Assessment of ecotoxicological effects of organic chemicals in soil

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ABSTRACT

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The trends in the development of laws and regulations for control of environmental pollution in some countries are reviewed. Attention is paid to the establishment of limit values for the content of polluting chemicals in soil by the Dutch authorities, using toxicity data for the environmental hazard estimation. Recently developed methods may lead to a diversification of the limit values for pesticides, resulting in higher values for herbicides as only toxicity data for soil animals are used. The requirements for ecotoxicological data in the registration procedure for pesticides are mentioned. Various aspects of internationally accepted tests for assessing effects of organic chemicals on plants, soil microorganisms and soil invertebrates are reviewed. The use of these tests in the procedures for terrestrial ecotoxicological hazard evaluation is discussed.

Keywords: review, soil pollution, limit values, organic chemicals, pesticides, effects, toxicity tests, plants, soil organisms.

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PREFACE

A literature review on legal criteria for soil pollution was started by W.J.M. Aben, research worker in the project "Organic chemicals in soil pore water and their bioavailability, as criteria for soil contamination" for his introduction in this research field. It was continued by N.W.H. Houx as the project leader, who narrowed the scope to the assessment of (eco)toxicological effects and their use for hazard assessment of soil pollution. Substantial financial support was given by Shell Internationale Petroleum Maatschappij, Den Haag. The project was started at the former Institute for Pesticide Research, Wageningen, at the end of 1987. After the reorganization of Agricultural Research at the end of 1988, the project was continued at DLO The Winand Staring Centre. We owe M. Leistra for his critical comments on this manuscript.

SUMMARY

Public concern about deleterious effects of organic chemicals on man and ecosystems resulted in the development of legal instruments to control proliferation of hazardous chemicals in the environment. Moreover, awareness is growing that possible damage to a healthy environment, caused by production, uses and discharge of wastes, should be an integral part of the cost/benefit evaluation to be made for all man-made products.

National and international laws and regulations dealing with special aspects of environmental pollution are more and more integrated in a coherent framework. Uniform procedures are being developed to set limit values for pollution of soil, water and air caused by emission of chemicals from production processes, purposeful applications or disposal after use. However, criteria and limit values for pollution, based on scientific knowledge, are often lacking.

The recent use by the Dutch authorities of an unvalidated risk assessment method, for setting limit values for "maximally acceptable risk" and "negligible risk" from pollutants in soil and water, and for setting "C-values" in the soil remediation procedure, is discussed. The extrapolation model is based on assumptions on ecosystem functioning and uses toxicity data from single species tests.

Requirements for data in the registration procedure for pesticides are extensive. However, research data on physico-chemical properties of a compound, its behaviour in the environment and its toxicity for a number of species of organisms are insufficient for evaluation of risks for ecosystems. A first need is an estimation of the real exposure of populations of organisms to the widely varying concentrations of the chemical in the field. Further, possible (in)direct effects on relations in ecosystems should be considered. Much of the basic ecological knowledge is missing yet.

Results from single species toxicity tests are often used for assessment of ecotoxicological effects. Only a few tests are accepted internationally. Great national and international effort is put into the development of new tests. However, the fundamental question is raised if it would be better to accept a restricted data set and to concentrate on improving predictive models. The environmental significance of laboratory data should rather be studied more intensively. Recent developments and trends in this research field are discussed.

1 INTRODUCTION

Pollution of the environment by chemicals is a topic in affluent societies, but it has probably more adverse effects on the major and less prosperous part of the world. The chemical revolution in the second part of this century has been beneficial to mankind, but has also its negative aspects. Dispersion of persistent chemicals and industrial wastes was simply considered as being harmless for man and environment. There was a great unconsciousness about detrimental effects of pollution. The price of a healthy environment was not taken into account in the total of product economics.

Already in the late 1950's worried individuals drew attention to unwanted effects of pesticides in the environment. Now highly sophisticated environmentalist groups exert pressure upon politicians to stop production and application of a large number of chemicals, used for so many years, because the risks are considered to be higher than the benefits.

This risk-benefit evaluation is not only economically important for manufacturer and user, but extends also to toxicological risks for man and organisms in the environment, being exposed to these chemicals involuntarily and unintentionally. Attention shifted from economical risks (manufacture, commercialization, profit from application) via toxicological risks for man (individual user and consumer) to risks from carcinogenic, mutagenic and teratogenic properties of chemicals for populations and finally to risks for the environment (threat for wildlife, ecosystems and loss of genetic diversity). This shift was caused by some large and frightening accidents at various places in the world, by better detectability of persistence of chemicals by increasing sophistication of chemical analyses and by growing insight into the toxicological consequences of long-term exposure to chemicals. Ecotoxicology is now recognized as a multidisciplinary science in which environmental chemistry, toxicology and ecology have to be combined.

If ecotoxicological criteria are the basis for an evaluation of risks of pollution, e.g. of soil, then the combined effects of variable exposure of communities of organisms to various chemicals in an actively interacting biogeochemical system have to be considered. Exposure depends on the fate of a chemical in that particular situation. Effects of chemical stress have to be weighed against those of natural stress or stress caused by human activities e.g. farming. Because of the complexity of this field of research no adequate expertise is available yet and gathering enough insight will require an enormous effort. Authorities could not wait to take action against the progressing pollution and tended to use criteria without a toxicological basis in setting limit values for contamination.

In this report a short survey of the successive laws and regulations for control of environmental pollution in some countries is given. Special attention is paid to developments in the establishment of limit values for the concentration of polluting chemicals in soil. Requirements of ecotoxicological data for registration of pesticides are briefly dealt with. Next, various aspects of the development of tests for assessing effects of organic chemicals on soil organisms and their use for terrestrial ecotoxicological risk evaluation will be discussed.

2 LAWS, REGULATIONS AND LIMIT VALUES

Increasing public concern over potentially adverse effects of chemicals on human health and the environment in the early 1960's triggered a host of regulatory activities by authorities. The fear of being involuntarily exposed to pest control chemicals in food and environment and of large scale chemical pollution of water bodies and land sites resulted in increasing political pressure for further development of requirements for registering pesticides and industrial chemicals. There was an urge for regulations to stop further polluting activities and to clean up heavily polluted sites. Most governments reacted with installing a Ministry of Environmental Affairs in the midst of longtime established Ministries of Industry, of Agriculture, of Public Health and of Economic Affairs. In most western countries these administrative departments compiled different regulations, depending on country, matter of concern and circumstantial factors, which were to some degree conflicting with each other and mostly lacking a sound scientific basis.

Laws

Examples of early legislation are the Pesticide Act of 1962 (revised in 1975) and the Surface Water Pollution Act of 1965 in the Netherlands and the Federal Insecticide, Fungicide and Rodenticide Act of 1975 and the Toxic Substances Control Act of 1976 in the U.S.A. Many other countries developed similar laws in that period. Under these laws the U.S. Environmental Protection Agency and comparable bodies or ad hoc working parties in other countries developed regulations and guidelines for registering new chemicals or special guidelines e.g. for pesticides. The enormous efforts of all these official bodies and their supporting scientific working groups resulted in a large number of laws and provisional bills with regulations serving special purposes as registration of new chemicals, prevention of surface or ground water pollution, clean-up of contaminated landsites or control of industrial or agricultural waste proliferation. They employed different criteria and limit values to be met.

Integration of laws and regulations into one framework for environmental protection was prompted by the growing awareness that pollution does not stop, neither at borders between the environmental compartments soil, water and air, nor at borders between states. In the Netherlands soil and groundwater pollution aspects of the Surface Water Pollution Act of 1965, the revised Pesticide Act of 1975, the Provisional Hazardous Substances Act of 1981, the Chemical Waste Material Act of 1983 and the Soil Decontamination Act of 1986 will be introduced into the framework of the Soil Protection Act of 1987. A different approach is followed in Germany, where the Bundes Immissions Schutz Gesetz (Federal Immission Control Act) will regulate all technical requirements for preventing pollution by past, present and future waste disposal under T.A. Abfall (Technical Information on Waste) and for preventing emission of chemicals to air under T.A. Luft.

Regulations
Pressure for international harmonization of requirements and guidelines, e.g. in the European Community (EC) or in the Organization for Economic Cooperation and Development (OECD), is also exerted by internationally active companies that are confronted with special regulations in various countries. Although the necessary legal basis for the European Common Market was founded in 1985, little progress has been made in the past years. Opposite stands taken by the EC's Environmental Directorate and its Agricultural Directorate, and more so by participating member states protecting their own interests, have been counterproductive.

Limit values
The intricacy of political decisions is well illustrated by the establishment of limits for pesticide contamination of soil, based on criteria for the process of making drinking water. The EC limit for individual pesticides in drinking water is based on the analytical chemical detectability (0.1 $\mu g\ dm^{-3}$) of organochloro pesticides around 1975, using gas chromatography with electron capture detection. The reasoning was that drinking water should be free from these man-made chemicals, regardless of their toxicity for man. For many other pesticides this limit of analytical detection is not yet achievable. The total concentration of pesticidal chemicals in drinking water should not exceed 0.5 $\mu g\ dm^{-3}$.

In the EC these limits apply also to both sources for drinking water: surface water and groundwater. In the Netherlands, where shallow groundwater tables occur in large areas, a discussion is going on at what depth these figures should be applied: at 1 m or at 10 m below the soil surface. The limit value for pesticides in soil at the chosen depth can be calculated from the value for ground water via the coefficient for the distribution of a pesticide between soil solids and pore water. Regulations under the Act on Chemical Substances and Products of 1987 in Denmark prohibit registration of pesticides if lysimeter or field tests show, that quantities exceeding 5 g ha^{-1} of the active ingredient and transformation products leach more than 1 m below the soil surface after the recommended applications.

No toxicological criteria for man or environment underlie these thus derived limits for the presence of pesticides in soil. Recently, however, (eco)toxicological criteria became the central point in the environmental quality standardization in the Netherlands. The principle, that all possible functions of soil have to be safeguarded against pollution, focussed attention on ecosystem functions as the most vulnerable. Concepts and procedures, based on effects of chemicals on ecosystems, are being developed for the integration of policies and objectives for the environmental quality for water and soil.

The assumption that the functioning of an ecosystem is safeguarded if the participating structural elements, i.e. the species, are not endangered, resulted in a proposal for a method of risk assessment for soil pollution, the Risico Analyse Bodemverontreiniging (R.A.B.-method) (Van Straalen, & Denneman, 1989). This method is still subject of study and modifications (Van de Meent et al., 1990). For the optimum application of this method, at least five toxicity data relevant on ecosystem level, e.g. the No Observed Effect Concentrations (NOEC) for reproduction, regarding species from at least three different ecosystem functions have to be used. These data are normalised for organic matter and clay contents of the soil. Based on the assumption that sensitivities of different species in an ecosystem for a pollutant are log-logistically distributed, these experimentally determined NOEC values are used to estimate the distribution of the NOEC's for all species in the ecosystem. Finally, the content of a pollutant in soil below which the NOEC distribution contains an arbitrarily chosen percentage (e.g. 5%) of the NOEC values for all species in the ecosystem is calculated as limit value, the Hazardous Concentration 5% (HC5). The reliability of the method decreases rapidly if less than five toxicity data are available. In this case safety factors (5 or 10) are introduced to compensate for the added uncertainty.

The concept of absolute protection of 95% of the species is in sharp contrast with the view by the international "Working Group on Pesticides and Beneficial Organisms" (Samsoe-Petersen, 1990). This Working Group has had almost 20 years of experience with testing pesticides on 18 species of arthropods used in integrated pest control programs. Populations of benificials, e.g. pollinators and predators and parasites of pest organisms, have to be safeguarded against detrimental effects of the pesticides that may still be needed. The Working Group has found that concentrations in field trials, lower than the assessed 50% NOEC for reproduction or predation by benificials in laboratory tests, are harmless for survival of the populations in the field. A reduction of these effect parameters with 80-99% is labeled as moderately harmful.

Soil organisms are subjected to natural stress, e.g. high or low temperature, flooding or drying of soil, anaerobic conditions or insufficient energy supply. Further may stress be caused by accepted human activities, e.g. soil tillage, harvesting, irrigation, use of fertilizers and monoculture cropping systems. Effects of both types of stress on soil organisms may be dramatic. Reductions by non-chemical stress to 10% of the average population occur under normal conditions and this does not seem to influence the functioning of ecosystems. Depressions of microbial functions in soil ranging from 50-99% do often not affect the recovery in 20 - 30 days after the end of a stress situation (Domsch, 1984).

Toxicity data adequate for application of the R.A.B.-method have only been measured for a few chemical substances. For all other substances a modified EPA-procedure (EPA, 1984; Van de Meent et al., 1990), producing indicative values only, can be used with safety factors ranging from 10 to 1000, depending on the quality and quantity of the data. If only measured or estimated acute toxicity data are available, a factor of 1000 is applied. A factor of 100 is applied if data for at least the food chain from algae, crustaceans to fishes can be used. A factor of 10 is applied if measured or estimated data on chronic toxicity or NOEC are available. Estimations via quantitative structure-activity relationships (QSAR) are acceptable.

Both methods are evaluated in a study to provide scientific support for setting quality objectives for water, sediment and soil (Van de Meent et al., 1990) and in a proposal for the 7th update for the C-values, the intervention levels for soil decontamination

(Denneman & Van Gestel, 1990). At contamination levels above the listed C-values, research for the need of soil remediation is obligatory.

The long-term quality objectives of the Dutch authorities for water and soil are "negligible risk" levels set at 1% of the "maximally acceptable risk" levels. This is done to compensate for the additional hazards, due to the potential presence of other toxic chemicals in soil, causing the so-called combination toxicity. This contrasts with the view of the international "Working Group on Pesticides and Beneficial Organisms" (Samsoe-Petersen, 1990) that toxicity tests in the laboratory present a worse case situation. The most susceptible life stages of the species are 100% exposed in laboratory tests, as there are no refuges.

For the "maximally acceptable risk" levels the HC5 is proposed, i.e. the concentration at which maximally 5% of the species in an ecosystem may be affected. As the (eco)toxicological basis for the C-values the HC50 is proposed, i.e. 50% of the species in ecosystems may be affected. However, for almost all organic chemicals studied, the indicative modified EPA-procedure had to be used (Van de Meent et al., 1990), because of lack of (eco)toxicological data. In addition, "maximally acceptable risk" levels for halogenated hydrocarbons and polycyclic aromatic hydrocarbons in sediments and soils were derived from those in water with the Equilibrium Partitioning method (EPA, 1989). With this method the content of a chemical in soil can be calculated from its concentration in water via the (estimated) distribution of the chemical between soil solids and soil solution. However, the uncertainties in the estimated parameters for this method are considerable (Boesten, 1991).

The modified R.A.B.-method and the modified EPA-procedures (Van de Meent et al., 1990) are used to set standards for the integral environmental quality policy for soil, sediment and water (VROM, 1991). The "negligible risk" levels for metals in soil are below the reference levels assessed in soils of (relatively) unpolluted nature conservation areas (Edelman, 1984; VROM, 1988). The setting of long term quality objectives for metals in soil at these reference levels is considered to be realistic, because metals are not degradable and the "negligible risk" level will never be achieved. However, "natural background" levels of metals in these nature conservation areas might have influenced the selection of species in that reference ecosystem by their toxic effects.

Justified by the assumption that most terrestrial organisms are exposed to pollutants via the soil pore water, "negligible risk" levels in soil have been derived from those in water. The long term quality objectives for polycyclic aromatic hydrocarbons (PAH's) in soil are set at the "negligible risk" levels for surface water via the equilibrium partitioning procedure. This is considered to be reasonable, because the few reference values determined for PAH's in soil are below these "negligible risk" levels. As measured coefficients for the equilibrium partitioning between soil and pore water are lacking for most other organic pollutants, estimated equilibrium partitioning data are used.

The "negligible risk" levels based on available NOEC values of lindane and dieldrin for terrestrial organisms, however, are below the "negligible risk" levels for these compounds derived via the equilibrium partitioning method. As the regulations under the Pesticide Act apply to all currently registered pesticides, no adjustments in the environmental quality standards for presently approved pesticides are foreseen. However, for pesticides to be registered in the future "negligible risk" levels, derived from ecotoxicological data will apply. For specific compounds this may result in lower standards for groundwater and soil, than the present ones, even those based on the standards for drinking water.

The use of ecotoxicological risk assessment for soil organisms for the levels of C-values in the Leidraad Bodemsanering (VROM, 1988) resulted in proposed C-values for metals maximally a factor 4 lower than the values in the preceding list. The proposed levels for organic chemicals, including pesticides are between one-fifth and ten times those in the earlier list. The proposed C-values for insecticides lie between 0.3 and 10 mg/kg soil (dry mass) and those for herbicides between 10 and 50 mg/kg soil. The ecotoxicological risk is only part of the risks considered for setting the standards for the C-values. Risks for public health and for proliferation of the contamination are also taken into account.

3 DATA REQUIREMENTS FOR PESTICIDE REGISTRATION

Early requirements for insecticide registration were effective control of insect pests in combination with low toxicity to user, farmworker, consumer and domestic animals. Data from tests with rats or other animals used in mammalian toxicity protocols, multiplied with a safety factor, were perfectly suited to set limits to protect individual humans. After some accidents in the field, fish and game populations had also to be protected. Analogous single species acute toxicity tests with fishes, birds and game mammals served equally well for that purpose. Toxic effects on crop plants, on pollinators and other beneficial insects and finally on microbial processes related to soil fertility had to be prevented to safeguard crop yield. Single species toxicity tests for plants and insects and tests for effects on microbial biomass or microbial activities in soil were developed. All these aspects of side effects of the application of pesticides had direct economical importance.

For protection of the environment against unwanted effects of pesticides, data are required on the physico-chemical properties of a compound, on its fate in the environment and on its toxicity to vertebrate and invertebrate animals and to plants. In particular for the soil compartment, data are required on persistence and leaching of pesticides in soils after their application and on their toxicity to plants and to some relevant non-target species of soil organisms. These data are inadequate for an evaluation of the risk from use of pesticides in agriculture for the terrestrial ecosystem because data on exposure and ecologically relevant effects are lacking.

It is generally accepted that the various functions of soil may not permanently be endangered by pollutants, including pesticides used for crop protection. Ecosystem functions are considered to be the most sensitive ones, but no clear criteria for unacceptable effects on ecosystems have been developed yet. Not only the extent of impairment is an important criterion, but also the time needed for recovery to a well functioning ecosystem, as measured against a matching ecosystem under natural stress, but not exposed to the chemicals (Domsch et al., 1983; Malkomes, 1985). Almost all internationally accepted tests on terrestrial organisms used for assessment of toxicological effects have death as effect parameter, which is ecologically unimportant (OECD, 1989). The OECD test on avian reproduction is the only internationally accepted test with ecotoxicological relevancy. There are only a few other reproduction or life cycle tests in use or under development for national registration purposes. However, route and actual extent of exposure, frequency of applications and combinations of chemicals for crop protection are not incorporated in these tests.

4 ECOTOXICOLOGICAL EFFECTS ASSESSMENT

The OECD Workshop on Ecological Effects Assessment in 1988 (OECD, 1989) was a recent forum for discussions on terrestrial ecotoxicology. Most OECD Member countries use a tier system of increasing complexity for assessment of ecological effects of chemicals, requiring ever more laboratory or field data. Three stages of assessment were discerned:

- The initial or screening stage, based largely on acute effects in single species tests;
- The intermediate stage, including chronic effects in single species tests;
- The comprehensive assessment stage, including life cycle tests and wider ecosystem effects.

Data requirements for effect assessment of pesticides are generally more extensive than those for industrial chemicals.

An important issue at the Workshop (OECD, 1989) was the inadequacy of current assessment procedures to predict the ultimate fate and possible effects of chemicals in the long term. Terrestrial organisms are exposed by air, water, soil and/or food and therefore no single concentration for terrestrial environments can be indicated. Information on persistence in the real environment, environmental transport, bioavailability and bioaccumulation is considered to be vital for predicting exposure. Environmental exposure needs to be better quantified and the uncertainties identified. Effects of long-term exposure are more likely to be seen in higher levels of ecological organisation. Current test methods are qualified as ecotoxicological rather than ecological. They assess primarily dose/effect relations for single species because methods to test effects of real exposure at multispecies or ecosystem levels are lacking. For the development of ecologically significant endpoints an increase in research into biology, comparative physiology and ecology of (terrestrial) biota is inevitable. A scientific basis for extrapolation of test data e.g. from species to species, from species to communities or ecosystems, and from acute to chronic toxicity, is lacking. A fundamental question was raised on the relative merits of ever expanding the existing battery of testing methods, by addition of more endpoints and more species. It would be preferable to accept a restricted data set and to concentrate on improving the accuracy of predictive models and the knowledge of the environmental significance of laboratory data.

Consequent on this workshop (OECD, 1989) a Detailed Review Paper on Terrestrial Ecotoxicology Test Guidelines for the OECD Updating Programme was written (Stavola, 1990), covering the state-of-the-art of terrestrial ecotoxicity testing. In the internationally accepted OECD guidelines only three avian tests, one earthworm and one plant test are mentioned. There are many more toxicity tests in use or under development by the members of the OECD, but the majority needs further research and ring-testing before they finally can be incorporated in guidelines. The Workshop agreed that ecological function, morphological and physiological structure and route of exposure should be considered in choosing suitable test organisms. Moreover, in any battery of ecotoxicological tests, it would be necessary to address the functional integrity of ecosystems as well as the damage by toxic effects to the survival of species.

The present report will be restricted to tests with plants, microflora and soil- dwelling invertebrates. Recent developments in tests for assessment of toxicity of chemicals for non-target terrestrial organisms will be reviewed. A placing of the different tests in a tier system is proposed. The use of basic toxicity data from tests with aquatic organisms for a first screening of chemicals for their ecotoxicological effects in terrestrial environments is discussed.

4.1 Plant testing

The current OECD Guideline 208 (OECD, 1984a) is a test for effects of chemicals on plant growth, originating from pesticide research on phytotoxicity of synthesized compounds. Effects of a single incorporation of chemicals in natural soil on seedling emergence and early stages of growth of a variety of cultivated plants are assessed. This 14 day test is used for pesticide and industrial chemicals and is currently under revision.

Considering the results of discussions in the OECD Workshop and information of researchers of EPA Environmental Research Laboratories and of other affiliations, Stavola (1990) recommended the following considerations for additional testing for effects on plants:
- other routes of exposure, such as by deposition on foliage and by vapour contact;
- a seed germination test, a vegetative vigour or a plant uptake and translocation test;
- a wild plant life-cycle test.

The EPA Office of Pesticide Programs (OPP) and the EPA Office of Toxic Substances (OTS) have protocols for vegetative vigour and early seedling growth after exposure via aerial routes (Stavola, 1990). Air-exposure tests should be considered for OECD guidelines as they seem to represent the more likely routes of exposure of wild plants to chemicals than incorporation into the soil.

Responses of the OECD Member countries (OECD, 1991) to the questionnaire of the Detailed Review Paper (Stavola, 1990) supported exploring the use of other exposure routes. Various suggestions were made for improvement of OECD Guideline 208. However, results of a ring-test in countries of the European Community indicate that the present plant growth test is very insensitive. As a consequence of the results of this ring-test, a higher plant growth test is abandonned in the EC's revised classification directive for chemicals (OECD, 1991).

Effects via aerial routes are always studied during the development of pesticides. These data might be made available by the pesticide industry for better evaluation of risks for the environment from the use of pesticides.

A revised OECD Guideline 208 is, in our opinion, only appropriate for ranking chemicals on a toxicity list for the Initial stage of Screening. There is no need for additional simpler tests for terrestrial plants, e.g. seed germination tests or vegetative vigour tests. However, basic data on acute phytotoxicity might also be derived from the revised OECD Guideline 201, (Multispecies) Algae Growth Inhibition Test (OECD, 1981), providing data for ranking the acute phytotoxicity of chemicals. These basic data might be appropriate for data requirements for both aquatic and terrestrial plants. Requirements for specific data for terrestrial plants for the Initial stage of Screening might then become superfluous. The test on early plant growth in OECD Guideline 208 is then redundant.

A protocol for a life-cycle study with the wild plant Arabidopsis thaliana has been developed and tested for OTS Guidelines (Stavola, 1990). This cruciferous plant grows to full maturity in a hydroponic culture under greenhouse conditions in 45 to 55 days. The test chemical is dosed to a nutrient solution and a number of effects are assessed during plant growth and at harvest. Another life-cycle test with a short life-cycle Brassica rapa cultivar, now under development at the Environmental Research Laboratory at Corvallis, is very promising (Stavola, 1990). This little plant has several advantages over Arabidopsis, e.g. development from seed to mature seed in 36 days. Preliminary data indicate two orders of magnitude higher sensitivity than current guideline tests (Shimabuku et al., 1991). In this Brassica test several effects can be studied, such as those on seed germination, vegetative vigour and reproductive success. The last criterion has ecological significance because of its consequences for the population level. Three OECD Member countries were in favour of the use of this life-cycle test with Brassica (OECD, 1991).

In our opinion a life-cycle test, like the Brassica test, should be positioned in the Intermediate Assessment stage, as it seems to become the most sensitive test in the guidelines. Moreover, it generates ecologically relevant data in only 35 days. However, full account has to be taken of the intricacies of growing plants and applying chemicals in a hydroponic system instead of in soil. This is an obvious example of an unrealistic exposure under unrealistic laboratory conditions for an ecologically relevant organism.

Uptake and translocation of pesticides in food and feed crops is routinely studied during their development for the market. These data might be made available for environmental risk evaluation. If the expected emission of industrial chemicals indicates exposure of plants, then this expensive test with radio-labeled compounds might also be required for industrial chemicals in the Comprehensive Evaluation stage. Presence of xenobiotics in vegetation can have ecological effects in the food web.

4.2 Microorganism testing

There are no OECD guidelines for testing effects of chemicals on soil microflora or soil enzyme activities. Germany, Denmark and the Netherlands use or investigate one or more tests for effects on functions of communities of soil microorganisms for pesticide registration. A synopsis of the German regulatory requirements on side-effects of pesticides on soil microflora and the tier system for testing was recently published (Ehle & Laermann, 1991). Examples of tests are soil respiration, nitrogen transformation, organic matter or glutamic acid degradation, and soil enzyme activities e.g. dehydrogenase, phosphatase and urease (OECD, 1989). In the last guideline of 1982 the US EPA withdrew all tests for effects of pesticides on microbial activities in soil that were proposed in the draft-guidelines of 1975 and 1978. Main reason was the inability to interpret the incongruous data on effects of chemicals on natural communities of soil microorganisms (Stavola, 1990). However, the OECD Workshop (OECD, 1989) concluded that tests on soil microflora to determine effects on soil functions should be considered for incorporation into testing requirements. Stavola (1990), on the contrary, concluded that more basic research is needed before a guideline can be implemented.

The methods recommended in the report of the 4th International Workshop in Basel on Tests for Assessing Side-effects of Pesticides on Soil Microflora (Bundesforschungsanstalt für Landwirtschaft, 1989) are widely accepted by European members of the OECD (OECD, 1991). It is not surprising that they support further research on development of an OECD Guideline for assessing effects of chemicals on soil microflora. However, it is agreed that development of such a test is not a priority. The OECD will co-ordinate activities regarding tests on microflora with similar activities of the International Standardization Organisation (ISO) in order to prevent divergence of methods.

It is disappointing that in spite of the long tradition of research on microorganisms in agricultural soils no internationally acceptable tests for assessment of ecological effects of pesticides on soil microorganisms could be developed (Malkomes & Woehler, 1983; Stavola, 1990). The question remains if tests with soil microorganisms deserve further attention in schemes for ecological effects assessment and hazard evaluation. Decades of intensive use of pesticides according to good agricultural practice under all climatic conditions all over the world have shown only transient and limited inhibitions or stimulations of soil microbial activities (Anderson, 1978; Malkomes, 1988, Schuster, 1988). The last author found only small and short-lived inhibition or stimulation of microbial activities after the sequential application of three herbicides, three fungicides and one insecticide to wheat and barley in field trials. Microbial biomass, dehydrogenase activity, cellulose degradation, ammonification and nitrification were measured as effect parameters. In parallel laboratory experiments, a maximum of 30% inhibition of some of the microbial effect parameters was found, while recovery before the next application was incomplete. Insufficient supply of nutrients to microorganisms in laboratory tests was supposed to cause this discrepancy. Indeed, microbial biomass, expressed as organic C, increased from 250 to almost 800 mg/kg soil in the field during the growing season, whereas it remained almost constant around 330 mg/kg in the laboratory tests over the same period.

The main reason that negative effects of pesticides on microbial activities in soil are transient, must be sought in the biodegradation of these synthetic organic chemicals, thus serving as sources of carbon, nitrogen, phosphorus or sulfur for microbial growth. Soil is in essence an oligotrophic environment with a largely dormant microbial community in a continuous need for substrate to flourish. By an unique evolutionary process soil microorganisms can readily pick up and utilize extrachromosomal genetic material via ubiquitous plasmids (Racke, 1990). If a plasmid contains a mutant gene encoding for a metabolic enzyme crucial in the degradative process of a specific xenobiotic structure, the microbial community can benefit from this new energy source made available by microorganisms, that incorporated the plasmid. Because of the acquired advantage to profit from a new energy source, these particular microorganisms proliferate to large populations capable of attacking the xenobiont. This phenomenon is known as enhanced or accelerated degration of pesticides after repeated applications to soils for crop protection.

This enhanced degradation of xenobiotics by microbial consortia in soils will mitigate their negative effects in terrestrial ecosystems, even if they are toxic for many organisms in the beginning. Assessment of ecological effects of chemicals on soil microorganisms, with all complications of interpretation of results from tests in the laboratory, does not seem to have priority and does not pay off for ecological risk evaluation.

4.3 Soil-dwelling invertebrate testing

OECD Guideline 207 (OECD, 1984b) describes acute toxicity tests with the compost worm Eisenia fetida. It involves a 48 to 72 hours contact test on filterpaper and a 14 days contact test in artificial soil for adult earthworms with LC_{50} as endpoint. This test is used in Europe for ecological effects assessment of chemical substances, including pesticides. Research groups in Europe and the US are highly active in developing more tests for soil-dwelling organisms, including sublethal, chronic, reproduction and field tests. Several species of earthworms, nematodes, springtails, spiders and mites are included. Often the OECD Guideline 207 artificial soil is used as medium.

The EPA Office of Pesticide Programs (OPP), the International Organization for Biological Control (IOBC), the European and Mediterranean Plant Protection Organization (EPPO) and the International Commission for Plant Bee Relations (ICBB) have all their own test guidelines for non-target insects and arachnids. A tiered testing scheme, from basic acute toxicity studies to full-scale field studies, on pollinators, on many predators and parasites of pest insects and on aquatic insects is used in the development of pest control methods and for pesticide registration purposes. There is a lively debate on the selection of species, effect parameters and endpoints. Basic data on ecology and functioning of soil ecosystems, on biology of the most appropriate surrogate species, on the fate of chemicals and on the exposure of soil-dwelling organisms to toxic substances in their typical habitat are missing. It is premature for OECD to consider writing a guideline for soil arthropods (OECD, 1989; Stavola, 1990). Stavola formulated the following questions:

- Should Eisenia, being a compost worm rather than a soil-dwelling worm, remain the preferred test species, or would another species be more ecologically relevant?
- Is there a need for assessment of chronic hazards from exposure to chemicals, and if so, which sublethal endpoints would be most appropriate?
- How useful and adaptable are tests on effects of pesticides on pollinators and beneficial insects for industrial chemicals?

Most OECD Member countries would like to improve the existing OECD Guideline 207. The controversy about the use of Eisenia is going on. Suggestions for using soil-dwelling species, e.g. Lumbricus, and for other endpoints were given. Responses of Member countries (OECD, 1991) on the last question indicated that an OECD Guideline for assessing effects of both industrial chemicals and pesticides on arthropods would be useful. In line with the outcome of discussions at the OECD Workshop (OECD, 1989) and recommendations in the Detailed Review Paper (Stavola, 1990), respondents on the questonnaire urged to co-ordinate the activities of all organizations involved in developments of tests.

Exposure of terrestrial organisms to xenobiotics may occur via air, water, soil or food and is much more complicated than that for aquatic organisms, where simple equilibrium partitioning processes seem to be predominant (OECD, 1989). Exposure mainly via the soil-water phase is likely for many other soil inhabiting organisms besides plants (Anderson, 1984; Van Gestel & Ma, 1988, 1990). The latter authors showed in their first paper that soil related differences in toxicity of chlorophenols to earthworms disappeared after correction for adsorption onto soil. They concluded that for earthworms toxicity and bioaccumulation of chlorophenols are dependent on the concentration in soil solution which may be predictable from the adsorption coefficient. Moreover, the thus derived bioaccumulation values for chlorophenols in earthworms, based on concentrations in soil pore water, were comparable to those for aquatic organisms. They extended their study with two more chemicals and two more soils (Van Gestel & Ma, 1990). They found significant correlations between the estimated LC_{50} values in soil pore water and the octanol/water partition coefficient. They indicate that the OECD artificial soil might become applicable as a standard soil for extrapolating data towards natural soils. However, in comparison to the organic matter in natural soils, the peat in the artificial soil has different properties for adsorption of organic chemicals.

In our opinion, their QSAR-approach might be an interesting substitute for toxicity tests with soil inhabiting organisms for use in the Initial stage of Screening. Pursuing in this research line for generating toxicological data for the first tier of terrestrial ecological effects assessment may be a much better investment than developing an endless series of new single species tests.

Toxicity tests on several species, with relevant functions in terrestrial ecosystems and tests with reproduction as parameter under realistic exposure conditions should be developed for the Intermediate stage of testing.

Studies in laboratory model ecosystems and field studies, linking fate of a chemical in the environment and exposure in the habitat with effects on population level, would be appropriate for the Comprehensive Assessment stage. We are aware, that there is still a long way to go, before a sound scientific base is acquired to develop these tests and, above all, to be able to interpret the results for ecological risk evaluation.

5 DISCUSSION AND CONCLUSIONS

Proliferation of pollutants in the environment transgresses the borders between states and between the compartments soil, water and air. Authorities in industrialized countries pursue the integration of regulations regarding pollution of the environment, developed with specific objectives under different laws, into one clear framework covering all aspects of protection of the environment.

There is a tendency of lowering limit values for contents of chemicals in soil. This is partly caused by use of methods for hazard evaluation, based on toxicity data for single species relevant for the (soil) ecosystem. A good example is the ready acceptance and recent use of the EPA procedures and the R.A.B.-method by Dutch authorities (VROM, 1991) for setting limit values for chemicals in environmental quality standardization of soil. However, basic knowledge on processes in the biogeochemical system is inadequate for a sound scientific support of procedures for assessment of hazards for the ecosystem. Moreover, exposure of organisms to chemicals in soil under realistic conditions is not taken into account.

The fundamental question is if the model for assessment of hazards from soil pollution for the ecosystem with the R.A.B.-method ever can be validated. Relevancy of criteria for no "observed" effect and of the arbitrarily chosen 95% protection level for sustainment of ecosystems is not known. The significance of effects from stress by toxic chemicals has to be weighed against effects from natural stress or from stress caused by accepted human activities.

The contribution of biologists studying (integrated) pest control and of soil microbiologists to concepts for ecological effects assessment is underestimated in our opinion. Contrary to enviromental toxicologists from other disciplines, who focussed on single species toxicity testing under artificial laboratory conditions, these "applied" scientists kept always the extrapolation of data from laboratory tests to field situations in their mind (Greaves et al., 1980; Bartha, 1982; Johnson, 1982; Samsoe-Petersen, 1990). Starting point of applied scientists seems to be: the useful application of chemicals should not affect the sustainment of a healthy ecosystem. The view of environmental toxicologists may perhaps be caricatured as: every chemical will have a deleterious effect on at least one single species and prove will be provided.

Further research is needed to develop a sequential testing scheme, in which finally environmental exposure is linked to ecologically relevant effects, that can be used in step-system hazard assessments. The extent of complexity of tests depends on the different objectives of the evaluation (registering of pesticides or of new non-pesticidal chemicals). Objectives and scope of each of the three stages in the ecological effects assessment, the Initial stage of Screening, the Intermediate Assessment stage and the Comprehensive Evaluation stage, have to be defined.

The use of data on physico-chemical properties of a chemical, on its fate in the environment and on its toxicity for mammals, for aquatic and terrestrial organisms has to be optimised. Quantitative structure-activity relations models may be useful tools, particularly for estimating data in the Initial stage of Screening.

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