Umwelt Bundes Amt
for our Environment

Recommendations for the Ecotoxicological Characterization of Wastes

26 February 2013

Publisher: Federal Environment Agency, Germany

Wörlitzer Platz 1

06844 Dessau-Roßlau

Internet: http://www.umweltbundesamt.de

Editorial: Section 1.5

Municipal Waste Mangement, Hazardous Waste, Focal Point to the Basel Convention

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State: 26 February 2013

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1 Introduction

This Recommendation is based on the results and experiences gained in an international inter-laboratory ring test on the ecotoxicological characterisation of wastes (Moser & Römbke 2007), the evaluation of analyses of the biological effects of mineral waste, an exchange of experience with CEN TC 292 WG7, and the ecotoxicological screening of a large number of wastes in mirror entries (Römbke et al. 2010a). In addition, numerous standards (e.g. of ISO), technical documents and research reports on the ecotoxicological assessment of complex environmental matrices (waste water, contaminated soil) were taken into account in its preparation, as were, in particular, the recommendations by CEN TC 292 WG 7.

A combination of chemical and biological test methods should be used for the ecotoxicological characterisation of wastes, since a comparison of the results of chemical analyses with existing threshold values is insufficient to derive the hazards posed by waste. Instead, an evaluation of the environmental hazards of waste is possible only by the use of biological test methods, as only these can mirror the effects of all bioavailable contaminants including their potential interactions as well as pollutants in waste which cannot be determined by chemical analysis.

The analysis of available tests of more than 100 waste samples showed that biological test methods produce well repeatable results for heterogeneous waste composites too. Therefore there is reason to assume, that not only effects of single contaminants, but also matrix effects, which are undetectable by chemical analysis, could be detected.

Goal of this recommendation is to support a reliable method base for application-orientated ecotoxicological analysis of wastes, which facilitates the comparability of test results. For this reason this Recommendation focusses on explaining the main outline and giving references to the necessary guidelines instead of going into detail about procedures.

2 Scope of application

This Recommendation contains, firstly, instructions for the selection and use of biological test methods suitable for estimating the ecotoxicological potential of waste and hence, its hazards for the environment, which are based on this criterion. The test strategies it proposes are recommendations and may be supplemented or modified in the light of specific properties of the waste and planned recovery scenarios. Secondly, it summarises standards and methodological experience facilitating an evaluation of the test results. The toxicity criteria indicated, were determined in scientific studies and specifically for the proposed test systems, reflecting the value above which, based on existing experience, a response in a test can be regarded as ecotoxicologically relevant. Further investigations are currently on-going to derive limit concentrations above/below which a waste can be classified as hazardous or non-hazardous.

As well as for the classification of waste, the results of biological analyses can also be used for risk assessment of waste to be recovered. Use of the test methods enables an assessment of potential adverse effects on aquatic and terrestrial ecosystems.

In previous years, as proposed in the first version of this guidance (Moser 2008), extended limit tests have been used for the classification of wastes. Extended limit tests have a shortened and simplified design which means that the waste sample is not tested in a dilution series until no toxicity is observed anymore. Instead, only the limit concentration and those two dilution steps immediately below and above the limit concentration are tested. This shortened assay has the

disadvantage of depending strongly on the limit concentration, which is set in advance and deemed fixed. Additionally, there is no differentiation between a strong or minor divergence of the limit concentration.

Deviating from this proposal, the utilization of a more detailed design is recommended. This design would aim to determine the dilution in which the chosen test parameter (of a given test species) is impaired by 50%. In addition, utilizing an EC50-assay helps harmonizing different law sectors (environmental risk assessment of chemicals, in particular pesticides, follows the same approach) and practices of waste testing in other European countries. France, for example, prefers the EC-assay (Pandard et al. 2006).

3 Legal background

In the European Union, wastes are classified according to the Waste Framework Directive (WFD) 2008/98/EC (EC 2008a). The European Waste List (EWL, Decision 2000/532/EC (EC 2000) and its amendments) is a harmonized list of waste types which groups wastes by source or generation process. In its applicable version the EWL is an EU-wide nomenclature system for. The European Waste List was transposed into German law in 2002 by the Waste List Ordinance (Abfallverzeichnisverordnung, AVV) and comprises 839 waste codes in 20 waste chapters including 405 wastes marked as hazardous and about 200 wastes in so-called “mirror entries”.

Mirror entries consist of pairs of entries of which one waste may be classified as hazardous or non-hazardous according to the type and concentration of the pollutants it contains. Wastes classified as hazardous are marked with an asterisk “*”. The majority of mirror entries refer to the term “hazardous substances” while some describe “hazardous properties” or the specific hazardous waste component (see Table 1).

To differentiate between hazardous and non-hazardous wastes in mirror entries, the European Waste List specifies 15 hazard criteria which are used for assessment and are based on European hazardous-substances legislation (Directive 67/548/EEC (EC 1967)). One of the hazard criteria (HP14 “ecotoxic” – substances and preparations which present or may present immediate or delayed risks for one or more sectors of the environment) describes the ecotoxicological potential or environmental hazards, as an intrinsic property of waste. In the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal, criterion H 12 (“ecotoxic - substances or wastes which if released present or may present immediate or delayed adverse impacts to the environment by means of bioaccumulation and/or toxic effects upon biotic systems”) likewise requires an assessment of the ecotoxicological potential of waste.

Table 1: Examples of mirror entries from the European Waste List

Example 1	
19 01 11*	Bottom ash and slag containing dangerous substances
19 01 12	Bottom ash and slag other than those mentioned in 19 01 11
Example 2	
10 08 10*	Dross and skimmings that are flammable or emit, upon contact with water, flammable gases in dangerous quantities
10 08 11	Dross and skimmings other than those mentioned in 10 08 10
Example 3	
06 03 11*	Solid salts and solutions containing cyanides
06 03 13*	Solid salts and solutions containing heavy metals
06 03 14	Solid salts and solutions other than those mentioned in 06 03 11 and 06 03 14

4 Scientific background

Biological test methods utilise the capacity of specific organisms to respond to exposure of pollutants under standardised conditions by a change in their vital functions. The sensitivity of biological test organisms to toxic waste constituents may vary significantly from species to species. The combination of different test methods in a so-called test battery must therefore include organisms belonging to different taxonomic groups and representing different trophic levels. The object here is to cover a sensitivity range as broad as possible and at the same time to get an impression of potential impacts on the ecological functions of both aquatic and terrestrial organisms. The test methods should comprise different effect criteria and cover both acute and chronic toxicity as well as genotoxicity. Test methods to be applied in enforcement practice must also meet various additional requirements, regardless of the test subject (chemical, composite, genetically modified organism. They must be standardised, sufficiently sensitive, easy to handle and economically feasible (Roembke et al. 2010b). In addition, it is essential that experience exists for each test system as regards specific requirements in the testing of waste and waste eluates.

The battery of tests proposed in this Recommendation enables a reliable, valid and reproducible determination of hazard criterion HP14 “ecotoxic” and thus allows wastes in mirror entries to be identified as hazardous in terms of that hazard criterion. This determination of intrinsic waste properties is not sufficient to define requirements for a disposal that is environmentally sound and compatible with the public interest or for proper and safe recovery, but instead will often have to be supplemented by further investigations. This may include, for example, the integration of possible exposure scenarios in a risk assessment when utilisation in an open system envisaged or in the permitting of waste treatment plants.

The use of biological test methods allows conclusions to be drawn as to the direct acute and chronic toxicity to the test organisms and, approximately, to biologically similar organisms. They do not, or not fully, cover hazards for the abiotic environmental spheres soil, water or the climate. The environmental hazards of persistent pollutants, which cause damage by accumulating in environmental compartments and food chains, can be determined by physical-chemical methods or suitable bioaccumulation tests (e.g. OECD 315; OECD 317). Waste constituents with global warming potential, on the other hand, can only be identified by chemical analysis.

The assessment of the published work on the ecotoxicological testing of wastes of the last 20 years, showed a continuous increase in publications. Nevertheless, a wide range of wastes and test methods (especially for the creation of eluates) is covered in these publications. The heterogeneity of the published data concerning the ecotoxicity of wastes complicates the comparison and simplification of the obtained results. However, it can be concluded that the current ISO-guidelines are suitable for the ecotoxicological characterization of a multitude of solid and liquid (slurry included) wastes. The results of the ecotoxicological tests differ significantly depending on the characteristics of the evaluated waste, which makes it possible to utilize them to differentiate between ecotoxic and non-ecotoxic wastes.

5 Methodology

This Recommendation is based on standard methods used originally for the testing of soil, waste water or chemicals and subsequently developed further for the ecotoxicological characterisation of wastes. These bioassays as well as supplementary guidance on sample collection and preparation are summarised in CEN 14735. For sample collection and preparation, further standard methods and technical instructions are available (see the compilation of ISO-test methods for the evaluation of contaminated soils or soil eluates (ISO 17616)). Up until today, there is no proposition for the standardization of procedures on the various levels of European and national norms and working instructions (e.g. LAGA PN 98). Some specific features of effects-related testing of waste are pointed out in the following section. This information cannot replace the description of the various methodological steps in the relevant standards.

5.1 Terms

5.1.1 Effect concentration (EC)

The ecotoxicity of waste is determined by biological tests, and expressed as an EC50. It is defined as the concentration of a dilution series in which the measured parameter complies 50% of the control concentration (ISO 11269-2).

5.1.2 Limit concentration

“Limit concentration” describes the EC50-value which determines whether a tested waste has to be classified as “harmful” according to the HP14 criterion. With reservations to the final draft, it is proposed in this guidance that the limit concentration should be defined as the concentration of the potentially harmful substance in waste, at which the EC50 of said substance is smaller than the concentration in a 10% dilution (Pandard & Römbke 2013). This proposal is applicable in solid matter and eluate testing.

5.1.3 Test design

The EC50-value is determined by a “Dose-Response-Design”, which has to consist of no less than five different dilutions. Waste eluates which are tested in aquatic systems are mixed with test-specific dilution water, as opposed to samples of solid waste originating from terrestrial systems which are mixed with the corresponding control medium (e.g. artificial soil, standard soil or quartz sand.) The dilution series to be tested mostly consist of nestled geometric sets on the basis of the numbers 2 and 3, although other factors can be set according to the range of the tested chemical (Table 2).

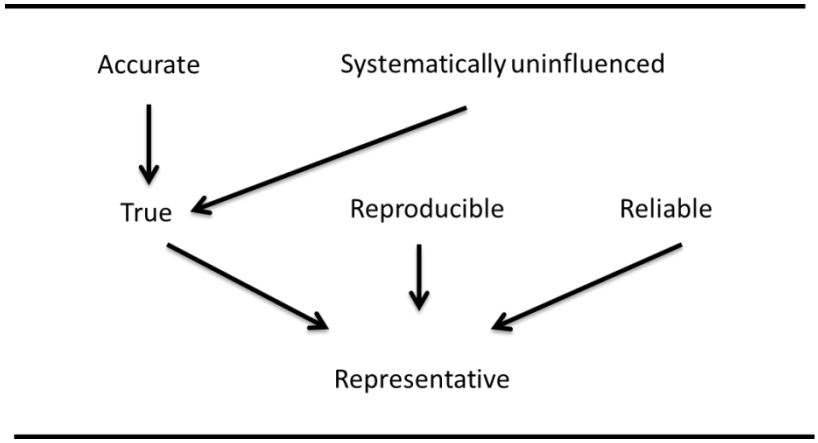
Table 2: Sample calculation for the sample concentration in the assays for the dilution-series

Series A			Series B		
LID-Value	Dilution	Sample concentration in test medium [%]	LID-Value	Dilution	Sample concentration in test medium [%]
2	1:2	50	1,5	1:1,5	66,667
4	1:4	25	3	1:3	33,334
8	1:8	12,5	6	1:6	16,667
16	1:16	6,25	12	1:12	8,334
32	1:32	3,125	24	1:24	4,167
64	1:64	1,562	48	1:48	2,084
etc.	etc.	etc.	etc.	etc.	etc.

5.2 Methodological requirements for the testing of waste

5.2.1 Sampling and sample preservation

Collecting representative samples from heterogeneous composite wastes still poses several difficulties. The same can be said for the chemical analysis, in which the creation of reproducible data for certain parameters; e.g. concentration of heavy metals, is comparatively difficult. The federal Working Group on Waste (LAGA) does not regard the sampling method for solid wastes as representative, but redefines it as a sampling method for the characterrization of waste (PN 98, page 5f). Goal of the characterizing sampling method for waste is to collect ascertained data about the material quality, via test results. For the LAGA the representativeness of attributes depends on the following aspects:



The attributes used are defined as follows:

Accurate:	Variance of measurement deviation is below a given limit
Systematically uninfluenced	Free of systemic (scale shifting) errors
True	Accurate and systemically uninfluenced
Reproducible	Repetition creates statistically identical results
Reliable	The risk of misjudgement is smaller than a default value

LAGA reacts to these requirements in the PN 98 by advising to increase the number of samples in order to model parameter-specific variations. This approach was incorporated in DIN 19698. For biological tests this means that all investigations have to checked concerning their reproducibility as well as their reliability. Assuming that the quality of the test material is comparable it is possible to compile the variance found in various analytical measurements and compare with a given threshold value. Regarding the reliability a value of 95% is striven for.

5.2.1 Sampling and sample preservation

The CEN standard 14735 "Characterization of waste – preparation of waste samples for ecotoxicity tests" refers to an unfinished (work in progress) standard of the CEN/TC 292/WG. It has not yet been concluded. The DIN has submitted the draft standard DIN 19698-1; "Testing of waste – Sampling of solid and set wastes – Part 1: Manual for the segment-oriented collection of samples of unknown heaps." in October 2012. This draft is based mainly on the LAGA PN 98 "Guideline for the physical, chemical and biological examination of wastes, in reference to its utilization/disposal". This approach does not coincide with the theoretical background, set in the European standards, e.g. DIN 15442 in CEN/TC 343 "Solid secondary fuels – sampling method".

In the light of the relevant literature, guidance documents and guidelines (Gy 1979, Gy 1992, LAGA PN 98, DIN 15442, DIN 19698, LAGA 2012) and with regard to a sufficient reliability and justifiable efforts, the following procedure is recommended for the sampling for the ecotoxicological testing of wastes

1. In a laboratory sample the mass above the 20th percentile of the sieve analysis should be represented by more than 20.000 particles.
2. Any sampling should ideally be performed at random points in time across the whole transversal section of the particle mass flow falling from a conveyor belt. In case this is not possible the sampling should be performed from the heap of waste.
3. When taking samples from a heap of waste, it should be secured that no phase separation did occur during the set-up of the heap.
4. Independently from the size of the basic population, 16 individual samples have to be taken.
5. The individual samples should be random samples. In other words: each particle of the basic population should have the same probability to be part of an individual sample.

6. Sampling from a heap of waste could be performed using a wheel loader. The 16 samples taken this way should be combined to a nearly two dimensional flat layer providing a height of 1 – 1.5 dm. Individual samples could be randomly taken from random coordinates of this two-dimensional layer.
7. All individual samples should be combined to one mixed sample, i.e. the laboratory sample.
8. A sample size reduction without a previous reduction in particle size is not allowed.

In addition, it should be noted that waste often undergoes chemical, physical and biological changes after having been sampled. Therefore, potential changes need to be taken into account, and sampling conditions should be such that the impact of such changes on the results of bioassays is minimised. The addition of preservatives (e.g. acids) for the purpose of delaying chemical and biological processes does not conform to the relevant standard.

5.2.2 Transport and storage of samples

The transport of waste samples should be as short as possible. Changes in the properties of the samples should be prevented. The transport period must be considered to be part of the storage period, and a transport period below 48h and/or a low temperature of 4°C ± 2°C must be adhered to. The samples should not be stored for longer than two months. Should longer storage be necessary, possible changes to the waste samples during storage have to be determined by accompanying physical, chemical or biological analysis of waste-specific parameters.

5.2.3 Pretreatment of samples

Any reduction in size of the waste particles ends up in producing new surfaces and, thus, in a modification of the ecotoxicological properties of the test material. Therefore, it should be avoided if possible.

For waste material with a d95 > 4 mm but with a relevant percentage in mass of material < 4 mm it is possible to get information on its ecotoxicological properties from the sieving fraction < 4 mm, taking the mass fraction into account. In the case of testing very heterogeneous materials with low mass fractions < 4 mm ecotoxicological testing seems to be inappropriate. However, material broken or grounded to a d95 < 4 mm can at least be used for a first assessment.

In case the original waste material consists mainly of particles > 4 mm, a sieve analysis has to be prepared using an appropriate amount of material. It is not necessary that this sieve analysis follows regulations like e.g. DIN 66165 in detail. Since such material contains coarse grain sieving by hand is sufficient to perform a suitable sieve analysis. Regarding sample mass and sieve dimensions it must be secured that in the coarsest fraction (d95+) contains more than 200 particles. An example of such a sieve analysis is shown in Annex A.

If materials with huge coarse grain content should be tested the coarse grain > 4 mm must be reduced in size, whereby the material should not be finely ground in any case. Cryogenic treatment may be necessary for this purpose. If the material has to be dried for sieving, the drying temperature may not exceed 40°C, as drying may alter various waste properties.

Non shredable and coarse materials, as well as tall single pieces which do not conform to the overall character of the sample, are to be removed during pre-treatment. Furthermore their

visual image has to be recorded photographically. Their number, weight and percentage of total sample weight have to be recorded as well. In general it has to be taken into account that sample preparation and pretreatment, depending on the test or analysis, will narrow the sample and therefore decrease confidence in the results. As the representativeness of the sample in relation to the examined property cannot be known in advance, information about the confidence of tests can only be gained by parallel-investigation. A ripple divider is recommended for sample rejuvenation in the course of sample preparation and pretreatment. As an alternative the method “coning and quartering” can be used (LAGA PN 98).

5.2.4 Preparation of waste eluates

A wide range of methods has been developed to characterise and evaluate leachable waste constituents. Leaching of water-soluble constituents from waste is considered a major mechanism for potential environmental hazards posed by waste. The aim of the leaching procedure, therefore, is to produce an aqueous extract which can be used to determine the ecotoxic properties of water-leachable waste constituents. Any laboratory method can only give an approximate picture of the complexity of leaching processes, so that simplifications need to be made in the test method. Furthermore, certain waste properties may affect the suitability of the leaching method. For example, waste samples that contain organic pollutants require an adaptation of the leaching method.

For the preparation of waste eluates, methods based on national or international standards should be used. DIN 12457-2 describes a suitable method for leaching of waste materials at a liquid to solid ratio of 10 litres of eluent per kilograms of solid. It applies to waste with a particle size below 4 mm with or without size reduction. This leaching method is also a component of CEN 14753 “Characterization of waste – Preparation of waste samples for ecotoxicity tests”.

According to current research projects and validation studies, another suitable leaching method is the production of eluate in a column, as done in the quick column test (DIN 19528). However, no experience exists as yet with the biotesting of such eluate and with possible limit concentrations for waste classification.

Eluates produced in a batch test or column test only represent the short-term water-available components of potential toxic waste constituents. Especially when performing a risk assessment for planned recovery in an open system, methods should be used that reflect the conditions of the recovery scenario. Each relevant route of entry into the environment should be taken into account in this assessment, and changes to the material over time should be evaluated or additional tests performed if necessary. Where test methods are prescribed by relevant secondary legislation, the leaching method should be chosen as appropriate for the intended purpose.

In addition to point 11.2.1 (Leaching Procedure) of CEN 14735, it is recommended to use a partition for the eluate which is between 100g and 200g dry weight in order to reduce the uncertainty of sample size reduction. The sample quantity used should be easily reproducible out of the sample division into halves. Division steps have to be documented, so that the necessary water ratio for a mixture of 1:10 is easily adjustable. If necessary potential uncertainties caused by sample preparation and pretreatment might be reduced by mixing several parallel conducted eluates (e.g. 4).

Since potential genotoxic waste properties represent a particular risk, a solid-phase extraction can be used to concentrate the eluate in addition to testing the aqueous eluate for genotoxicity (Ehrlichmann et al. 2000).

5.2.5 Storage of waste eluates

The waste eluates must be used in the test as soon as they are produced (recommended maximum storage period: 48 hours; maximum storage period: 72 hours). Waste eluates may not be stabilised by the addition of preservatives. Freezing may cause eluates to undergo irreversible changes and therefore, is permitted only in exceptional cases. If freezing is unavoidable, this has to be documented in the test protocol.

5.2.6 pH of waste eluates

The waste eluates must be tested in the bioassays without pH adjustment, whereby it must be borne in mind that the pH of the test mixture, due to dilution, may differ significantly from that of the waste material to be evaluated. The decisive factors for this are the chosen range of dilutions and the buffering capacity of the waste. If toxic effects are observed at dilutions whose pH does not permit or significantly influences the survival of the test organisms, the ecotoxicity tests may be repeated with adjustment of the pH in conformity with the relevant standards. It is important to note that an acid or alkaline eluate may exhibit an ecotoxic effect due solely to its non-neutral pH and that this may affect the classification of the waste.

5.2.7 Control medium

Ecotoxicity testing of waste requires the use of a dilution medium which does not affect the response of the test organisms and does not interact with the sample. Since the requirements to be met by the dilution medium depend upon the test organisms, suitable dilution substrates are described in the test methods. The same medium must be used for both the control and the dilution series. The production of test mixtures also depends on the waste type to be tested. CEN 14735 summarises procedures for the production of test mixtures.

6 Test strategies

The assessment of wastes is aligned on assessment under hazardous-substances legislation. Waste can thus be classified based on sufficient knowledge of its composition in terms of hazardous substances. Every waste which, based on its (known) composition, is to be classified and labelled in accordance with hazardous-substances law (H1 to H13) is considered hazardous waste.

In addition, if the composition of the waste is unknown or complex, biological test methods may be applied for the following purposes:

- to identify hazardous wastes in mirror entries in the Waste List Ordinance according to the criterion HP14 (Chapter 6.1),
- to carry out a basic ecotoxicological characterisation of wastes (Chapter 6.2),
- to assess the risks of waste recovery measures. (Chapter 6.3).

6.1. Identification of ecotoxicological wastes in mirror entries in the Waste List Ordinance

6.1.1 General

If the concentration levels in the eluate or waste as determined by chemical and physical analysis do not permit classification under hazardous-substances legislation, if insufficient knowledge exists about the composition of the waste, or if the waste constituents have not been classified under hazardous-substances legislation, then tests have to be performed for the hazard criterion HP14. These tests should be conducted in several steps (Fig. 1).

Waste-evaluation begins with the utilization of accessible information, which means that an accumulation method which is in concordance with the CLP regulation (classification, labeling and packaging of substances and mixtures) for acute and chronic parameters in aquatic systems is used (European Regulation EC 1272/2008 (EC 2008b)). If sufficient data for every waste-component is available, waste-evaluation can be executed in this manner which renders ecotoxicological testing unnecessary. In all other cases, the experimental determination of the ecotoxicity is required. An evaluation as “not ecotoxic” does not automatically lead to classification as non-hazardous waste, since all other hazard criteria specified in the Waste List Ordinance must also be evaluated, irrespective of the ecotoxicological assessment.

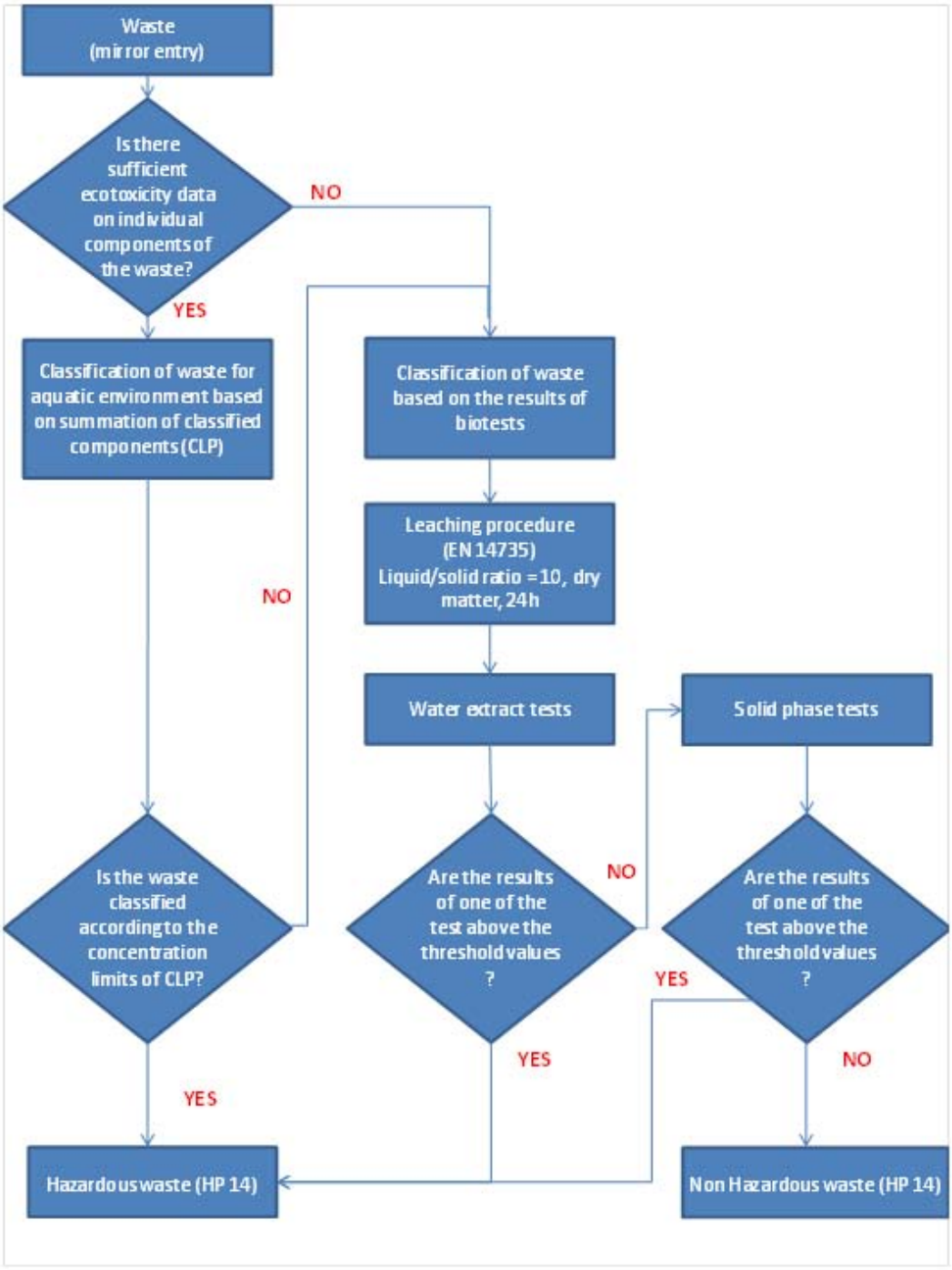


Fig. 1: Flow chart for the waste-classification in accordance with HP14 (Pandard & Römbke 2013)

6.1.2 Test strategy

Practical implementation of ecotoxicological tests is determined by CEN 14735, and starts with the examination of waste eluates (Fig. 1), which are mixed with a control-substrate (reconstituted water). In general, pH-values are not adjusted. Tests can only be repeated if the pH-value of the composite is threatening the survival of the test specimens. However, the result cannot change waste-classification but is mainly used for cause identification. The test results are designated as ECx (=effect concentrations). The waste is classified as dangerous and the tests are discontinued if the EC is below the limit-concentration. If the EC is above the limit concentra-

tion, terrestrial tests are conducted. Waste can only be classified as nonhazardous, if all test-results are below the limit concentration. So far, published data of ecotoxicological waste tests does not show a consistent advantage of aquatic tests in respect of test-sensitivity (Römbke et al. 2009). Therefore, the proposed test strategy encompasses both aquatic and terrestrial tests. Nevertheless, due to practicability reasons it focusses on eluate tests which are less time consuming and expensive.

6.1.3 Selection of test methods

The battery of tests shown in Table 3 is based on the results of a European inter-laboratory comparison for the ecotoxicological characterization of wastes (esp. Pandard et al. 2006, Roembke et al. 2010b) and describes the minimum set of methods to identify potential biological effects of environmentally dangerous waste constituents. The most suitable tests were selected according to the following criteria:

- Eluate- as well as solid tests should be represented (CEN 14735);
- For practicability reasons, the number of tests per compartment was set to three;
- Test species should represent the most important groups (microbes, plants, animals), in order to include several taxonomic and physiological groups as well as trophic levels.
- Fish tests were not included for reasons of animal protection and because of the large quantity of eluate needed.
- Only standardized ISO- and/or CEN- methods are considered.
- Sufficient experience about the execution of waste-evaluation tests has to be available in order to produce robust results;
- Endpoints must be of sufficient sensitivity;
- Furthermore, the selected test battery should characterized by high practicability and low effort (specifically with reference to short test durations, low demand of equipment, and a high number of laboratories in Europe able to perform these tests).

According to present knowledge, the aquatic and terrestrial tests, summarized in Table 3, are the ones most suitable to fulfil the above-mentioned criteria and have already been used to test wastes. Other, not yet standardized, test-procedures could replace or supplement the existing procedures, if the necessary experience is available.

For the performance of ecotoxicological tests it is assumed that the physicochemical properties of the waste or waste eluate are such as to allow use of the relevant bioassays. Particular properties such as strongly coloured eluate or waste samples with high nutrient content may make it necessary to extend the battery of tests.

Table 3: Test battery for the classification of wastes in mirror entries according to the criterion HP14

Methods for testing waste eluates	
Description and duration of the tests	**Reference**
Determination of the inhibitory effect on the light emission of *Vibrio fischeri* (luminescent bacteria test) (0,5 h)	ISO 11348-1/2/3
Fresh water algal growth inhibition test with *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata* (72 h)	ISO 8692
Determination of the inhibition of the mobility of *Daphnia magna* (Cladocera, Crustacea) – Acute toxicity test (48 h)	ISO 6341
Methods for the testing of solid wastes	
Contact toxicity test using the dehydrogenase activity with *Arthrobacter globiformis* for contaminated solids (6 h)	ISO 18187
Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the emergence and growth of *Brassica rapa* (14 d)	ISO 11269-2
Testing the quality of soil and effects of chemicals on behavior – Test with earthworms (*Eisenia fetida* or *Eisenia andrei*) (48 h)	ISO 17512-1

6.1.4 Evaluation of test results

In order to evaluate the test results, it is determined if the EC50 ≤ the concentration in a dilution with 10% test substrate (ECx-approach) (see ISO 17616 (2006)). Therefore, the fixation of a certain dilution as limit-concentration was dropped (Deutsches Institut für Bautechnik 2008). If the EC50 is ≤ the concentration in a 10% dilution, there is a distinct negative effect on the test organisms.

The assessment of the genotoxic potential of wastes is partially covered by the HP 11 “mutagenic” criterion. However, it cannot be used to evaluate the complete waste-sample but only to differentiate between the potential of the individual substances in the sample. As there can be unknown substances in the sample and interactions between those substances, one of the following tests with the waste eluate should be conducted to determine the genotoxic potential: umu-test (ISO 13829), or Ames test (ISO 11350). This evaluation is performed independently from the other tests (Römbke et al. 2009).

6.2 Detailed ecotoxicological characterisation of waste

6.2.1 General approach

Besides performing tests in order to differentiate between wastes in mirror entries, it may be necessary to subject wastes to a more detailed, basic ecotoxicological characterisation. This more comprehensive testing will be important in particular if waste is classified as hazardous according to HP14 on the basis of solid contents, but the waste holder wishes to demonstrate the absence of ecotoxicological risks (e.g. for wastes containing heavy metals in elementary form).

Basic ecotoxicological characterisation can also be used to assess wastes which cannot clearly be classified as non-hazardous due to their source or composition. The test strategy proposed below should also be used when the testing of wastes in mirror entries is not possible or has led to unclear results due to specific waste properties (e.g. wastes with high nutrient contents or strongly turbid eluates). The ecotoxicity of waste should be evaluated using the ECx-approach, which means the study of dose-effect relationships. Fig. 2 shows the general strategy for the characterization of waste, and explains the gradual advancement which ensures the cost- and time optimized analysis (especially the discontinuation after establishing effects).

Fig. 2 Test strategy for the identification of hazardous wastes

6.2.2 Selection of test methods

The biological test methods were selected on the basis of the method compilation by CEN TC 292 WG7 and the experience gained in a European inter-laboratory comparison (Moser & Römbke 2009). The proposed test battery comprises methods with terrestrial test organisms for testing of solid waste and methods with aquatic test systems for testing of waste eluates including a method to determine the genotoxicity of the eluate (Table 4). It has to be noted that the acute earthworm test is no longer part of the test battery, due to lacking sensitivity.

Table 4: Battery of tests for the identification of hazardous wastes

Methods for testing of waste eluate	
Description	**Reference**
Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test	ISO 6341
Fresh water algal growth inhibition test with *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata*	ISO 8692
Determination of the toxicity of water constituents and waste water to duckweed (*Lemna minor*) – Growth inhibition test	ISO 20079
Determination of the genotoxicity of waste and waste water using the umu test	ISO 13829
Water quality – Determination of the long-term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea)	ISO 10706
Methods for testing of waste samples	
Description	**Reference**
Avoidance test for testing the quality of soils and effects of chemicals on behaviour (*Eisenia fetida* and *Eisenia andrei*)	ISO 17512-1
Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the growth of *Brassica rapa*	ISO 11269-2
Toxicity test with *Arthrobacter globiformis* for contaminated solids	ISO 18187
Effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: Determination of acute toxicity using artificial soil substrate	ISO 11268-1
Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants	ISO 11267

6.2.3 Evaluation of test results

For basic characterisation, the waste sample is tested in a series of dilutions in order to determine the EC50-value (Table 5). The tested waste is classified as hazardous if ecotoxic potential was demonstrated in at least one of the tests performed. This also always applies if the eluate was determined to display genotoxic potential. Until specific information about this test system is available, a limit concentration of ≤ 10% test-substrate is proposed to be used (Pandard & Römbke 2013).

Table 5: Battery of tests for the basic characterization of wastes

Type of test	Test organism	Reference	Parameter
Eluate	*Desmodesmus subspicatus, Pseudokirchneriella subcapitata*	ISO 8692	Growth
	Daphnia magna	ISO 6341	Immobilisation
	Lemna minor	ISO 20079	Growth
	Salmonella typhimurium	ISO 13829	Gene induction
	Daphnia magna	ISO 10706	Reproduction
Solid material	*Eisenia andrei, E. fetida*	ISO 17512-1	Behaviour
	Brassica rapa	ISO 11269-2	Growth
	Arthrobacter globiformis	ISO 18187	Enzyme activity
	Folsomia candida	ISO 11267	Reproduction

7 Ecotoxicological characterisation for assessing the risks of waste management scenarios

7.1 General

Biological test methods can be used to assess the environmental hazards of waste. Although the test methods are applied under highly standardised and usually non-field conditions, it is possible to extrapolate biological effects determined in the tests to biota potentially affected by open-system recovery measures. In considering risks associated with waste recovery, it is especially important to adapt the test strategy to the conditions likely to be encountered. The test strategy must seek to cover the main exposure pathways and to determine associated environmental effects by using primarily suitable leaching methods and bioassays. Defining such a test strategy requires more knowledge and experience than is required for the classification of waste and it cannot therefore be regarded as a strategy for the routine testing of waste.

7.2 Test strategy

The complexity of the test strategy depends on the type of waste and the envisaged recovery method (Figure 3). If a basic ecotoxicological characterisation of the waste was performed prior to risk assessment (see Section 6.2), the resulting findings can be integrated into the test strategy. It is particularly important to choose a suitable leaching method and to tailor the series of bioassays to the test objective. A compilation of test methods suitable for the testing of waste can be found in Annex B to ISO 14735. The scope and composition of the requisite test battery is geared to the indicator components and properties of the waste. For a risk assessment of wastes sufficiently differentiated dilution series should be tested since not all wastes exhibit a clear dose-effect relationship. It must be made clear that this test strategy is only one element of such assessment of waste; even waste that is classified as non-ecotoxicological may not meet the requirements for safe recovery.

A deviating approach might be followed by waste holders or the competent authorities when devising a test strategy in the fundamental assessment of waste destined for recovery. Therefore, it is suggested that experience and findings from such comprehensive testing be ex-

changed in the relevant bodies (e.g. the Joint Working Group of the Federal States on Waste) and/or a report on this should be prepared.

Fig. 3 Test strategy for an ecotoxicological characterisation as part of an environmental risk assessment of wastes

7.3 Selection of test methods

The set of tests for risk assessment of wastes must cover test organisms from three trophic levels (decomposers, consumers and producers), for the testing of both the eluate and the solid material. The tests must cover the exposure pathways identified as relevant for the recovery, i.e. the water-leachable component has to be tested using aquatic test methods and the direct toxicity of the waste has to be determined in tests with the solid material. It is particularly important to use test methods with long exposure periods to determine chronic effects. Table 6 presents a list of biological test methods which have already been used successfully for the testing of waste. (chronic tests, belonging to waste classification and detailed characterization (see Chapters 6.1.3 and 6.2.3) are not listed again). Although additional test methods such as the fish-egg test (OECD 212) for aquatic samples or the test using predatory mites (OECD 226) for solid samples can in principle be used, the suitability of those tests for wastes should be clarified in advance.

Table 6: Compilation of biological test methods for the testing of waste

Methods for testing of waste eluates	
Description	**Reference**
Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test	ISO 6341
Fresh water algal growth inhibition test with *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata*	ISO 8692
Determination of the toxicity of water constituents and waste water to duckweed (*Lemna minor*) – Growth inhibition test	ISO 20079
Determination of the genotoxicity of water and waste water using the umu test	ISO 13829
Determination of the long-term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea)	ISO 10706
Pseudomonas putida growth inhibition test	ISO 10712
Determination of the chronic toxicity to *Brachionus calyciflorus* in 48 h	ISO 20666
Determination of the chronic toxicity to *Ceriodaphnia dubia*	ISO 20665
Methods for testing of waste samples	
Description	**Reference**
Avoidance test for testing the quality of soils and effects of chemicals on behaviour – Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia Andrei*)	ISO 17512-1
Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the emergence and growth of higher plants	ISO 11269-2
Contact toxicity test with *Arthrobacter globiformis* for contaminated solids	ISO 18187
Effects of pollutants on earthworms (*Eisenia fetida*) - Part 2: Determination of effects on reproduction	ISO 11268-2
Effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: Determination of acute toxicity using artificial soil substrate	ISO 11268-1
Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants	ISO 11267
Effects of pollutants on Enchytraeidae (Enchytraeus sp.) – Determination of effects on reproduction and survival	ISO 16387

7.4 Evaluation of test results

The results of the bioassays have to be evaluated in the context of the relevant recovery scenario. Here too, the effect thresholds defined in the test methods may be used. A generally valid limit concentration cannot be applied here, since the exposure and its relation to the measured effects, (expressed for example as an EC50) is decisive for the assessment of recovery in an open system. Depending on the problem to be investigated, it may also be important to extrapolate the findings from biological effect analysis to a relatively long period. This underlines the need to determine chronic effects or the associated limit concentrations in long-term studies.

8 References

8.1 Guidelines

CEN 12457-2: Characterisation of waste - Leaching - Compliance test for leaching of granular waste materials and sludges - Part 2: One stage batch test at a liquid to solid ratio of 10 l/kg for materials with particle size below 4 mm (without or with size reduction). Brussels.

CEN 14735: Characterization of waste – Preparation of waste samples for ecotoxicity tests. Brussels.

DIN 15442 Feste Sekundärbrennstoffe – Verfahren zur Probenahme. Berlin.

DIN 19528. Elution von Feststoffen - Perkolationsverfahren zur gemeinsamen Untersuchung des Elutionsverhaltens von organischen und anorganischen Stoffen für Materialien mit einer Korngröße bis 32 mm - Grundlegende Charakterisierung mit einem ausführlichen Säulenversuch und Übereinstimmungsuntersuchung mit einem Säulenschnelltest. Berlin.

DIN 19698-1: Abfalluntersuchung – Probenahme von festen und stichfesten Abfällen – Teil 1 Anleitung für die segmentorientierte Entnahme von Proben aus unbekannten Haufwerken, Entwurf. Berlin.

DIN 66165 Teil 1 Partikelgrößenanalyse Siebanalyse Grundlagen. Berlin.

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[EC] European Commission 2008b. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, Official Journal of the European Union L 353, 31.12.2008, p 1-1355.

ISO 6341 Water quality - Determination of the inhibition of mobility of *Daphnia magna* (Cladocera, Crustacea) - Acute toxicity test. Geneva.

ISO 8692: Water quality — Fresh water algal growth inhibition test with *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata.* Geneva.

ISO 10706: Water quality – Determination of long term toxicity of substances to *Daphnia magna* (Cladocera, Crustacea). Geneva.

ISO 10712: Water quality – *Pseudomonas putida* Growth inhibition test. Geneva.

ISO 11267: Soil Quality - Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. Geneva.

ISO 11268-1: Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) - Part 1: Determination of acute toxicity using artificial soil substrate. Geneva.

ISO 11268-2: Soil Quality - Effects of pollutants on earthworms (*Eisenia fetida*). Part 2: Determination of effects on reproduction. Geneva.

ISO 11269-2: Soil quality – Determination of the Effects of Pollutants on Soil Flora. Part II: Effects of Chemicals on the Emergence and Growth of Higher Plants. Geneva.

ISO 11348-1/2/3: Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). Geneva.

ISO 11350. Water quality – Determination of the genotoxicity of water and waste water – Salmonella/microsome fluctuation test (Ames fluctuation test). Geneva.

ISO 13829: Water quality - Determination of the genotoxicity of water and waste water using the umu-test. Geneva.

ISO 16387: Soil Quality - Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.). Determination of effects on reproduction and survival. Geneva.

ISO 17512-1: Soil Quality - Avoidance test for evaluating the quality of soils and the toxicity of chemicals. Test with Earthworms (*Eisenia fetida/andrei*). Geneva.

ISO 17616. Soil quality – Guidance on the assessment of tests applied in the field of ecotoxicological characterization of soils and soil materials. Berlin.

ISO 18187 Soil quality — Quality of solid samples — Solid contact test using the dehydrogenase activity of *Arthrobacter globiformis*. Geneva.

ISO 20079: Water quality – Determination of toxic effect of water constituents and waste water to duckweed (*Lemna minor*) – Duckweed growth inhibition test. Geneva.

ISO 20665: Water quality — Determination of chronic toxicity to *Ceriodaphnia dubia*.. Geneva.

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OECD 226: Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil. Guideline for the testing of chemicals. Paris, France.

OECD 315: Bioaccumulation in sediment-dwelling benthic oligochaetes. Guideline for the testing of chemicals. Paris, France.

OECD 317: Bioaccumulation in terrestrial oligochaetes. Guideline for the testing of chemicals. Paris, France.

8.2 Publications

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Römbke, J., Jänsch, S. Meier, M., Hilbeck, A., Teichmann, H., Tappeser, B. 2010b. General recommendations for soil ecotoxicological tests suitable for the Environmental Risk Assessment (ERA) of Genetically Modified Plants (GMPs). IEAM 6: 287-300.

Annex A: Sieve analysis

Waste materials containing a huge mass content of particle sizes > 4 mm provide a limited applicability for eco-toxicological testing because most testing procedures require a particle size of d95 < 4 mm. Normally, particle size reduction like comminution or crushing is avoided during sample preparation due to the risk that freshly fractured surfaces might significantly influence the eco-toxicological properties of the material.

If an eco-toxicological testing for such kind of material is intended it is reasonable to develop a rough sieve analysis of the original material in order to get information on the representativeness of the sample. It is not necessary to conduct a complete sieve analysis according to e.g. DIN 66165 as the main focus is put on larger grain sizes > 4 mm while a classical sieve analysis defines particle sizes down to the µm range.

For the typical sample sizes of 10 to around 50 kg a hand sieving using a sieve area of 3 dm * 5 dm has proven of value. Typically we use the following set of round hole sieves:

4 mm / 5 mm / 10 mm / 15 mm / 20 mm / 30 mm / 50 mm / 100 mm

For a reliable characterization of large particle sizes we ensure that even the largest particle fraction (d95+) contains more than 200 particles.

Using this procedure the following exemplary results are achievable: