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Selecting a battery of bioassays for ecotoxicological characterization of wastes

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Abstract

This study was conducted in France within the context of waste classification (Hazardous Waste Council Directive 91/689/EEC), and focused on "ecotoxic" property (H14). In 1998, an experimental test strategy was developed to assess ecotoxicological properties of wastes using a battery of six standardized bioassays. This combined direct and indirect approaches integrating two solid-phase tests: emergence and growth inhibition of *Lactuca sativa* (14 days), mortality of *Eisenia fetida* (14 days) and four standardized tests performed on water extracts from wastes: growth inhibition of *Pseudokirchneriella subcapitata* (3 days), inhibition of mobility of *Daphnia magna* (48 h), inhibition of reproduction of *Ceriodaphnia dubia* (7 days), inhibition of light emission of *Vibrio fischeri* (30 min).

This study aimed to set up preliminary conclusions on relevancy of this experimental test strategy, based on data obtained since 1998. Results were analyzed from the combined use of Hierarchical Cluster Analysis, Principal Component Analysis and Nonlinear Mapping. These multivariate analyses clearly showed that it was possible to reduce this number of tests without changing the typology of the wastes.

A battery of bioassays including one solid phase test and two tests performed on water extracts (*L. sativa*, *V. fischeri* and *C. dubia*) was found as an optimal solution for characterizing the toxicity of the studied wastes. This optimal battery represents a good basis for determining the H14 property.

Keywords: Ecotoxicity; Bioassay; Waste; Hazard; Test battery; Multivariate analyses

1. Introduction

The management of urban and industrial wastes has become a high priority. The quantity of wastes produced regularly increases with the standard of living of populations. The landfilling of wastes, which still

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remains one of the major ways of disposal, can be a potential source of contamination of soils and ground waters by percolation waters.

Some years ago, European legislation on waste policy considered that only safe and controlled landfill activities should be carried out within the European Union. This involved that the disposal of wastes by landfill should be monitored and managed to prevent or reduce potential adverse effects on the environment and risks to human health. Specially, the mixing of hazardous wastes with non-hazardous wastes should be avoided.

In 1994, European Union then established a list of wastes (Commission Decision 94/3/EC) and a list of hazardous wastes (Council Decision 94/904/EC) that were modified on several occasions. The last major change (Commission Decision 2001/118/EC) included a significant number of mirror entries (e.g., a waste from power paint, identified by the six-digit code 08 01 04, was previously considered as non-hazardous; now in the revised list, the same waste may be classified hazardous (08 01 11[c]) or not (08 11 12) depending, whether or not, it contains hazardous substances).

In the European Union, the concern and need for assessing the potentially harmful effect of wastes were already expressed in the Hazardous Waste Council Directive 91/689/EEC which defined a set of 14 properties allowing waste classification. They are derived from the Council Directive 67/548/EEC on dangerous substances. For some of these properties (e.g. explosive, oxidizing, flammable), tests methods, developed for chemicals in the Annex V to the Commission Directive 67/548/EEC or in subsequent Directives adapting Directive 67/548/EEC to technical progress (Commission Directives 88/302/EEC and 92/69/EEC), are also applicable for waste characterization. However, H14 or "ecotoxic" property (substances and preparations which present or may present immediate or delayed risks for one or more sectors of the environment (Council Directive 91/689/EEC)) does not refer to specific methods.

The Annex III of the Council Directive 91/689/EEC only indicates that test methods to be used have to be in compliance with the Annex V of the Council Directive 67/548/EEC and its further modifications, according to the work and recommendation of OECD. This statement is not sufficient for a clear evaluation of this property.

The H14 property was considered by experts as a conclusive factor for deciding the fate of more than 80% of hazardous wastes (Méhu et al., 2004). Notwithstanding the preponderance of this property, no procedure has been yet validated to assess the ecotoxicological characterization of wastes. Moreover, the European Waste List contains more than 250 mirror entries, for which the classification as ecotoxic or non-ecotoxic is left open, thus reinforcing the necessity of developing a strategy for assessing the hazardous properties of wastes.

Generally, hazard can be estimated using two approaches: a chemical-specific approach and a toxicity-based approach. In the former case, chemical analyses are performed and results are compared to threshold values. In the latter situation, toxicity is directly measured using biological tests.

The determination of pollutants in complex mixtures of unknown composition, a common situation with many wastes, but not restricted to such matrices, does not allow a relevant estimation of toxicity. For such samples, the toxicity-based approach is usually recognized to be the best method for assessing a potential toxicity. The main advantage of performing bioassays is their integrative character (Thomas et al., 1986). Indeed, they integrate the effects of all contaminants including additive, synergistic and antagonistic effects. They provide valuable information on the bioavailable fraction of the contaminants only; they also integrate the effects of all contaminants, including those, not considered or detected by chemical analyses (Vasseur and Férard, 1993). Usually, two methods can be applied for selecting bioassays in order to establish a test battery: (i) an "a priori" method, in which the selection is made, independently of the results, according to decision criteria such as standardization of the method, ecological relevance of test organisms, or cost (e.g. Keddy et al., 1995; Van Gestel et al., 1997), (ii) an "a posteriori" method, in which the selection is made after analyzing test results obtained on a large series of bioassays (e.g., Rojickova-Padrtova et al., 1998; Clément et al., 1996).

Most of the publications dealing with the selection of test batteries are based on the latter strategy. However, in that case, the use of multivariate analyses remains unexploited (e.g., Clément et al., 1996; Manusadzianas et al., 2003, Ren and Frymier, 2003) while they provide invaluable information on the characteristics of the toxicity data through powerful graphical displays, which are easy to interpret (Devillers and Karcher, 1991).

In 1998, a battery of six standardized ecotoxicity tests was proposed by the French Ministry of Environment in order to assess the H14 property (MATE, 1998). Since that date, this experimental test strategy was applied on various industrial and domestic wastes (organic and inorganic chemical processes, tanneries, iron and steel industries, non-ferrous hydrometallurgy, water treatment facilities, etc.). Such an experimental