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DISCRISET: A battery of tests for fast waste classification – Application of tests on waste extracts

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ABSTRACT

The Hazardous Waste Directive (HWD, Council Directive 91/689/EC, 1991) provides a framework for classification of hazardous waste, based on 15 Hazard (H)-criteria. For complex wastes the HWD foresees the application of toxicity tests on the waste material itself to assess its toxic properties. However, these proposed test methods often involve mammalian testing, which is not acceptable from an ethical point of view, nor is it feasible economically. The DISCRISET project was initiated to investigate the use of alternative chemical and biological fast screening tests for waste hazard classification. In the first part of the project, different methods were reviewed and a testing strategy was proposed to minimize time and cost of analysis by a tiered approach. This includes as a first tier chemical analysis followed by a general acute toxicity screen as a second tier and as a third tier mechanistic toxicity tests to assess chronic toxicity (genotoxicity, hormone disturbance, teratogenic effects, immunologic activity). In this phase of the project, selected methods were applied to 16 different waste samples from various sources and industries. The first tier chemical tests are recommended for the full characterization of the leachate fraction (inorganics) but not for the organic fraction of samples. Here the chemical characterization is only useful if toxic content is known or suspected. As second tier the fast bacterial test Microtox is validated as a general toxicity screen for the organic fraction (worst case organic extract). Samples that are not classified in tier 1 or 2 are then further investigated in the third tier by the mechanistic toxicity tests and tested for their potential chronic toxicity: immune activity (TNF-α upregulation) is indicative for corrosive, irritating or sensitising effects (H4/H8/H15), reproductive effects (H10) are indicated by hormone disturbance and early life stage abnormalities in fish larvae when exposed to the extracts and mutagenicity and carcinogenicity (H7, H11) are indicated by SOS response induction and increased mutation frequency in the Ames test when exposed to the extracts. Results indicate that the combination of chemical tests and bioassays allows important hazardous properties to be addressed and the tiered approach ensures that the tests are performed quickly and economically. The suggested strategy provides a solid and ethical alternative to the methods described in the HWD and is a vast improvement on the current, arbitrary classification.

1. Introduction

The hazardous waste directive (HWD, Council Directive 91/689/EC, 1991) provides a framework for the classification of waste. Waste is classified by its hazardous properties as defined in the HWD-Hazard (H)-properties: physical (H1 explosive, H2 oxidising, H3 flammable) and toxicological hazardous criteria (H4 Irritant, H5/6 harmful or toxic, H7 carcinogenic, H8 corrosive, etc.) (Table 1). These properties can be attributed to individual waste compounds, but for complex waste with unpredictable composition, the hazardous properties should be measured directly on (extracts of) the waste material (as recommended by HWD). The recommended methods in the HWD for the evaluation of toxicological and ecotoxicological properties are those used for the hazard assessment of chemicals (Council Directive 67/548/EC). However these involve mammalian testing which is not acceptable from an ethical point of view for hazard assessment of waste and not feasible from an economical point of view.

The DISCRISET project was initiated to investigate the application of existing alternative tests for hazard assessment of chemicals to waste materials for classification purposes. In the previous phase of the project, a number of existing assays were reviewed and their suitability for assessment of complex waste samples

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Table 1
HWD – list of hazardous properties.

H	Criterion
H1	Explosive
H2	Oxidising
H3a	Highly flammable
H3b	Flammable
H4	Irritant
H5	Harmful
H6	Toxic
H7	Carcinogenic
H8	Corrosive
H9	Infectious
H10	Toxic for reproduction
H11	Mutagenic
H12	Release of (very) toxic gases
H13	Leachate with hazardous properties
H14	Ecotoxic
H15	Substances and preparations capable by any means, after disposal, of yielding another substance, e.g. a leachate, which possesses any of the characteristics listed above

was discussed (Weltens et al., 2008, 2009, in press). This review resulted in a list of tests (Table 2) which comply to the following conditions:

(a) the classification has to be based on total concentrations and based on the 15 hazard properties described in HWD;
(b) assays should take only a minimum of time (preferentially less than 48 h) and should be as cheap as possible to allow batch controls and to prevent waste from piling up on the site (avoiding odour and/or space problems);
(c) a high level of standardisation is necessary to allow the results to be compared to preset limit values.

Moreover, a tiered test strategy was proposed, aiming to avoid unnecessary testing and minimising the time and costs of waste assessment by a tiered approach (Fig. 1). The first tier consists of a targeted chemical analysis of the inorganic fraction of the waste. This step is also useful for the organic fraction if the composition of the waste is known or if there is a strong indication about the potential toxic substances present in the waste. In these cases the analyses allow the concentrations of the analyzed elements and compounds to be compared to existing HWD limit values. Exceeding these limits results in immediate classification and further analysis is then no longer required. When targeted analytical approaches cannot provide the necessary chemical information bioassays are applied on extracts of the waste materials.

The second tier consists of a fast (bacterial) test for general toxicity that enables a first triage by recognising the samples that have a very high intrinsic toxicity and can be classified as hazardous based on this test result alone. Microtox, a well validated bioassay which takes only 30 min, was suggested for this purpose. A validation study was started to confirm the screening abilities of this test (Weltens et al., in press).

The last tier of the strategy consists of a battery of biotests performed in parallel and evaluating different types of mechanistic toxicity. Selected tests identify samples with genotoxic content or endocrine disruptive substances as well as partially recognising teratogenic and irritative effects.

Ecotoxicity tests were also performed in this phase. Although these are not based on mechanistic toxicity but measure general toxicity, these tests are included in this last phase as they tackle a specific hazardous property of waste (H14).

In this study we will use complex waste samples selected from a wide range of sources to test the robustness of the proposed testing strategy. For some of the industries represented here, such as the wood, paint and textile industries, toxic properties of waste or effluent have been described in literature (Giorgetti et al., 2011; Orrego et al., 2011; Ghisari and Bonefeld-Jorgensen, 2009). Often however, results indicate that toxicity varies between sites and samples, confirming the need for a waste assessment tool which can be implemented for routine waste classification. Analyses will be performed on worst case extracts of the waste to comply to current legislation, which states that classification should be based on the intrinsic toxicity of the waste, as opposed to the bioavailable fraction, which is better evaluated using water eluates (Tigini et al., 2010; Vaajasaari et al., 2003; Charles et al., 2011; Ma et al., 2010).

Here we report on the results of the selected assay candidates on 16 waste samples from various sources. The suitability of the selected tests for waste assessment is discussed and the implementation in the proposed testing strategy is evaluated.

2. Materials and methods

2.1. Samples

Initially seven filter press samples from the water treatment plant of a tank cleaning company were tested in a pilot study. In

Table 2
Bioassays used for waste toxicity assessment in this project.

Hazard	General toxicity (H5/H6)	Reproductive effects (H10)	Corrosive, irritating, sensitising (H4/H8/H15)	Genotoxicity Mutagencity (H7/H11)	Ecotoxicity (H14)
Chemical classification (risk phrases)	R20/21/22 R23/24/25 R26/27/28	R60/61/62/63	R36/37/38 R42/43	R45/46/47 R49	R50–58
Bioassays	Microtox	Teratogenicity	TNFa upregulation	Ames toxicity	Algae growth inhibition
	Fish larvae EC50	Fish larval mortality/macroscopic abnormalities		Vitotox	
	Cytotoxicity			DNA damage (BGPA)	Daphnia immobilisation Fish larval mortality
	Vitotox toxicity Ames EC50 Stress responses (BGPA) Algae growth inhibition	Hormonal disturbance CALUX			Microtox