reference community defined approach is based on a correlation between the most important soil properties and the occurrence of organisms at uncontaminated sites deduced from literature. Currently, the knowledge about the biology of soil organisms and their dependency from certain soil properties is not very well studied, but experiences from aquatic ecosystems using the same approach are very promising (e.g. BEAST (Canada): Reynoldson *et al*. 1995).

Additionally, some improvements are necessary to ameliorate the evaluation of ecotoxicological field investigations. For example, they suffer from the fact that often the data essential for test result interpretation were not measured at the same time and place. When measuring biological effects the exposure situation and the main soil properties as well as climatic conditions should be characterised simultaneously.

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Table 1 - Some representative existing protocols with microorganisms

Tests	Measured parameters	Guideline	Source
Nitrogen transformations			
• Nitrification test	Nitrification (IC100; DT50tret-DT50ct > 1 day)	NEN 5795	NNI, 1988
• Denitrification test	Denitrification (EC50, EC90, NOEC)	MATS 07	Pell, 1993
• Nitrogen fixation test	Heterotrophic N fixation (EC50, EC90, NOEC)	MATS 09 & 10	Martensson, 1993a & b
• Nitrogen fixation test	Cyanobacterial N fixation (EC50, EC90, NOEC)	MATS 12 & 13	Martensson, 1993c & d
• Ammonium oxidation test	Ammonium oxidation (EC50, EC90, NOEC)	MATS 05	Torstensson, 1993b
• Nitrogen conversion test	NH4+, NO3-, NO2- (% in relation to control)	BBA VI, 1-1	BBA, 1990
• Nitrogen mineralization test	N mineralization (ICx, NOEC)	ISO 14238	ISO, 1997b
Enzymatic assays			
• Dehydrogenase test	DHA (% inhibition in relation to control)	BBA VI, 1-1	BBA, 1990
• Phosphatase test	Acid or alkaline phosphatase activity (% inhibition in relation to control)	MATS 14	Sjoquist, 1993
• BIOLOG test	Community structure/physiological capacity		Garland, 1997
Respiration methods (incl. Biomass determinations)			
• Soil respiration test	SIR, vitality of microb. pop. (% inhibition in relation to control)	MATS 17	Palmborg & Nordgren, 1993
• Soil respiration test	Short term respiration (% inhibition in relation to control)	BBA VI, 1-1	BBA, 1990
• Soil respiration test	SIR, biomass	ISO 14240-1	ISO, 1997a
Bioluminescent bacteria tests			
• *Pseudomonas fluorescens*	Reduction on light emission		
• *Vibrio fischeri* (light emission reduction)	Reduction on light emission (LID)	ISO/DIS 11348	ISO, 1995a
• *Vibrio fischeri* (growth test)	Reduction on light emission (LID-Growth)	DIN draft	

NOTE: Regarding enzymatic assays more methods are available (including mainly oxido-reductases and hydrolases), but only some were used to evalute pollution effects.

Table 2 - Most representative effect assessment tests with plants

Tests	Effect parameters	Guideline	Source
• Plants, growth test	Emergence, growth (EC50, LC50)	OECD 208	OECD, 1984b
• Phytotoxicity test	Emergence, growth (EC50)	BBA (draft guideline)	BBA, 1984a
• Seed germination/ root elongation test	Seed germination, root growth (EC10, EC50)	EPA CFR 40-1-R	EPA, 1992a
• Early seedling growth toxicity test	Root, shoot and total plant growth	EPA CFR 40-1-R	EPA, 1992b
• Root elongation test	Root growth (ECx)	ISO 11269/1	ISO, 1993
• Seed germination test	Emergence, growth (NOEC, LOEC)	ISO 11269/2	ISO, 1995b
• Whole plant test	Total plant, root and shoot growth (EC50)	ASTM STP1115	Pfleeger et al., 1991
• "Life cycle test"	Germination, growth, photosynthetic systems flower development, reproduction	ASTM (draft guideline)	ASTM, 1997
• Chronic plant test	Emergence, growth, shoot length, number of flower buds and seed pods	ISO (draft guideline)	Kalsch & Römbke, 2000

Table 3 - Most relevant tests with invertebrates

Tests	Effect parameters	Guideline	Source
Single species tests			
• Nematode Test	Reproduction (EC50, NOEC)	NIRSP	Kammenga & van Koert, 1992
• Entomophagous Nematode Test	Survival, parasitism	IOBC	Vainio, 1992
• Acute Nematode Test	Survival, parasitism		Donkin & Dusenberry, 1993
• Nematode chronic toxicity test	Abundance		Niemann & Debus, 1996
• Earthworm acute test	Survival (LC50)	OECD 207	OECD, 1984a
• Earthworm subsacute test	Survival (LC50), biomass, behaviour	FDA 4.12	FDA, 1988
• Earthworm acute and sublethal test	Survival (LC50), biomass, behaviour	ASTM 1676-97	ASTM 1997
• Sublethal toxicity on earthworms	Survival (LC50), biomass, behaviour	*SECOFASE*	Kula & Larink, 1998
• Earthworm reproduction test	Reproduction (NOEC)	ISO 11268 - 2.2	ISO, 1996
• Earthworm field test	Abundance, number of species	ISO 11268 - 3	ISO, 1999b
• Enchytraeid reproduction test	Reproduction (ECx, NOEC)	ISO 16387, OECD 220, ASTM 1676-97 (drafts)	Römbke et al., 1999
• Enchytraeid sublethal test	Growth, fragmentation	*SECOFASE*	Rundgren & Axelsson, 1998
• Collembola sublethal toxicity test	Survival, body length	*SECOFASE*	Wiles & Krogh, 1998
• Collembola reproduction test	Reproduction (NOEC)	ISO 11267	ISO, 1999a
• Isopod growth test	Survival, growth, feeding rate (LC50, ECx)	*SECOFASE*	Hornung et al., 1998
• Isopod reproduction test	Survival, reproduction, oorsoption (LC50, ECx)	*SECOFASE*	Hornung et al., 1998
• Oribatid mites test	Survival, reproduction, feeding rate (LC50, EC50, NOEC)	*SECOFASE*	Van Gestel & Doornekamp, 1998
• Predatory mites test	Survival, reproduction	EPPO 151	OEPP/EPPO, 1990
• Predatory mite sublethal test	Survival, reproduction		Krogh, 1995
• Staphylinid beetle test	Survival, reproduction	IOBC	Samsøe-Petersen, 1992

• Staphylinid generation test	Hatching, survival	BBA VI, 23-2.1.10	BBA, 1994
• Staphylinid test (adults)	Hatching, number of eggs (ECx)	*SECOFASE*	Metge & Heimbach, 1998
• Staphylinid test (larvae)	Survival, hatching wt, development time (LC50, EC50, NOEC)	*SECOFASE*	Metge & Heimbach, 1998
• Staphylinid semi-field test (larvae)	Survival, hatching wt, development time (LC50, EC50, NOEC)	*SECOFASE*	Metge & Heimbach, 1998
• Carabid test	Survival	IOBC	Chiverton, 1988
• Carabid test	Survival, feeding rate	BBA VI, 23-2.1.8	BBA, 1991
• Carabid semi-field test	Survival, feeding rate		Heimbach et al., 2000
• Centipede test	Survival, growth, locomotor activity respiration rate (LC50, ECx)	*SECOFASE*	Laskowski et al., 1998
• Millipede test	Survival, development, reproduction (LC50, ECx)	*SECOFASE*	Tajovsky, 1998
• Parasitic wasp test	Survival, parasitism, reproduction	EPPO 142/1& 142/2	OEPP/EPPO, 1989
• Spider test	Survival, food consumption, behaviour	BBA VI, 23-2.1.9	BBA, 1993b
• Spider test	Survival, food consumption	IOBC	Inglesfield, 1985
• Honeybee test	Survival (LD50)	EPPO 170	OEPP/EPPO, 1991
Multiple species tests			
• Nematode competition test	Ratio between species (LOEC, NOEC, ECx)	*SECOFASE*	Kammenga & Riksen, 1998
• Predatory mite - Collembola test	Survival, reproduction		Schlosser & Riepert, 1992
• Predatory mite - Collembola test	Survival, growth, reproduction of mites (LC50, ECx)	*SECOFASE*	Krogh & Axelsen, 1998

Table 4 - Major effect tests with vertebrates

Tests	Effect parameters	Guideline	Source
Acute			
• Avian single dose test	Survival (LD50)	EPA E 71-1	EPA, 1982a
• Avian dietary test	Survival, body weight behavioural changes, feeding	OECD 205/ EPA E 71-2	OECD, 1984c EPA, 1982a
• Bird uptake test	Survival, body weight behavioural changes	BBA VI, 25-1	BBA, 1993a
• Acute oral toxicity (i.e. rats)	Survival (LD50)	OECD 401	OECD, 1987a
Acute dermal toxicity	Survival (LD50), irrigations, behaviour	OECD 402	OECD, 1987b
Acute inhalation study	Survival (LD50), toxic effects, behaviour	OECD 403	OECD, 1987c
Chronic			
• Avian reproduction test	Survival, number of eggs, morphological changes of the eggs, effects on the young birds	OECD 206/ EPA E 71-4	OECD, 1984d EPA, 1982b
• One-generation reproduction toxicity study	Various reproductive endpoints	OECD 415	OECD, 1983a
• Two-generation reproduction toxicity study	Various reproductive endpoints	OECD 416	OECD, 1983b

CHAPTER 5

DOSE /RESPONSE RELATIONSHIPS AND METHODS FOR SETTING ECOTOXICOLOGICAL THRESHOLDS AND QUALITY OBJECTIVES

5.1 Introduction

The purpose of setting ecotoxicological thresholds or quality standards is to derive concentrations in the environment that are safe for all organisms. It is unknown whether a discrete threshold actually exists for populations or ecosystems but at least we need to arrive at concentrations at which the effect on the environment is small enough to be acceptable. There are in principle two ways to derive thresholds for individual chemicals from single-species data:

1) use of (fixed) assessment factors to cover uncertainties in extrapolating from laboratory tests (short duration, few species) to the field situation (chronic exposure, many species).
2) fit a continuous distribution through the available toxicity data and calculate the concentration at which the theoretical percentage of species exposed above their NOEC or EC 50 is small enough (e.g. five percent).

These approaches are sometimes called deterministic and probabilistic which is confusing as both methods are deterministic in the sense that one answer is derived from the input data. We will therefore stick to the terms "assessment factors" and "species sensitivity distributions" or " statistical extrapolation" and reserve the term probabilistic for stochastic calculations (e.g. see chapter 9).

Setting environmental quality standards, both for the aquatic and terrestrial compartment is typically based on information on single-species toxicity data for aquatic species, soil fauna, and aquatic and terrestrial plants. Toxicity data for soil microflora (i.e. effects on enzymatic activities and soil processes) are usually determined in natural soils with their complex microbial community. The acute and chronic laboratory data, respectively, are transposed to field situations using extrapolation approaches as mutually agreed upon within the scientific and the political community. Conventions on how to use the different methodologies comprise a scientific background concerning species sensitivities distributions, differences in single species and ecosystem responses to an impact as well as knowledge on the representativeness of selected species and the social/political background concerning the objective and level of protection. However, the social decisions are also triggered by scientific knowledge and recommendations. For example, the setting of soil quality standards should ensure the intactness of all soil functions as well as the structure and diversity of the soil ecosystem. These parameters have to be defined scientifically. Moreover, environmental quality objectives and standards are typically developed for individual chemicals, while ecosystems are usually exposed to complex mixtures of contaminants. Defining the effects of mixtures on living organisms is a still open problem.

This chapter tries to give an overview of existing extrapolation approaches, recommendations on their applicability and an outlook on the improvement. Finally, the possibility of using toxicity data for individual chemicals for developing quality objectives for complex mixtures will be explored.

5.2 Data quality and (un)certainty of input

In the tests serving to determine the ecotoxicological effects of chemical substances on terrestrial organisms, various concentrations of the test substance are added to an artificial substrate or to a soil, which is defined by chemical-physical properties (e.g. particle size distribution or organic carbon content). As significant variations in sensitivity towards a chemical substance may already be found for different species of a genus and as substance bioavailability is governed by the chemical/physical properties of a substrate or soil as well as the organism tested, the determined Ec_x is restricted to the selected test conditions. In standardized test systems, test organism as well as test substrate and test soil, respectively, are prescribed. This allows a comparison of different test substances on the basis of different ecotoxicological test procedures.

The single set-ups differ only in the concentration of the test substance. Therefore, the reliability and precision is high and clear dose-response relationships can be obtained, which allow the calculation of an EC_x or NOEC. As the test conditions can be fixed in detail, the reproducibility is high. The ecological relevance, however, is comparatively low.

Dose-response analysis

Most ecotoxicity tests are performed with several exposure levels to allow the assessment of the dose-response relationship. Acute toxicity data are usually described with a log-logistic or log-normal curve after which an EC50 or LC50 is determined (the estimated exposure at which a 50% effect is observed). In chronic studies, it is common practice to derive a no-observed effect concentration (NOEC) as the highest dose at which the effect is not significantly different from the control. The criticism of this approach is devastating (Hoekstra & Van Ewijk, 1993a; Laskowski, 1995; Van der Hoeven et al., 1997; Crane & Newman, 2000) but the use of the NOEC nevertheless persists. The NOEC has properties which are undesirable, among which:

- No *significant* effect does not mean that there is *no* effect or that the effect is small. In fact, the effect percentage at the NOEC may be as high as 47% (Hoekstra & Van Ewijk, 1993b) and in extreme cases up to nearly 100% (Crane & Newman, 2000).
- The NOEC depends strongly on the selected test concentrations as it has to be a tested concentration. This also means that there is no way to provide a confidence level for the NOEC.
- The less accurate the test (smaller sample sizes, high variation), the higher the probability of a higher resulting NOEC.

Not only is the use of the NOEC not protective, the associated effect level is highly variable and based on study design and variation in the tested population. Several alternatives to the NOEC exist which do not have the above-mentioned weakness:

- Regression analysis or bootstrap procedures to estimate an EC5 or EC10. This procedure is consistent with the well-accepted LC50 estimation but the estimates are located in the tails of the distribution and thus depend on the selected dose-response model.

- The bounded-effect concentration (Hoekstra & Van Ewijk, 1993b); the concentration at which *at most* 10% effect occurs (with 95% confidence).
- A true no-effect concentration based on a (more complex) mechanistic model (Kooijman & Bedaux, 1996).

In view of its disadvantages, the concept of the NOEC needs at least re-evaluation. The NOEC is however firmly anchored in existing test protocols and regulatory frameworks (De Bruijn & Hof, 1997). A discussion on the most appropriate (or most acceptable) alternative is needed and current test protocols need to be adapted.

Consideration of food chains and food webs in derivation of quality objectives
Currently, most approaches to derive quality standards for soil focus on soil living organisms. Concepts to include organisms feeding on the terrestrial fauna and flora such as birds and mammals use information from feeding experiments, and transfer and exposure scenarios. The latter should account for the feeding behaviour of the species to be protected, contaminant concentrations in food as well as transfer of the chemical from soil to food. Several suggestions thereof are published but are not commonly agreed so far.

5.3 Extrapolation methods

5.3.1 Assessment Factors

State-of-the-art:

The most pragmatic approaches are deterministic either applying fixed assessment factors (modified EPA-Method, FAME, method according to Slooff et al., 1986) or using regression and correlation analyses.

<u>Fixed assessment factors</u>
The assessment factor approach has been suggested and further modified mainly by the U.S.EPA (U.S. EPA, 1984) and is therefore currently referred to as the *(modified) EPA method*. The method applies fixed assessment factors to the lowest results of single species laboratory tests. Factors are derived by assuming constant differences between acute and chronic toxicity, between responses by laboratory single species and species under field conditions. A further assessment factor can be applied depending on the size of the data set and the quality of the available information. The OECD workshop on extrapolation of laboratory aquatic toxicity data to the real environment (OECD, 1991) recommended the following fixed assessment factors:

Available information	Assessment factor
Lowest acute L(E)C50 value or QSAR estimate for acute toxicity	1000
Lowest acute L(E)C50 value or QSAR estimate for minimal algae/crustaceans/fish	100
Lowest NOEC value or QSAR estimate for chronic toxicity	10[a]
Lowest NOEC value or QSAR estimate for chronic toxicity for minimal algae/crustaceans/fish	10

[a]This value is subsequently compared to the extrapolated value based on acute L(E)C50 toxicity values. The lowest is selected.

The assessment factor approach has also been recommended by the EU (Technical Guidance Documents) [TGD, 1996] for a screening of concentrations of no environmental concern (PNEC) for New and Existing Chemicals as well as by the CSTE (CSTE, 1994) for the derivation of water quality criteria. The latter committee introduced the nomenclature *„factorial application method“ (FAME).* In most of the EU Member States FAME is also applied to terrestrial organisms either for a preliminary derivation of soil quality standards or as a method being complementary to the probabilistic approaches (see below) with the following fixed factors being suggested:

Available information	Assessment factor
Acute tests for – e.g. - plants,earthworms, microbes	1000
NOEC for one chronic test	100
NOEC for two chronic tests of two trophic levels	50
NOEC for three chronic tests of three trophic levels	10
Field or model ecosystem	case-by-case

With respect to birds and mammals (secondary poisoning) the following factors are suggested:

Available information	Assessment factor
LD_{50} for birds and mammals	Not acceptable for extrapolation
LC_{50} for birds	1000
NOEC (28 day repeated dose test)	100
NOEC (90 day repeated dose test)	30
NOEC for chronic studies	10

A further deterministic approach which could - in principle - be used to derive quality standards tries to make use of the entire set of ecotoxicity data. The *method according to Slooff et al. (1986)* has been developed for the aquatic compartment and aims at the derivation of a $NOEC_{ecosystem}$ by using statistically determined correlations between single species tests and data for field and model ecosystems. The lower limit of the 95% confidence interval of the lowest test value is equal to $NOEC_{ecosystem}$. Equations are:

$$\log NOEC_{ecosystem} = - 0.55 + 0.81 \log L(E)C50_{ss} \ (r=0.77)$$

$$\log NOEC_{ecosystem} = - 0.63 + 0.85 \log NOEC_{ss} \ (r=0.85)$$

ss = single species

The approach has not been used for the derivation of quality standards.

<u>Regression and correlation analyses (variable assessment factors):</u>

The extrapolation factors as used in FAME are assumed to cover certain extrapolation steps. Instead of a fixed factor 10 for each step, information from literature or factual databases can be used. This will lead to frequency distributions of assessment factors which can be used in several ways:

- Take the 95th percentile of each distribution as a new, data-based assessment factor. Disadvantage of this approach is that the multiplication of these assessment factors leads to a higher protection level than 95 percent.
- Combine the distributions for each extrapolation step into a distribution of an overall assessment factor (analytical or through Monte Carlo simulation). The 95th percentile of this overall distribution can then be used as a total assessment factor.
- Use all the distributions in an overall probabilistic risk assessment. Important advantage is that sensitivity analysis will show if the uncertainty in an extrapolation step is important, thus providing an aim for further testing.

These types of data-driven approaches make better use of the large body of available literature. The disadvantage is that this approach seems to be very accurate and scientific whereas our knowledge on ecosystem effects is still limited. Examples of these approaches can be found in the human health area (Slob & Pieters (1998) and Vermeire et al. (1999). A preliminary example in probabilistic risk assessment of ecosystems and predators is given in Jager et al. (1997).

Evaluation of the approaches and recommendations:

Assessment factors are not usually based on thorough scientific argumentations but on precautionary principles. Some of the assumptions are at least debatable such as the fixed ratio between acute and chronic effect concentrations and the prerequisite that protection of the most sensitive species will also protect the ecosystem structure and function. However, due to the obvious arbitrariness and its lack of pretension, it is recommended to use assessment factors, especially in cases of limited effects data. In cases where sufficient data are available, it is recommended to use the statistical extrapolation alongside the extrapolation with factors. Nevertheless it should be noted that assessment factors should not be used to predict toxicity thresholds, rather, they provide a screening method to derive levels that are "safe enough". Appropriate effects data should always be preferred above extrapolation, and assessment factors should be used in a flexible manner. A critical evaluation of the assessment factor approach is given by Chapman et al. (1998).

With respect to birds and mammals, the effects data are translated from a dosis to environmental concentration basis using the animals' daily intake of food and the BCFs for fish and earthworms. There are some ideas of correction for differences beween lab experiments and field effects (e.g. for differences in metabolism and caloric content of the food) (Jongbloed et al., 1994); Traas et al., 1996). The corrections however have not been generally agreed.

5.3.2 Species-sensitivity Distributions

State-of-the-art:

An alternative to the use of assessment factors is to use the variability in sensitivity of the test species as a measure of the variability of all species in the ecosystem. By fitting a continuous distribution (e.g. log-normal or log-logistic) through the data (usually NOECs), an exposure level can be derived at which a theoretical percentage of the species is fully protected, i.e. is exposed below its NOEC. Generally, it is considered acceptable when the NOEC is exceeded for less than 5% of the species (see also (OECD, 1992; Health Council of the Netherlands, 1988)). The use of species sensitivity distributions should in our opinion not be seen as an ultimate representation of ecosystem sensitivity. The approach is still based on laboratory toxicity data for single species. Rather, this approach must be seen as a statistical extrapolation to derive a concentration that is safe enough from the available data. This approach has several convenient properties:

1) The difference between the lowest NOEC and the PNEC depends on the amount of data and the spread in the data (when sensitivity differences in the tested species are large, the PNEC is low to account for the existence of even more sensitive species).

2) A confidence interval around the PNEC can be calculated which depends upon the number of species tested (see Aldenberg & Jaworska, 2000).

3) It uses selected data for different species, not just the lowest.

Several approaches exist which were discussed in the OECD (OECD, 1992):

Approach I

This method was first proposed by (Kooijman, 1987) using (chronic) LC_{50} laboratory data. A log-logistic species sensitivity distribution was assumed and hazardous concentrations (HC) can be calculated. The method estimates a safety factor in such a way that the most sensitive species is protected from lethal effects. However, the HC depends on the number of species: the higher the number of species the lower the HC since the probability of introducing extremely sensitive (and extremely insensitive) species increases with the increasing number. A prerequisite of the method is the specification of the number of species of the considered community, which introduces an element of arbitrariness. For protecting the most sensitive species an extremely high safety level (low value for HC) is often needed.

Modifications to the approach of Kooijman were made by several authors (Van Straalen & Denneman, 1989; Wagner & Løkke, 1991; Aldenberg & Slob, 1993), using NOEC data instead of chronic LC_{50}-values and refining the statistics (Approach II-IV).

Approach II

The approach suggested by van *Straalen & Denneman (1989)* is also based on a log-logistic sensitivity distribution but uses NOEC-values and is independent of the number of species introduced into the system. Species should be selected according to their representativeness for the terrestrial ecosystem, i.e. the ecological function (primary producers, consumers and saprotrophs), the exposure routes (via pore water, particles or air) and the anatomical design of the test organisms should also be considered.

Approach III

The *Aldenberg & Slob (1993)* approach further refines the statistics by accounting for the uncertainty in the estimates depending on the number of data. The one-sided 95%

confidence limit and the one-sided 50% confidence limit of the Hazardous Concentration form the mean and standard deviation of a sample of toxicity data.

This approach is also applied in the derivation of environmental quality standards in the Netherlands and is advised in the TGD for New and Existing chemicals (EC, 1996) as an additional method to support the FAME method derived information.

Approach IV

Wagner & Løkke (1991) derived a method assuming a log-normal species sensitivity distribution and also considering NOEC data for representative species (DIBAEX method, distribution based extrapolation method).

Evaluation of the approaches:

Advantages of the distribution-based methods are multiple: they are more "scientific" than the use of arbitrary assessment factors. The distribution methods make use of different data and not just the lowest NOEC-value. Major differences in the sensitivity of the tested species give rise to a lower, precautionary, PNEC. The use of statistics makes calculations of confidence intervals around the PNEC possible.

On the other hand, disadvantages cannot be neglected: the form of distribution (log-logistic or log-normal) is rather arbitrary. This will especially affect the estimates in the (extreme) tails of the distributions. Generally, there is insufficient data available to estimate a reliable distribution. The NOEC-values form a poor basis for the distribution because of methodological problems of their derivation (see Section 5.2), but also because laboratory species are not a random sample from the ecosystem to be protected. The ecological impact of a certain percentile remains unknown. The use of eloquent statistics may suggest a larger degree of accuracy than warranted.

In order to overcome typical disadvantages of the species sensitivity distribution approaches such as assuming an – to some extent – arbitrary distribution, a possible development may be the application of a bootstrap method to estimate the hazardous concentration (for x% of the species) without assuming a specific sensitivity distribution (Newman et al., 2000)

Probably most serious is the lack of (quality) data for terrestrial risk assessment. The main problem with this approach is the discussion how much data points are needed to have a representative sample and at which taxonomic level. Clearly, a distribution made up of 1 earthworm and 10 plant species would usually not be satisfactory. In principle, two data points suffice for a sensitivity distribution. When few data points are used, the confidence interval around the PNEC will be large, thus reflecting a high uncertainty. We therefore recommend to report confidence intervals with the calculated PNEC as shown by Aldenberg & Jaworska (2000) and adapted by Verbruggen et al. (2000, in prep) for the median sensitivity (HC_{50}) of species.

The data should preferably cover several phyla or, when many data are available, different (super) classes. Including more than one value from a phylum/class may lead to severe bias when sensitivity differs between taxonomic groups (e.g. 10 NOECs for plants may yield a sensitivity distribution which is not representative for the entire terrestrial system). For chemicals with a mode of action targeted at a specific taxonomic group (e.g., herbicides, insecticides), inclusion of all phyla in the distribution is less obvious. In that case, it is advisable to construct a distribution for species of this specific group separately and focus on their protection.

Options which may be considered to resolve a lack of data are:

1. Use of equilibrium partitioning to include aquatic toxicity data in the distribution for terrestrial species.
2. Extrapolate LC_{50}s to NOECs using information, extracted from databases

Recommendations for improvement:

More work and discussion is needed in this area before it can be applied in routine risk assessment. Nevertheless, these approaches are promising in the sense that they attempt to implement scientific knowledge in the effects assessment of chemicals.

Another topic to pinpoint is the meaning of representativeness of species and their sensitivity as well as taxonomical biases by the selection of test species. Forbes & Forbes (1993) summarized: “In practice, 'representatives' are selected from different taxa and trophic levels. This may result in the estimation of a toxicity distribution that is biased and thus one in which the variance is either under- or overestimated. For example, selecting a very sensitive species, a moderately sensitive species and a tolerant species, as is often suggested, will overestimate the true community variance because the extreme values are sampled in greater proportion than they actually exist. Conversely, selecting a sensitive species from each of the several taxa will underestimate the community variance. Ideally, the selection should be random. However, it may be difficult to design sampling schemes in which random rather than representative samples are selected unless the species composition of the community, ecosystem or statistical population of interest has been determined”.

At this moment, we cannot make strong recommendations about which approach to follow or which taxonomic grouping to make as experience is currently insufficient. However, we advise to use assessment factors and sensitivity distributions side-by-side to gain experience. With the sensitivity distributions we advise to calculate confidence intervals.

In case mammals and birds are to be included in the same distribution as soil-dwelling organisms, representative test-endpoints and exposure scenarios to assess the organism´s daily uptake have to be selected carefully and agreed upon. Furthermore, it has to be stated on what basis the organisms´s exposure has been determined: total concentration in soil, concentration in pore water, and internal concentration, respectively. As long as the bases are different, effect data are not comparable. This holds true for all types of biological soil testing.

5.4 Validation of mathematically derived quality data using multispecies and field data

It is uncertain whether the extrapolated values in fact represent concentrations of no concern for the terrestrial environment and thus protect the fraction given by the mathematical equations. In order to calibrate or even validate the extrapolation methods used, Emans et al. (1993) compared NOEC-values derived from multispecies field or semi-field experiments and extrapolated values. The extrapolation methods according to the Aldenberg & Slob, Wagner & Løkke and the modified U.S. EPA method were also tested. It was concluded – with some reservations – that data from single species tests can be used to obtain „safe“ extrapolated values. This was tested for the aquatic compartment only. Best correlations were observed for the comparison of experimental data with the Aldenberg & Slob and Wagner & Løkke methods with a protection level of 95% and a confidence level of 50%. FAME is a more conservative method but usually also safe enough.

Heimbach (1998) showed for pesticides that the laboratory reproduction test with the earthworm *Eisenia fetida* was at least 5 to 10 fold more sensitive than the field test. A comparison is also possible for the results of the publication of Römbke et al. (1994): when applying the FAME-method to laboratory test results the extrapolated concentrations are in a range where no effects can be observed in terrestrial microcosm or field studies. However, in this case laboratory and field studies were performed independently from each other and not with the objective to calibrate extrapolation methods – and thus with completely different application rates. In a recently-summarised validation study, toxic effects of zinc on community endpoints were determined at an experimental field plot and a contaminated field site using soil-dwelling organisms (Posthuma et al., 1998). For zinc, no or weak responses were observed at HC5 level, while measurable effects were present at HC50. This was confirmed by data from the literature.

In a recent comparison for 11 chemicals, Versteeg et al. (1999) concluded that the geometric mean NOECs from model ecosystems corresponded to affected fractions of 10-52% (percentage of species exposed above their NOEC). The 95% protection level (or HC5) was a fairly good predictor of the lower 95% confidence limit of the model system NOEC. These findings suggest that despite the conceptual uncertainties the HC5 is sufficiently protective for ecosystems (at least model ones). It should be noted that the authors had at least 6 chronic NOECs available for each chemical.

Outlook / recommendations

For the terrestrial ecosystem a final and valid comparison of extrapolated laboratory data and NOEC values derived from field or semi-field experiments still has to be performed systematically. Up to now correlations between extrapolated laboratory data and field data are mainly performed for aquatic test results. For the terrestrial environment only limited data exist. Mainly literature data are available where terrestrial laboratory and field tests are performed under different aspects but not under the common objective to calibrate extrapolation methods. Thus, more test series should be designed comprising laboratory, microcosm and field studies and also considering the same – or at least comparable – test organisms, endpoints and application rates.

5.5 Quality objectives for mixtures

Theoretical bases for the assessment of toxicological response to chemicals in combination: concentration addition and independent action.

In order to describe the toxicological behaviour of a mixture of chemical substances, two different approaches can be used: the Concentration Addition (CA) and the Independent Action models (Greco *et al*, 1992). The reference equation for the independence model, for a binary mixture, is the following:

$$f_{ab} = f_a + f_b - f_a f_b \qquad [1]$$

where f_a, f_b and f_{ab} are the fractions of total possible effect produced by the individual toxicants "a", "b" and their combination respectively. For a multiple mixture, the model can be written as follows:

$$f_{(1,2,...n)} = 1 - [(1 - f_1) * (1 - f_2) * .... (1 - f_n)] \qquad [2]$$

Equations 1 and 2 describe the combination of probabilities for independent events. Thus, the model represents the combined action of toxicants acting independently, with different modes of action.
The additivity model is described, for a binary mixture, by the following equation:

$$\frac{C_a}{EC_{x,a}} + \frac{C_b}{EC_{x,b}} = 1 \qquad [3]$$

where C_a and C_b are the actual concentrations of two toxicants in a mixture producing X% effect, $EC_{x,a}$ and $EC_{x,b}$ are the concentrations of each toxicant alone which would produce X% effect (for example EC_{50}). For a multiple mixture the equation can be written as follows:

$$\sum_{i=1}^{n} \frac{C_i}{EC_{x,i}} = 1 \qquad [4]$$

This model applies to substances with the same mode of action. In this case, however low the concentration of a toxicant is (even a small fraction of the individual quality objective), it could theoretically contribute to the total effect.

The combined assessment of additive and independent toxic chemicals. Need for regulation.

Quality objectives or criteria for the protection of the environment, as well as PNEC are usually developed for individual chemical substances. As early as the late seventies the problem of mixtures was recognised by the EIFAC/FAO (EIFAC, 1987). The need for quality objectives for mixtures of chemicals was stressed by research demonstrating that very low levels of chemicals may still be active in a mixture, as they are additive at concentrations as low as 0.02 of the LC_{50} (Könemann, 1981).
For substances which behave in agreement to equation 4, a quality objective for the mixture (QOm), derived from individual quality objectives (QOi), was proposed by Calamari and Vighi (1992) according to the following algorithm:

$$QO_m = \sum_{i=1}^{n} \frac{C_i}{QO_i} < 1 \qquad [5]$$

where Ci is the actual concentration of each individual substance and n is the number of substances in the mixture.

In order to apply a common quality objective, chemicals should be grouped on the basis of a known similar mode of action. Unfortunately, for a large numbers of chemicals, knowledge on the toxic mode of action is not available or is inadequate.
In these cases, it was suggested that QSAR studies could be applied to gain indicators of a similar mode of action. The general hypothesis is that chemicals with similar structure patterns and responding to the same QSAR model would have a similar mode of action. Moreover the molecular structure of chemicals may be studied by means of chemometric approaches in order to evaluate structural similarity and to assess links between structural and toxicological similarities.

How to select additive chemicals.

A procedure for the study of structural and toxicological similarities among chemicals is schematically described in Fig. 1.

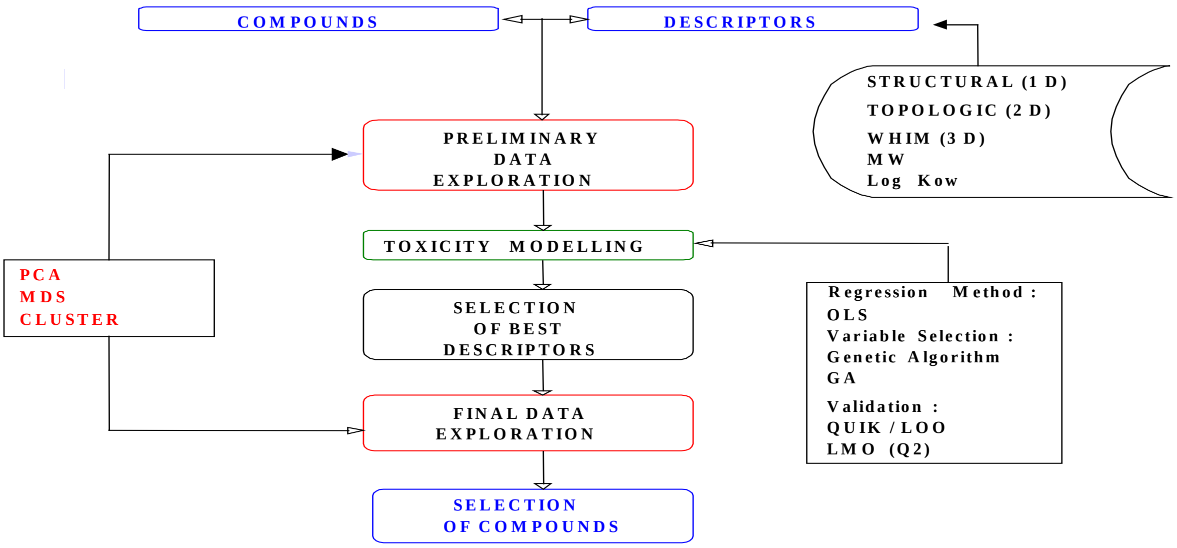

Figure 1. Scheme of the procedure for studying structural and toxicological similarities among chemicals (modified from Gramatica et al. 2000)

As a first step, chemical structures are described by a large set of more than 170 molecular descriptors. This allows a preliminary exploration of structural similarities, using all descriptors, based on Principal Components Analysis, MultiDimensional Scaling and Hierarchical Cluster.
The next step is the development of QSAR models on a set of toxicity data for individual chemicals. The selection of most suitable independent variables among the huge numbers of molecular descriptors, for developing good predictive toxicity models, was made using the Genetic Algorithm procedure (Leardi et al., 1992).
Finally, the conclusive data exploration for the selection of structurally and toxicologically similar compounds is based on the best-selected descriptors. This last exploration allows evaluating molecular similarities as a function of a selected set of descriptors relevant for the toxic effect. Therefore, similarity is evaluated by structural patterns related to the biological activity.

A complete description of the procedure and of the results of the chemometric approach is reported by Gramatica et al (2000).
The combined use of the chemometric approach and QSAR models has proven a powerful tool for the study of molecular similarities in relation to the biological effect of chemicals.

How to evaluate the risk for mixtures of chemicals.

It can be assumed that mixtures actually occurring in the environment are seldom composed of similarly and/or dissimilarly acting chemicals. Although it has been suggested, to group the constituents of such a mixture into subgroups of similar mechanism of action and to assess their contribution to the overall toxicity independently (Ankley et al, 1996), concrete approaches as well as experimental evidences are lacking so far.
From theoretical calculations and experimental findings, it may be concluded that with respect to the precautionary principle, CA could be a valuable tool for the hazard assessment of multiple mixtures. The results demonstrate that this model may indeed provide a reasonable worst-case estimation of mixture toxicity for regulatory purposes, as already put forward by Boedeker et al. (1993).

Even if we can assume a good general predictability of the toxicity of chemical mixtures, one major question within the context of quality objectives remains: What happens if the components are present in concentrations below their individual quality objectives? Is there still a combined effect expectable and detectable?
A quality objective, conceptually comparable with a Predicted No Effect Concentration (PNEC), is a theoretical “no effect” figure, which cannot be determined experimentally. The current procedures to estimate QOs or PNECs are based on the use of application factors (more or less stringent depending on data availability, uncertainty evaluation, etc.) to experimental NOECs (CSTE/EEC, 1994; EC, 1996). NOECs are defined as the highest concentration actually tested within a specific test procedure, where the response of the exposed organisms cannot be significantly distinguished from the response of untreated control organisms. As the prediction of mixture toxicity is either based on effect concentrations of the individual components in the case of CA or on their individual effects if IA is used (see eqs. 1 to 4), it is evident that NOECs are not directly usable for the analysis of the predictability of combined effects at low concentrations of toxicants.
Furthermore, the NOEC approach has been heavily criticised, mainly for the following reasons:

(1) The NOEC is based on the statistical failure to detect an effect. This does not prove that there is no effect in reality. Therefore, NOECs do not describe “safe” concentrations.
(2) The actual value of the NOEC of a given chemical is heavily dependent on the actual design of the biotest.
(3) As the NOEC is based on a case-by-case comparison with untreated controls, it is impossible to calculate confidence limits or any other statistical measure to give an indication of the quality and precision of the determined NOEC value.

As an alternative to the NOEC approach, so-called point estimates are discussed. Within the context of the analysis of the biological potency of a chemical or a chemical mixture, this means the estimation of a concentration, provoking a small effect (ECx-estimates). The weaknesses of the NOEC-approach can be overcome by this approach. Nevertheless, the determination of ECx values is experimentally and statistically more demanding. Furthermore, most of data already obtained for environmental pollutants are based on the NOEC approach. Advantages and disadvantages of NOECs and ECx estimates are discussed in detail within workshop reports from the SETAC (SETAC, 1996) and the OECD (OECD, 1998) and the references cited therein.

Both theoretical predictions and experimental results indicate the need for QOs for mixtures for CA chemicals (defined on the basis of either a known mode of action or a chemometric predictive analysis). On a theoretical basis, however low the concentration of a chemical is, even lower than an experimental NOEC, it must be added, in terms of Toxic Units (TU, i.e. concentration normalised by toxicity), to other similarly acting chemicals and will contribute to the total TUs of the mixture.
The problem is more complex for IA chemicals. In this case, the response of a mixture is calculated in terms of “combination of effects” and not of “addition of concentrations”. Conceptually, the combination of “not significantly detectable” effects is a delicate problem. The assumption of the EC_{01} as a suitable figure is a possible, even if arbitrary, solution, giving the possibility of combining a quantitative effect, although theoretically estimated (Vighi et al., 2000).

Nevertheless, the PNECs of the individual pollutants may be orders of magnitude below their NOECs. A QO or a PNEC for individual chemicals, determined by the application of arbitrary safety factors, cannot be assumed as scientifically sound “zero effect level”, even if, for regulatory purposes, they could be assumed as safe enough in order to protect the environment. Are they also safe if the chemical is part of a mixture of IA compounds? Can a concentration as low as a PNEC significantly contribute to the toxic response of a mixture of IA chemicals?
These questions have relevant implications from both the scientific and regulatory point of view. On the scientific side, the problem is the evaluation of a “zero effect level” (if any) for xenobiotics.
As for regulatory problems, it would be relevant in evaluating the need for developing mixture quality objectives applicable to non concentration-additive chemicals.
In order to experimentally assess the combined effects with the components being present only at the PNEC concentrations in a standard bioassay, the mixture has to be composed of a rather high number of chemicals. It is therefore difficult, if not impossible, to compose such a mixture entirely of strictly similarly or dissimilarly acting chemicals. To overcome this limitation, the QSAR/chemometric approach described above may be a suitable tool. Nevertheless, the toxicological characterisation of the high number of constituents requires a tremendous experimental effort. Therefore, experimental evidence is lacking at the moment.

Outlook / recommendations

So far, mainly mixtures of specifically acting and toxicologically well-known components have been analysed, using ratios of the components based on their relative toxicity. In contrast, in the aquatic and terrestrial environment, the mixture types and their ratios are mainly dependent on factors such as production volumes, distribution behaviour, persistence etc. Therefore, a future direction could be the analysis of more heterogeneous mixtures, and with mixture ratios reflecting actual exposure situations within the environment.
A possible suggestion for a pragmatic approach for covering this gap could be:
- to evaluate realistic exposure scenarios with respect to mixtures likely to occur:

- to assess how relevant are combination effects (for single species and biological communities) due to mixtures that are typically found in the environment;
- to evaluate if toxicity of mixtures found in the environment is predictable on the basis of known toxicity of individual pollutants;
- to develop schemes for producing QOs for “priority” mixtures recognised as a matter of concern.

Finally, it must be underlined that most information available at present on ecotoxicology of mixtures refers to the aquatic environment. Thus, there is the need for more information specifically oriented toward the terrestrial environment.

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CHAPTER 6
EXPOSURE ASSESSMENT

6.1 EXPOSURE THROUGH SOIL

6.1.1 How chemicals may reach soil.

Potentially dangerous chemicals may be intentionally or unintentionally applied onto soil. The first kind of application refers to pesticides, nutrients and chemicals present in sludge disposed onto soil.
Nutrients usually produce negligible risk for the soil environment. They are contaminants for the aquatic compartment (eutrophication of surface water, nitrate in groundwater).
Pesticides are applied in known amounts according to agricultural practices, thus the load for the soil compartment can be easily estimated. Nevertheless, in relation to application patterns, different situations may occur.

a) Chemicals directly applied on soil. This is the case of most herbicides, mainly applied in pre-emergence, and of insecticides, nematocides or other active ingredients used for disinfecting soil. In these cases, the amount of chemical reaching the soil compartment corresponds exactly to the agronomic application rate:
b) Chemicals applied on the crop. These are mainly insecticides and fungicides. Although losses should be minimised, for obvious economical reasons, significant amounts may reach soil in function of the type of crop, application patterns, etc. A realistic estimate is in the range of 20 to 50% of the total application rate (Ganzelmeier et al., 1995).
c) Chemicals reaching soil by drift. A significant amount of pesticides may reach the areas surrounding directly treated fields by drift. Quantitative attempts to estimate drift losses are present in the literature (Ganzelmeier et al. 1995). An example is shown in table 6.1.

Sludge from treatment plants may be applied on soil as organic fertilisers. Moreover, soil can be used as disposal for waste sludge. In the first case, even if domestic sludge is usually applied, significant amounts of potential contaminants (metals, organics) may be present. In the second case, the presence of contaminants, in quantitative as well as qualitative terms, is extremely high.
To quantify the load of contaminants to soil, detailed knowledge of sludge composition is needed. Moreover, additional information is needed in order to assess the bioavailability of contaminants.
The same applies for animal manure applied as organic fertiliser, often containing relevant amounts of contaminants (metals, therapeutics, etc.).

Unintentional emissions of chemicals may occur as a consequence of accidents in human productive activity (industry, transport of chemicals, mining, etc.). Accidents may produce extremely high concentrations of contaminants, generally on relatively small areas, but the extension of the pollution may also be large, as a function of the gravity of the emission.
Very relevant examples in Europe are dioxin pollution after the Seveso accident in Italy (Pocchiari et al., 1983) and metal pollution in the Doñana Park in Spain.

In intensive industrial areas, chemical spilling on soil may occur as a more or less regular consequence of the productive activity.
Soil pollution may also occur as a consequence of eluate spilling from inadequately protected solid waste disposal.
An additional source of soil pollution may be the use of contaminated water (e.g. domestic sewage) for agricultural soil irrigation.

Table 6.1. Losses of pesticides by drift as a function of the distance from the treated crop. Data, for the main types of crops, represent the amount of deposition as percentage of the agronomic application rate (modified from Ganzelmeier et al., 1995)

Distance from the crop (m)	**Field crops (corn, wheat, etc.) f+s**	**Tall growing crops: Grapevine**		**Tall growing crops: Fruit crops**		**Hops**	**Vegetables Ornamentals Small fruit (pedestrian sprayer)**	
		f	**s**	**f**	**S**	**f+s**	**h<50cm**	**h > 50 cm**
5	0.6	1.6	5.0	20	10	12.5	0.6	5
10	0.4	0.4	1.5	11	4.5	9.0	0.4	1.5
15	0.2	0.2	0.8	6	6	5.0	0.2	0.8
20	0.1	0.1	0.4	4	1.5	4.0	0.1	0.4
30	0.1	0.1	0.4	2	0.6	2	0.1	0.2
40	-	0.1	0.2	0.4	0.4	-	-	0.2
50	-	0.1	0.2	0.2	0.2	0.3	-	0.2

Remarks:
Basic drift values in % relative to the application rate in l/ha or Kg/ha
f = early growth stages
s =late growth stages
- = values not defined

Finally, chemical input to soil may derive from atmospheric (wet and dry) deposition. In this case, the amount per unit of surface is usually low, at least in comparison with other sources, but the areas involved may be extremely wide. Atmospheric transport and deposition are the major causes of global and remote area contamination by Persistent Organic Pollutants (POPs) (Wania and Mackay, 1996).

6.1.2.Fate of chemicals in soil.

The fate of a chemical applied on soil depends on its physico-chemical properties, accounting for the partitioning among soil phases (air, water, organic and inorganic solid matrices), and on degradation patterns.

Chemicals may be lost through water transport by leaching or runoff. Leaching and runoff potential may be estimated as a function of the water solubility and, for non-polar chemicals, of the organic carbon sorption coefficient (Koc). For polar compounds (anionic or cationic), the affinity for the solid matrix of soil must be estimated through the soil sorption coefficient (Kp) including also the interactions with the inorganic soil component.

Several simple indexes have been developed in order to qualitatively estimate the leaching potential of a chemical, such as, for example, the GUS (Groundwater Ubiquity Score) index based on Koc and degradation half-life (Gustafson, 1989). Vighi and Di Guardo (1995) give a review of these indexes.

A quantitative assessment of the distribution of a chemical among soil phases and a prediction of losses through leaching and runoff can be made through suitable multimedia models (Cowan et al, 1995).

Specific models, mainly based on the fugacity approach (Mackay, 1991), have been developed to describe partitioning into the soil system. Because soil is not a well-mixed compartment, soil modelling needs approaches quite different in comparison with models developed for describing distribution in the aquatic environment. In some models (ChemCAN: Mackay et al., 1996; HAZCHEM: ECETOC, 1994) the approach is based on the use of a fixed diffusion-path length (typically 0.05 m) independent of the chemical being evaluated. These models are suitable for studying distribution in a relatively thin soil layer (typically 0.1 m). In other cases (CalTOX: McKone, 1993) soil layers are designated to represent the zone between the soil surface and the top of the saturated zone. As an alternative, a chemical-specific soil depth has been proposed for use in multi-media models. This approach is applied in SimpleBox 2.0 (Brandes et al., 1996) and is described in the SETAC multimedia book (Cowan et al., 1995). The effective soil depth is defined by the actual penetration depth of the chemical due to leaching and diffusion (the depth is taken at which degradation equals the vertical movement in soil).

Several models have been developed for calculating losses from soil trough leaching. A selection of leaching models suitable for application at European level has been made by the FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) Working Group (FOCUS, 1995). In a second step, environmental scenarios, representative of different environmental conditions from South to North Europe, have been developed for the application of leaching models (FOCUS, 2000).

Different approaches have also been developed for calculating runoff losses. Some methods are not real models, but algorithms proposed to calculate the percentage of chemical lost through runoff (OECD, 1998). Others are fugacity-based models developed for predicting concentrations in the soil and surface water compartments (SoilFug: Di Guardo et al., 1994).

The most commonly used models have been experimentally calibrated and validated and the reliability is, generally, good (FOCUS, 2000; Barra et al., 2000). Nevertheless, some of them require a large amount of input data for the description of the environmental scenarios, thus the practical applicability in real, site specific, conditions may be sometimes difficult.

A further route of chemical transport from soil is erosion. In this case, chemicals strongly bound on soil, are mechanically taken away by surface water flux. Erosion depends on soil characteristics (geo-pedology, slope, plant cover, etc.) and on water fluxes. It may be predicted by means of physical and hydrological transport models.

Losses by volatilisation may be extremely variable and are often underestimated by multimedia models. According to many authors, volatilisation can be considered one of the most important ways by which chemicals are lost from soil. It has been proved that, for non soil-incorporated pesticides, up to 90% loss may occur in a few days, even for relatively non-volatile chemicals (Glotfelty et al., 1984).

It has been recently demonstrated (Otto et al., 1998, 1999) that volatilisation losses of pesticides applied on dry soil follow two separate patterns:

- in a first phase, if soil is kept dry, partitioning among phases does not occur and soil behaves as an “inert” matrix; in this phase, pesticide flux is only a function of the vapour pressure and of some properties of the substrate, according to Hartley’s law (Hartley and Graham Brice, 1980); losses as high as 60-70% of the application rate were measured after a few days for low volatile chemicals (alachlor, terbuthylazine);
- after the first rain partitioning among phases occurs, losses are strongly reduced and can be described by multimedia partitioning models.

Besides this particular behaviour, an additional problem in the prediction of volatilisation from soil is the difficulty to define a precise quantification of the air balance above soil. As a consequence, modelling air losses, at present, is less accurate in comparison with water transport.

Persistence and degradation patterns

Another significant loss mechanism of an organic chemical in the soil system is represented by abiotic (hydrolysis, photolysis) and biotic (microbial) degradation processes. Higher organisms also may metabolise compounds, but they play a minor role in the biodegradation of organic chemicals. It can be assumed that, quantitatively, microbial degradation is the most important environmental process that can cause the breakdown of an organic compound in soil (Alexander, 1978).
Microbial degradation can take place both in aerobic or anaerobic conditions. In anaerobic conditions (i.e. paddy field) the ultimate degradation leads to CH_4.
Most organic pollutants are biodegraded by means of cometabolic processes (Alexander, 1979). Cometabolism is defined as the degradation of a compound that does not provide a nutrient or energy source for degrading organisms but is broken down during the degradation of other substances.
The persistence in soil is in general expressed as a half-life (DT50) which is the time needed for degrading 50% of an organic compounds.
The characterisation of the rate of degradation for each process involved (photolysis, hydrolysis biodegradation) and consequently the half-life of chemicals, depends not only on the intrinsic properties of the chemical, but also on the nature of the surrounding soil environment. Factors such as soil pH, temperature and sunlight incidence (which varies diurnally and seasonally), humidity, redox potential, nature of the microbial community and bioavailability can influence the lifetime of any organic compound. For instance sulphonylurea herbicides (weak acids) are degraded mainly by means of hydrolysis, which is particularly active when these herbicides are in neutral form. It derives that, in acidic soils the half-life of bensulfuron-CH_3 (pK = 5.2) is 11 days, whereas in neutral soil it is higher than 150 days (Roberts et al., 1988).

On this basis, it is difficult to estimate the environmental persistence of a chemical and the characterisation of the reactivity is an important problem in environmental modelling and for mass balancing of a compound. So it is impossible to obtain a single and reliable half-life; with this perspective Mackay and co-workers (1997) proposed a semi-quantitative classification scheme for organic chemicals in terms of their persistence (Table 6.2). According to the table, for example, a chemical with a half-life within the range 12 to 40 days, is included in class 5, with a half-life of 550 hours. This classification offers the advantage to provide a single figure, even if relatively arbitrary and approximate, useful as input information for modelling.

In risk assessment procedures, the persistence of substances is principally considered in the evaluation of the exposure. A first problem comes from the lack of knowledge about how to extrapolate available information from biodegradation laboratory tests to natural conditions. Another problem derives from the transformation products that may be very persistent under natural conditions and in some case more toxic than the starting compound. This problem is mentioned in the EU Directive 91/414/EEC concerning the placing of plant protection products on the market in relation to the data requirements and evaluation of degradation studies submitted by applicants. In the accompanying Annex of the Directive the necessity of performing a further evaluation of any relevant metabolites deriving from the degradation of the active ingredient considered is indicated.

Table 6.2 Classification of organic chemicals persistence (Mackay et al., 1997)

Class	Mean half-life (hours)	Range (hours)
1	5	<10
2	17 (~ 1 day)	10-30
3	55 (~ 2 days)	30-100
4	170 (~ 1 week)	100-300
5	550 (~ 3 weeks)	300-1,000
6	1,700 (~ 2 months)	1,000-3,000
7	5,500 (months)	3,000-10,000
8	17,000 (~ 2 years)	10,000-30,000
9	55,000 (~ 6 years)	>30,000

The question now is whether a metabolite is relevant. A possible answer could be given by considering, among all the major metabolites (reaction products that are formed in amounts of ≥10% of the applied amount of a.i.), those that are effectively relevant for the compartment considered. On this basis a major metabolite occurring in a soil degradation study will require further assessment for risk (relevant metabolite) unless one of the following conditions apply:

a) it is CO_2 or an inorganic compound, not being a heavy metal;
b) it is an organic compound, which consists of an aliphatic structure, with a chain length of less than 4, and of which the atoms are only C, H, N or O, not being an aldehyde, an epoxide, a nitrosamine or a nitrile.

An opinion on this issue was adopted by the Scientific Committee on Plants (SCP) after discussion at a joint SCP-CSTEE working group.

6.1.3 Input to biota from soil. Bioavailability of chemicals.

It is generally accepted that the total amount of a chemical in soil is a rather inappropriate measure for hazard and risk assessment. It is the “bioavailable fraction” that reflects the effective portion, which is taken up by organisms and which causes adverse effects. Although the importance of bioavailability is clearly accepted, implementation into risk evaluation is less straightforward, also due to the numerous definitions. In this report, the bioavailable fraction will be seen as:

“The amount/percentage of a compound that is actually taken up by an organism as the outcome of a dynamic equilibration of organism-bound uptake processes, and soil particle related exchange processes, all in relation to a dynamic set of environmental conditions.” (Eijsackers et al., 1997)

Or shorter: “*That fraction of the total amount of a chemical that can be taken up by a (specific) organism in a (specified) time period.*”

This definition implies that the total amount of a compound in a soil compartment can be subdivided into an unavailable and an available portion. The magnitude of these fractions depends on such environmental parameters as organic carbon content, organic chelating agents (e.g. humic substances, organic carbon), inorganic ligands, cation exchange capacity, pH and redox potential. Furthermore, bioavailability differs between species as a result of different lifestyles, physiology and/or morphology. Bioavailability is governed by the dominant uptake routes. Several uptake routes are possible for organisms: over the skin through contact with pore water, after ingestion of food or via specific uptake of drinking water (e.g. via the ventral tube of springtails). In all cases, it appears that chemicals are not taken up directly from the solid phase, but that a water phase plays a role, at least as an intermediate phase. Regarding morphology of organisms, a crude distinction can be made between "soft-bodied" (nematodes, earthworms, bacteria, plants) and "hard bodied" (insects, isopods, spiders) organisms. In the first group, uptake of chemicals from pore water over the outer skin seems to dominate whereas this is less obvious for the second group. The hard bodied species are in less direct contact with the soil and exposure via ingestion or water uptake (via specialized organs) will become more important. The quantitative consequences of these considerations are however unclear as little experimental evidence is currently available.

<u>Organic chemicals</u>

The available evidence for soft bodied organisms supports the idea that uptake is governed by partitioning of the chemical between pore water and the phases inside the organism (mainly lipids) (equilibrium partitioning -EP-). Organic matter is the main abiotic property of soil influencing uptake as hydrophobic interactions with organic carbon tend to dominate sorption. For neutral organic chemicals, normalisation to organic matter will suffice in most cases as the hydrophobic interaction with the organic carbon in the soil will explain most of the sorption. This sorption can be predicted from Kow (Sabljic *et al.*, 1995) with reasonable accuracy although the error can be an order of magnitude for high Kow values. This variation will be partly caused by differences between chemicals but also reflects differences between soils (e.g. in quality of the organic matter). Furthermore, Koc-Kow relations may fail to predict bioavailability in field situations as a result of sequestration (“ageing”) (e.g. (White *et al.*, 1997).

Especially for earthworms, a lot of experimental work on accumulation is available. Even though earthworms are able to take up chemicals from food (Belfroid *et al.*, 1994), this does not seem to lead to higher body residues than expected on the basis of EP (Jager, 1998). Dietary uptake of organic chemicals seems to be a passive diffusion process from the water in the gut contents to the internal tissues of the organism (Gobas *et al.*, 1993). Uptake from the gut contents can only lead to higher body residues when:
1. the fugacity of the ingested material is higher than in the external medium (either by selective uptake, through digestion of organic material, or through compaction of gut contents),
2. and, the direct exchange with outside porewater is relatively slow.

When these conditions are met, the organism can reach higher body residues than expected on a basis of EP. For earthworms, it is unlikely that these conditions are met (as reflected in the BCF data set collected by Jager, 1998). However, care must be taken in case the food sources are specifically contaminated (e.g. in case of pesticide spraying in orchards where leaf litter may contain high residues). The models needed to assess this exposure route are, however, lacking. Uptake and translocation by plants is also clearly porewater-driven (see e.g. Trapp & McFarlane, 1995).
For hard bodied organisms, the second condition is probably easily met as the opportunity for diffusive exchange with pore water is limited. However, comparative studies suggest that earthworms usually accumulate chemicals to a larger extent than do arthropods, on a lipid basis (Van Brummelen et al., 1996; Pathirana et al., 1994). Although further experimental work on the different uptake routes and their quantitative consequences is needed, for now, earthworms may be taken as a worst-case for bioavailability of organic chemicals. These organisms are likely to encounter the highest body residues although this does not necessarily imply the highest toxic effects as sensitivity will also differ between taxonomic groups.

Metals
For metals, the situation with regard to bioavailability is more complex. On the abiotic side, sorption and speciation are more complex and difficult to predict for field situations. On the abiotic side, several metals are essential for the functioning of organisms and mechanisms exist to regulate the internal levels of these chemicals; for other metals, detoxification mechanisms are developed in some species.

Taking all processes and factors into account, it becomes obvious that the bioavailable fraction of a metal compound is not a constant but multifactorially influenced (Hammel, 1998):

Total metal amount in soil

Mobilization,
influenced by soil substrate and substance

Environmentally available portion

Species specific,
influenced by morphology and behaviour

Bioavailable portion

Species specific,
influenced by physiology

Effective portion

It is often assumed that the free metal ion is available for uptake. Although uptake may be in the form of free metal ions, this does not necessarily mean that measuring or calculating the free ion concentrations solves the issue of bioavailability. It may be the case that accumulation is governed by labile bound metal. For example in the case of plants, accumulation is often best predicted by the 0.01 CaCl2-extractable fraction. Uptake is probably in the form of free ions but the subsequent supply from weakly bound fractions limits the uptake rate. In many studies, differences between accumulation or toxicity decrease when exposure is expressed as porewater or CaCl2-exchangeable concentrations (see e.g. Posthuma et al., 1998, Peijnenburg et al., 1999). There are however notable exceptions. Vijver et al. (subm.) exposed the springtail Folsomia candida to field soils and observed a better correlation of accumulation with the total metal concentrations than soluble concentrations.

Consequences for risk assessment and quality criteria

Bioavailability needs to be dealt with in the risk assessment and the derivation of soil quality standards. Several approaches to do so are currently in use. For (neutral) organic compounds, normalisation to a standard soil based on organic matter will usually suffice (see e.g. in the Netherlands: Crommentuijn et al., 1994).

For metals, the situation is more complex. Currently-used test methods have been optimized with regard to the substrate for culturing test species. These methods have been developed for a comparative assessment of the toxic potential of a chemical but not for a site specific risk assessment. Although it is known that the characteristics of a soil alter the bioavailable fraction of a substance and therefore the toxic potential of the compound, the bioavailable – i.e. the effective – fraction in the test substrate or soil is usually unknown. As a result, effect data are referenced to the total content which makes results from different test systems incomparable. However, comparability is needed for PNEC-derivation. A concept has been developed (Hammel, 1998) to make differently generated effect data comparable in order to allow for the derivation of PNEC-derivation. The concept comprises:

- consideration of the chemical´s distribution between the 3 soil phases (particles, pore water, soil air),

- consideration of the fact that only the bioavailable fraction will cause biological effects ,
- consideration of the habitat of test species and thus the main exposure route.

There is, however, currently insufficient evidence to apply such a concept in a quantitative manner for all species in the soil ecosystem. For metals, a standardisation based on organic matter and clay is currently used in the Netherlands for the derivation of quality standards (VROM, 1994) although the inclusion of at least pH seems warranted (Posthuma et al., 1998). However, the recent EU risk assessment report on zinc (EU, draft 1999) concluded that the currently available normalisation procedures lack sufficient scientific validity to be used in risk assessment and decided not to normalise the data.

Consideration of background values of metals and ubiquitous compounds

A recently discussed concept in terrestrial risk assessment of metals and metal compounds tries to account for both the bioavailable portion of the natural background and of the anthropogenically impacted amount (Struijs et al., 1997 and Crommentuijn et al., 1997). The authors try to differentiate between the active or bioavailable and the inactive or unavailable fractions of the background concentration. Allowing a limited addition of effect to the natural background effect is proposed. An “acceptable added risk” is related to a “maximum permissible addition” by combining ecotoxicity data, the effect inducing natural background concentration and the risk limit chosen by environmental policy makers as tolerable if there are no background phenomena. The method requires that in addition to the total background concentration, the fraction of the background that is bioavailable also has to be known. This approach has been applied in the EU risk assessment report for zinc (EU, draft 1999). The final realization of the concept even foresees regional differences in the metal speciation and thus in the bioavailable fraction based on spatial geological and hydrological differences.

Although this generally is a central and promising aspect which needs to be considered in the environmental assessment of metals and in the derivation of environmental quality standards, the information required, such as the spatial variability of the bioavailable fraction of the background, is so far not available. Thus, pragmatic assumptions still have to be made. The assumptions lead to some uncertainties and generalizations in currently derived environmental quality standards, by not sufficiently reflecting geological variability and differences depending on habitat characteristics. There is no scientific controversy regarding the variation of bioavailability between sandy and loamy soils, and general dependence on clay minerals and organic matter in soils. It is also well known that metal bioavailability depends on the parent rocks.

Recommendations

For organic chemicals, uptake from pore water is the most likely route of exposure. Although other routes are possible (especially via ingestion), focussing on pore water seems to provide a worst case estimate in most cases. Available concentrations may be predicted on the basis of Kow-Koc relations although measured values are preferable in view of the prediction error (easily a factor of 10) and possibilities for sequestration (“ageing”) in field situations.

As long as approaches for metals are not finalized and generally agreed upon, preliminary concepts such as the added risk approach or the operationally determined bioavailable fractions (extraction techniques that are used to mimic pore water concentrations that are expected to be available for biota) should be used. This information may be combined with information on organisms' exposure routes and habitat.

6.2 EXPOSURE THROUGH AIR

6.2.1 How chemicals may reach the atmosphere.

Air pollution may be a consequence of a direct emission of chemicals in the atmosphere by human activities (industry, combustions, vehicular emissions, etc.).
Moreover, chemicals applied on soil or discharged in water may reach the atmosphere through volatilisation. As previously described, in particular conditions, volatilisation from soil may strongly contribute to air pollution, even for low volatile chemicals.
Assuming a partitioning equilibrium among the various components of soil (solid organic and inorganic matrices, pore water, air), volatilisation depends on the Henry's law constant of a chemical (the ratio between vapour pressure and water solubility) and can be described by multimedia partitioning models.

6.2.2 Fate of chemicals in air

Air is the most mobile among environmental compartments and is the major responsible of long range transport of chemicals.
In particular, the atmosphere is responsible for global pollution by POPs. POPs can be transported to remote areas, fi the Arctic, via several pathways and in different media. However, the atmosphere in particular is considered responsible for long range transport of chemicals and thus for global pollution by POPs. Atmospheric transport pathways for semi-volatile organics are often divided into two types: ”one-hop” and ”multi-hop” pathways, although this is a simplification of events. ”One-hop” compounds would be emitted to the atmosphere, transported, and deposited on the surface never to return to the atmosphere. This type may apply for relatively non-volatile POPs, which tend to be particle-associated at low temperatures. The dispersion of such compounds would simply be defined by its initial source distribution, its lifetime in the atmosphere, and atmospheric circulation. The pathways of these constituants follow the arctic haze from mid-latitudinal sources into the Arctic (Barrie 1986, 1994, 1996). A compound that has a tendency to re-enter the atmosphere after initial deposition on the earth`s surface can move through the environment in a series of ”hops” due to repetitive revolatilization. Most organochlorines fall into this group. The polar regions are potentially cold traps for these compounds. Wania and Mackay (1993, 1995) and Strand and Hov (1996) have developed models to simulate this process and have been able to to quantitatively reproduce observed patterns and concentrations of contaminants in the different compartments of the Arctic (Wania and Mackay 1996).

Degradation patterns in air depend on several mechanisms such as photodegradation and different processes of chemical reaction. Photochemical degradation is an abiotic degradation process occurring on organic compounds based on the absorption of electromagnetic radiation coming from sunlight. Several transformations of organic chemicals such as dechlorination, ring cleavage or oxidation are photochemically mediated by the action of the sunlight. This process plays a prominent role in the atmospheric compartment, nevertheless it is also important in the first layers of surface water and on top of vegetation and soil. The rate of photodegradation in the atmosphere is seasonally limited by the sunlight incidence (Neilson et al., 1991), whereas in aqueous environments, the process is governed by the degree of eutrophication that could prevent the penetration of the light. Photolysis takes place by means of two different mechanisms:

1. Direct photolysis when the reaction proceeds after the absorption of light and the compounds and is directly excited by this absorption process.
2. Sensitised photolysis when the light is absorbed by a compound that can react with a second one leading to degradation of the latter.

Literature data on chemical persistence in the atmosphere must be evaluated very carefully in order to avoid major misinterpretations. Well-known persistent chemicals are often reported to have an astonishingly short half-life in air, even when highly reliable literature sources are consulted. For example, DDT half-life figures in air as low as a few hours are reported (Howard et al., 1991), and it is demonstrated that atmospheric transport is the pathway responsible for DDT pollution in the Arctic and in Antarctica (Wania and Mackay, 1996).

The main reason for this apparent discrepancy is the fact that air half-life data generally refer to experimental conditions, where chemicals are maintained in the gas phase. In the real environment, chemicals are mainly associated with solid particles (hydrophobic POPs) or to aerosol (more water-soluble chemicals) and this strongly reduces reactivity and degradation velocity.

6.2.3 Exposure of living organisms.

The uptake of chemicals from air to living organisms occurs through gas exchange surfaces: leaves in plants, respiratory systems and skin in animals.

For terrestrial plants, uptake of foliage from the atmosphere (bioconcentration) is the major uptake pattern. The bioconcentration factor (BCF) can be estimated from the n-octanol /air partition coefficient (Koa) (Bacci et al, 1990; Paterson et al., 1991). Nevertheless, there are many controversial aspects in the relationship between BCF and Koa. A very few experimental Koa data are available. Usually Koa is calculated as a ratio between Kow and Kaw :

Kow/Kaw = (Co/Cw)/(Ca/Cw) = Co/Ca = Koa

The calculation is mathematically correct, but has been verified experimentally on a few chemicals and should be confirmed on a wider range of molecular properties. As an example, for very soluble chemicals, high Koa values can be calculated even with low Kow and medium vapour pressure.

The relationship between Koa and BCF in plants has been verified, with sound experimental bases, on a relatively few chemicals and on experimental Koa (Paterson et al., 1991).

In general, there is a good agreement between Koa and BCF for values of log Koa<9 although the slope of this relation seems to vary between different plant species (Bohme et al., 1999). Above this log Koa, the BCF reaches an apparent maximum, caused by a

lack of equilibrium (McLachlan, 1999). At very high values of log Koa (above 11), BCF again starts to increase as a result of particle-bound deposition. Despite the apparent validity of this concept, inter-species variation and environmental factors preclude a generic estimation of BCFs from air with any degree of accuracy (Bakker, 2000).
Thus, there is the need for more experimental data on Koa and for a better definition of the relationship with BCF.

For terrestrial animals, bioconcentration from the atmosphere plays a minor role in comparison with bioconcentration from water in the aquatic environment, at least if the respiratory route is considered. Nevertheless, an additional and not negligible exposure route could be by contact with chemicals adsorbed on particulate matter. It must be underlined that highly lipophilic chemicals are mainly present in the atmosphere in the particulate phase.

Finally, a direct deposition of chemicals on living organisms may occur. Direct deposition is limited to the area where a chemical is intentionally applied or unintentionally emitted, and to the surrounding area, where the chemical can be transported by drift immediately after emission. Standard procedures have been developed in order to calculate deposition on plants and animals, considering application or emission rates, emission patterns, etc. (Hoerger and Kenaga, 1972; EPPO, 1994).

6.3 EXPOSURE THROUGH FOOD INCLUDING DRINKING WATER

Transfer of chemicals into the trophic chain may occur through direct chemical deposition (see above), or through uptake by animals and plants by bioaccumulation and biomagnification.

Main conditions for biomagnification are the following:
- the chemical must have a high affinity for a storage system in the living organism; for most organic chemicals the storage system are lipids, but some biomagnifiable substances may be stored in other structures, as, for example, methylmercury in proteins, lead in bones;
- the chemical must be persistent, with a low potential for metabolism and excretion;
- losses of the chemical in the passage through the various levels of the trophic chain must be low (high Trophic Transfer Coefficient: TTC>1).

In general, biomagnification potential is higher if bioaccumulation through food is higher than bioconcentration through respiratory systems. Therefore, the condition for biomagnification would occur more in terrestrial than in aquatic ecosystems.

Nevertheless, it must be taken into account that in terrestrial plants, hydrophobic chemicals are often concentrated in wax or in other structures hardly assimilable by herbivores. On the contrary, chemicals bioconcentrated in algae are easily transferred to aquatic animals. Moreover, aquatic ecosystems are more studied than terrestrial ones. Therefore biomagnification is better known and studied in aquatic ecosystems.
Relevant biomagnification phenomena have been observed in terrestrial (or, more in general, pulmonate) top predators of the aquatic trophic chains, such as birds or marine

mammals (Bacci, 1993). This confirms the hypothesis that gill respiration tends to reduce biomagnification.

A further, not negligible, exposure route for terrestrial organisms is through water.

Plants may bioconcentrate chemicals through root adsorption from soil pore water. Even if, for most chemicals, root adsorption is less relevant in comparison with foliar uptake (Bacci et al.,1990), in some cases, root uptake and translocation is not negligible. Models have been developed in order to calculate bioconcentration in plants from water (Topp et al., 1986, Briggs, et al., 1983).

Soil dwelling animals too, may bioconcentrate chemicals from soil pore-water. Models for calculating the bioconcentration factor for earthworms are proposed by Connell and Markwell (1990) and Jager (1998).

Moreover, terrestrial animals may also bioconcentrate chemicals from drinking water. Usually, the uptake from drinking water is calculated from the concentration in water and the amount of water ingested, by assuming, as a worst case, that chemicals are totally taken by animals (this is, for example, the usual procedure adopted for calculating human exposure from drinking water). More realistic models could be developed based on partitioning of the chemical as a function of its hydrophobicity, nevertheless, at present, such simple models are not available in the literature.

6.4. METHODS FOR ASSESSING PEC IN THE TERRESTRIAL ENVIRONMENT

Several procedures have been developed to calculate PEC in various compartments of the terrestrial environment in order to perform the risk assessment for terrestrial organisms (EPPO, 1993, 1994; SETAC, 1995; EC, 1996). In general, more attention was paid to pesticides, as chemicals intentionally applied on soil, but suitable procedures were also developed for other kinds of emissions.

Earthworms, bees and birds are the most commonly used risk indicators for the terrestrial environment. With regard to relevant exposure routes, it has been assumed that earthworms are primarily exposed via soil (FOCUS, 1996), for bees oral and contact exposure are both considered relevant (EC, 1994), while birds are assumed to be exposed mainly through the intake of residues in their food (for pesticides; treated plants, seeds or insects). ECPA (European crop protection association, EPPO 1993; 1994) has proposed to ignore exposure via water, drifting spray, other prey, inhalation etc. for birds; furthermore, they also propose a method by which one can calculate residues on various food stuffs immediately after spraying of the pesticides.

Exposure via soil is most important for earthworms and other soil-dwelling organisms. Simple methods have been recommended for the calculation of pesticide concentrations in soil immediately after application (FOCUS, 1996; EC, 1996):

$$\text{PEC soil} = A \times (1-f\ int)/100 \times depth \times bd$$

A = application rate (g/ha)
f int = fraction intercepted by crop canopy

depth = mixing depth (cm)
bd = dry soil bulk density (g/cm3)
As a standard scenario one assumes a bulk density of 1.5 g/cm3 and a mixing depth of 5 cm for applications to the soil surface, or 20 cm where incorporation is involved. Unless better information is available, the fraction intercepted is assumed to be 0 for applications to bare soil, or up to 0.5 for applications when crop is present. Using these assumptions, the concentrations in soil immediately after a single application becomes:
PEC soil (mg/kg)- A/750 assuming no incorporation or interception
A/1500 assuming no incorporation but 50% interception
A/3000 assuming incorporation but no interception
For multiple applications a simplifying worst case assumption of additive soil residues could be made.

Concentration trend in function of time can be calculated according to soil half-life (DT50), with the following equation:

$$PECt = PECi \times e^{-kt}$$

Where: PECt = concentration at time t
PECi = initial concentration
k = ln2/DT50

Finally, a Time Weighted Average (TWA), i.e. an average concentration during a given exposure time, can be calculated as follows:

$$TWA = PECi\ (1- e^{-kt})/kt$$

For repeated emissions, TWA can be calculated as follows:

$$TWA = [PECi_1\ (1- e^{-kt}) + PECi_2\ (1- e^{-k(t-t1)})] / kt$$

Were $PECi_1$ and $PECi_2$ are the initial PEC corresponding to the different emissions, and t_1 is the time between the two emissions.

A comparable procedure is proposed by the Technical Guidance Document on risk assessment for new and existing substances (EC, 1996) for calculating PEC on a local basis. Moreover, the TGD suggests procedures for calculating PEC on a regional and continental basis, using multimedia fate models based on the fugacity concept, such as the Generic Model proposed by Mackay et al. (1992) or the SimpleBox (van De Meent, 1993). To apply these models, it is important to define realistic scenarios, either for the environmental characteristics or for emission patterns. There are two different possibilities:

- to define standardised regional environments with agreed input parameters;
- to define more particular scenarios on the basis of country-specific environmental parameters.

The second approach may result in a better estimation of realistic PEC, but, obviously, requires a careful assessment of environmental data.

For birds, exposure is mainly through intake via food. Various models are available for estimation of exposure concentration.

Among these methods the one based on the concept of the *Total Daily Intake* (TDI), that is the total amount of pollutant daily ingested by birds and mammals (mg/kg b.w.), is frequently used in the risk assessment procedures. The assumptions for the evaluation of TDI are representative of a worst-case scenario. Above all it is considered that both birds and mammals ingest exclusively contaminated food. Furthermore, it is assumed that the food consumption of small birds and mammals reaches about 30% of their body weight, whereas decreases up to about 10% for higher animals. It is possible to calculate the TDI considering data proposed by Hoerger and Kenaga (1972) on the pesticide residues concentration (PRC) typically present on a plant after a treatment. For instance for a small birds the TDI is calculated by means of the following procedure:
PRC (mg/Kg) = Rate of application (kg/ha) x 29 (when seed and forage cultivation are considered)

$$\text{TDI (mg/Kg b.w.)} = \text{PRC} \times 0.3$$

Standard procedures to calculate exposure for bees and other beneficial insects are not suggested in official documents. Application rate is usually assumed as a rough exposure indicator for risk assessment on bees. A procedure for estimating exposure through pollen, assumed as the major exposure route for pollinator insects, is proposed by Villa et al. (2000).

All these approaches are simple standard procedures for calculating realistic PEC figures for a preliminary comparative risk assessment for existing and new chemicals.
More precise approaches may be used for a site-specific assessment. Suitable models may be selected on a case-by-case basis depending on the type of chemical, emission patterns, characteristics of the area under study, etc.

These approaches may give better results but they require an appropriate and detailed description of the environmental scenario, and often a complete data set is hardly available.

6.5 COMPARISON/USE OF EXPOSURE PREDICTION AND DATA OBTAINED IN MONITORING PROGRAMMES

It is obvious that exposure assessment can be based on experimental data, produced by means of environmental monitoring, or on theoretical data, predicted by means of suitable models. Both approaches have values and limitations, and comparison between experimental and predicted data is not always possible.

If land use and emission data are known, predictive models are a vital tool for a preliminary assessment of exposure. On the other hand, predicted data have a certain degree of approximation. In general, models accepted by the international scientific community are theoretically sound and reliable. The variability of the results depends mainly on the reliability of the environmental scenario. For site-specific applications, collecting all information required by the models, with the required precision, is sometimes difficult and difficulties increase with the scale of the application and of the unomogeneicity of the territory (Barra et al., 2000).

Experimental data are essential for validating and calibrating predictive models, but experimental monitoring has many disadvantages:

- experimental monitoring is expensive and time consuming;
- selection of chemicals to be monitored is often based on criteria not related to the real probability of environmental occurrence (public concern, availability of analytical methods, etc.); the lack of positive findings in monitoring data, does not necessarily mean that the chemical is not present in the environment, but that the chemical has not been analysed;
- monitoring site selection may be misleading too: usually, monitoring takes place at sites where high concentrations are expected and thus may be biased;
- monitoring data represent single points in space and time; nothing can be extrapolated without knowledge on distribution and fate patterns;
- finally, monitoring is an *a posteriori* approach and cannot be used for preliminary and preventive assessment.

As a consequence, environmental exposure assessment should derive from the combination of experimental and predictive approaches. Preliminary predictions are essential for a proper planning of cost effective experimental monitoring and for the interpretation of data. A few, suitably planned, experimental data are needed for validating theoretical predictions.

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CHAPTER 7
RISK CHARACTERISATION

7.1 Value and limitations of TERs as a tool for risk characterisation

Risk characterisation can be defined as the qualitative or quantitative estimation of the probability, frequency and severity of a known or potential environmental effect liable to occur.
It follows that risk characterisation depends on three components:
- quantification the effects (dose/response relationships, hazard characterisation);
- quantification of exposure and assessment of environmental concentrations;
- characterisation of potentially exposed systems (populations, communities, ecosystems) in terms of distribution, ecological relevance, sensitivity and vulnerability, etc.

A first step for risk characterisation is the calculation of a Toxicology/Exposure Ratio (TER) defined as the ratio between a toxicological end point (LC50, LD50) or a Predicted No Effect Concentration (PNEC), and a Predicted Environmental Concentration (PEC).

According to the TGD on risk assessment for new and existing substances (EC, 1996), standard procedures for calculating TERs for the terrestrial environment are proposed on the basis of PEC for soil and food (see chapter 6) and of toxicological end points on selected organisms taken as representative of the terrestrial ecosystems (mammals, birds, bees, other arthropods, earthworms, plants) (see chapter 3).

Major limitations of the approach are the following.
- TERs are generally based on single-species toxicity test data. Also PNEC values, assumed as predicted no effect concentrations for the environment, are derived from single species, short or long term, data. Therefore, they could be of reduced meaning in describing effects on structure and functions of the ecosystem.
- Due to the need for a general applicability, PECs are calculated for fixed worst case scenarios, with a lack of environmental realism.
- The third component of the risk characterisation, i.e. the environmental system potentially exposed, is not taken into account. Risk is referred to a general, hypothetical terrestrial environment.
- Secondary poisoning through the terrestrial food chain is generally assessed by assuming simple deposition of chemicals on food (seeds, leafs, insects) or estimated bioconcentration potential. No assessment is made of transfer through the trophic chain and biomagnification processes.
- In some cases, due to the difficulty of assessing exposure, risk characterisation is not based on real TER. For example, a hazard quotient (HQ) defined as the ratio between application rate (in grams/hectare) and LD_{50} (in μg/bee) to evaluate risk for pesticides to bees.

If one considers the objectives for which this approach has been developed, these cannot be considered as real limitations. Indeed, this approach is useful for practical purposes in all the cases where, for the sake of transparency, simplified and easily applicable and comparable tools are needed, either on new chemicals before their marketing, or for existing chemicals for a preliminary comparative assessment. The obtained TER values are then compared with given triggers, in order to assess the level of concern.

It is also noteworthy that proposed standard procedures for risk characterisation of new and existing chemicals are suitable for assessing the risk for direct effects on living organisms due to chemical emissions. They are less effective for assessing risk due to indirect effects on structure and functions of the ecosystem, as well as long term risk due to chemical transfer through the trophic chain as a consequence of bioaccumulation and biomagnification processes (Figure 1)

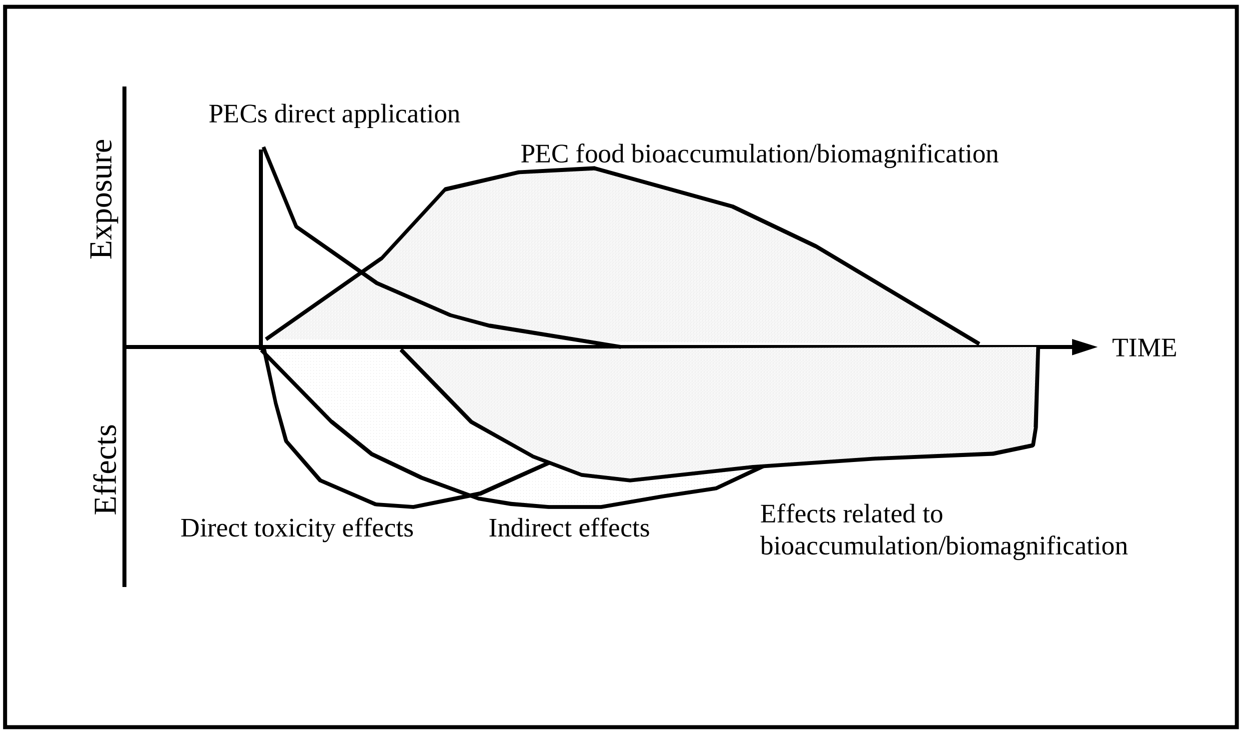

Figure 1. Relationship between different exposure routes and effects on natural ecosystems (modified from Tarazona, 1999).

7.2 Risk characterisation at higher hierarchic levels

In chapter 3 multispecies experimental approaches, from laboratory to field, have been described. In order to evaluate to which extent these approaches can be used for risk characterisation, it must be underlined that ecotoxicological testing represents a compromise between simplicity and ecological realism (Figure 2).
Single species tests have the advantages of high simplicity and reproducibility but they provide little information about the real risk for the ecosystems. On the contrary, testing at higher hierarchic levels provide better information but the results are less reproducible and comparable.

Tests on biological communities are generally developed for organisms behaving on the same trophic level (producers, consumers, decomposers). They may give information on different end points related either to functional characteristics (biomass, primary or secondary production, etc.) or to community structure (species composition, changes in biodiversity, etc.). For the aquatic environment, some more or less standardised testing procedures have been developed, mainly on microorganisms (bacteria, algae, protozoans) (Blank et al, 1988; Cairns and Pratt, 1988). At present, for the terrestrial environment, standard procedures for community testing are not yet available, even if several methods have been developed (see chapter 3).

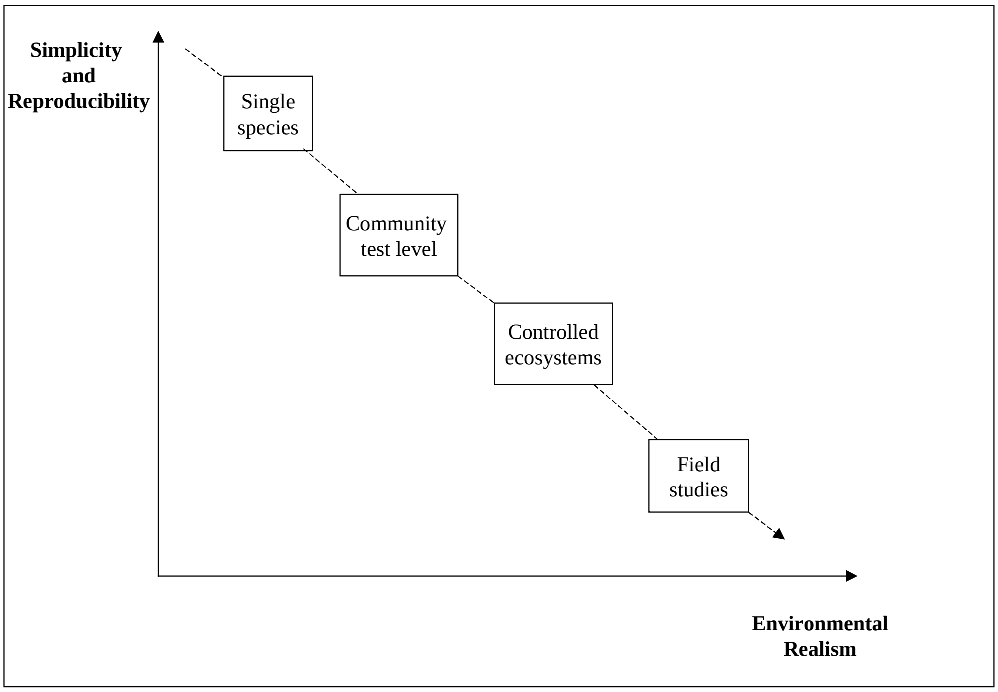

Figure 2. Relationship between simplicity and ecological realism in ecotoxicological testing.

Tests on controlled ecosystems in the laboratory (microcosms) or semi-field (mesocosms) level, may provide information on the interactions between different trophic levels, as well as on the transfer of chemicals through the trophic chain. In this case possibility of standardisation and reproducibility are very limited due to the difficulty of controlling all environmental (biotic and abiotic) conditions. Ecosystem tests must be planned case by case, in conection with the objectives of the study.

Finally, important tools for environmental risk assessment are field studies. They can be performed by studying natural populations and communities or indicator organisms. Community structure changes can be studied by comparing actual conditions with those assumed as reference natural conditions. Also in this case, standard procedures have been developed for the aquatic environment (e.g. several types of Biotic Indexes for rivers) but are still lacking for the terrestrial ecosystems.

Another relevant approach to field studies is represented by the use of biomarkers as an early warning diagnostic tool for assessing ecosystem health (Depledge, 1994). A number of biomarkers techniques have been developed for measuring several types of end points (biochemical, physiological, behavioural, etc.) on terrestrial organisms, in particular on vertebrates. The usefulness of this approach for assessing the potential hazard for natural populations and ecosystems has been extensively investigated and demonstrated (Peakall and Shugart, 1993). Nevertheless, at present, it is still difficult to define precise cause-effect relationships between the measure of a given biomarker response and the real meaning in terms of effects on population dynamics or community structure. Therefore, more research is needed in order to completely exploit the potential of biomarkers as a tool for quantitative risk characterisation.

It can be concluded that higher tier assessment using laboratory, semi-field and field studies on organisation levels higher than single species represents a vital tool for environmental risk characterisation. It is particularly relevant and useful for site-specific assessment; nevertheless, the use of these approaches for regulatory purposes may be problematic and should be carefully evaluated.

7.3 Biomagnification risk for persistent and bioaccumulable chemicals

As mentioned above, transfer through the trophic chain and biomagnification may be studied by means of experimental approaches at higher hierarchical level, but for preliminary and preventive assessment predictive approaches are needed.

A first, preliminary, step for predicting possible risk for the trophic chain is the assessment of the bioconcentration potential. For animals, it is related to octanol/water partition coefficient (logKow). For aquatic animals many highly significant relationships have been experimentally found between bioconcentration factor (BCF) and log Kow. For terrestrial animals, comparable relationships are not available but as a rough preliminary classification, the following scheme is usually accepted:
negligible bioconcentration potential: log Kow<3
low bioconcentration potential: 3<log Kow<3.5
high bioconcentration potential: log Kow>3.5.

As described in chapter 6, predictive approaches have also been developed for bioconcentration in terrestrial plants, based on octanol-air partition coefficient (Koa) or on more complex models.

Theoretical approaches for predicting biomagnification are more complex in comparison to those currently used for assessing bioconcentration, because biomagnification is not only determined by equilibrium partitioning processes. In this case, metabolic patterns of the chemicals, which may be different in different groups of living organisms, must be known. Moreover, the level of assimilation of the chemical in the transfer from lower to higher trophic levels must also be known.

It may be concluded that general predictive approaches can be used for a very preliminary assessment of the potential risk for biomagnification. For a more precise assessment, suitable procedures, based either on models or on experimental data, should be selected on a case by case basis, taking into account the structure and functions of the trophic chain considered. In this case too, as for fate models, the development of realistic trophic chain reference scenarios for the terrestrial environment would be extremely useful.

7.4 Risk assessment for areas of high ecological value

Certain areas of Europe and elsewhere have received special levels of protection. This level of protection is required to guarantee different environmental values including special landscape conditions, ecosystems, endangered populations or species. In certain cases, the protection aims at keeping the conditions of the area as naive as possible, such as in the case of National Parks. In other cases, the program tries to harmonise human, mostly agricultural, activities with the special protection of certain species, such as that regarding birds. The risk assessment should consider these particular concerns.

Ideally, specific risk assessments should be conducted for each area of high ecological value. These risk assessments should require a proper problem definition and a specific analysis plan considering the particularities of the ecological value to be protected. Particular attention should be given to the indirect hazards, i.e., effects on non-target ecological reports which nevertheless play an essential role in the overall assessment. This is particularly important when the value to be protected is or includes the population of certain species. The lack of direct effects (low toxicity or low potential for exposure) does not guarantee the lack of effects because indirect effects on other species affecting the food supply or the habitat conditions will result in equivalent problems.

Several aspects, including the examples listed below, should be considered:

- Effects on food supply: some species feed almost exclusively on a restricted group of food items (represented in extreme conditions by a single species). The reduction of rabbit populations will significantly affect their predators.

- Effects on habitat: similarly, the habitat of certain species is related to the physical conditions offered by other species.

- Behavioural patterns. Metallic metals are expected to be of low bioavailability, however, aquatic birds can ingest lead shot when looking for grit and the metal becomes available at the low pH value of the stomach, representing a risk not only for the bird itself but also for their predators.

- Landscape effects: The effects of chemicals, i.e. fluoride, on trees can produce a dramatic change not only on the tress but on the whole forest system.

- Over-population effects: A reduction in predatory species, i.e. related to biomagnifiable chemicals, can increase herbivorous populations and therefore the pressure of herbivores on plant species.

- Therefore, the hazard identification phase should be particularly concerned with the detection of all relevant indirect relationships.

Additional consideration should be given to the uncertainty and the acceptability of the risk. The first issue is mostly scientific while the second mostly reflects regulatory and social concerns. Assuming that a risk is, by definition, the likelihood of an effect to occur, it seems to be reasonable that the level of likelihood which is considered to be acceptable will depend on the magnitude of the effect. In any case, the risk characterisation should consider the results and the uncertainty in the assessment, while the ultimate decision on acceptability is related to the risk managers.

Finally, it should be clearly stated that the ecological risk assessment is expected to cover effects on populations and higher levels of the biological organisation. In certain cases, such as highly endangered species, this protection should be expanded to the protection of individuals. In such cases, the ecological approach in not enough and a specific risk assessment, aiming at the identification of effects at the individual level, should be required. The human risk assessment constitutes the best example for this kind of assessment and can be used as the basis for the development of a proper conceptual model.

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CHAPTER 8

RECENT PROPOSALS ON HAZARD AND RISK ASSESSMENT FOR THE TERRESTRIAL ENVIRONMENT

Ecological hazard and risk assessment is currently one of the "hot issues" for environmental toxicologists and discussion forums and panels are continuously organised by regulatory bodies, industries and scientific institutions and organisations. The "terrestrial environment" is receiving much more attention and specific meetings, particularly for the soil compartment, are relatively frequent.

This chapter tries to summarise the conclusions and recommendations produced in some of these meetings and bodies. Obviously, this chapter is not an exhaustive recompilation of all the papers published on this issue, but a selective presentation of those considered more relevant for this opinion selected from those available to the CSTEE. In doing this selection, those representing collective recommendations produced at the international level and those representing a regulatory use of scientific knowledge have been chosen. The proceedings of scientific Symposia or Congresses publishing individual papers without general conclusions agreed by the participants have not been included.

The final list included a set of workshops presented at the European Union and Member State level, those reporting opinions of the OECD and a set of documents produced by the Society of Environmental Contamination and Toxicology, resulting from ad-hoc expert meetings organised in co-operation with regulatory bodies.

In each case, the proposals and recommendations, sorted chronologically, will be presented and discussed in this chapter.

- **OECD PROPOSALS**

Following a formal request from the secretariat, the OECD has submitted three reports for the consideration of the CSTEE. Each is discussed separately.

DISCUSSION PAPER REGARDING GUIDANCE FOR TERRESTRIAL EFFECT ASSESSMENT. DECEMBER 1994

The paper was prepared as an initiative of the OECD Hazard Assessment Advisory Body to initiate a discussion regarding the foundation and prerequisites for the elaboration of effect assessment schemes and Test Guidelines for the terrestrial environment.

The main conclusions are summarised below.

> .../...
> The terrestrial environment may be comprehended as consisting of two parts, the soil compartment and the compartment above soil. Chemicals may reach the soil compartment by all of the above mentioned routes of emission, while the above-soil compartment is mainly exposed via the application of pesticides and atmospheric deposition and irrigation. Industrial chemicals emitted to the atmosphere and/or the ground and/or surface water, are expected to be regulated by emission standards, and these standards are considered to be sufficient also for the protection of terrestrial environments. Thus, evaluation of ecotoxic effects on the soil compartment is considered relevant for pesticides and industrial chemicals that are not readily degradable, sorptive and/or lipophilic, while the evaluation of effects on the above-soil compartment is only considered relevant for pesticides (and some biocides).
>
> .../...
>
> Due to the general lack of data on terrestrial ecotoxicity, the use of aquatic effect data for relevant species as surrogates is discussed. This approach could be considered relevant when no toxicity data on terrestrial organisms are available and the alternative is thus a complete lack of assessment. However, the use of aquatic effect data requires a transformation of the effect concentrations in water to effect concentrations in soil. Such transformation methods are based on even more assumptions than the calculation methods described above and certainly need validation.
>
> .../...
>
> The purpose of terrestrial effects assessment is to evaluate possible effects on terrestrial ecosystems and their species and to estimate Predicted No-Effect (PNEC). In chapter 4, endpoints and organisms of concern for such evaluations are discussed. It is concluded that despite the complexity of terrestrial ecosystems, single species tests measuring lethal and sublethal effects are preferred for the initial effects assessment. For a refined evaluation, consideration could be given to tests with two-several species and single species toxicity tests, as for the initial stage; but using exposure conditions in the laboratory resembling field conditions. For a comprehensive evaluation, (semi-)field studies investigating structure and function of ecosystems will be valuable.
>
> Organisms to be investigated or tested should be selected carefully. Criteria for the selection of test species are proposed. The test organisms should be exposed to chemicals via water, air, contact and/or food. They should represent primary producers, herbivores, carnivores, degraders and pollinators, as well as different reproductive strategies (K- and r-strategists). They should also comprise micro-organisms, plants and animals, including arthropods (preferably insects) and, finally, they

should include "key" species selected from ecological, economic or conservational criteria.

.../...

For the discussion, it is assumed that five would be a suitable number of species for the assessment and the principles for selecting test species are illustrated by a Proposal for each of the soil and above-soil compartments. For practical reasons, organisms from uncultured environments and reproductive K-strategists are not expected to be included in the tests considered for development. For the evaluation of effects in the soil environment, the combination of test species comprises degrading micro-organisms, plants (OECD 208), degrading annelids (OECD 207), a carnivorous and a herbivorous insect/mite. For the above-soil compartment, a test set including plants (spray exposure), three different arthropods (herbivorous aphid, mite or weevil; carnivorous parasitic wasp or predatory mite; pollinating honey bee), and finally a herbivorous mammal (OECD 4xx) is proposed. These combinations are intended to maximise the ecological and taxonomical representativeness of species to be included in a "base set". Reference is made to ongoing work regarding selection of test species (especially the OECD-initiated Dutch project) and it is emphasised that the final choice of combinations of species for the terrestrial "base set" should also be based on comparative studies of the sensitivity of the species considered. Moreover, it is stressed that Test Guidelines for arthropods would gain from being developed for groups of species rather than for specific species.

Considering the diverse exposure conditions in terrestrial environments, new Test Guidelines should ideally allow for investigation of the separate routes by which the species in question are exposed. Moreover, the exposure concentrations should be maintained constant during the test period. Research in the field of bioavailability of chemicals in soil (including the characterisation of relevant parameters) is needed before a final selection of standardised soil(s) can take place, and the recommendations given should be considered as preliminary. For initial effects assessments, inert substrates not influencing the availability of the test substance should ideally be used. Sand could be a good choice for soil dwelling organisms, and glass plates could be used as the surface for organisms living above the soil. However, as some tests (e.g. micro-organisms) cannot be performed with inert substrates, the use of standardised soils is recommended for these. For refined effects assessment, more "natural" substrates and exposure conditions should be used and it is proposed that one or a few reference or standard soils should be chosen. Co-ordination with the research, and selection of standard soils for (pesticide) fate studies and with the aquatic sediment testing research is strongly recommended. Finally the importance of validation of the interpretations of results from laboratory studies is stressed. For this field studies as well as validations based on existing databases are suggested.

REPORT OF THE OECD WORKSHOP ON ENVIRONMENTAL HAZARD/RISK ASSESSMENT

As part of OECD's Hazard Assessment Programme, a Workshop on Environmental Hazard/Risk Assessment was held in London on 24th-25th May 1994. It was hosted by the UK Department of the Environment (DoE) and chaired by Dr Norman King of the DoE. The Workshop objectives were to:

(i) improve awareness and understanding in OECD Member Countries of the various environmental hazard/risk assessment schemes in use, or in advanced stages of development;

(ii) identify similarities and differences in the various approaches and the reasons for these differences;

(iii) recommend further work for OECD in building consensus on environmental hazard/risk assessment procedures, whilst at the same time avoiding duplication of work done in other fora.

These objectives were designed to contribute to the longer-term goal of encouraging the mutual use and eventually the mutual acceptance of hazard/risk assessments of chemicals among OECD Member Countries and others. The Workshop focused on environmental hazard/risk assessment for the regulation of chemicals, i.e. new and existing chemicals (including detergents) and pesticides. Hazard/risk assessments of effluents, accidents, etc. were not addressed.

The Workshop was organised around a series of Plenary Sessions and three Working Groups, on Environmental Fate and Exposure, Aquatic Effects, and Terrestrial Effects. The Working Groups were asked to address a set of questions on topics which covered important aspects of the hazard/risk assessment process. The topics included such areas as whether different chemical types needed to be treated differently, the structure of schemes (e.g. are tiers used?), data required, tests to be used, the use of models, approaches to extrapolation, identification of uncertainties, and the use of expert judgement.

The conclusion and recommendations are summarised below:

> A particularly important outcome of the Workshop was the agreement that the scientific principles involved in risk characterisation and risk assessment of general chemicals and of pesticides are fundamentally the same. Any differences in assessment for these two types of chemicals will relate to details in the application of the assessment process rather than in the principles applied.
>
> It was recognised that most hazard/risk assessment schemes have a tiered structure, enabling a progressive refinement of exposure/effects

ratios. The Workshop agreed that this structure is highly desirable. It also agreed with the concept of having harmonized sets of base tests for initial risk assessments for the aquatic and the terrestrial environments, but felt that further testing at higher tiers should be done on the basis of potential exposure. A distinction was made between terrestrial effects testing for the initial assessment of pesticides and of general chemicals. It was recognised that, because of the nature and use of pesticides, some terrestrial effects data will always be needed at a base set level. However, for general chemicals, it cannot automatically be assumed that there will be exposure in the terrestrial environment. Effects data should therefore only be required after an initial comparison of information on fate and potential toxicity.

The Environmental Fate and Exposure Group agreed that reliable monitoring data, when available, should take precedence over predictions from models in the risk assessment of chemicals (with the exception of new chemicals on which monitoring data cannot be available). However, exposure models were viewed by the Workshop as being essential tools that play an important role in the exposure assessment process. The Workshop agreed that the estimation of Predicted Environmental Concentrations (PEC) using models will, in general, be the most cost-effective approach. It was recognised that work is needed on the harmonization of model selection and application, whilst allowing for geographic specifications.

The Workshop recognised that there are difficulties and uncertainties involved in the various extrapolations made during risk characterisation and assessment, and in the application of expert judgement. Clear, transparent reporting of risk characterisations and assessments was therefore viewed as being essential in order that the assessments can be understood and possibly used by others.

Summary of Recommendations for Further Work

The Workshop identified work needed on the development of Test Guidelines, in addition to that needed on risk assessment procedures. High priority was given to the development of: (1) guidance for the testing of difficult substances (e.g. poorly soluble substances, mixtures) in aquatic tests; (2) guidelines for assessing the effects of chemicals in aquatic sediments; and (3) a set of standard terrestrial effects tests.

A number of recommendations were made in relation to work on assessment procedures for environmental fate and exposure and aquatic and terrestrial effects. These included:

Environmental fate and exposure - the development of practical instruments such as an emission database and emission scenarios to estimate releases, guidance for determining rate constants and other parameters derived from laboratory tests for incorporation into models, and harmonized models for predicting environmental concentrations.

Aquatic and terrestrial effects - work on extrapolation techniques and assessment factors, e.g. (1) harmonization of assessment factors used in aquatic effects assessment; (2) development of guidance on the extrapolation of data obtained on single substances to preparations and mixtures; and (3) derivation of assessment factors for terrestrial effects assessment.

General - All Working Groups recommended the development of guidance on: (1) criteria for assessing the suitability of non-standard data; (2) the quantification and reporting of uncertainty in risk assessment; and (3) the consistent, transparent reporting of a risk assessment such that it can be understood and used by others.

Finally the Workshop recognised the need for the efficient use of resources and the importance of transparency in risk assessment procedures. Risk assessment can easily become so information-hungry that higher tiers become difficult and disproportionate in terms of resource use. It was recognised that cost-benefit and animal welfare are factors that should be considered when requesting additional tests, and that the setting of criteria for ending the risk assessment process may be required. With regard to transparency, the importance of communicating risk assessment results to the public, who ultimately determine the acceptability of risks, was highlighted. In addition, the importance of providing readily understood (and hence more useable) results to those involved in risk management and risk reduction was stressed.

FINAL REPORT FROM THE MEETING OF THE OECD TERRESTRIAL EFFECTS WORKING GROUP, MAY 1995.

The Terrestrial Effects Working Group met on 18-19 May to make proposals for a standard set of tests for assessing the effects of chemicals on terrestrial organisms and to identify discussion issues relating to the development of a classification system for the terrestrial environment.

The main conclusions/recommendations are summarised below:

The Working Group agreed that in general, terrestrial testing should be exposure-driven: If exposure can be completely ruled out, then there is no need for testing. If exposure is possible, then tests should be targeted at the compartment of concern.

The group did not reach any conclusion on the possibility to predict effects on terrestrial organisms from other available data (aquatic and mammalian toxicity, physical-chemical properties). The feeling was that this could be useful for some general chemicals but would probably be of limited use for pesticides.

The question of considering the terrestrial environment as two compartments (soil and above soil compartment) was unresolved.

The group considered that dose-response information is preferred, although they did recognise that there may be cases, or particular types of test, where this is not necessary.

The Working Group strongly recommended the development of testing strategies for terrestrial effects assessment of general chemicals.

The Working Group agreed that test selection should be driven by the information needed for risk assessment for the terrestrial environment. It should then be decided if, and how, information from these tests could be used for classification.

For industrial chemicals priority should be given to the development of tests applicable to the soil compartment. The group agreed to include a plant test but could not reach an agreement on the inclusion of a test with an arthropod in addition to the test with an annelid.

The Working Group felt that the use of QSAR should be considered, but recognised the need of more work in this area.

Regarding classification, the Working Group developed a proposal for a terrestrial effect testing scheme an how this may lead to classification.

- **USEPA GUIDELINES FOR ECOLOGICAL RISK ASSESSMENT**

The basic document, published in 1998, represents the general guidelines for Ecological Risk Assessment. These guidelines produced by the USEPA Risk Assessment Forum are quite general, offering an excellent background with a clear scientific basis. Although they are not restricted to the ecological risk assessment of chemical substances, and use the general term of "stressors", the recommendations can be easily applied in the arena of chemical pollution as well as in other areas. In addition, the guidelines are not restricted to the regulation and risk assessment of the Life-Cycle (or part of it) of individual chemicals as are the European guidelines revised in this document. Nevertheless, several examples deal with this issue with problem definitions relatively similar to those considered in Europe, and allow comparisons.

The recommendations, being general, do not offer particular considerations for terrestrial ecosystems, but the scientific basis included in these guidelines can be perfectly applied. Differences in the terminology and structure of the assessment must be considered, and the particular connections between the EU guidelines and the specific pieces of the regulation which tries to support each guideline must be fully understood before trying to extrapolate the recommendations included this document.

In May1999 the ECOFRAM (Ecological Committe on FIFRA Risk Assessment Methods) Terrestrial Workgroup published the ECOFRAM Terrestrial Draft Report which covers the ecological risk assessment of pesticides. This document offers an extensive guide for the application of the USA regulation of pesticides, following a tiered approach under scientifically sound methodologies. The document is too specific to be included in this opinion but it demonstrates that the current state of the art of terrestrial ecotoxicology, when properly used, can offer a sound basis for the development of hazard and risk assessment schemes for the terrestrial environment.

- **INTERNATIONAL WORKSHOP ON HAZARD IDENTIFICATION SYSTEMS AND THE DEVELOPMENT OF CLASSIFICATION CRITERIA FOR THE TERRESTRIAL ENVIRONMENT**

Madrid, 4th to 6th November, 1998

This event was organised by the Spanish Ministry for the Environment, Dirección General de Calidad y Evaluación Ambiental, in co-operation with the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and supported by the European Chemicals Bureau, Unit: Toxicology and Chemical Substances, Institute for Health and Consumer Protection, DG-Joint Research Centre, European Commission.

This meeting was organised after agreement by the **Commission Working Group on the Classification and Labelling of Dangerous Substances (Group on Environmental Effects)** and represents a step forward in the discussions of this group to develop criteria for the classification of substances regarding their hazard for the terrestrial environment.

The objective of this workshop was to provide a forum to discuss the concerns of protecting the terrestrial environment in the framework of hazard identification, and also the problems and benefits related to the development of a specified terrestrial classification system, considering terrestrial physical-chemical and toxicological parameters in addition to the aquatic ones.

The introductory paragraphs of the proceedings state that the meeting followed the initiatives and efforts co-ordinated by the Nordic Council of Ministries to develop criteria for the R-phrases which cover the terrestrial environment. These initiatives were recognised by the Environmental Effects Group and were included as a specific topic. After several discussions and the presentation of more than twenty documents, the Group accepted the proposal of the Spanish Ministry of the Environment to organise this Specific Workshop in Madrid.

The workshop included two meetings of the Core Working Group, constituted by the members of the ECB Classification and Labelling groups, and an open session with the additional participation of invited experts from industry and academia.

Delegates from all Member States, with the only exception of Ireland and Luxembourg, participated in the Core Group meetings, and the open sessions counted representations from several industrial organisations, covering general chemicals, metals, pesticides, petroleum hydrocarbons, etc., and a selected representation of independent scientists, including Professor P. Chambers, vice-chairman of the CSTEE.

Conclusions and recommendations agreed at this Workshop

The following conclusions and recommendations are reported in the Workshop's proceedings:

> The coreworking group was invited to comment on the different presentations to discuss possible approaches for the development of a classification system for the terrestrial environment. Main contributions are reported in the following chapters and an overlview of the different classification proposals presented during the workshop is included in Annex I of these proceedings.
>
> As one of the main outcomes of the workshop, and as a starting point to facilitate further discussions, the main issues agreed during the meeting can be summarised as the following- **"Workshop Conclusions and Recommendations":**
>
> 1. There is an urgent need for the development of an EU (and, if possible OECD) harmonised classification system for the terrestrial environment,
>
> 2. The overall concern is the protection of the structure and function of the terrestrial ecosystems. Ultimately, the classification system should protect the terrestrial ecosystem as a whole. However, the development of such a system should follow the step-wise and a pragmatic approach (e.g. starting the discussions for the soil compartment). Further definition of the protection goal may be required in order to aid development of specific criteria.
>
> 3. Development of this system must consider classification of preparations when developing criteria for substances.
>
> 4. For each category, more than one level of hazard is required. This conclusion reflects the need to identify not only the hazard but also the level of hazard, being probably analogous to the current aquatic system.
>
> 5. The classification should cover all types of land-use (agriculture, forests, natural habitats,...).
>
> 6. Data concerning chemicals dangerous to the terrestrial environment should be gathered.
>
> 7. The classification system should cover all types of chemicals. If some specific uses require provision to users of additional labelling information (i.e. to inform farmers on potential risk and risk management measures, e.g. regarding the compatibility of pesticide formulations to bees), this additional information should be covered by the regulations dealing with that specific use (e.g. Council Directive 91/414/EEC for pesticides).
>
> 8. More information is desirable before establishing the final set of criteria, the categories and the specific number of "levels of hazard" for each category.

9. Classification for terrestrial hazard should be on the agenda for the coming working-group meetings on Environmental Classification and Labelling.

Priority issues of concern and development needs.

Among different opinions collected during the workshop we could conclude the above-mentioned general ideas.

The development of a terrestrial classification system should be clear and transparent, and especially, understandable for users and consumers.

There was a general agreement that the main aim of the new terrestrial classification system is to protect the ecosystem as a whole, classifying all types of chemicals and covering all types of land-use. Basically the feeling was that "the tree" and "sterile land" included in the symbol "N" (Dangerous to the Environment), need to be covered by the Classification and Labelling system, considering also the toxicity data available for the terrestrial system.

In general, there is a lack of toxicity data on terrestrial organisms. However, a large amount of information on mammalian toxicity is provided for the classification for human health effects should also be considered. The convenience or not of using these data twice will need some further discussions.

The complexity of the terrestrial system makes it impossible to isolate/differentiate clearly compartments of concerns. A division between soil and above-soil compartments is very artificial, also because some organisms would be potentially exposed from both compartments. In addition, these compartments are obviously associated with other environmental systems as the ground water system. Therefore, it is necessary to keep in mind the concern of ground water pollution through processes of soil leaching and/or percolation.

There is a general need for more data on different terrestrial organisms in general and on soil organisms in particular.

- **Proposals from the SETAC workshops: (metals, beneficial arthropods, higher tier assessment, ...)**

SETAC, the Society of Environmental Toxicology and Chemistry, is playing a significant role in the development of hazard and risk assessment methodologies, including the development of recommendations for its regulatory use.

SETAC has been particularly active in the development of guidelines for the registration of pesticides in Europe, and several workshops in co-operation with the EU have been held. The proceedings of these workshops are available. In addition, several general books on ecological risk assessment have been published by the Society, but, following the rationale expressed above, those presenting individual works and opinions instead of agreed conclusions and recommendations will not be considered in this chapter.

Three specific topics have been selected: one on metals and two on pesticides.

TEST METHODS FOR HAZARD DETERMINATION of METALS and SPARINGLY SOLUBLE METAL COMPOUNDS in SOILS

A SETAC Workshop on Hazard Assessment of Metals in Soils was held in June 1999, in San Lorenzo de El Escorial, Spain. The aim of the meeting was related to the general need to produce recommendations on the available methodologies to identify the hazard of metals for soil dwelling organisms. In addition, a very specific regulatory use of hazard identification and the classification of substances according to the EU regulation, were considered. The following questions were addressed by 31 experts from 11 countries including regulators, academics, industry representatives and consultants

- How should existing standardised soil toxicity tests be modified to accommodate the particular properties of metals and inorganic substances (such as required micronutrients, incorporation into soils of sparingly soluble substances, species adaptations and acclimatisation, etc.)?
- What additional standardised tests are required to characterise hazard to important biotic components of the soil ecosystem?
- How should the hazard assessment information be integrated with exposure information (i.e., environmental chemistry, bioavailability, interaction with other naturally occurring metals or metalloids, etc.) to provide an assessment of risk to terrestrial environments, for development of universal soil criteria, or for comparative hazard identification?

The proceedings of this workshop are not yet available, but a summary has been published by SETAC (Fairbrother et al., 1999). The following conclusions are stated:

Convening this workshop was very timely, as potential hazards of metals and other substances to the soil ecosystem are coming under increasing scrutiny. During the past five years, Canada and many European countries have developed soil criteria for assessment and remediation of contaminated lands, and Australia and the US EPA are working towards this goal. These criteria depend upon information about hazards to plants, soil invertebrates, and (in some cases) soil micro-organisms. Additionally, the EU is beginning the process of developing a hazard classification system for materials in commerce relative to their potential to cause adverse effects in the terrestrial ecosystem. Standard hazard identification protocols are needed for this effort, if the goal of a comparative ranking is to be achieved.
The Workshop participants recognised the need to standardise methods for hazard identification and testing across all substances, but clearly identified specific properties of metals that require different approaches than those used for testing organic compounds. Mixing and equilibration of metals in soils during test set-up is a major point of difference. The concept of a "transformation protocol" with short- and long-term tests run

before and after the transformation period was cautiously endorsed by the Workshop participants, with the caveat that additional work is needed to define the length of the transformation period for various substances, appropriate leaching and storage conditions, and so forth. The objective of the transformation protocol is to simulate weathering of metals in the environment to ascertain whether these naturally persistent compounds might change in bioavailability over time. This would be equivalent to evaluating environmental persistence of organic substances for the purposes of hazard classification.

Soil type also is a major consideration for testing of metals, and was discussed at length by the Workshop participants. Consensus and closure on this issue were not achieved. It was acknowledged that some widely occurring soils were not represented, in particular those of a low organic matter status such as found in arid environments or soils rich in iron and aluminium such as in the tropics. The use of a standard artificial soil matrix was endorsed by the Plant Work Group, particularly for the purpose of hazard identification and ranking. The Soil Invertebrate Work Group preferred using natural soils that met specifications such as those developed for EUROSOILs. The use of natural soils also was endorsed by the Soil Micro-organism Work Group, as the proposed tests utilise indigenous organisms rather than the addition of cultures to a soil matrix. The Chemistry Work Group also supported using defined natural soils, but pointed out the necessity of different soil parameters for maximising bioavailability of different types of metals (cationic metals versus anionic metals). Thus, the use of artificial versus natural soil and the number and types of soils required still remain unresolved for hazard identification and ranking. There was general agreement, however, that ecological risk assessment requires hazard information developed from natural soils representative of the area under consideration as our ability to extrapolate toxicity data across soil types is limited.

The Plant and Soil Invertebrate Work Groups proposed standard species and described detailed test guidelines (to be included in the Workshop Technical Report). The Soil Micro-organism Work Group recognised that there are no accepted standard methods for determining hazard to microbial communities. Several microbial function tests were recommended, but additional information will be required to standardise the methods and to put the results into an ecological context.

Workshop participants identified many areas where short-term research will be required to formalise test methods. Moreover, although the focus of this workshop was on developing methods to measure effects to organisms from direct soil exposure, it is acknowledged that the overall objective is to evaluate hazard for the terrestrial ecosystem. Therefore, there is a need to identify and suggest further tests for soil ecosystem function, as well as test for above-ground organisms such as foliar and aerial invertebrates and methods for identifying potential hazard to vertebrates from food chain exposure or direct soil ingestion. Determination of the potential for metals to biomagnify in the food chain is much more complex than for synthetic organic compounds, as organisms have evolved various mechanisms to use, exclude, or take up these naturally occurring substances. Finally, the workshop participants strongly endorsed the concept that measurement endpoints chosen for all tests should be ecologically relevant for both acute and chronic effects.

ESCORT

Two workshops on the risk assessment of beneficial (ground and foliar dwelling) arthropods have been organised in the frame of the regulation of pesticides in the EU. The outcome of the first workshop was published by SETAC in 1995 while the second workshop was held only a few months ago and no summary or conclusions have been published yet.

The recommendations of the first workshop were quite specific for the registration of pesticides and it is difficult to extrapolate these conclusions for a general hazard and risk assessment scheme. The conclusions/recommendations for the second workshop are not yet available.

Opinion of the CSTEE

A clear movement on the procedures to address the complexity of the terrestrial environment can be observed looking at the dates these documents where produced. The oldest document, from the OECD, includes a initial statement establishing two clearly distinguished compartments: soil and above soil. This statement is no longer supported as a fundamental concept, and it is interesting to see that even groups of experts discussinng specifically on the soil compartment express the need to cover the terrestrial ecosystem as a whole, and not only the soil comparment, as concluded at the SETAC meeting on metals. The terrestrial environment is specifically defined as the interphase between air and soil, and most organisms interefere with both compartments. Plants can be exposed simultaneously through atmospheric deposition and through soil, the number of "pure" soil dwelling invertebrates is relatively low, and the exposure of vertebrates to chemicals *via* soil (dermal contact, inhalation, soil consumption) is considered a key element in the development of soil quality criteria.

The CSTEE proposes to use, alternatively, distinctions based on the exposure routes, i.e.,:

- Exposures via soil
- Exposures via food
- Exposures via atmospheric deposition
- Other exposure routes (i.e., ingestion of contaminated water).

For each route, the relevant taxonomic groups must be identified (i.e, micro-organisms, plants, invertebrates and, in some cases vertebrates, for soil exposures; invertebrates and vertebrates for food exposures; etc.). This approach is considered to be in line with the current state of the art and with the opinions expressed in most recent documents.

Similarly, the distinctions related to the class of chemicals (industrial chemicals, pesticides, biocides) are no longer considered absolute statements. The highest concern and the need for specific exposure scenarios for chemicals intended to be discharged in the environment, such as pesticides and some biocides is clearly acceptable. But the same industrial chemicals should also be included in this category. The scientific literature also demonstrates that other industrial chemicals can represent a real risk for the terrestrial ecosystem from exposures other than *via* soil.

The CSTEE agrees with the statement that the same scientific principles must be used for the risk assessment and risk characterisation of industrial chemicals and pesticides. As previously pointed out, the EU regulations have not followed this approach, and major differences between the approaches recommended by the different guidelines can be observed, particularly for the terrestrial environment.

The CSTEE also agrees with the recommendation of tiered approaches and with harmonised sets of base test for the aquatic and terrestrial environment. However, in the opinion of the CSTEE the initial hazard and risk assessment for the terrestrial environment must always be a requirement, and not only after an initial comparison of information on fate and potential toxicity as suggested in some reports. Under a cost/benefit balance, the use of exposure-driven schemes is reasonable. However, a base data set for the terrestrial environment is required in almost all cases, because:

(a) exposure of the terrestrial environment can be very low, but in most cases it is not completely ruled out. For chemicals of high concern (highly toxic, persistent and bioaccumulable), very low emission levels can suppose an unacceptable risk. Toxicity data are required even for a preliminary assessment (some chemicals are toxic at concentrations below the detection limit from chemical analysis); and
(b) unless total banning, the risk of accidental emissions cannot be ruled out, and at least an initial hazard assessment for the terrestrial environment is required.

The scientific aspects related to the conclusions of the Madrid workshop are considered sound. The information presented in previous chapters clearly shows that the current state of the art can provide scientific support to those aspects of the classification scheme needing technical advice. As expressed previously, the CSTEE also agrees with the need to cover the terrestrial environment as a whole for hazard identification schemes.

It is also considered appropriate to develop general hazard identification schemes for all chemicals. Specificities should be considered within the system in the broadest way allowed by scientific information. The CSTEE recognises that metals have several differences, as expressed at the SETAC workshop, including the presence of background concentrations, the lack of degradation, the role of soil characteristic and time evolution on their bioavailability. However, these specificities are not exclusive for metals, i.e., background concentrations can also be relevant for other substances including organic chemicals of natural origin; the lack of degradation is an obvious property for all elements, soil properties are critical to determine the bioavailability of all substances (although differences in the key properties for metals versus organic chemicals are obvious), and the changes of bioavailability over time have been, for example, considered the critical aspect for a proper ecological assessment of several pesticides which are considered persistent in soil due to strong binding to soil particles.

The proposal for soil invertebrates is probably the most controversial of those expressed at the SETAC workshop. The use of four different species and, exclusively, long-term tests with reproductive endpoints, even for hazard identification, is quite ambitious. The CSTEE recognises the scientific value of this approach, however, in terms of the cost/effect relationship and the testing strategy it should be recognised that the proposal represents a considerable jump in the requirements. Possibilities for a tiered approach and for cost effective bioassays, i.e. combining several species in a single test, should be considered.

PREVIOUS OPINIONS OF THE CSTEE REGARDING THE HAZARD AND RISK ASSESSMENT FOR THE TERRESTRIAL ENVIRONMENT.

Several opinions adopted by the CSTEE have included comments on the hazard and risk assessment of chemicals for terrestrial ecosystems. These previous opinions have been grouped and sorted by the most relevant issue. The following items have been identified:

Non-soil exposures:

The CSTEE has already agreed that the concern for the effects of industrial chemicals on the terrestrial environment is not restricted to the contamination of soil. The Risk Assessment Reports submitted to the CSTEE clearly demonstrate that, for several High Production Volume Chemicals, exposures via air are particularly relevant in the risk assessment for the terrestrial environment (**Opinions on the results of the Risk Assessment of: DIMETHYL SULPHATE; HYDROGEN FLUORIDE; ACRYLALDEHYDE).**

Extrapolation of aquatic toxicity data and use of the partitioning equilibrium model:

The CSTEE has considered this method as scientifically valid but with several restrictions. The extrapolation must be done on a case-by-case basis after an in-depth consideration of the toxicological profile of the chemical, its mechanisms of action, and its physical-chemical profile. In fact it has been considered acceptable for some substances but not for others. The extrapolation should not be accepted for chemicals with specific mechanisms of action such as pesticides and biocides, and therefore real toxicity data on soil dwelling organisms are required even for the initial risk assessment of these substances (**Opinions on the results of the Risk Assessment of: CUMENE; ALKANES, C10-13, CHLORO {SCCP}; 4,4'-METHYLENEDIANILINE; PENTABROMODIPHENYL ETHER; Opinion on the "TECHNICAL GUIDANCE DOCUMENT IN SUPPORT OF THE DIRECTIVE 98/8/EC CONCERNING THE PLACING OF BIOCIDAL PRODUCTS ON THE MARKET: GUIDANCE ON DATA REQUIREMENTS FOR ACTIVE SUBSTANCES AND BIOCIDAL PRODUCTS - FINAL VERSION, 7TH OF DECEMBER 1999"; Opinion on the report by WS Atkins International Ltd (vol. B) "Assessment of the risks to health and to the environment of arsenic in wood preservatives and of the effects of further restrictions on its marketing and use")**

Use of species-distribution curves and probabilistic approaches:

The use of species-distribution curves for the derivation of PNEC values and the use of probabilistic risk assessment have been considered valid and scientifically sound (**Opinion on Cadmium. The Final Report by WS Atkins International Ltd. Based on: The Final Report (September 1998) & Additional Assessment September 1998): "Assessment of the risks to health and to the environment of Cadmium contained in certain products and of the effects of further restrictions on their marketing and use").**

Bioavailability and consideration of the effects of soil characteristics on toxicity:

Previous opinions address the need for considering the bioavailability of the chemicals and the effects of the soil characteristics on the toxicity of the substance. This point has been stressed particularly for metals and other elements such as arsenic. (**Opinion on Cadmium. The Final Report by WS Atkins International Ltd. Based on: The Final Report (September 1998) & Additional Assessment September 1998): "Assessment of the risks to health and to the environment of Cadmium contained in certain products and of the effects of further restrictions on their marketing and use"”; Opinion on the report by WS Atkins International Ltd (vol. B) “Assessment of the risks to health and to the environment of arsenic in wood preservatives and of the effects of further restrictions on its marketing and use”).**

Uncertainty factors for higher tier studies:

The use of an uncertainty factor of 1 for field studies has been considered acceptable when the available information was considered to cover the most sensitive species/systems. (**Opinion on “Risk assessment underpinning new standards and thresholds in the proposal for a daughter directive for tropospheric ozone”**)

SUMMARY

The documents discussed in this chapter demonstrate that the hazard and risk assessment for the terrestrial compartment is receiving enhanced attention regarding both the scientific problem and the use of scientific results as the basis for regulatory issues. It should be clear that the documents discussed here only represent a brief overview of the current developments in the regulatory use of terrestrial ecotoxicological data, and hundreds of additional documents can be found elsewhere.

The main complexity for the terrestrial environment is the identification of the relevant relationships between potential exposure routes and environmental receptors. Simplifications, such as the distinction of two sub-compartments, soil and above ground, are frequently used to facilitate the identification of these relationships. However, it has been recognised that these distinctions are artificial, and must only be considered as pragmatic approaches keeping in mind that the real goal is the protection of the ecosystem and that this goal cannot be achieved by the exclusive consideration of the soil compartment. Even for specific "soil regulations" such as soil quality objectives, the assessment for the terrestrial compartment cannot be made on the sole basis of the soil compartment. The establishment of ecotoxicological thresholds or acceptable levels of contamination must considered, in addition to soil dwelling organisms, the hazard and risk for other terrestrial organisms, exposed directly (i.e. soil ingestion) or indirectly (i.e. through the food chain or via volatilisation) to the contaminated soil.

Mention must also be made of the perfect agreement observed between the generic views expressed in this opinion and those formulated previously by the CSTEE in relation to specific issues. This coherence is an additional argument to consider that the state of the art terrestrial ecotoxicology, although requiring continuous improvement, is sufficient to produce the basis for a scientifically sound regulation.

CHAPTER 9

THE CURRENT STATE OF THE ART AND ITS POSSIBILITIES FOR DECISION MAKING

9.1 HAZARD COMPARISON AND CLASSIFICATION. SCIENTIFIC BASIS FOR THE IDENTIFICATION OF DANGEROUS SUBSTANCES

According to the information presented in this opinion, it can be concluded that the main remaining problem for the development of a proper classification scheme for the terrestrial environment is the combination of effects representing the different exposure routes relevant to the terrestrial environment in order to identify the characteristics of chemicals which cause adverse effects on terrestrial ecosystems.

Two additional problems have been frequently mentioned as difficulties in developing sound criteria:

- Lack of standardised effect assessment protocols, and
- Lack of toxicity data.

However, these aspects seem to be of low actual relevance considering the current state of the art.

The information included in chapter 4 shows that there is a significant set of toxicity tests available that are either standardised, under standardisation, or under inter-calibration. These tests cover the different taxonomic groups and exposure routes at different degrees.

Similarly, the review conducted by the ECB on the available data included in the European data base IUCLID (Allanou et al., 1999) suggests that some terrestrial toxicological information is available for about one third of high volume industrial chemicals. Although the problem is still evident in terms of the application of the criteria, the large majority of chemicals will still remain without classification due to lack of data, a 30-35% of availability is clearly enough for the development of sound criteria.

Therefore, the main problem associated with the identification of substances dangerous to the terrestrial environment is the need to combine hazards associated with different exposure routes, in an exercise, such as hazard identification, which by definition does not cover the exposure assessment.

The final criteria for classification and labelling should be based on the social concern, the regulatory goals, cost/benefit analysis, etc. However, the scientific principles of hazard assessment can also contribute in supporting this development. There are at least three different mechanisms by which chemicals can cause adverse effects in the terrestrial environment.

1. Adverse effects related to the presence of the substance in the soil
2. Adverse effects related to the presence of the substance in food items

3. Adverse effects related to the presence of the substance in air including air-ground and air-biota interfaces.

The first group can be described by:

- Toxicity of the substance for soil dwelling organisms (including vertebrates exposed to contaminated soil), modulated by the persistence of the substance in the environment and particularly in soil.

The second group can be described by:

- Oral toxicity of the substance, particularly for vertebrates, modulated by the potential for bioaccumulation and the persistence of the substance.
-

The third group can be described by:

- Inhalation toxicity and toxicity to the aerial part of plants, relevant for gases and volatile compounds.
- Contact toxicity, particularly for plants and invertebrates, modulated by the potential for atmospheric deposition.

A similitude among terrestrial vertebrates and human beings exposed through the environment can be clearly observed. Typical examples are effects related to contact and inhalation toxicity via contaminated soil. Although the level of exposure is obviously higher for certain species (mostly reptiles and mammals) these differences are covered by the higher level of protection required for human beings. Therefore, to avoid duplications in holistic assessments, these aspects do not need a specific assessment if a cross-reference to the hazard identification for human health effects is included.

The danger of all three hazards is also related to the mobility of the substance inside and among compartments. Mobility has two opposite effects; it can contribute to the distribution of the chemical and therefore its potential to affect a larger area, and at the same time this distribution contributes to the dissipation of the substance.

Tools for the assessment of each hazard are available, although additional efforts are still required to improve their capability and standardisation. In fact, several classification proposals for each hazard have been presented inside and outside Europe (USEPA 1985, Poels and Veerrkamp, 1992; EMEA 1997; Tortesson et al., 1997; Vega et al, 1999).

For a proper use of the available information it is suggested to define the hazard category according to the toxicity of the chemical. In a first approach all toxic substances should be considered as dangerous. Those that are in addition persistent and/or have potential for bioaccumulation should be regarded as possessing a higher degree of danger. High mobility can be regarded as an indication of higher hazard for highly toxic and/or persistent/bioaccumulable substances. Specific criteria should be required for determining the cut-off values for each selected property, in other words the levels of toxicity (for each relevant assay), persistence, bioaccumulation potential, and mobility which determine that the potential of hazard of a chemical is high enough to require classification. It is recognised that these levels must be set considering basically the goal of the classification system and its use as a regulatory tool and using basic hazard assessment concepts to support the selection. The revision of the scientific literature on the effects produced by chemicals on terrestrial systems can facilitate this development (Tarazona et al., 1996; Fresno and Tarazona, 1997).

This general and simple scheme can be refined at a higher tier level considering additional properties of the chemical. This tiered approach combines simplicity (substances can be easily classified on the basis of their toxicity and a few additional parameters) with a proper use of the scientific information when available. In this way, and under a case-by-case basis, it can be decided that a particular chemical fulfilling the initial criteria do not require classification because additional properties suggest that the identified hazard is, in reality, of low relevance. For the terrestrial environment the capability to reach the target ecological receptor in the case of intended or accidental releases to the environment should be essential for this higher tier level. For example, a very rapid degradation in air indicates that the hazard associated with the presence of the substance in air is expected to be of very low relevance, while a lack of bioaccumulation potential indicates that oral exposure should be restricted to the deposition of the chemical on food items (and consumption of contaminated water and soil when relevant). Equivalent situations have been considered in the EU criteria for the classification related to the aquatic environment. For example, a toxic or harmful chemical does not require classification if its chronic toxicity is low, or a non-biodegradable chemical is considered of low persistence when a rapid hydrolysis to non-toxic metabolites can be demonstrated.

The key issue is therefore the harmonisation of the classification criteria. Harmonisation is required among the three hazard types relevant to the terrestrial environment and between the terrestrial and the aquatic compartment.

Following the recommendations expressed in chapter 3, this harmonisation could be supported by a combination of the environmental relevance of the expected hazard and the statistical analysis of the distribution curves on the toxicity of substances for different taxonomic groups. The first point can be achieved establishing generic scenarios, i.e. in terms of a fixed surface. The information compiled in the TGD can provide the required data on relative weights, sizes and relationships among the different abiotic and biotic components of a generic terrestrial system.

A central issue in the development of criteria is that these criteria have to be as valid for complex mixtures of chemicals as for single well-defined substances. From a regulatory point of view the hazard identification for complex mixtures is required due to different reasons including:

A. Several "substances" requiring a legal classification and labelling are in reality complex chemical mixtures
B. The need to classify preparations
C. The environment is threatened by hundreds of emissions including hundreds of different chemicals and several hazard assessment approaches require the estimation of synergistic effects.

The possibility to assess the effects of a complex mixture on living organisms has been described in Chapter 5.5. Obviously, the experimental and predictive methods for the assessment of mixture toxicity cannot be applied to the huge (near to infinite) number of theoretically possible mixtures of contaminants.

The use of hazard identification for classification and labelling (points A and B) requires the establishment of transparent criteria for the classification of mixtures. Two essential points can be considered: the combination of dangers related to different types of hazards and the combination of toxicity and fate (persistence-bioaccumulation) properties. Hazard identification principles can be used to support the final regulatory decision.

For setting quality standards and effects assessment in risk analysis (point C), an important step is the assessment of those mixtures that are more likely to occur in the environment, based on comparison of chemical emissions and on the environmental fate of individual components.

A list of “priority mixtures” should then be developed, comparable to the priority lists compiled for individual chemicals of environmental concern. At present, this is under development for the aquatic environment, in the frame of the European Research Project BEAM (Bridging Effect Assessment of Mixtures to Ecosystem Situations and Regulation) which started in Spring 2000 and is scheduled for a three-year period (Grimme et al., 2000). In this field too, a gap exists between aquatic and terrestrial ecotoxicology.

9.2 INFORMATION SUPPORTING THE DERIVATION OF QUALITY STANDARDS

The derivation of soil quality standards or criteria suffers, again, from clear distinctions among the different environmental compartments. The aquatic compartment, and specifically the water column, received priority at European level in the early 70s, environmental air quality standards several years later, and currently, the derivation of soil quality standards is still a national responsibility not harmonised among Member States.

The methodology for the derivation of soil quality standards has considered different scientific bases and therefore has produced large conceptual differences. Annex includes, as examples, the criteria employed by The Netherlands and Germany.

From a risk analysis perspective, the derivation of quality standards can mostly be integrated in the effect assessment part, with additional contributions from the exposure assessment for including the fate and behaviour of the chemical in the compartment and the transfer among compartments, and considering the principles of risk characterisation when setting the acceptability patterns. The methodological approach employed for the derivation will establish the role of each phase in the whole process.

When the criteria focus exclusively on one compartment and its organisms, excluding the interaction with other organisms (i.e., deriving soil quality criteria considering exclusively soil dwelling organisms) the procedure basically comprises the initial decision on the risk acceptability and an effect assessment exercise, determining the best assessment endpoints according to the available information and the ecological relevance.

However, when holistic criteria are considered, the complexity of the process required a full risk assessment exercise but moving in the opposite direction. In other words, instead of assessing the actual or potential risk of the activities which employ a certain chemical, the process starts establishing the level of acceptable risk and moves backward to determine the maximum concentrations to achieve this level of risk.

These holistic approaches are clearly recommended. They are particularly important when setting soil quality criteria. The criteria should be protective for soil dwelling organisms and soil functions, but also for herbivorous animals consuming plants growing on that particular soil, for the predators of these animals, for surface water bodies located in the proximity of the contaminated area, or for groundwater. Most of these factors are also essential when considering the protection of human health and therefore can be implemented to also cover the environmental concerns.

From a regulatory perspective, the way in which all these concerns are covered (holistic criteria or independent criteria for soil, food, groundwater, etc.) should be adapted to the specific needs and strategies.
The proposed Water Framework Directive represents a good example of the harmonisation between selection of quality standards and risk assessment methodologies. The proposed method employed for the derivation of ecological quality standards is equivalent to the PNECaquatic organisms derivation included in the TGD. Harmonisation is also observed for the particular case of pesticides, where the acceptability criteria based on chronic toxicity are equivalent to an assessment factor of 10, which is the factor recommended when a full chronic data set (which is in all cases required for pesticides) is available. Obviously, this harmonisation should be maintained in the future, and considering that the TGD is currently under revision, mechanisms to guarantee this agreement should be included.

The CSTEE recommends the harmonisation of the soil quality criteria within the EU. It is obviously recognised that issues such as background concentrations, soil characteristics, or intended uses, must be considered in this process and setting a single value for all soil in Europe is unrealistic. Local and regional conditions must be obviously considered when setting soil quality criteria, and from a scientific point of view, differences should be related to environmental and ecological conditions not on political borders. A tiered approach, starting from a generic worst-case assessment as currently employed for lower tier risk assessment of industrial chemicals and pesticides, should be developed at the EU level.

Criteria should also be related to the intended soil uses. The relevance of different ecological receptors as a function of the intended use can be included in the criteria development. For example, the framework developed by the Spanish National Institute for Agricultural Research includes three main soil uses which are related to the relevance of ecological receptors:

- Industrial soils require the protection, at least, of basic soil functions, including vegetation cover, but not biodiversity, therefore effects on sensitive species can be accepted if other species are able to keep their function.
- Residential soils, including gardens, require the protection of soil functions and biodiversity. Terrestrial vertebrates are also relevant ecological receptors in this case, although no complex food-chains are expected, and therefore only secondary poisoning must be included. The dimensions of the contaminated area can be used to consider the likelihood for a vertebrate to obtain its food from the contaminated area.
- Recreational, forest and agricultural soils require complete protection including biomagnification through a complex food chain.

In addition, the protection of surface and groundwater from run-off and leaching should be considered in all three cases.

The first tier assessment can suggest either a single value, equivalent to the derivation of a single PNEC value in the TGD, or a function based on soil characteristics. In a second step, regional conditions, including background levels, weather conditions, soil characteristics etc., should be considered. Setting eco-regions showing equivalent conditions for the key parameters appears as a proper approach for getting specificity while keeping transparency. Finally, the third step requires a local perspective, which, whenever possible, should be based on a real risk analysis of the contaminated site, including realistic exposure estimations, instead of a single derivation of local quality criteria.

It is therefore concluded that the development of guidelines for the ecological risk assessment for terrestrial ecosystems will also allow the development of harmonised soil quality criteria, and the inclusion of ecologically relevant inter-compartment routes when setting ecological quality objectives for air and water.

9.3 INFORMATION SUPPORTING RISK ASSESSMENT

9.3.1 Comprehensive Risk Assessment

From the extensive review presented in the previous chapters, it follows that a risk assessment for the terrestrial environment can be performed at different levels of complexity and precision, relative to the objectives, the scale of application, etc. Nevertheless, in order to provide the information needed for environmental regulation on new and existing chemicals, simplified standard procedures must be applied. These must be capable of providing a pragmatic and transparent decision-making tool.

Assessing the risk for terrestrial ecosystems requires some specific conditions during the risk analysis. These special requirements affect all parts of the risk assessment. The major particularities and difficulties are summarised below.

1) Hazard identification and quantification

The complexity of the terrestrial environment increases the relevance of the hazard identification phase of comprehensive risk assessment. For the aquatic compartment, the effects assessment for water column organisms is required in all cases, and the needs for an assessment of sediment dwelling organisms can be easily decided based on the physical-chemical properties of the chemical (binding capacity). This decision is taken on the basis that the chemical has potential for reaching surface waters. However, for the terrestrial environment, the potential for reaching, soil, air, food items, etc., depends primarily on the lifecycle of the chemical, and particularly on the production, use patterns and disposal conditions. Secondarily it also depends on the behaviour and fate properties of the chemical.

Therefore, the hazard identification phase becomes essential, and must be scheduled to identify the relevance of all potential hazards. For comprehensive risk assessments, a tiered approach should be established, and well defined potential hazards should be considered.

According to the information presented in previous chapters, the CSTEE considers that proper conceptual models should be established for different uses and disposal conditions. As clearly observed for pesticides, this aspect is particularly important for all chemicals with uses or disposal conditions associated with significant environmental releases. This condition includes pesticides, several biocides, veterinary products, and several industrial chemicals such as those included in products intended to be used by the general public.

Additionally, selection criteria for chemicals with particular fate properties should be established. These models should identify those chemicals which are expected to reach terrestrial organisms independently of the use and disposal patterns. These possibilities include persistent and bioaccumulable chemicals as well as those which are expected to partition into the sludge in the wastewater treatment plants and therefore can appear in soil amendments.

2) Exposure assessment

As previously pointed out, the holistic assessment of terrestrial ecosystems requires the consideration of a complex matrix of exposure routes. There are two additional aspects that will require greater attention in the future and are related to the needs for covering non-constant exposure levels in the assessment, e.g. due to changes in the concentrations over time or non-homgeneous distribution within the compartment.

The estimation of expected changes in the PEC versus time is obviously an important issue for all assessments. For the aquatic compartment, two basic simplifications are considered. When continuous emissions are of concern, a constant PECsurface water (usually estimated as the maximum PEC, i.e assuming the highest potential emission and the lowest expected dilution in the water body) is assumed. For intermittent emissions, either peak concentrations (assuming that only short-term exposures are relevant) or a time dependence related to the dissipation rate of the chemical, is normally accepted. Pragmatic approaches, such as assuming first order kinetics for dissipation and estimating the time-weighted averages for the PEC according to the exposure time of the bioassay, are generally used for pesticides.

These simplifications are not always possible for the terrestrial compartment. The main problem regards the relationships among different exposure routes. For example, when estimating the concentration of a pesticide or biocide in plants, the direct deposition on the plant surface will produce an initial peak, which will be followed by a reduction in the concentration related to the further dissipation of the chemicals from the plant surface. In the meantime the chemical has reached the soil and the absorption of the chemical from the soil into plant tissues will produce an increase in the concentration in the plant. The latter will achieve a maximum value days or weeks after application.

The second aspect regards the lack of homogeneity within the compartment. Risk assessments applied to the aquatic compartment mostly accept the simplification of an homogeneous distribution within the water body. This simplification requires some pragmatic assumptions such as excluding the mixing zones from the assessment. Similarly, pragmatic approaches have been assumed for sediments and soil; most cases assume homogeneous distribution within the top centimetres of sediment or soil.

However, a terrestrial ecosystem requires a significant land surface, and the assumption of homogeneous distributions in soil, food or air for point source emissions is largely unrealistic. For a first tier assessment, it is always possible to consider a single worst case assumption, but the risk refinement requires exposure estimations considering a non-homogeneous distribution of the chemical in the compartment. Several possibilities should be explored to assess these particular issues. These include the estimation of concentration gradients for point emissions.

Some compartments, such as food items, can only be addressed using complex relationships. PECfood, even for the same trophic level, will largely depend on behavioural patterns. Predators will obtain their food from a relatively large area, with different levels of contamination, and their diet will thereafter comprise several food items, each exposed through a different route. Even for a low tier assessment, the decision on how much food is obtained in the contaminated area is crucial, and obviously a higher tier assessment requires complex considerations. Probabilistic assessments are expected to be an essential element for the further development of these aspects.

Obviously, a similar problem can be observed for the aquatic compartment when food chain transfer is included in the assessment (e.g. Carbonell et al., 2000). However, this aspect is only rarely considered in the aquatic compartment while it is an essential route for terrestrial ecosystems.

3) Effect assessment

In addition to those aspects described in previous chapters, two special issues should be identified, related to the exposure conditions of the toxicity tests.

Most standard toxicity tests using water or food exposures are designed to keep a constant exposure level during the test. Renewal or flow-through conditions are required when testing chemicals which disappear rapidly from the water column. This is obviously not possible in the case of soil exposures, and therefore the exposure assessment of soil dwelling organisms includes a non-constant exposure level. The fate of the chemical during the tests can be very different from the expected fate in real soils, and the effect assessment should address these issues.

The second aspect focuses on the assessment of long-term effects and the required exposure times for observing chronic effects. As clearly demonstrated for endocrine disrupters, a short-term exposure can produce long-term effects. For specific mechanisms or targets, exposure in the critical period (hours, days, weeks) might provoke the same effects as the exposure during the whole life-cycle. Whenever possible, the long-term effect assessment tools should be able to identify the critical period in order to consider this aspect in the risk characterisation. As a starting point, the current guidelines on long-term toxicity tests on mammals, birds, fish and other organisms should be carefully evaluated to determine the realistic exposure times for the different endpoints measured during the test. For example, in the bird reproduction test, the effects on the shell thickness and egg resistance should be related to the time lasting from the initiation of parent exposure to starting egg production, or even to the shorter period related to the calcification of the egg surface when immediate effects on calcium metabolisms are expected. However, the whole test duration, which includes several more weeks for checking effects on embryo development and chick survival, is usually considered. Similarly effects on egg development should be related to the exposure of the parents before egg production, because the subsequent exposure of the parents does not affect the levels of the chemical inside the egg.

This is a need not only for the environmental effect assessment, but also for the human health assessment, and is considered a key issue for the development of integrated risk assessment.

4) Risk characterisation

Risk characterisation requires the selection of several combinations of exposure versus effects. Therefore, several trigger values for the TER on each toxicological end point or PNEC/PEC ratios for each compartment must be calculated.

For a lower tier assessment, the general procedure is conceptually similar to those applied for the aquatic ecosystem. Nevertheless, at present, many gaps exist for the terrestrial ecosystem. In particular:

- The set of toxicology tests and of indicator species is not clearly defined as for the aquatic ecosystem. In some cases, only a generic group (e.g. beneficial arthropods) is indicated;
- Standard testing procedures are only available for a reduced number of indicator organisms;

- Models and calculating procedures for assessing exposure are not available or not sufficiently validated for all environmental compartments;
- Due to the variability of conditions of the terrestrial environment, in general more heterogeneous in comparison with freshwater aquatic environment, suitable environmental scenarios are not sufficiently defined.

In addition, the problems of time/concentration relationships and compartment heterogeneity already mentioned under exposure and effect assessment items must be considered.

When there is not harmony between the expected rates in the environment and those observed/expected in the toxicity tests, procedures for solving these differences must be implemented. Time-weighted averages are usually employed to compare the continuous exposure of the toxicity test (through water or food) with the expected dissipation of the pesticide after application. However, particularly for long-term exposures, the selection of the time employed to determine the average is crucial. This time should be the time-span of the critical receptor/endpoint. This selection requires knowledge on the mechanisms of action which is normally not available.

In soil toxicity tests, the dissipation can be either more or less rapid than under real conditions (and likely more rapid than in certain real conditions and less rapid than under other, also real, conditions). The behaviour of metabolites can also be different. In long-term tests including recovery, such as microbial tests, the recovery can be related to the dissipation of the chemical from test media. Obviously, the recovery of a population related to cessation of exposure requires a different assessment than the recovery of an exposed population. Further information on proper risk characterisation strategies to cover all these issues is required.

Finally, higher tier assessment must consider the heterogeneity issue in terms of time patterns, exposure and effects. Probabilistic assessments offer a possible way of addressing this compartment.

In addition, even comprehensive risk assessment could be needed for more site-specific conditions, such as the protection of particularly valuable areas (see chapter 7.4) or the assessment of the impact of a specific emission in a specific environment or territory.

In these cases, even if the general structure of the procedure remains the same, standard approaches (indicator species, testing procedures, environmental scenarios) may not be suitable and ad hoc procedures, representative for the site-specific conditions, may be developed.

The approach described refers to the preliminary risk assessment performed for general, regulatory purposes. Nevertheless, a risk assessment could be needed for more site-specific conditions, such as the protection of particularly valuables areas (see chapter 7.4) or the assessment of the impact of a specific emission in a specific environment or territory.

In these cases, even if the general structure of the procedure remains the same, standard approaches (indicator species, testing procedures, environmental scenarios) may not be suitable and ad hoc procedures, representative for the site-specific conditions, may be developed.

9.3.2 Probabilistic Risk Assessment

Introduction

The measures of "risk" that is used in the general framework of risk assessment is a point estimate; usually the quotient of the results of exposure and effects assessment (e.g. PEC/PNEC or a TER). These quotients are, however, not true risk levels. Risk is generally defined in the scientific community as the magnitude of an "impact" times the probability that this impact will actually occur (e.g. in the case of flying by aeroplane, risk is the number of expected casualties times the frequency of a plane crash). Unfortunately, we cannot usually define a risk in this strict sense in chemical risk assessment because the probabilities are not routinely quantified, but first of all, because impacts are not properly defined. We can only indicate how many times a certain "(no-)effect level" is exceeded at a certain exposure level. Because the dose-response relationship for the protection targets is unknown, the absolute magnitude of the risk quotient cannot be interpreted and chemicals cannot be properly compared on this basis. For ecosystems, our scientific knowledge is still too limited to predict the nature and the extent of the impacts that chemicals may have (Power & McCarthy, 1997). Furthermore, probabilities are not quantified; the risk assessors rely on deterministic point estimates. These point estimates may be efficient in a first stage to focus on the most important contaminants and emission sources (Bartell, 1996) but the disadvantages are numerous. It is impossible to determine where the point estimate lies in the range of possibilities, the point estimate gives a false sense of accuracy and it ignores variability in the population (see detailed discussion in (Thompson & Graham, 1996). In the scientific community, it is broadly accepted as a necessity to provide confidence intervals when presenting secondary data. As a logical consequence, uncertainty analysis is broadly accepted as a necessity when presenting model results in a scientific manner. The risk manager, however, has to deal with the legal aspects and a decision must be reached within certain time constraints. A series of probability distributions, although very scientific, does not seem to be an obvious help in this process.

Risk assessments in the initial or screening stages are performed with relatively small data sets. This implies that the results from such an assessment must be accompanied by a fair amount of uncertainty caused by measurement errors and lack of knowledge. Examples are the use of QSAR estimates instead of measured data (e.g. in partition coefficients, degradation rates or bioaccumulation factors) and the extrapolation of laboratory effects to field populations or ecosystems. These sources of uncertainty can, in principle, be diminished by further research in a more refined risk assessment. Another source of uncertainty is the natural variability of the environment and the organisms. In contrast with the previous source, variability cannot be decreased by research, it can only be characterised more accurately. The influence of uncertainty and variability should, however, not be mixed but must be considered separately (Hoffman & Hammonds, 1994). A pragmatic and transparent approach to visualise variability is to perform calculations for alternative plausible scenarios (e.g. see Jager et al., 2000). There are more sources of uncertainty in risk assessment which are usually ignored as they are extremely difficult to quantify. A model is a simplification of reality and the simplifications themselves also constitute a source of uncertainty. Furthermore, the decisions about the system and situation to be modelled (e.g. the selection of the protection targets) can be considered a source of uncertainty.

Quantitative uncertainty analysis

Up till now, risk assessors have reacted upon this sense of uncertainty by introducing worst-case assumptions in the methodology. This is a potentially dangerous situation as a multiplication of worst cases could eventually lead to unrealistic assessments which is neither transparent, nor efficient when it is inducing unnecessary further testing or risk reduction measures. At least from a scientific point of view, it is advisable to quantify this uncertainty and take it explicitly into account in the decision-making process. Quantitative uncertainty analysis is a tool to deal with uncertainties in a more systematic manner. There are numerous studies where the power of quantitative uncertainty analysis is demonstrated in the chemical risk assessment domain (e.g. (McKone & Ryan, 1989; De Nijs & De Greef, 1992; Traas *et al.*, 1996; Copeland *et al.*, 1993). Nevertheless, the application of uncertainty analysis to decision making is far from routine as virtually all decisions are still based on point estimates of exposure and effects. One of the reasons for the reluctance of regulators to accept probabilistic risk assessment is the lack of proper guidance and policy (Finley *et al.*, 1994) although regulators in the U.S. have already started addressing these issues (see e.g. (EPA, 1997). In the area of pesticide risk assessment, a scientific committee advising the EPA extensively discussed probabilistic methods and how to implement them into regulatory practice (ECOFRAM, 1999). In their recommendations they also conclude that "… the Workgroup recognizes and endorses the tremendous value of probabilistic approaches."

With uncertainty analysis, parameters are not characterised by a single value (point estimate) but by probability distributions. The effect of these distributions on the model's results is calculated, leading to a probability distribution of the risk estimate. Probabilistic methods are well defined mathematically and are well established in other disciplines including physics, chemistry, biology, engineering, economics and finance as their distributions represent reality better than point estimates (see (Burmaster, 1996). Using probabilistic methods is not only closer to the truth but also acknowledges that some chemicals can be assessed with greater confidence than others. All relevant information can be included and sensitivity analysis can be used to identify the main sources of uncertainty, thus offering an efficient way to ask for further testing. A risk manager can then base decisions upon an "acceptable" level of certainty. In this way, the probabilistic approach places the responsibility for determining who should be protected and how much with the risk manager, where it belongs (Thompson & Graham, 1996). In effect, decisions or cut-offs can be based on the costs of errors of type I (rejecting a harmless substance) and type II (accepting a harmful substance) and the expected effects of the chemical (e.g. for an endocrine disruptor one may desire more certainty than for a narcotic compound). It is of utmost importance that the user of the end results of a probabilistic risk assessment is aware of the uncertainties that are accounted for; otherwise, interpretation is impossible. This implies that transparency in the methods and the presentation of the results is very important.

Recommendations

A more scientific and defensible risk assessment in the future will require the tools for a quantification of the uncertainties. However, several issues need to be resolved before uncertainty analysis can be routinely applied to risk assessment:

1. Agreement and guidance on which uncertainties to include in an assessment and which (default) distributions to take for input parameters.
2. Discussion how to implement probabilistic methods into the risk assessment scheme (e.g. how to combine distributions for effects and exposure or which percentiles to take from a distribution) (e.g. see (Jager *et al.*, 1997; ECOFRAM, 1999).
3. Probabilistic risk assessment requires more effort from the risk assessors and the risk manager. They have to familiarise themselves with stochastic variables and equations.
4. Example risk assessments will help to increase the familiarity with these methods (e.g. see (Jager *et al.*, 2000). As guidance is lacking, risk assessors must be advised to attempt a probabilistic risk assessment, alongside a standard deterministic one, to gain experience with these methods and to start the discussion on harmonisation.

Not only do probabilistic methods allow for a more scientific approach but also a more transparent risk assessment, clearer risk communication, use of all available data, and last but not least, the possibility to identify the main sources of uncertainty (which are likely candidates for further testing). Of course, uncertainty analysis is not a topic specific for the terrestrial compartment and activities have to be harmonised with the developments in aquatic and human health risk assessment.

The effects assessment remains a critical stage when we want a probabilistic risk framework that is scientifically justifiable (Jager *et al.*, 1997). The uncertainties in this part need to be addressed but the concept of the PNEC or TER forms a problem in itself as no attempt is made to quantify ecosystem damage. Further study in this area is desirable. It must be noted that the use of species-sensitivity distributions (SSD) offers better opportunities than the use of assessment factors to address uncertainties in the effects assessment (see also Chapter 5). The theoretical fraction of species exposed above their NOEC may be interpreted as a kind of risk level and uncertainty in this figure can be quantified (Aldenberg & Jaworska, 2000). However, as a harmonised guideline is lacking at this moment, risk assessors must be encouraged to attempt these methods, next to the standard PNEC derivation, to gain experience.

In principle, uncertainties should be quantified in all tiers of the risk assessment process. However, the level of detail of this analysis can vary with the purpose of the assessment, e.g.:

- Initial tier of generic assessment: focus on uncertainty in chemical-specific parameters and use default distributions for the parameters. This may result in broad risk distributions. When the probability of PEC exceeding PNEC is unacceptable, sensitivity analysis will show where refinement or further testing is required in the next tier. Address variability with some additional scenarios.
- Refined tiers of risk assessment: include a more detailed assessment of variability and more accurate representations of sensitive uncertainty distributions for the specific chemical.
- Quality standards: a fixed value is probably preferable to a distribution for a quality standard. However, consideration of the uncertainty can be incorporated in a fixed standard; e.g. by selecting a low percentile from the distribution.

9.3.3 Comparative risk assessment

General tools for preliminary risk assessment

The criteria used to decide the acceptability of environmental risks are generally based on the concept of toxicity-exposure ratio (TER). This ratio should be calculated for each of the environmental compartments at risk (ground water, surface water, soil) so as to choose critical thresholds as triggers for the need of further information. TERs may also be used for making comparisons with appropriate "safety factors" representing the acceptable limit for the different components of the environment in terms of risk.
A different approach that has often been utilised is the ranking of chemicals in terms of their environmental hazard by prespecified criteria. In general, the proposed systems (Sampaolo & Binetti, 1986; Swanson & Socha, 1997) are based on a development of a score for a set of physico-chemical, toxicological and ecotoxicological properties of the substances considered. The scores are then combined through an algorithm in order to obtain a numerical index useful for comparative purposes.
Many examples of risk indexes have been proposed in the literature (CLM, 1999).
In particular, comparative indexes for pesticide risk, specific for the terrestrial environment, have been proposed by Finizio et al. (2000).
The indexes are fully based on the information required by annex VI of Directive 414/91/EEC for placing plant protection products on the market (Uniform principles). Different indexes are developed for the terrestrial hypogean and epygean systems. For each system two different time-space scales are considered. The short term at local scale indexes are referred to a risk posed by a pesticide immediately after a treatment to the three different systems. On the contrary, other indexes, in a broader time-space scale context, are finalised to evaluate the pesticide impact in a medium period and in a wider area than the treated one.
The indexes are based on exposure indicators (rate of application, environmental distribution, bioaccumulation and soil persistence) and on the effects (i.e. EC_{50}, NOEL) that these substances can exert on non-target organisms assumed as representative of the environmental systems, according to Directive 414/91/EEC.

As a general procedure for the development of the indexes, a Predicted Environmental Concentration (PEC) is calculated using simple dilution models or more complex models.
Once a PEC is obtained, TER's are calculated using toxicity data for the selected bioindicators. To each TER value a subscore is assigned, that is weighted in function of its role, arbitrarily determined, in the overall risk evaluation and then combined by means of algorithms to get a single synthetic score.

Examples of indexes developed for the risk assessment of pesticides for the terrestrial environment are reported in the appendix

Comparative risk assessment on a local, site-specific, basis

Risk indexes like those described above are a useful tool for preliminary comparative approaches without any reference to local site-specific scenarios.

Comparable approaches could be used on a local basis if realistic environmental scenarios are developed.
These scenarios are particularly relevant for exposure assessment in order to calculate more realistic PEC. The type of information needed for developing suitable scenarios depends on the procedure adopted for the assessment of exposure (i. e. quality and quantity of input data required by exposure models. As a general rule, data required are:

- land use
- meteorological data
- soil characteristics
- water and air balance
- characterisation of chemical emissions (quantity, time, point or diffuse, etc.).

Moreover, a characterisation of the potentially exposed biological systems should be needed (vulnerability, quality, ecological relevance, etc.).

A compromise between a general risk index and a site-specific assessment could be the development of realistic regional scenarios, applicable, as a worst case, to different geographic areas. An example of this approach is the proposal of the FOCUS (FOrum for the Coordination of pesticide fate models and their USe) group (FOCUS, 2000). The group developed nine different scenarios, from South to North Europe, suitable for the application of leaching models for the prediction of groundwater pesticide contamination. At present, comparable scenarios are not available for terrestrial ecosystem risk assessment.

More detailed approaches could be developed for mapping the risk on the territory by using Geographical Information Systems (GIS) for the description of the variability of environmental conditions (Calliera et al., 1999).
The steps of the procedure are the following

- collection and inclusion in a GIS of data needed for the description of the territory;
- identification of Uniform Geographic Units (UGU) assumed as homogeneous in function of data required by exposure models;
- collection of physico-chemical and ecotoxicological data of the chemicals;
- application of suitable models and calculation of PEC for the different UGUs;
- application of a hazard index based on PECs and ecotoxicological data;
- collection and inclusion in a GIS of data for the characterisation of ecosystems and development of an Ecosystem Sensitivity Index (ESI);
- mapping the risk on the basis of hazard index and ESI.

A scheme of the procedure is shown in figure 3. This kind of approach is still being applied for mapping the risk for surface waters and is under development for the terrestrial ecosystem.
Among the three main boxes indicating input data, “Ecosystem characterisation” is, at present, less clearly defined and some more work is required to define suitable criteria for the terrestrial environment. As a general rule, the following steps could be proposed:

- to define the main ecosystems present in a given territory (woods, grassland, hedges, river banks, etc.), mapping their distribution and extension;
- to characterise the main features of the biological community for each ecosystem and to define the relevance and the mobility of some key populations (birds, pollinator insects, etc.);

- to assess the level of protection that should be attained on the basis of many factors describing the present status of the system (vulnerability, level of “naturality”, alterations due to human impact, naturalistic value, etc.);
- to integrate all this information in order to develop an ESI.

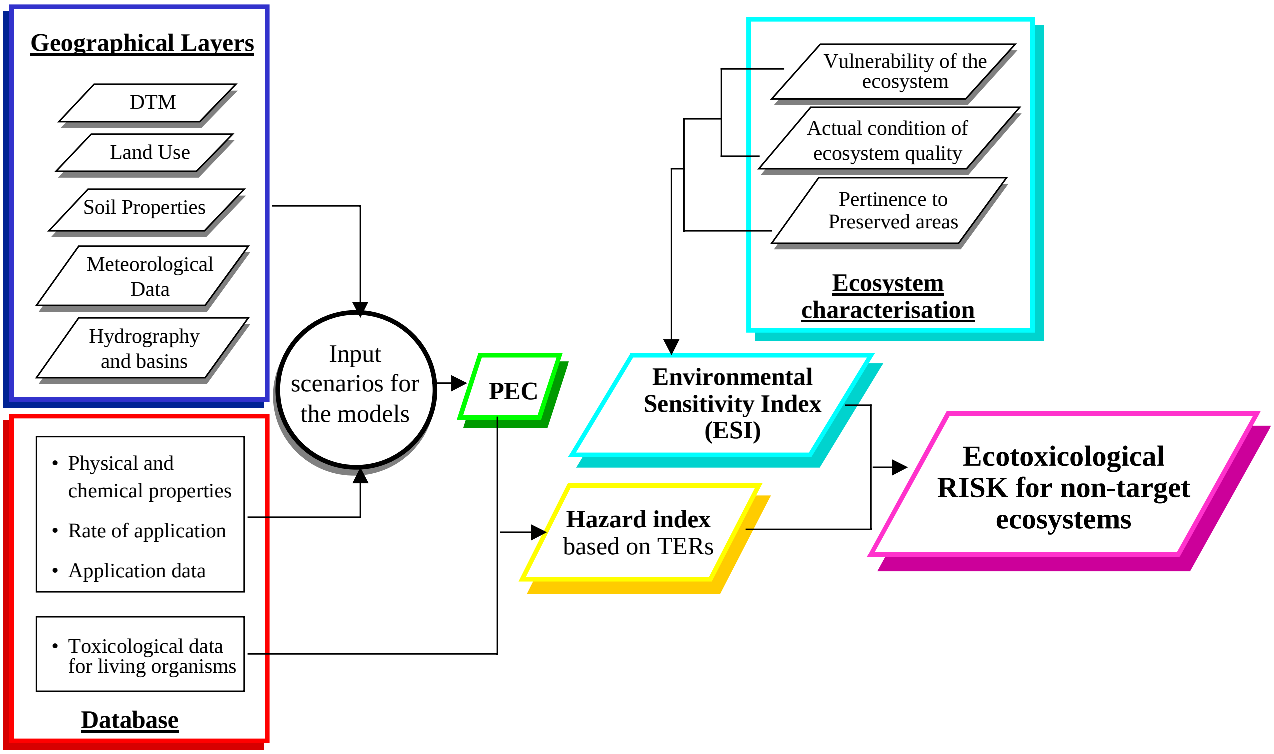

Figure 3. Scheme for mapping pesticide risk for non-target ecosystems on the territory.

APPENDIX

Examples of risk indexes developed for the terrestrial ecosystem (from Finizio et al. 2000)

Short Term Pesticides Risk Index for the Hypogean Soil System (PRIHS-1)
This index refers to evaluating the effects on non-target organisms with hypogean habits immediately after a pesticide treatment.
PEC is calculated assuming that the product spreads uniformly on a surface of 1 ha and on a layer of 5 cm.
As non-target organisms representative of the hypogean system, earthworms, beneficial arthropods and mammals have been selected among terrestrial test organisms indicated by the Uniform principles.
Table 1 shows the scores and weights assigned to the different intervals of categories in which the possible TER values have been subdivided.

The final score of the chemical, ranging from 0 to 100, can be obtained by means of the following algorithm:

$$\text{PRIHS-1} = (A \times 5.5) + (B \times 5) + (C \times 2) \qquad (2)$$

Table 1 – PRIHS-1: TER categories with relative scores and weight for non-target organisms representative of the hypogean soil system

Earthworms (A)		**Beneficial Arthropods (B)**		**Mammals (C)**	
(EC50/PEC)	**SCORE**	**% EFFECT (MRA)**	**SCORE**	**(LD50cut./PEC)**	SCORE
>1000	0	(2xMRA) = 0%	0	>1000	0
1000 – 100	1	0% <MRA <30%	2	1000 – 100	1
100 – 10	2	MRA >30%	4	10 – 100	2
10 – 1	4	(0.5xMRA)>30%	8	10 – 1	4
<1	8			<1	8
	W = 5.5		**W = 5**		**W = 2**

Long Term Pesticides Risk Index for the Hypogean Soil System (PRIHS-2)
Unlike the previous index, in this case it must be considered also the period of time and the persistence of the substance. Then the PEC is calculated as follows:

$$PEC_{LT} = PEC_{ST}\,(1 - e^{-kt}) / kt \qquad (3)$$

where:
PEC_{LT} = Predicted Environmental Concentration in the soil after a certain period of time;
PEC_{ST} = Predicted Environmental Concentration immediately after the treatment (cf. previous index);

t = period of time considered for the assessment, usually equivalent to the duration of the toxicological test (i.e. 14d for earthworms, 730d for mammals).
k = ln2/DT50
Among relevant organisms, microorganisms have been included assuming that their role is higher in the long term.

Table 2 reports the scores and weights assigned to the different intervals of categories in which the possible TER values (or effects levels) have been subdivided.
The final score of the chemical can be obtained by means of the following algorithm:

$$\text{PRIHS-2} = (A \times 4) + (B \times 5) + (C \times 2)\ (D \times 1.5) \quad (4)$$

Table 2 – PRIHS-2: TER categories with relative scores and weight for non-target organisms representative of the hypogean soil system

Earthworms (A)		**Micro-organisms (B)**		Beneficial Arthropods (C)		**Mammals (D)**	
(NOEC/PEC) (14d)	SCORE	**% EFFECT**	**SCORE**	**% EFFECT**	**SCORE**	**(NOEL/CD) (2 years)**	**SCORE**
>1000	0	(2xMRA) = 0%	0	(2xMRA) = 0%	0	>1000	0
1000 – 100	1	0% <MRA <25%	2	0% <MRA <30%	2	1000 – 100	1
100 – 10	2	MRA >25%	4	MRA >30%	4	10 – 100	2
10 – 1	4	(0.5xMRA)>25%	8	(0.5xMRA)>30%	8	10 – 1	4
<1	8					<1	8
	W = 4		**W = 4**		**W = 3**		**W = 1.5**

Short Term Pesticides Risk Index for the Epygean Soil System (PRIES-1)
The index should allow evaluating the risk of epygean non-target organisms immediately after a pesticide treatment.

Table 3 reports the risk classification interval for the selected non-target organisms together with their relative scores and weights for calculation of PRIES-1 index.

Table 3 – PRIES-1: Risk classification intervals, scores and weight for epygean non-target organisms

Bees (A)		**Birds (B)**		**Beneficial Arthropods (C)**		**Mammals (D)**	
(HQ)	SCORE	**(LD50/TDI)**	**SCORE**	**% EFFECT**	**SCORE**	**(LD50/TDI)**	**SCORE**
<0,1	0	>1000	0	(2xDMA) = 0%	0	>1000	0
1 – 0,1	1	1000 – 100	1	0% <DMA <30%	2	1000 – 100	1
10 - 1	2	100 – 10	2	DMA >30%	4	10 – 100	2
100 - 10	4	10 – 1	4	(0.5xDMA)>30%	8	10 – 1	4
>100	8	<1	8			<1	8
	W = 5		**W = 4**		**W = 2**		**W = 1.5**

The final score is obtained as follows:

$$\text{PRIES-1} = (A \times 5) + (B \times 4) + (C \times 2) + (D \times 1.5) \quad (5)$$

Long Term Pesticides Risk Index for the Epygean Soil System (PRIES-2)
The index evaluates the risk for the epygean soil system when a wider time-space scale is considered. In relation to the variability of possible environmental scenarios, a PEC cannot be calculated, then this index is of qualitative nature due to impossibility to obtain a more quantitative TER. Scores are assigned to a number of exposure and effect selected parameters (Tables 4 and 5). As exposure parameters, besides application rate,

persistence and bioconcentration potential (expressed as log Kow), the affinity for the soil and air compartment expressed as percent distribution calculated by means of the standard Fugacity Level I model (Mackay, 1991).

Among the relevant organisms, plant, not included in PRIES-1, has been added. It has been assumed that, in the treated area, crop is not affected (by definition of a plant protection product), while outside the treated area, an effect on other plant species is likely to occur.

Toxicity and exposure parameters are then combined through an algorithm (eq. 6) for the final calculation of the index:

$$\text{PRIES-2} = \left(\frac{\sum_{i=1}^{5} Ti}{5}\right) \text{ x } \frac{(A+S)}{2} \text{ x B x P x MRA} \qquad (6)$$

Table 4 – PRIES-2: Scores assigned to the exposure parameters

Persistence (P)		Bioaccumulation **(B)**		**Air Affinity (A)** **Fugacity Level I**		**Soil Affinity (S)** **Fugacity Level I**		**Application Rate (MRA)**	
DT50 (d)	**SCORE**	**(log Kow)**	**SCORE**	**%**	**SCORE**	**%**	**SCORE**	**g/ha**	**SCORE**
<10	1	<2,5	1	<0,01	1	<1	1	<10	0,5
10 – 30	1.5	2,5 - 3,5	1.1	0,01 - 1	1,5	1 - 10	1,5	10 – 100	1
>30	2	>3,5	1,25	1 - 10	2	10 -30	2	100 – 500	2
				>10	2,5	>30	2,5	500 – 1000	3
								>1000	4

Table 5 – PRIES-2: Scores assigned to the effect parameters

Plants (T1)		**Bees (T2)**		**Beneficial Arthropods (T3)**		**Birds (T4)**		**Mammals (T5)**	
FITOT.	**SCORE**	**NOEL (µg/bee)**	**SCORE**	**NOEL (g/ha)**	**SCORE**	**NOEL (mg/Kg diet)**	**SCORE**	**NOEL (mg/Kg diet)**	**SCORE**
+	4	<0.1	4	<10	4	<0.1	4	<0.1	4
-	0	0.1 - 1	3	10 - 100	3	0.1 - 1	3	0.1 - 1	3
		1 – 10	2	100 - 500	2	1 - 10	2	1 - 10	2
		10 – 100	1	500 - 1000	1	10 - 100	1	10 - 100	1
		>100	0.1	>1000	0.1	>100	0.1	>100	0.1

The main problem for a complete application of the indexes concerned the availability of the information on both physical-chemical and toxicological properties of pesticides, in particular for some non-target organisms (i.e. microorganisms, beneficial arthropods).

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Chapter 10

Conclusions and Recommendations

Ecotoxicological Risk Assessment procedures represent the necessary bridge between a descriptive approach of ecosystem quality and the decision-making requirements for the protection of the environment.

There is a large consensus, both in the scientific community and in the regulatory and political community on the usefulness of tools capable of attaining a reasonable compromise between a sound scientific approach and the need for simple, transparent and pragmatic decision-making tools. Nevertheless, many scientists underline some important weaknesses of the commonly used approaches.

Power and Adams (1997) indicated some items considered as relevant within the scientific community to assess value and weakness of ecotoxicological Risk Assessment procedures.

1. Validation. There is a lack of validation approach of the Risk Assessment procedure as a whole, as well as of some specific steps or methods (models, extrapolations, etc.)
2. Objectivity. Ecotoxicological Risk Assessment procedures are generally considered as objective and transparent enough, even if expert subjective judgement is sometimes needed.
3. Management or science. It is generally agreed that a reasonable compromise between management and science is attained.
4. Quantitative method. A lack of quantitative assessment of uncertainties is often recognised.
5. Appropriate use. Generic Ecotoxicological Risk Assessment, even if less precise and not site specific, is considered more versatile and adaptable. Some consider Ecotoxicological Risk Assessment to be suitable at the level of population and community, but not for ecosystems.
6. Limitations. Too many assumptions to be controlled and validated. Difficulty in obtaining basic information (e.g. chemical emission patterns).
7. Ecology. Too simplified to reflect ecological complexity and realism.
8. Major needs. Methodological harmonisation. Validation. Definition of appropriate fields of application. Defining environmental baselines (reference conditions).

This is particularly true for the terrestrial Risk Assessment procedures, which are presently at a level of development and standardisation significantly lower compared to the aquatic environment:

However, the scientific efforts to improve our basic knowledge on the effects of chemicals on terrestrial ecosystems allow an optimistic evaluation of the current and future situations. Efforts have been particularly significant in two specific areas: the development and standardisation of methods for the assessment of the fate, behaviour and toxicity of chemicals in the terrestrial compartment, and in the development of protocols for the risk analysis of specific groups of chemicals such as industrial chemicals including metals, pesticides, etc.

According to the current state of the art, summarised in this opinion, the CSTEE considers that although additional efforts are required, the available information is sufficient to develop scientifically sound criteria for the hazard and risk assessment of the potential effects of chemicals on terrestrial ecosystems.

Although the conditions for the environmental releases of different chemicals are obviously associated with their particular life cycle conditions (production-formulation-use-disposal, etc.), it should be recognised than once a chemical has reached certain environmental compartments, the observed adverse changes will be related to the concentration achieved and the physical-chemical and toxicological properties, not to the chemical type or use. Therefore, a similar scientific basis should be applied to the different legislation related to the hazard and risk assessment of different types of chemicals.

In order to achieve the required transparency in the process, harmonisation of the use of scientific principles in different legislation and technical guidance documents is needed. This harmonisation does not necessarily imply the use of the same structure or the same Margins of Safety in all legislation. Each piece of legislation has specific connotations which should be considered. In addition, risk analysis is mostly considered as a tiered approach and the Margins of Safety should be adapted to the uncertainty associated with each tier inside the assessment. Nevertheless, when different criteria are applied, a clarification on the rationale for such differences, and particularly if they correspond to scientific (i.e., uncertainty) or social-political issues (social concern; application of the Precautionary Principle), is required.

Coherence is regarded as a basic principle for legislation. Considering that technical guidance supporting the different EU legislation on hazard and risk assessment of chemicals in Europe is currently under revision, and given the complexity of the terrestrial environment, instruments to guarantee coherence in the use of scientific information throughout the legislation should be established.

The following general recommendations are expected to facilitate this process:

1. The final goal for the environmental hazard and risk assessment must be the evaluation of ecosystem effects. Effects on the soil compartment are only part of the expected effects and other receptors (plants, ground and foliar invertebrates, terrestrial vertebrates) should be considered in the assessment.
2. This recommendation applies for all legislation, even legislation related to a single compartment, such as those producing maximum acceptable levels for soil or air. For example, a soil ecotoxicological threshold must guarantee the protection not

only of the soil community, but also of the terrestrial system associated with the soil which can be exposed, for example, via food chain transfer.

3. Therefore, the evaluation of the terrestrial compartment requires the consideration of several hazards, corresponding to the combination of exposure routes and relevant ecological receptors. Although for pragmatic reasons it can be justified to conduct parallel assessment for each hazard or hazard type, a common and harmonic rationale for setting acceptability criteria should always be considered.
4. Similarly, the connections between the terrestrial and aquatic systems should also be considered, in terms of both transfer of the chemical and ecological relationships.
5. The relevance of the different effect assessment tools and protocols should be assessed establishing programmes for the comparison of results from laboratory, semi-field and field studies.
6. A much higher integration between the legislation related to the control and assessment of the environmental effects of chemicals and that related to the protection of biodiversity is needed. When required, the risk analysis should consider the specific protection of endangered species and areas of high ecological value.
7. A further research effort of ecotoxicology and environmental chemistry in the terrestrial area is required to support the balanced use of scientific information in the decision-making process.
8. Tiered approaches are considered suitable for achieving a proper equilibrium between scientific information, uncertainty and precaution. Tiered protocols, commonly used in risk analysis, can also be valuable for hazard identification.

Specific recommendations

Hazard Identification

The complexity of the biotic and abiotic relationships within terrestrial ecosystems makes hazard identification a key issue for both hazard and risk assessment exercises. The assessment of relevant effects for terrestrial ecosystems can only be achieved by the identification of a set of relevant hazard types which should be considered. Nevertheless, the need for listing relevant hazard types should be understood as a part of an analysis to identify the different types of effects that are damaging to the ecosystem as a whole. Any potential hazard type represents, in reality, a potential effect on the structure and/or functioning of the whole ecosystem. Therefore, the use of different hazard types for the terrestrial effect assessment should not be interpreted as establishing different protection goals. Ecosystem protection can only be achieved protecting all relevant hazards.

Transparent protocols should be developed in order to identify which potential hazards are relevant for each chemical. Relevant hazard types are represented by a combination of exposure routes (soil, air, food) and taxonomic groups (micro-organisms, plants, invertebrates, vertebrates).

A tiered approach is suggested. Hazard identification should be initially based on basic intrinsic properties of the chemical such as toxicity for different taxonomic groups, persistence in different environmental compartments and potential for bioaccumulation. This initial assessment should be able to identify all potential hazard types that could be potentially relevant for each particular chemical assuming that it is able to reach ecological receptors. At a higher tier level additional aspects should be considered in order to identify which ecological receptors are really expected to be reached.

- For hazard assessment applications, such as classification or quality criteria development, the information related to the exposure potential is restricted to some intrinsic properties which give information about the expected fate and behaviour in the environment. The usual parameters inform on the persistence and bioaccumultion potential using standardised assessments (e.g. biodegradation and the bioaccumulation factors). Others can inform on the likelihood of specific routes of exposure (i.e., a very fast degradation in air indicates that atmospheric deposition is not expected to be of concern; a high metabolic rate in vertebrates indicates that food chain biomagnification is not expected) allowing the identification of certain hazards which are of low relevance for that particular chemical.
- In risk assessment additional exposure conditions, related to the use pattern and life-cycle assessment of the substance can also be included in the higher tier evaluation. Therefore certain potential hazard types can be identified as of low relevance when the chemical is not likely expected to reach the ecological receptors relevant for these hazard types.

These general suggestions can be split into the following specific recommendations:

1. The scoring (classification) of the potential danger of chemicals to terrestrial ecosystems should be established as a combination of a set of different hazard types (constructed on the basis of toxicity to different organisms modulated by persistence and bioaccumulation potential. Harmonisation among the criteria selected for each terrestrial hazard type and between terrestrial and aquatic compartments is regarded as an essential issue. In a second step some hazard types initially identified as potentially significant could be regarded as not relevant due to additional properties related to the environmental fate and behaviour of the chemical.
2. Quality objectives should be derived for each compartment after a proper hazard identification. A fixed minimum level of protection and certainty cannot be recommended by statistical / mathematical considerations but has to be agreed politically when deriving quality criteria. The transfer of the chemical among compartments (soil-air, soil-biota, soil-water, air-soil, air-biota, biota-biota, ...) should be included in the hazard identification. The ecological quality objectives established for each specific compartment (soil, air, water) should consider not only the biotic community of this compartment, but also the other relevant taxonomic groups potentially exposed through direct and indirect routes.
3. In risk assessment, the identification of the potentially relevant terrestrial hazards should be based on both the properties of the chemical and the likelihood for the different exposure routes. Relevance should be decided after comparisons between toxicity and exposure likelihood. Low exposure likelihood in not equivalent to low environmental concentration level. The establishment of fixed exposure thresholds (concentrations below which no risk is assumed) without considering the toxicity of the chemical is not acceptable on scientific grounds.

Effect assessment tools

Some key recommendations to improve the existing effect assessment methods can be taken from the words written above. Special attention will be given not to the existing and recognized official national or international guidelines, but to those protocols or approaches that can have a key role in an integrated test strategy for the soil medium.

1. *New laboratory test guidelines using more species and embracing more trophic levels and life-history strategies*.

On several groups, the following is required:

• Microorganisms – More data is needed in order to elect a cost effective and sensitive test battery. Relevance should be given to nitrogen mineralization methods and to those that can describe the physiological status and structure of the microbial community.

• Plants – Concentrate efforts on the development of plant generation guidelines.

• Soil invertebrates – Improvements and standardization is required on existing protocols with soil nematodes, isopods and oribatid mites (on the saprophagous group) and on gamasid mites and carabid beetle larvae (representing predators).

2. *Bioaccumulation studies and guidelines using terrestrial organisms*.

• Further development is needed on existing bioaccumulation test protocols using plants, enchytraeids, earthworms and isopods in order to develop guidelines using these organisms

• Moreover, issues like bioavailability of pollutants, effects of ageing on these parameters and the generalization of the Equilibrium Partitioning Theory need to be investigated urgently.

3. *Multispecies tests.*

• Standarisation and validation is needed on existing mesocosm tests. Efforts should be concentrated on a more realistic mesocosm type with the possibility to measure both fate and effect parameters (e.g., TMEs).

• Further development of field tests using communities of soil organisms. Efforts should be concentrated on the experimental design and on data analysis, using up-to-date statistical methods to detect changes in community structure induced by the chemicals (e.g., newly developed multivariate methods).

• Not necessarily a multispecies test, *in situ* tests in soil ecotoxicology need further development. Similarly to what is happening in aquatic ecotoxicology, where *in situ* testing is gaining importance every day, also for the terrestrial environment, these tests can give important information at individual and population level. The work done so far with earthworms and isopods needs to be continued and the use of other organisms should be encouraged.

4. *Guidelines on methods related to soil processes.*

• Already quite often used, existing proposals for litter decomposition tests need to be formulated as a test guideline (an issue which is urgent due to the fact that such tests can already required as part of the registration process for pesticides in the European Union). The minutes of two workshops, held under the auspices of the BBA in Germany, could be used as a starting point (Kula and Guske, 2000). Regarding litter decomposition, and in order to achieve standardization and to overcome some drawbacks of the method, efforts should be concentrated on methodological aspects (e.g., proper decomposing material and packaging).

In addition, the usefulness of bait-lamina tests as an addition or alternative to the long-lasting decomposition studies should be investigated, especially for screening purposes.

5. *Development of strategies and guidelines to evaluate the ecotoxicological potential of contaminated soils*

• Besides the use of *in situ* tests, the adaptation of existing guidelines to be used with natural soils is urgent.

• Under this last point, efforts should be concentrated on the adaptation and validation of existing guidelines/protocols using several soil types (a European gradient should be appropriate; e.g. soil series comparable to the EURO-Soils (Gawlik and Muntau 1999) identified for fate tests could be used for this purpose).

• Also, special emphasis should be given to the definition and the use of the most appropriate control, since, in most cases, a non-contaminated soil with the same properties as the soil to be assessed can not be found

• Finally, the strategy for testing individual chemicals briefly outlined in the last point should be adapted for soil quality assessment, taking especially the low usefulness of acute tests in the latter case into consideration (due to their low sensitivity). This also includes the relationship between laboratory tests and site-specific methods like field tests and community approaches.

From the specific perspective of improving toxicity tests, three main issues can be identified as priority needs:

1. Development of a sensitive test battery to cover effects on soil quality, combining effects on soil dwelling organisms and soil processes.
2. Establishment of criteria for the appropriate use of the vertebrate bioassays required for the human health assessment as part of the ecological risk assessment, including the incorporation, if required of additional end-points of ecological relevance. <u>The assessment of ecological relevance should also be required for specific issues such as endocrine disrupting chemicals. Specific recommendations regarding adequation of mammalian toxicity tests have already been produced by the CSTEE.</u>
3. Special attention should be given to the development of bioassays on invertebrates and plants using non-soil exposures and the identification of relevant end-points for these hazards.

Use of assessment factor approaches

Assessment factors are usually not based on thorough ecotoxicological argumentations but on precautionary principles and mathematical approaches. Some of the assumptions are at least debatable such as the fixed ratio between acute and chronic effect concentrations and the prerequisite that protection of the most sensitive species will also protect the ecosystem structure and function.

However, due to the clear advantages of assessment factor approaches which are transparency, the obvious arbitrariness, the ease-of-use and lack of pretension, it is recommended to use such methods especially in the case of limited numbers of effect data sets.

Use of distribution based approaches

Advantages of the distribution based methods are multiple: the distribution methods make use of all available data and not just the lowest NOEC-value; large differences in the sensitivity in the tested species give rise to a lower, precautionary, PNEC; the use of statistics makes calculations of confidence intervals around the PNEC possible.

However, disadvantages cannot be neglected such as the rather arbitrary form of distribution (log-logistic, log-normal, etc.), which will especially affect the estimates in the tails of the distributions. Generally, there is insufficient data available to estimate a reliable distribution and the use of eloquent statistics may suggest a greater degree of accuracy than warranted.

More work is needed in this area before it can be applied in regular risk assessment. Nevertheless, these approaches are promising in the sense that they attempt to implement scientific knowledge in the effects assessment of chemicals.

It is recommended to – at least in addition to the assessment factor approaches – apply the distribution-based approaches. A strong recommendation for the minimum requirements with respect to the size and the composition of the data set cannot be given, but the CSTEE recommends to calculate the confidence intervals of the calculated PNECs and advises decision-makers using these methods to be aware of potential bias caused by the over-representation of certain taxonomic groups.

Use of NOEC-values

The NOEC has properties which are undesirable. For example, the NOEC depends strongly on the selected test concentrations as it has to be a tested concentration. This also means that there is no way to provide a confidence level for the NOEC. The less accurate the test (smaller sample sizes, high variation), the higher the resulting NOEC.

In view of its disadvantages, the concept of the NOEC needs at least re-evaluation. The NOEC is, however, firmly anchored in existing test protocols and regulatory frameworks. A discussion on the most appropriate (or most acceptable) alternative is needed and current test protocols need to be adapted. The CSTEE is aware of the ongoing efforts at different levels to consider the replacement of NOEC/NOAEL by more appropriate endpoints in both toxicity and ecotoxicity tests and encourages this debate.

Calibration of extrapolation approaches

For the aquatic system, the (numerical) validity of sensitivity distributions has been indicated. For the terrestrial ecosystem a final and valid comparison of extrapolated laboratory data and NOEC values derived from field or semi-field experiments still has to be performed systematically: recent comparisons are either based on aquatic test results or on terrestrial laboratory and field tests which are performed under different aspects but not under the common objective to calibrate extrapolation methods. Thus, test series should be designed comprising laboratory, microcosm and field studies and also considering the same – or at least comparable – test organisms, endpoints and application rates.

Risk assessment and management of environmental catastrophes

Risk assessment can significantly contribute to the management of environmental catastrophes involving the emission of toxic chemicals and/or wastes into the environment, such as accidents of industrial or mining installations, transport accidents, etc. Due to the particularities of these events and the need for very rapid decision making, specific protocols for the risk assessment and management of major accidents should be implemented. The CSTEE considers it essential to cover environmental catastrophes with integrated risk assessment schemes, covering simultaneously Human Health and Environmental risks. In addition, environmental catastrophes are not restricted to the terrestrial environment and a holistic approach for aquatic and terrestrial systems is required. Therefore, the scope of these protocols is wider than the aim of this opinion and should be addressed in a further specific action.

Needs for research

All issues related to the hazard and risk assessment of terrestrial ecosystems still require a considerable research effort. Some critical research needs are considered below:

- Acquisition of basic knowledge on the relevance of different taxonomic groups and endpoints for the protection of terrestrial ecosystems. Key issues regarding these basic needs are:
    - Development of protocols for the risk assessment of effects on terrestrial plants from non-soil exposures.
    - Studying the ecological relevance of effects on ground and foliar dwelling invertebrates
    - Use of information obtained from toxicity tests on mammals and setting the ecological relevance of specific effects such as endocrine disruption, teratogenicity and genotoxicity.

- Standardisation and further development of testing protocols and methods. Including three main issues:
    - Standardisation of bioassays on the soil microbial population
    - Evaluating the capacity of the efficacy and sensitivity assays used for testing herbicidal activity as generic effect assessment tools.
    - Development of standardisable cost/effective multispecies systems.

- Development of guidelines for the assessment of potential exposure routes in the terrestrial environment focusing on
    - Standardisation of methods for the evaluation of the fate and behaviour of chemicals in soil and the atmosphere (particularly atmospheric deposition.
    - Selection and development of suitable models for calculating exposure levels in all compartments

  - Development of suitable general and site-specific environmental scenarios

- Development of suitable protocols and conceptual models for the ecological risk assessment of chemicals according to their intended use and disposal conditions, giving priority to:
  - Biocide categories representing a significant emission to soil and/or the atmosphere
  - Pharmaceutical products
  - Industrial products with a significant use by the general public
  - Non biodegradable chemicals with high absorption potential on the sludge of waste-water treatment plants.

- Establishing methodologies to compare the relative relevance of the different hazards in the final level of danger for the terrestrial system. Including:
  - Development of a tiered approach for the identification of potential terrestrial hazards and its implementation in a tiered testing strategy for biocides and notified chemicals.
  - Development of guidance for the use of monitoring data on environmental compartments and biota in the evaluation of existing chemicals.

- Development of basic knowledge on possibilities for handling variability and uncertainty during the risk analysis including the use of probabilistic estimations.

ANNEX.

Ecological soil quality standards.
[Information supporting quality standard derivation]

Several national approaches exist for the setting of environmental standards with respect to ecological soil quality.

Integrated Environmental Quality Standards in The Netherlands

The National Institute of Public Health and the Environment (RIVM) derives Environmental Risk Limits (ERLs) for the protection of ecosystems by commission of the Dutch government. ERLs are based on an analysis of existing ecotoxicity data by RIVM and serve as advisory values to the government which sets the final environmental quality standards (EQS).

Three types of ERLs are used, with different levels of protection that correspond to their use in environmental management:
- the Ecotoxicological Serious Soil Contamination Concentration for soil (Ecotox SCC),
- the Maximum Permissible Concentration (MPC), and
- the Negligible Concentration (NC).

ERLs are determined according to methods that are well documented (Crommentuijn et al., 2000a,b, Sijm et al. 2000, submitted). Below, a brief description is provided on how ERLs are derived, followed by their use in environmental policy. Further details should be taken from these detailed publications.

Deriving ERLs

Toxicity endpoints are selected that may affect species at the population level, in general survival, growth and reproduction. When less than four chronic NOECs are available, fixed assessment factors are used to estimate ERLs. If at least four chronic NOECs are available from four different taxonomic groups, statistical extrapolation is used (Aldenberg & Slob 1993). By describing the NOEC data with a normal distribution of the log NOECs, a so-called Species Sensitivity Distribution (SSD) is obtained that is used in ERL derivation. Special procedures have been developed for
- metals (Crommentuijn et al. 2000b),
- toxic substances expected to be bioaccumulative (i.e. with a logKow of > 3)
- substances that are expected to pose a risk to humans, such as very volatile substances (Sijm et al., 2000).

Ecotox SCC. *The Ecotox SCC represents a level in the soil or groundwater when adverse effects on species diversity threaten both the ecotoxicological functioning and the structure of a soil ecosystem. Serious soil contamination is therefore set at a level where 50% of the species and/or 50% of the microbial and enzymatic processes are possibly threatened, corresponding to the median (50th percentile) of the SSD for the selected toxicity data. Further details can be found in Swartjes (1999).*

MPC. The MPC is supposed to protect all species in ecosystems. Pragmatically, a cut-off value is set at the 5^{th} percentile of the SSD, the Hazardous Concentration for 5% (HC_5) of the species (Aldenberg and Slob, 1993). For soil, an HC_5 for microbial and enzymatic processes is derived in addition to an HC_5 for species. The lower of the two is chosen as the HC_5 for soil.
MPCs are determined for the individual compartments of water, soil, and sediment. To account for intercompartmental exchange processes, harmonisation of ERLs is included.

NC. The NC represents a value causing negligible effects to ecosystems. In contrast to the ECOTOX SCC and the MPC, the NC is not based on a Hazardous Concentration but is derived by dividing the (harmonised) MPC by 100. This factor is applied to take into account the possible combined effects of the many substances encountered in the environment.

Use of ERLs: ecosystem protection.

The Ecotox SCC, the MPC and the NC are derived by RIVM but the final EQS are set by the Dutch government. Table 1 shows the relation between ERLs and EQS. The Intervention Values for soil, groundwater and sediment are based on the lower value of two underlying SCCs: one based on ecotoxicological risk assessment, the other based on human risk assessment.

Table 1. *Environmental Risk Limits (ERLs) and the related Environmental Quality Standards (EQS) that are set by the Dutch government in The Netherlands for the protection of ecosystems.*

ERLs	**EQS**		
	Water	*Sediment*	*Soil/ Groundwater*
ECOTOX SCC*	-	Intervention Value	Intervention Value
MPC*	MPC	MPC	-
NC*	Target Value	Target Value	Target Value

*:
ECOTOX SCC = Ecotoxicological Serious Soil Contamination Concentration,
MPC = Maximum Permissible Concentration
NC = Negligible Concentration
- = Not used (in environmental policy)

When levels in the environment exceed any of the individual EQS, distinct actions follow which will be briefly explained in the following sections.

Intervention Value and Target Value: soil pollution

The Intervention Value (based on the SCC) is used for the risk assessment of historically polluted sites and for curative purposes. When the Intervention Value (IV) for soil or groundwater is exceeded, a potential unacceptable risk to man or the environment is assumed. In principle there is a need for soil clean up but a subsequent

actual risk assessment is required. The actual risk assessment determines the urgency to clean up the site.
Target Values (based on the NC) indicate the soil quality at which the risks of adverse effects are considered to be negligible. In order to prevent unnecessary soil pollution Target Values are embedded in specific regulations.

MPC and Target Value: water and sediment pollution

For water, the MPC should not be exceeded when based on average water quality. When the MPC of a substance is exceeded, the compound is regarded as 'substance of concern', and as such is recommended for regular monitoring in relevant water bodies and/or effluents. The Target Values indicate the final level to be reached in The Netherlands on the longer term, preferably within a decade. A long-term strategy to reach the Target Value is the responsibility of regional authorities, and should be laid down in their water management plans. National or supranational (e.g. EU) policy objectives may provide further boundary conditions for the regional strategies.
For sediment, the EQS include the Intervention Value, the MPC and the Target Value. The MPC and Target Value are used to evaluate the quality of the sediment compartment and are used in the same way as described for the water compartment. In addition, the Intervention Value, MPC and Target Value are embedded in a system to evaluate the classification of the dredging material from harbours to differentiate between different classes of material.

Soil quality standards in Germany:

Soil quality standards are defined in the Federal Soil Protection Act (1998) and Soil Protection Ordinance (1999) as well as in lists of contaminants and values prepared by the single Lands of Germany taking into account special problems of the regions. To give a general overview on the national situation in the following, only the Act and Ordinance are regarded.
The purpose of the Act is to protect or restore the functions of the soil on a permanent sustainable basis. The following soil functions are considered:

1.natural functions
- as a basis for life and a habitat for people, animals, plants and soil organisms,
- as part of natural systems, especially by means of its water and nutrient cycles,
- as a medium for decomposition, balance and restoration as a result of its filtering, buffering and
- substance-converting properties, and especially groundwater protection,

2.functions as an archive of natural and cultural history and

3.functions useful to man as
- a medium that holds deposits of raw materials,
- land for settlement and recreation,
- land for agricultural and silvicultural use,

- land for other economic and public uses, for transport, and for supply, provision and disposal.

Three different values are defined:

Precautionary values: values which, if exceeded, shall normally mean there is reason for that concern for a harmful soil change exists, taking geogenic or wide-spread, settlement-related pollutant concentrations into account.

Trigger values: values which, if exceeded, shall mean that investigation with respect to the individual case in question is required, taking the relevant soil use into account, to determine whether a harmful soil change or site contamination exists

Action values: values which, if exceeded, shall normally signal the presence of a harmful soil change or site contamination, taking the relevant soil use into account, and to mean that measures are required.

Trigger values and action values are defined with respect to soil use (playground, residential area, park and recreational facility, industrial and commercial real properties, agriculture, vegetable garden and grassland) and pathways (soil – human being, soil – useful plant and soil – groundwater). Presently values for selected soil contaminants are defined with respect to the protection of human beings (pathway: soil – human being, soil – useful plant). It is under consideration whether the protection of soil organisms and of nutrient cycles is already included. Data from laboratory and field tests as well as extrapolation methods mentioned in the previous chapters are used.
Precautionary values cannot be differentiated in this way. These values have the objective of maintaining multi-functionality and therefore have to comprise all possible soil uses. A differentiation however is performed with respect to chemical and physical soil properties. For heavy metals different soil types and pH-values are considered. For organic chemicals the values are differentiated with respect to different humic contents (> and < 8 %).

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