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STRATEGIES IN ECOTOXICOLOGICAL RESEARCH

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Strategies in ecotoxicological research

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SUMMARY

This paper discusses the type of knowledge we should generate if xenobiotics are to be released into the environment in a responsible way. Alternative strategies, ranging from single species to ecosystem tests all have their limited merits. If small effects of xenobiotics are tolerated, we must obtain a better insight into the dynamics of uncontaminated ecosystems in terms of the physiological behaviour of the organisms involved. The principal task of ecotoxicology in this context is to give a description of the effects or the expected effects of a xenobiotic if released into the environment.

INTRODUCTION

At present, we are usually aware that chemicals may have direct or indirect adverse effects if they are released into the environment. However, it does not always seem to be possible or economically attractive to develop and apply clean technologies on the basis of complete recycling of materials or prevention of wastes. The application of such technologies is strongly delayed by the common impression that chemically induced disturbances of the ecosystem are to a certain degree local and temporal without obvious long lasting adverse effects. The responsible use of this supposed resistance of ecosystems to chemical disturbance requires a considerable amount of knowledge to quantify effects or expected effects. This paper discusses single-species and multispecies tests that are used or can be used to generate the necessary knowledge.

SINGLE-SPECIES TESTS

The first type of test that has been developed in ecotoxicology is the single-species test, in which (cohorts of) individuals are exposed to chosen concentrations of a compound during a chosen exposure time and some response is compared with the blank. Some characteristic of the concentration-response function, usually the concentration showing a response half the size of that of the blank (EC50, LC50, LD50) or the no-observed-effect concentration (NOEC) is taken as a measure for toxicity of the compound. Such measures are subsequently used to infer

concentrations in the environment that could be considered acceptable, i.e. those expected to give no long lasting adverse effects.

Originally, these tests comprised acute tests, i.e. short exposure times, on the survival of aquatic species like fish and daphnids. Later extensions included longer exposure times (called chronic tests), sensitive stages in life cycles (larval development), other types of (sublethal) response, e.g. reproduction, growth, respiration rates, other species and habitats (microbial populations, plants). Considerable variation in results obtained by different laboratories with a particular test encouraged standardisation of test procedures. This standardization is best developed in the oldest tests.

The advantage of single species tests above alternative approaches is that these tests are relatively inexpensive, so allowing application on a routine basis and that it is in principle possible to optimize replicability of results by standardizing test procedures to a certain degree (especially for soluble compounds).

However, the methodology for single species tests is far from completely developed. So far it has concentrated on the empirical description of the survival probability as a function the concentration of test compound. I consider the statistical problems concerning parameter estimation (for NOEC and LC50) and optimal design of experiments as largely solved. The growing data base has facilitated the formulation of so-called quantitative structure-activity relations (QSAR), which quantify the relationship between the LC50 of structurally related compounds and lipophilicity down to a point at which solubility becomes too low. Such knowledge is very useful in designing experiments.

It is a limitation that the basis of the descriptions is empirical only, but in view of the great variety of toxicity mechanisms involved, it is not likely that the concentration-response descriptions will ever link up with underlying mechanisms. One of the major obstacles is that different concentrations of a particular compound can induce different mechanisms of death. Relatively few studies relate the accumulation-elimination behaviour of compounds with effects. Such a link is essential for the sensible introduction of exposure time as a factor determining toxicity. Survival models on the basis of a k-compartment accumulation-elimination process have been proposed (Kooijman, 1981) but insufficiently tested and rarely applied. It is doubtful whether it is possible to improve on this description in a way that is not specific for a particular compound or species of test organism. A characteristic of the concept "death upon reaching a threshold body concentration" is that the scatter in threshold levels among individuals, which is graphically visible as the slope of the concentration-response function, is independent of the exposure time. This is difficult to check in practice because for most compounds it is hard to obtain concentrations showing partial survival. For some pesticides it is possible, however, and this concept proved not to apply. For other compounds like salts of heavy

metals, the 1-compartment accumulation-elimination model provides very reasonable description for the LC50-time behaviour.

Uncertainty in the relation between acute and chronic toxicity necessitates chronic testing, because chronic toxicity is the only one that is relevant in practice. For all tests, but especially for chronic tests, it is necessary to be able to offer the test-organisms a stress-free environment, apart from the compound to be tested of course. One of the more serious problems of toxicity testing is to provide such an environment. It usually involves one or more "magic" factors in the food or in the medium, reflecting our lack of knowledge about physiological details that are relevant. There are many examples of extreme toxicity, like N-containing compounds for the cyanobacterial pest *Microcystus aerogunosus*, that could be related to deficiencies in test media. In general it is necessary to be able to culture the test species, which severely restricts the number of suitable species. There still occur many biological errors in toxicity testing, like ignorance with respect to the manner of exposure. Aquatic insects for instance hardly take polar compounds directly from the water and are usually difficult to feed in chronic tests. Nevertheless, they are used in acute tests. In my opinion, the principal field of development of single species tests should be in life-cycle tests with extra attention for exposures via soil and air.

The methodology for sublethal effects is poorly worked out for several reasons. Reproduction seems to be a sensitive as well as an ecologically relevant test criterion. One problem here is that at low concentration, compounds usually stimulate reproductive output rather than reduce it. Since we do not know the mechanism, it is hard to classify such an event as adverse. Here, again, we lack the necessary physiological knowledge about the test species. The number of seeds, eggs or young highly depends on nutritional input, which is usually difficult to standardize. From a ecological point of view, the total accumulated reproductive output is not the most relevant, but the time at which the first few offspring appear. This complicates the statistical analysis of the results. The statistical problems become even more obvious as soon as additional responses like growth are to be observed in view of dependencies between these variables: The acquisition of nutrients from the environment depends on size, and so on growth, which fuels the reproductive output. Here we are in need of stochastic models for the size dependent uptake and energy partitioning, not only in the blank, but also in the stressed situation to identify partial effects with a specified certainty.

Many toxicity tests on more subtle sublethal effects have been proposed. The difficulty here is the "so-what" question, concerning the ecological significance of the results. To a lesser extent, this also applies to the effects on survival and reproduction. The point is that individuals are taken from blank cultures. So, the no-effect-individuals not only have to resist the compound but also the abrupt change in its concentration, which is not always a realistic representation of exposures in the environment. We hardly know anything about processes of adaptation and

selection for resistance to non-pesticides.

APPLICATION OF RESULTS

The translation of results of single species toxicity tests into acceptable concentrations in the environment is at the limit of scientific capability. First we have the problem of defining what is acceptable, which involves many non-scientific elements. Thinking of acceptable concentrations as those inducing "small" effects and of effects as deviations from blank behaviour, we have the problem that the blank behaviour of real world ecosystems is largely unknown. Last but not least, we never have sufficient toxicity data and none on the more subtle effects, like those involving interspecies interactions.

The usual situation is that only results of acute toxicity tests are available for very few species of test organisms (that frequently do not even occur in the target ecosystem). If the LC50 values are low, the usual conclusion is not, curiously enough, that such a compound should never be released into the environment, and so making any further toxicity research redundant. Instead, the conclusion is that for such compounds chronic tests are required. The resulting LC50 values are usually divided by 1000 for non-degradable compound or 100 for degradable compounds to arrive at "safe" concentrations. These rules of thumb lack any scientific underpinning, however.

In an attempt to improve a little on this situation, I proposed the concept of a hazardous concentration for sensitive species (HCS) that could be calculated from chronic LC50 values of at least two test species (Kooijman, 1987). It is chosen such that the LC50 value of the most sensitive species in a community of a certain number of species exceeds that concentration by a specified probability. Its derivation is based on the assumption that the LC50 values for both the test species and for the community species can be conceived of as independent random trials from a log-logistic distribution. It accounts for the uncertainty in the parameter values of this empirical distribution, which we can only infer from the few LC50 values we have for a particular compound. As long as the number of species in the community is large, its exact value proves not to be very relevant. The motivation of this approach stems from the observation that the variation of sensitivities among species, as expressed in their LC50 values, is quite considerable and does not follow obvious patterns. Further improvement on this concept is likely to make use of patterns in sensitivity of related species, as a logical counterpart of the QSAR. To my knowledge, little data in the open literature are available for this purpose.

The application of the results of single species toxicity tests is one of the weakest elements in efforts to determine small or no-effect concentrations in ecosystems. Here knowledge about emission, transportation and transformation of compounds should be combined with

effects on ecosystems in a way that allows for the disturbance often being local and temporal. Very few studies incorporate all these considerations. The situation is even more problematical because in practice compounds are rarely pure, but frequently consist of complicated mixtures. It has been shown that the toxicity of combinations of chemicals, which might deviate by a factor 2 from expectation based on separate toxicities. Although this is relevant for drugs, I do not consider this as a major ecotoxicological problem in view of the uncertainties involved, which can easily amount to a factor 100. What I do consider as a major problem in this respect is the judgement of individual independent emissions into the same ecosystem, especially if the norm allows small effects instead of no effects.

MULTISPECIES TESTS

The motivation to supplement single species tests with multispecies tests originates from the incompleteness of the results (irrelevant species test organisms of too small a number, no interaction among species etc.) and the need of a shortcut for problems relating to the manner of exposure; such tests seek to combine elements of transportation, transformation and effects in integrated systems. The general idea is to mimic the target situation and observe what happens.

Although in this way a number of weak points in the single species approach can be circumvented to some extent, some new problems arise. Depending on the set-up of the tests, there usually exists a conflict between costs, replicability of results (i.e. standardization) and the degree of correspondence with the target situation. The latter is obviously less than perfect; It is not possible to dose the compound in a realistic way, to include the species of larger body size and to run the experiments long enough while still mimicking the target ecosystem. The latter problem is analogous to the problem of stress-free environments in single species tests, but much more serious. We still fall short of detailed knowledge about the factors that drive ecosystems and lack the technical ability to provide these factors in multispecies tests. The judgement of the degree of mimicking is a problem in multispecies testing, due to the lack of an adequate frame of reference. Probably related to uncertainty about the exact nature of driving factors, the behaviour of experimental ecosystems usually show sizeable variation among replicates. Statistical analysis of results is severely hampered by lack of adequate stochastic models for ecosystem behaviour and the usually low number of replicates. Even if these problems are solved, the recognition of small effects will remain difficult, i.e. the probability on an error of the second kind is high.

In view of the great variety of multispecies tests that has been proposed, particularly with respect to the degree of complexity, it is not easy to discuss them as a whole. Some seek to simulate a particular process only. I consider tests on specific microbial activities, like degradation and N-fixation, as the most valuable supplements of single species tests.

Such tests can combine elements of standardization while preserving an "acceptable" degree of mimicking the target situation. For other processes, this is much more problematic. We are here confronted with different conceptions about ecosystems and protection aims.

Some research workers are of the opinion that one should not protect particular species, but ecosystem functions like primary production. If we would apply this idea, say, to the waterways of Holland, we probably conclude that nothing is wrong with the primary production there. Yet, even satellite exposures show that this production is mainly done by a very few species of cyanobacteria, instead of a variety of plants. I do not argue in favour of such a protection aim. A further problem with the function criterion is that the concept of ecosystem function is intimately connected with the concept of an ecosystem structure that randomly fluctuates around some equilibrium. (A function is any rate related to a material and energy cycle; the structure is the set of species together with their abundances.) The existence of (static) equilibria is still a matter of debate on theoretical grounds and even more so on practical grounds, because experimental ecosystems usually show a rapid evolution during the experiments. Protection aims and ecosystem concepts already play a role in the composition of the sampling programme. Since it is not possible to monitor fluctuations in abundances in all species, or to measure all possible rates, one can easily fail to notice effects in variables that have not been investigated.

ECOTOXICOLOGY

My discussion of the single species and the multispecies approach in toxicity testing and leads to the conclusion that both provide valuable information, but both have serious limitations with respect to the aim of giving reliable estimates for concentrations in ecosystems that could be considered "safe". The single species approach has the best prospect to be used on a routine basis providing a multipurpose data base, while the multispecies approach can be made more relevant for a particular pollution event, but carries the risk of missing effects that could prove to be disastrous. If one wants to develop ecotoxicology as a part of science, instead of trying to solve a practical problem by guess work, one can only be satisfied if both approaches lead to roughly the same estimates for "safe" concentrations. For this purpose it is necessary to supplement information from routine toxicity tests such that sensible predictions at ecosystem level become possible and, at the same time, to try to identify the principal mechanisms operating in experimental ecosystems under stress. The idea is that, if both approaches correspond in a number of case-studies, we have a better idea of how to supplement and adapt routine toxicity tests.

The problems one encounters in comparing results of single-species and multispecies tests can be classified into physical chemical and physiological ecological ones. The first category involves problems concerning exposure to the compound, which includes transportation,

transformation (speciation and degradation) and accumulation through food chains. In routine aquatic toxicity tests for instance the concentration is usually kept constant and the amount of complexing agents is very low, which means a high bio-availability. In multispecies tests, the opposite is true. The administration of the compound is usually only once at the start of the experiment and it is rarely possible to recover more than a tiny fraction of the applied compound at the end of the experiment.

The physiological ecological problems are more complex. The core is the relation between properties of individuals and the behaviour of populations consisting of such individuals. Probably due to the complexity of the subject, very little is known about this relationship. Its vital importance for ecotoxicology lies in the fact that compounds change the physiological properties of individuals,like the elevation of routine metabolic costs and/or a reduced reproductive output, sublethally while the significance of such a change is at population level (Kooijman *et al.*, 1987). This relationship also plays a crucial role in related problems, e.g. those concerning the consequences of the release of genetically modified organisms, and various problems in fundamental ecology.

In conclusion I want to state that ecotoxicology as a science is still in its infancy and that its maturation is bound to that of ecology. In view of the worldwide increase in chemical disturbance of the environment, we have no choice but to try to speed up its development. At the same time, we are in urgent need of clean technologies, not in the least in agricultural industries.

REFERENCES

The papers below contain many other references to relevant work.

KOOIJMAN, S.A.L.M. (1981). Parametric analysis of mortality rates in bioassays. *Water Research* 15, 107-119.

KOOIJMAN, S.A.L.M. (1987). A safety factor for LC50 values allowing for differences in sensitivity among species. *Water Research* 21, 269-276.

KOOIJMAN, S.A.L.M., HANSTVEIT, A.O. & HOEVEN, N. VAN DER (1987). Research on the physiological basis of population dynamics in relation to ecotoxicology. *Water Science and Technology* 19, 21-37.