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# EARTHWORM TOXICOLOGICAL TESTS, HAZARD ASSESSMENT AND BIOMONITORING. A METHODOLOGICAL APPROACH

MARCEL B. BOUCHÉ
*Laboratoire de zooécologie du sol (INRA) CEPE/CNRS, Montpellier, France*

## 1. Introduction

The aim of this paper is not to cover the wide research field indicated by the title, but to discuss how to improve the design of our research by a better connection between toxicological tests (laboratory), hazard assessment (field) and biomonitoring. There is confusion on the degree by which laboratory experiments can be compared with field trials. To try to obtain information by an inadequate method limits the improvement of the method and obscures the interpretation of results. In this brief account, I present schematically, remarks encouraging the reader to look at papers where the arguments are given more in details (*e.g.*, Bouché, 1984a, b).

## 2. Toxicological tests

Tests often labelled 'ecotoxicological tests' have basically to answer the question: what is the relation between chemical dose(s) and effect(s) on earthworms.

- Such a test cannot be designed with references to 'natural conditions', because these conditions at the earthworm level are poorly known and probably not reproducible.
- Such a test cannot be made with one species 'representative' of all earthworms. We have no criteria to check the representativeness of a single species.
- Such a test has to be *reproducible*, *i.e.*, must be made using standard products (repetition in the same laboratory), and by synthetic products (readily available between laboratories), with an unique strain of a precisely known species of earthworm which can be used as a standard (*i.e.*, easy to rear and to exchange between laboratories).
- Such a test must give comparable results between chemicals, *i.e.*, must be usable to assess consequences of chemical use on earthworm communities, and consequently on soil properties. Results of tests are needed to interpret observed field hazards or to predict potential risks from new chemicals. Comparability between chemicals made possible a classification of chemical on one scale of toxicity (Fig. 1). To obtain comparable results, the test must use a neutral medium where earthworm crawl and eat, must have a long enough duration for spontaneous chemical change to occur (*e.g.*, hydrolyse) and a biological process leading to the toxic effect (*e.g.*, cholinesterase inhibition leading to death).

A comparison of various technical tests has been made (*e.g.*, Edwards, 1983; Goats and Edwards, 1988; Heimbach, 1985). Among the convenient tests only one medium is synthetic, and permits reproducible and comparable results: the *Artisol* (described

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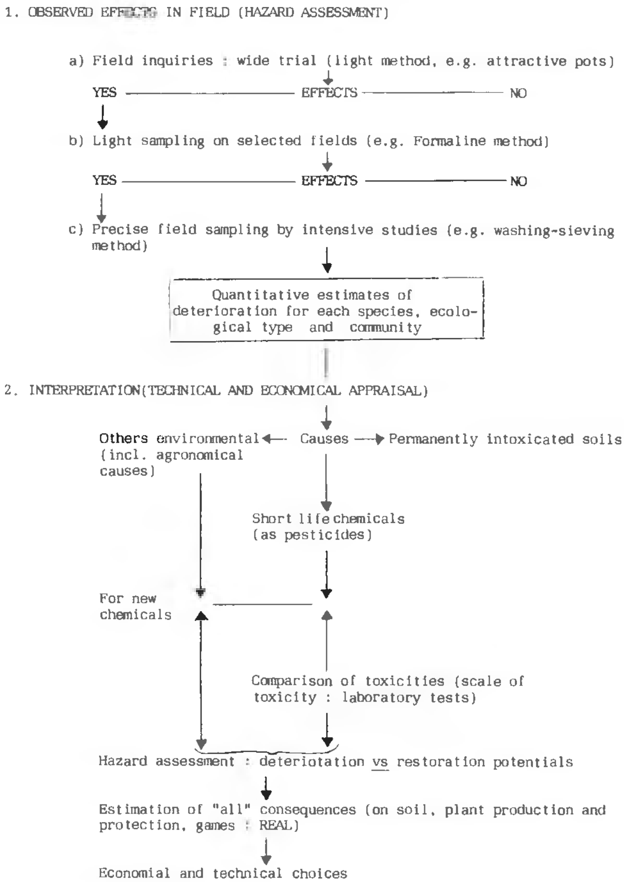


*Fig. 1.*

by Ferrière *et al.* 1981) following the AFNOR procedure (AFNOR, 1984). Among potentially standard earthworm species the *Eisenia fetida* species-complex was adopted, because it is easy to rear and to transport. On the basis of the work of André (1963) and others, I defined *Eisenia fetida andrei* Bouché, 1972, at the subspecies level because of predictable resistance against this change, while I wrote 'il s'agit très vraisemblablement d'une bonne espèce'. In fact, classical taxonomy has been forgetting this species and Jaenike (1982) 'rediscovered' this species before Sheppard (1988). This important species is now used in both vermiculture and toxicological tests so we have to characterize the strain (*Eisenia andrei*, Bouché, 1972, P2342) by electrophoresis (Robotti, 1982) to be sure we work on the same population.

## 3. Hazard assessment

A toxicological test gives an answer only in artificial and controlled laboratory conditions on the effect on mortality of one species. In no circumstances can such a test predict directly the hazard to an earthworm community of chemicals; field hazard must be observed in the field.

Conversely, changes in community structure and numbers in field are not dependent directly on toxicity of any one chemical. The behavior of any chemical interferes with its toxicity in soil and plants (adsorption, infiltration, retention by above ground parts), depends on climatic factors (rain, temperature), depends on biological activity (*e.g.*, biodegradation), and on its chemical properties (stability) or others factors such as agricultural practices (sowing, plowing). In turn, earthworm risks vary very much with the behavior (*e.g.*, surface, activity, lethargy) of each stage of each species, this behavior depending on climate, food availability, photoperiod, and agricultural practices. Changes in structure and level of communities also reflects other ecological or agronomical parameters (*e.g.*, cultivation, use of other pesticides). Some plot experiments could give precise results, and more information on processes (Goats and Edwards, 1988). However, the use of plot experiments is restricted to only critical compounds because experimental costs are high and the results are local (*e.g.*, soil, earthworm community, culture and climate are local).

One suspects that so-called harmless chemicals act dramatically against earthworms in the field through sublethal effects such as those described by Reinecke *et al.* (1985). On the other hand, a chemical is often associated with other agronomic stresses (cultivation, other chemical) in a crop rotation system. The state of one community results of the combined effects of such stresses and its ability to recover from the stress.

To improve our ability to predict hazards of new chemicals before approval of use, we need to interpret the effects of present use of classical chemicals in normal field conditions. From this background, we can hope to make by comparison some hazard assessment of new chemicals.

To reduce cost of such studies we have to devize a research strategy:

i. First tests could be very simple, by easy-to-make inquiries relevant to many agronomical and/or environmental conditions. The Lofs-Holmin's pot method seems to be, with some improvement for standardization, a good candidate test.
ii. If some community deterioration is suspected from the first stage test, a semi-quantitative sampling, using the formalin method, could be used in selected treated fields.
iii. Very carefully selected situations could be studied more carefully by destructive quantitative procedures such as digging, sieving, washing procedures (Bouché and Beugnot, 1972).
iv. The interpretation of earthworm community deterioration needs a close knowledge of earthworm species, of their ability to recover, on other climatic, edaphic, human factors acting on this community. Indefinite toxicity of chemicals could be observed by a global soil toxicity test as described by Callahan (1988) or by the electrophysiological detection of Drewes *et al.* (1988) both used as sensor of pollutants. Nevertheless, in many agricultural circumstances, chemicals potentially responsible for some community deteriorations are degraded. The knowledge of climatic events, techniques of field spraying, physical and chemical properties and the position in the *scale of comparative toxicity* give the possibility to interpret the causal agent of deterioration. Conversely, this general

knowledge about the agents of deterioration can help to predict the effects of new chemicals before their legal approval.

## 4. Estimation of consequences of chemical use

In agricultural sites, a pure conservationist position for survival of earthworms is not possible. Some use of chemicals (pesticides, fertilizers) is often a critical need to obtain high yields. Conversely, usually classical pesticide field trials demonstrate the use of a chemical, but take mostly as parameters the yield and effects on a few pests. Destruction of earthworms is not taken in account, in the short term (*e.g.*, release of nitrogen from earthworm bodies increasing yield), mid-term (*e.g.*, no burying of dead leaves, increasing fungal inoculum in subsequent years), or, long term (destruction of soil structure, infiltration decrease, erosion increase) (Bouché, 1974, 1984c).

In fact, we have to take in account all consequences of chemical use, not only the positive ones (such as pest reduction, short time yield increase) or the negative ones (as biological perturbation, long term effects). For earthworms, this can be made thanks to an integration of the role of earthworms in a simulation model, which has been developed for eight years (REAL, Bouché, 1980). At first, it was a conceptual model to select available techniques (or to improve them) to obtain field data to feed later an operational model in term of energy, matter, soil material movements, due to each of the main ecological types of earthworm. All data has been gathered from fields, to avoid assumptions and non-confirmable results. This model is now reaching slowly the operational stage and gives the possibility to estimate from field data the pedological, ecological and plant physiological consequences of any changes for 'all' earthworm species. The model requires humidity, temperature and latitude (photoperiodism) data, plus the influence on structure and level of population of the agricultural technique being studied.

## 5. Biomonitoring

The study of environmental risks could be improved by the use of earthworms. It is possible to detect the bio-availability of chemicals in soils in the way it was done for PCBs (Tarradellas *et al.*, 1982) or heavy metals (Ma, 1980) from earthworms in natural communities. The introduction of a 'standard' species as *Eisenia fetida* has been done by Marquenie *et al.* (1985). The observations are dependent on:

1. The level of chemical in the soil.
2. The food choices of a particular earthworm stage-species (Ferrière, 1980).
3. The availability of the chemicals in earthworm food and skin contact.

A standard bioaccumulation system, using *Artisol*, and *Eisenia andrei* Bouché, 1972, was used to demonstrate a constant ratio of bioaccumulation after equilibrium for heavy metals. Detoxication of soil by earthworms could be also potentially detected by the same standard process (Bouché, 1984b).

## 6. Conclusions

In ecological studies, earthworm toxicity must be taken as a question at its right level. They are the largest invertebrate biomass of land surfaces. Because of their great usefulness in agrobiocenoses and natural systems, they have to be protected by a careful choice of chemicals applied to lands. We have techniques available today to make toxicity tests, hazard assessments and technico-economical appraisals: *i.e.*, it is possible to balance between the need of a pesticide and the requirements for earthworm activities in soils.

Because of their wide natural dispersal, their close relation with other main biomasses (*i.e.*, microorganisms, roots, other soil animals), necromasses (humus compound, litter) and their ability to ingest soil minerals compounds (from sand to clay), those animals give us an excellent tool to detect pollutants, to measure their bioavailability in soil, and/or to compare earthworm uptake with a standard bioaccumulation method.

Closely associated with the 'fast' turn-over (Coleman *et al.*, 1983) of elements, earthworms re-cycle most of them rather quickly. Possible chemical transformations at their level, as detoxification, are poorly understood but could be of significance.

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