OECD GUIDELINES FOR TESTING OF CHEMICALS

SUMMARY OF CONSIDERATIONS IN THE REPORT FROM THE OECD EXPERT GROUP ON ECOTOXICOLOGY

Test Guidelines for Ecotoxicological Testing were prepared in draft form by the OECD Expert Group on Ecotoxicology (List of Participants appended). The drafts were issued in December 1979 from the Lead country (Netherlands) in a report, which included detailed considerations of the Group's approaches, objectives and general principles in ecotoxicological testing and a compilation of existing test methods. The following test constitutes a summary of the considerations and is presented as background information to users of OECD Test Guidelines.

GENERAL

Some of the guiding principles for the measurement of concentration-effect relationships in ecotoxicology can be summarised as follows:

Damage to the biotic part of an ecosystem may affect both function and structure of the system. Function includes the complex relationships through which the ecosystem is maintained. This is possible, however, only when the material basis, i.e. proper structure of this system, is present. In the functioning of ecosystems, trophic relations, involving energy and nutrient transfers, which are part of natural cycles, are of prime importance.

In this respect, the ecologically functional distinction between primary producers, secondary producers, consumers and decay organisms is useful in visualising important biotic elements of these natural cycles. Environmental disturbances will generally manifest themselves as changes in relative numbers or as changes in the biomass of elements of the system or as both. Biodepressive effects (mortality, decrease of growth and reproduction) and biostimulatory effects (eutrophication, immigration, etc.), which may both be harmful to the ecosystem and as such undesirable, may be distinguished.

The chemical under consideration will be transported to the receptor (the biotic system) via water, air or soil. In this respect the frequently-made (and practical) distinction between water, air and soil pollution is not of ecotoxicological significance, the identity of the medium

of transport being of minor importance. In assessing the potential effects of chemicals in practice, however, this distinction is of paramount importance, as different methods are used for determining effects in each of these media. To these transport media "food webs" (including the simplified form "food chain") can be added as a separate entity because of specific phenomena, such as biomagnification and the formation of more or less toxic metabolites.

An holistic point of view simplifies our problem to a certain extent in that it implies that basically only the concentration-effect relationships between potentially harmful substances and biotic systems that are representative of certain functional types in the environment need be studied. In Table 1 a summary is given of the relative importance of the environmental compartments to the ecosystem. Air, water and soil are not well-defined entities; the crosses in the table give only rough indications.

Table 1: The relative importance of the environmental compartments air, water and soil in relation to some of their properties

	content of biotic systems (habitat)	transport medium for pollution		risk from chemicals to populations present	sink for pollution	global uniformity of composition	dilution
		distance of transport	volume transported				
air	+	+++	++	+	+	+++	+++
water	++	++	+++	+++	+++	++	++
soil	+++	+	+	++	+++	+	+

+ slight; ++ moderate; +++ considerable.

From this table it may be observed, for instance, that their relative importance depends on whether the environmental compartments are looked at as a habitat, as a transport medium or as a sink for chemicals.

For the assessment of environmental effects, ideally the whole ecotystem should be considered; for practical and scientific reasons, however, one is restricted to the analysis of the effects on subsystems (except where retrospective studies are possible).

One of the main difficulties in ecotoxicology is deciding whether or not some observed change is ecologically significant, and this may involve difficulties for extrapolation. This problem is made more difficult because of the abilities of biotic systems to repair damage and to adapt themselves to adverse conditions. Ecologically irreversible changes are considered to be undesirable effects of pollution; reversible effects may, however, also be objectionable, although they may be tolerated under certain circumstances.

It may be assumed that any impairment of the function and structure of a biotic system, especially at the level of communities should be considered as an undesirable pollution effect. From this point of view persistent and/or irreversible effects, are of primary importance in ecotoxicology. It should be borne in mind that some irreversible effects, which are harmful for individual organisms, may be less important at the level of the community. The ecological importance of an effect may differ widely between species, e.g. because of differences in reproduction rate or life cycle span.

Important effects include, for example, the main biological events of germination and birth, maturation, reproduction and death, which ultimately determine the biomass. Other effects, such as impairment of respiration, increase in mixed function oxidases, changes in pigmentation, behavioural changes, etc., can be considered as secondary effects that may indirectly influence ecologically important parameters.

"DOSE" IN ECOTOXICOLOGY

In toxicological testing one generally tries to determine dose-effect relationships. This can be done for terrestrial animals to which the substance tested can be administered orally or by injection. In aquatic ecotoxicological testing this is virtually impossible, particularly for small animals, because the test substance will be administered with the water; concentration-effects are then determined.

Dose-effect relationships could, however, be studied in aquatic ecotoxicology by determining analytically the amount of the test compound present in the test organisms. This, however, is not generally done.

In extrapolating the results of ecotoxicological tests the fact that the test compound may have been changed in the environment should be taken into account. In the following the word "dose" will be used to indicate amounts administered directly to or present in the test organism, while the amounts present in the test medium or in the environment will be called "environmental concentrations".

The actual environmental concentration depends in the first place on the amount added, but it is greatly influenced by the physical-chemical properties of the chemical and the particular transport medium involved (air, water, soil, food web) in which the biotic system is exposed.

Physical-chemical properties, such as volatility and solubility, determine the distribution among the different environmental compartments. In addition, the chemical may be affected by environmental factors before it reaches the living system to be affected. Factors such as physical-chemical stability (resistance to oxidation, hydrolysis, radiation, etc.), adsorption and biodegradability will determine what fraction of the initial environmental concentration will be active in the immediate vicinity of this final living system.

Finally, the dose to which an organism at risk in a system may be exposed is influenced in two ways by processes of bioaccumulation:

- the internal concentrations of chemicals in aquatic organisms are seldom the same as the external concentrations, and substances which are readily absorbed but only slowly lost or released can build up to very high concentrations;

- chemicals may also reach the receptor organisms via food chains or food webs; this is typically the major route for terrestrial animals and, again for chemicals rapidly absorbed but only slowly lost, can lead to enhanced exposure levels at sensitive critical sites.

Although the phenomenon of "biomagnification" via the food chain, resulting from sequential bio-accumulation may be less important than used to be thought, exposure through

food must be taken into account in determining the "Predicted Environmental Concentration" (PEC), a parameter that is important in the interpretation of data derived from toxicity tests.

From the above it will be clear that, for the proper planning of ecotoxicological testing, certain key information on physical-chemical properties and on the persistence of the chemical under investigation is desirable, not only because of problems of interpretation of concentration-effect relationships but also for sound operational reasons. For example, different test systems will be necessary for the testing of volatile or hydrophobic compounds. It is important, furthermore, to check biodegradability before (aquatic) toxicity tests are carried out since biodegradability not only influences the concentration actually present, but may sometimes lead to erroneous results because the oxygen depletion may kill the organisms while the compound is not or only slightly toxic. For the purpose of ecotoxicological evaluations, therefore, the gathering of such data prior to the testing is to be preferred, although sometimes one will want to deviate from this logical order.

TYPES OF TEST

In ecotoxicology the most difficult situation is that which involves the prediction of the impact of very low concentrations of a chemical, maintained for long periods, and affecting communities of organisms, giving rise to sublethal and interspecific phenomena. For such difficult situations one would want ideally to carry out chronic tests with multi-species systems, but no standard test systems of such a nature are available. This would, moreover, be an enormous task, even where feasible, if all environmental chemicals had to be evaluated accordingly. Because of such factors it is generally agreed that a stepwise procedure is to be followed, starting with simple tests for all compounds but terminating with more complex multispecies and field tests for those few compounds which are of great practical value but which have been indicated as being potentially dangerous to the environment.

The following types of test should be considered:

(a) short term tests with single species:

(b) long term tests with single species;

(c) tests with multi-species systems;

(d) tests with infra-organism systems (e.g. cell or tissue cultures).

It is impossible to go into details of all of these types of tests, but the following comments can be made.

Initial testing is aimed at finding indications of the nature of the effects (qualitative screening) and at obtaining a first estimate of the concentration ranges within which these effects occur. In ecotoxicology this means that ideally effects on the function of the system need to be taken into account. If relative biomass of the constituent species of a system is taken as the ultimate basis for a proper functioning, then the parameters mortality, reproduction, assimilation and growth should be a matter of concern. They are the only parameters which can be measured reliably and routinely in currently available tests and which can be interpreted. It may be preferable to use the terms "short term" and "long term" instead of the terms "acute" and "chronic" in ecotoxicological testing.

Special care in the selection of types of ecotoxicological tests should be taken to ensure that concentrations in the test environment are kept constant during long test periods; they should at any rate be known or checked periodically. Generally, for aquatic organisms we distinguish among:

- static tests in which the test chemical is added once to the test system, no flow occurs and the test medium is not changed during the experiment;

- semi-static tests in which test medium and test compound are periodically replaced;

- flow through (continuous or intermittent flow) tests in which test medium and test compound are supplied at a constant rate and concentration to the test organism.

This last test system simulates to some degree natural conditions better than a static test does, while, moreover, there is a greater degree of assurance that the concentrations to which the organism has been exposed during the test period have remained constant.

In reviewing the types of ecotoxicological tests available at present, it is obvious that tests with aquatic organisms are abundant, whereas tests with terrestrial organisms are scarce. Since most of the pollutants eventually are found in water, and also for practical reasons such as ease of testing, aquatic animals are used for a first indication of environmental effects of chemicals. In a more detailed analysis, however, the organisms living in terrestrial habitats must not be overlooked.

In ecotoxicological testing we should be concerned with both structure and function of the total biotic environment in assessing the ecotoxicological effects of chemicals. "Structure" is related among other things, to the relative biomasses of co-existent species and "function" to the dynamics of the geo-chemical cycling as well as of the energy input.

SELECTION OF APPROPRIATE TESTS

It is not possible to select a small number of species that can be considered to be representative for any taxonomic group; therefore any choice made on this basis must be arbitrary. A choice based on ecologically or physiologically different functional types appears to be more appropriate. Considering this it should be stressed that all tests serve only as models and consequently have shortcomings. Prediction of all possible environmental risks cannot be expected; this implies that any scheme will only lead to a correct assessment in, hopefully, a majority of cases.

In ecotoxicology the response of populations and communities to stress is more important than the responses of individuals since, however, chronic studies on multi-species systems under natural conditions have not yet become available, a practical approach requires a stepwise procedure starting with single-species tests under laboratory conditions and establishing concentration/effect relationships within definite time limits.

A stepwise procedure might involve three steps; the basic level, the confirmatory level and the definitive level.

At the basic level simple tests should be used indicating possible effects on a few functionally important types of organisms. Ecological function is considered to be of greater importance than any suggested "representativeness" of particular species for particular environmental compartments.

The following tests have been proposed for inclusion in the basic level: "Alga, Growth Inhibition Test"; "Daphnia sp., 14-day Reproduction Test (including an Acute Immobilisation Test)"; and "Fish, Acute Toxicity Test".

For reasons of occupational safety and handling of the compound and before the basic level tests are performed, it is necessary to have available safe handling precautions and a summary of results from human safety evaluation of new chemicals.

The Group of Experts wished to stress that, because of the nature of the "science" of ecotoxicology, the imperative prescription of one standard test species to be used in environmental testing is irrational. To do so would suggest an accuracy which does not exist and, moreover, would be contrary to the philosophy of model systems in ecotoxicological testing.

The reason for using more than one test species is that the variation between species in sensitivity to one type of toxic chemical need not necessarily coincide with the variation in sensitivity to other types of chemical.

It is also not practical to prescribe only one test species to be used all over the world, as that species may not be available everywhere. The Expert group is of the opinion that a thorough knowledge of and experience with the optimal conditions of breeding and testing species indigenous to the area of concern is more valuable than a rigorous international standardisation.

At the confirmatory level tests should be used which yield more complete information if suspicion as to the acceptability of a chemical has been previously raised at the basic level. Suspicion need not necessarily be based only on the results of ecotoxicological tests. In general, these tests may be ones of a more complicated nature, related to specific or additional environmental compartments. Also chronic exposure and specific routes of contamination should be considered depending upon the chemical properties and the degree of environmental contamination expected.

Testing at the definitive level may be needed in some special cases, e.g. where appreciable environmental concentrations of the chemical are likely to be involved and/or some indication of possible environmental hazard exists. It may be necessary to assess the effects in experimental systems more closely approaching something like natural conditions, especially with regard to interspecific relations and the functioning of multi-species systems. At present suitable multi-species tests are not readily available.

QUANTITATIVE ASPECTS OF TESTING

In ecotoxicological testing it is desirable that some indication of the range of effects to be expected is obtained. As chemicals may cause different effects in different organisms at divergent concentrations, an indication of the broad magniture may be sufficient. It is more important to carry out tests with several species of organisms, perhaps from different taxa, in

order to get some indication of the natural variability than to carry out a very precise test with only one species of organism.

In general, very high precision cannot be achieved in ecotoxicological tests because of the variation which exists naturally within populations of organisms. Standardisation of test conditions is, in general, possible, but many of the so-called standardised methods are highly arbitrary in their assumption as to the parameters that are to be standardised and, therefore, have little relevance from an ecotoxicological point of view. One of the parameters that appears to be amenable to standardisation is that of the degree of variation in the test organism, and inbred populations may be used to reduce this.

Because of the variability within populations, data obtained can only be expressed in terms of the particular probability at some stated confidence level, at which the event o concern might be expected to occur. This type of unavoidable uncertainty which results from the inherent variability, but which can be treated statistically, should not be confused with that due to bad experimentation. The rules of "Good Laboratory Practice" (GLP) apply to ecotoxicological testing.

In multi-species tests high precision will never be achieved because of the multifactorial nature of the variability of such systems and the different requirements of the component species of organisms. At best, one can achieve some compromise between an experimentally feasible standardisation and an approximate representation of natural conditions. At present it can be stated that these tests, although possible, require much more development before they can be used effectively. Such a development is very desirable.

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