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TESTING OF 24 POTENTIALLY HAZARDOUS WASTES USING 6 ECOTOXICOLOGICAL TESTS

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ABSTRACT

The ecotoxicological characterization of wastes according to the European Waste List (EWL) is part of their assessment as hazardous or non-hazardous. Despite inclusion in national laws no methodological details have been fixed concerning the hazard property HP 14 ("ecotoxic").This paper intends to discuss the classification of wastes by ecotoxicological testing, using 24 representative samples of solid wastes (identified by their EWL number) with different properties. They were sampled according to standard methods, and, with one exception (galvanic sludge), ecotoxicologically tested. No chemical investigation of the samples was performed but they were characterized according to their properties in the ABANDA data base. Nearly all of these wastes were "mirror entries" in the EWL (i.e., they can be hazardous or not depending on the concentration of hazardous substances). For the ecotoxicological characterization three aquatic tests with eluates (genotoxicity, Algae, Daphnia) as well as three terrestrial tests with solid wastes (bacteria, plants, earthworms) were conducted. All investigations were performed as limit tests with three dilution steps. Algae, plants and terrestrial bacteria were the most sensitive organisms. Since no waste eluate showed any indication of genotoxicity, the genotoxicity test should be replaced by the luminescent bacteria test (ISO 11348-3). Proposals for toxicity criteria as well as hazard classifications were taken from the literature but they were modified according to own experiences. Using these concentration limits for the classification whether these wastes are ecotoxic or not, and using different versions of the hazard classification approach, 15-19 waste samples out of 23 waste samples were classified as ecotoxic (64-83%). It is proposed to perform a plausibility check of the respective HP 14-classification. The procedure used in this contribution (i.e. sampling of the wastes, their ecotoxicological testing as well as their hazard classification) could form the basis of a standardized hazard classification approach as proposed recently in the literature. In summary, this works confirms that ecotoxicological tests are practical and sensitive in order to be used for the ecotoxicological hazard classification of very different wastes.

1. INTRODUCTION

In the European Union, the hazard properties of wastes have to be determined following Commission Regulation (EU) N° 1357 (EU 2014). However, until quite recently it was not specified how the HP 14 property ("ecotoxic": waste which presents or may present immediate or delayed risks for one or more sectors of the environment) has to be assessed, but this situation has changed (see Council Regulation (EU) 2017/997). In fact, two approaches are possible:

1. Evaluation according to the classification of chemical mixtures (Classification, Labelling and Packaging CLP approach - EC 2008).

   The hazard of a waste is calculated based on its composition, i.e. adding-up the concentrations of all chemicals with chronic aquatic toxicity (Council Reg. 2017/997 (EU 2017)). Details on the pros and cons of this approach are, for example, given by Wahlström et al. (2016). However, wastes contain many, often unknown chemicals, and even in case they are known, it is not certain that ecotoxicological data are available for them (Eurelectric 2016). In addition, no interactions between the individual waste components are considered. However, according to the recent Council Regulation in case ecotoxicological tests were performed with a respective waste their results will prevail (EU 2017).
2. Ecotoxicological testing of wastes, using standard ISO (International Organization for Standardization) methods.

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Originally, this approach was developed for the assessment of contaminated soils (see e.g. ISO 15799 (2002f) and ISO 17616 (2008b). In short, it consists of a battery of aquatic and terrestrial tests, whose results are assessed together. Recent discussions focus on the selection of the appropriate leaching method as well as on questions regarding the interpretation of the results (Wahlström et al. 2016). Recently, this approach has been (slightly modified) taken over in the Technical Report "Guidance on the use of ecotoxicity tests applied to construction products" products (CEN/TR 17105 (2017)).

Such an ecotoxicological test battery has been successfully used for different waste materials in France (Pandard et al. 2006), Germany (Römbke et al. 2009; Moser et al. 2011) and in particular in an international ring-test (Moser and Römbke 2009). One outcome of this ring-test was to use three test methods for waste eluates and three tests for solid wastes, but there was still some doubt which test methods exactly should be included in the test battery. For reasons of acceptance and data quality standardized test methods should be used (preferably, either ISO, CEN (Comité Européen de Normalisation) or DIN (Deutsches Institut für Normung)). It is also recommendable for any test battery to cover species from the three main trophic organism groups (microbes, plants and animals) in order to cover a wide range of physiological and ecological properties. Finally, the number of three test methods per compartment (aquatic/eluates and terrestrial/solid wastes) seems to be a good compromise between general coverage and practicability (i.e. the efforts in terms of time and costs are manageable).

However, a broad comparison of the toxicity of many different waste types investigated with exactly the same methods had not been done so far. Therefore, a project was funded by the German Federal Environmental Agency (UBA), whose results are summarized in this contribution. The aims of this work can be summarized as follows:

- Evaluation of the suitability (i.e. practicability, sensitivity, reliability and robustness) of six standardized test methods when used for the hazard classification of 24 waste samples which differ strongly in their physico-chemical and toxicological properties;
- Comparison of different assessment options using the whole data set from this exercise (including recommendations for a specific option);
- Comparison of the results of these tests with those tests proposed by Pandard and Römbke (2013); note that they recommend the luminescent bacteria test (ISO 11348-3 (2007)) but in this contribution a genotoxicity test was used instead of the bacterial luminescent test (see also Figure 1 and Chapter 4.2). This proposal is based on discussions in Workgroup 7 of CEN/TC 292/WG 7 "Characterization of waste - Ecotoxicological properties". It consists of an attempt to combine the two approaches for assessing the ecotoxicological hazard of wastes.

So far it is not known how many wastes would be classified as hazardous when their classification is based on ecotoxicological tests. The work presented here is intended to provide a first answer to this question by testing a high number of very different wastes in parallel.

2. MATERIAL AND METHODS

2.1 Tested wastes and their properties

In close co-operation with the German Federal Environment Agency (UBA) the wastes to be studied were identified using the following criteria:

- Not classified as hazardous;
- Economically relevant, mainly in terms of their amount;
- Broad coverage of the List of Wastes (EC 2000);
- Problematic due to variable composition with potentially hazardous properties;
- Difficult to classify as ecotoxic regarding the HP 14 property.

When applying these criteria, it quickly became clear that – for different reasons – it was often difficult to get the wastes we had selected, partly because they were not available in Germany or the owners were reluctant in providing them. In Table 1, the outcome of the selection process is listed. Wastes already classified as hazardous according to one of the other 13 hazard criteria, were – with one exception – not considered, since in these cases the fulfilment of the property HP 14 would not change the already existing classification as being "hazardous". It was planned to test all 24 wastes listed here, but when getting the waste classified as Code No. 110110 it was realized that it was in fact a highly condensed but still fluid galvanic sludge. Since this sample could not be tested in ecotoxicological tests, the final number of tested waste samples was 23.

Ideally, waste samples are characterized chemically and physically. However, due to the heterogeneous composition of these materials this is a very exhaustive and expensive exercise. Therefore, the origin of these waste types was compiled from the waste owners, while information on major contaminants were taken from a database named ABANDA (organized by the German state of North-Rhine-Westphalia) (Table 1). However, this information is not specific for the individual sample tested.

2.2 Sampling of wastes

Collecting representative samples from heterogeneous composite wastes still poses several difficulties. Here the definitions provided by the German "Federal Working Group on Waste (LAGA)" were used, which focus on the material quality of a waste as part of its characterization (LAGA 2004). A compilation of the currently available documents and recommendations in the context of waste testing are combined in the method collection of the LAGA-Forum "waste testing" (LAGA 2012). Thus, the following procedure was used for the sampling for ecotoxicological testing of wastes (Römbke and Ketelhut 2014).

In order to minimize the risk that an analytical result is

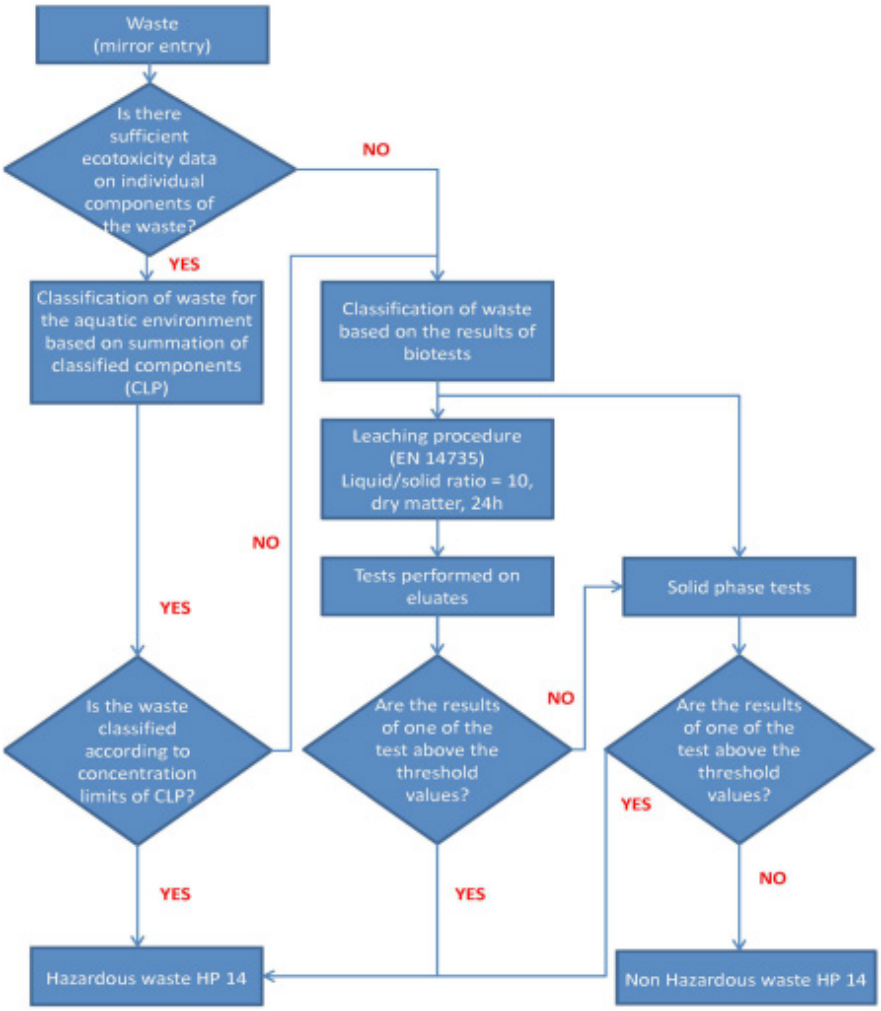

FIGURE 1: Flowchart for the assessment of the HP 14 property (Pandard and Römbke, 2013).

strongly influenced by singular particles samples should offer a tight spectrum of particle size. This could be proven by sieve analysis which is recommended to be done for any sample taken from heterogeneous waste materials:

- In a laboratory sample the mass above the 20th percentile of the sieve analysis should be represented by more than 20.000 particles.
- 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.
- 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.
- Independently from the size of the basic sample, at least 16 single samples have to be taken.
- 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.
- Sampling from a heap of waste could be performed using a wheel loader. The 16 samples taken should be combined to a two-dimensional flat layer providing a height of 1-1.5 dm. Samples could be randomly taken from random coordinates of this two-dimensional layer.
- All individual samples should be combined to one mixed laboratory sample.
- A sample size reduction without a previous reduction in particle size is not allowed.
- The addition of preservatives (e.g. acids) for the purpose of delaying chemical and biological processes does not conform to the standard CEN 14735 (2005).

The duration of the transport of waste samples was as short as possible (i.e. less than 48 h) and the samples were not stored for longer than two months (temperature: ≤ 8°C).

2.3 Test organisms and test performance

2.3.1 Aquatic tests

The aquatic test methods used in this study are briefly described in Tables 2-4.

2.3.2 Terrestrial tests

The terrestrial test methods used in this study are briefly described in Tables 5-7.

TABLE 1: Wastes tested, classified according to the Code of the European Waste List (EC 2000). * = waste classified as hazardous since it fulfills one of the 14 hazard criteria. One sample could not be tested (given in italics). Conc. = concentration. Note that most of these wastes could be classified as either hazardous or non-hazardous (i.e. "mirror entries"), with two absolute non-hazardous exceptions and one absolute hazardous exception. For "mirror entries", the most relevant code has been underlined according to their origin and contamination, when possible.

Code	Waste type	Origin and Contamination
01 05 05*	Oil containing drilling mud and wastes	Soil soaked with mineral drilling oil; probably high PAH conc.
06 03 15*/ 06 03 16	Metallic oxides	Waste from titan dioxide production; pH ca. 4; Cd, Cr, Cu, Ni, Zn content possible
08 01 15*/ 08 01 16	Aqueous sludges containing paint or varnish	Coating and point remnants from a car body shop, high Zn content, probably low biocide conc.
10 01 16*/ 10 01 17	Fly ash from co-incineration	Fine dust from electric filters in a coal plant. Very high Pb, Cu, Zn conc.
11 01 09*/ 11 01 10	Sludges and filter cakes	Waste water concentrate from a print shop (fluid galvanic sludge)
12 01 16*/ 12 01 17	Waste blasting material	Waste blast dust plus paint remnants from the body of a ship; Pb, Th, Zn conc. high
17 01 06*/ 17 01 07	Mixtures of concrete, bricks, tiles and ceramics	Mixed construction waste. Low PAH conc.
17 02 01/ 17 02 04*	Wood	Old wood from construction sites; Pb + Zn contamination, paint remnants
17 05 03*/ 17 05 04	Soil and stones	Soil from construction sites
17 05 05*/ 17 05 06	Dredging spoil	Dredged material from Hamburg harbor. Probably contaminated by organo-tin-substances.
17 05 07*/ 17 05 08	Track ballast	Stoney material from rail tracks. High Cu, Pb, Zn + PAH conc.
17 08 01*/ 17 08 02	Gypsum-based construction materials	Fine gypsum material from construction waste sites, partly mixed with paper remnants
17 09 03*/ 17 09 04-A	Mixed construction and demolition wastes (we got two of these samples; this was identified as A)	Mineral, metallic and woody mixture from waste containers, very heterogeneous, but no contaminants.
17 09 03*/ 17 09 04-B	Mixed construction and demolition wastes (we got two of these samples; this was identified as B)	As sample 17 09 04-A, but higher plastic content and less insulation material
19 08 02	Waste from de-sanding	Material from waste water channels, mixed with polymer flocking agent. High PAH, Cu, Zn contamination
19 08 13*/ 19 08 14	Sludges from other treatment of industrial waste water	Filter press sludges from a painting plant; no specific contaminants
19 10 03*/ 19 10 04	Fluff-light fraction and dust	Selected light material from a reprocessing plant. High conc. of various heavy metals, incl. Hg
19 12 05	Glass	Origin: TV screens. High Pb conc.
19 12 06*/ 19 12 07	Wood (wastes from the mechanical treatment of waste) (we got three of these samples; this one did not get an additional identifier)	Wooden ULD pallets; maybe low Cr conc.
19 12 06*/ 19 12 07	Wood (wastes from the mechanical treatment of waste) (furniture) (we got three of these samples; this one was identified as A)	Construction and furniture material, 5 years stored; maybe low Cr conc.
19 12 06*/ 19 12 07	Wood (wastes from the mechanical treatment of waste) (mixed) (we got three of these samples; this one was identified as B)	Community storage pile, age unknown maybe low Cr conc.
19 12 11*/ 19 12 12	Other wastes (including mixtures of materials) from mechanical treatment of wastes	Mainly styrol-based plastic particles, few organic or metal parts; extremely heterogenous; high conc. of heavy metals possible, mainly Zn
19 13 01*/ 19 13 02	Solid wastes from soil remediation	Soil material strongly contaminated by PAH and mineral oil, but also Pb, Zn, Cu, Cr and PCB
20 01 37*/ 20 01 38	Wood	Wooden bulk trash, maybe low Cr conc.

2.4 Control and mixture media in the tests

Ecotoxicity testing of waste requires the use of a dilution medium which does not affect the response of the test organisms and does as little as possible interact with the sample. The same medium must be used for both the control and the dilution series (see also CEN 14735 (2005)). Depending on the ecological requirements of the test species and the requirements listed in the ISO-standard different control and mixture media were used.

2.4.1 Aquatic tests

The aquatic organisms were tested with eluates, which were prepared according to CEN 12457-2 (2003), i.e. with a

TABLE 2: Umu genotoxicity test (ISO standard 13829 (ISO 2000).

Test system:	*Salmonella choleraesius subsp. choleraesius (formerly: Salmonella typhimurium)* TA 1535/pSK1002
Test duration:	4 h
Test parameter:	Comparison of the induction of the umuC-gene in comparison to spontaneous activations in the negative control
Threshold value:	Induction rate (IR) ≥ 1.5
Test medium:	Tryptone, glucose, ampicillin (TGA) medium
pH (control):	7.0±0.2
Temperature:	37 ± 1°C
Light conditions:	Darkness
Test vessels:	96 well microtitration plates (optical clear)
Volume / vessel:	380 µl
Validity criteria:	Minimum growth in the negative control = 140 FNU (formazine nephelometric units)
Reference chemical / Positive control:	4-Nitro-quinolin-N-Oxid, 2-Aminoanthracen

TABLE 3: Green algae growth test (ISO standard 8692 (ISO 2004a).

Test system:	*Pseudokirchneriella subcapitata*
Test duration:	72 h (permanently shaking)
Test parameter:	Growth in comparison to control
Threshold value:	25%
Test medium:	Mixture of four nutrient stock solutions in water
pH (control):	8.1 ± 0.2
Temperature:	21-24°C (fluctuations < 2°C)
Light conditions:	60-120 µE*$m^{-2}s^{-1}$ permanent light
Test vessels:	300 ml Erlenmeyer flasks (microtiter plates)
Volume / vessel:	100 ml water
Validity criteria:	Increase of cell density in the controls by a factor of 67 after 72 h (i.e. growth rate ≥ 1.41); increase of pH ≤ 1.5 during the test; coefficient of variation in the control ≤ 5%
Reference chemical / Positive control:	Potassium dichromate or 3,5-Dichlorophenol?

TABLE 4: *Daphnia magna* test (ISO standard 6341 (ISO 2012).

Test system:	*5 juvenile Daphnia magna* (age 2-26 h) per replicate
Test duration:	24 h
Test parameter:	Immobilization of the water flea
Threshold value:	20%
Test medium:	Reconstituted water according to OECD 203 (1992)
pH (control):	7.5-8.0
Temperature:	20 ± 2°C
Light conditions:	Permanently dark
Test vessels:	50 mL vessels without lid
Volume / vessel:	20 mL eluate/water mixture or water (controls)
Validity criteria:	Mortality in the control ≤ 10%
Reference chemical / Positive control:	Potassium dichromate

solid/liquid dilution ratio of 1:10. The elution medium was distilled water and an end-over-end tumbler was used. After 24 h, the eluate was centrifuged for 20 min at 17000 x g and finally it was filtered (< 0.45 µm). In the Luminescent Bacteria test the control culture medium is TGA, consisting of tryptone, glucose and ampicillin. In the Algae test the control growth medium is a mixture of four nutrient stock solutions in water, which are defined as follows: No. 1: five macro-nutrients, No. 2: Fe-EDTA; No. 3: seven trace elements; No. 4: $NaHCO_3$; all of them in specific concentrations. In the *Daphnia* test the test medium is reconstituted water, which is a mixture of four nutrient salts in deionized water (Calcium chloride, magnesium sulfate, sodium bicarbonate, and potassium chloride) in specific ratios.

TABLE 5: *Arthrobacter globiformis* test (ISO standard 18187 (ISO 2016)).

Test system:	*Arthrobacter globiformis* (freeze-dried)
Test duration:	< 1 d (Incubation time 2 h)
Test parameter:	Dehydrogenase activity
Threshold value:	30%
Test medium:	Mixtures of Quartz sand and waste material
pH (control):	5.0-7.5
Moisture:	20% (up to 33% possible)
Temperature:	30 ± 1°C
Light conditions:	Dark
Test vessels:	24-well microplate
Volume / vessel:	0.6 g weighed in a micro-well
Validity criteria:	Relative fluorescence increases by a factor > 5 during a measuring time of 0 to 60 min. Coefficient of variation for the average slope of relative fluorescence in the negative control replicates is less than 15%
Reference chemical / Positive control:	Benzalkonium chloride (BAC) (600 mg/kg) causes effects between 30% and 80%

TABLE 6: Higher plant test (ISO standard 11269-2 (ISO 2004b)).

Test system:	*Brassica napus* (turnip), 10 seeds per replicate (4 replicates per dilution step)
Test duration:	14-21 d after 50% of seeds in the control emerged
Test parameter:	Determination of the emergence rate within the first week. At the end of the test determination of the fresh weight and visible damages
Threshold value:	30%
Test medium:	Mixtures of LUFA 2.3 standard soil and waste material
pH (control):	Not specified
Moisture:	On demand
Temperature:	25 ± 10°C
Light conditions:	Light/dark cycle: ca. 16/8 h; Light intensity: 13000 ± 5000 lx
Test vessels:	Plastic pots, diameter about 10 cm
Volume / vessel:	900 g soil / soil-waste mixture (fresh weight)
Validity criteria:	Emergence rate in the control: > 70%
Reference chemical / Positive control:	EC50 (boric acid): 80 - 330 mg/kg soil (dry weight) for the endpoint shoot weight (see also Becker et al. 2011)

TABLE 7: Earthworm avoidance test (ISO standard 17512-1 (ISO 2008a)).

Test system:	10 adult *Eisenia fetida* (biomass 250 – 600 mg/worm) per test vessel; 5 replicates per dilution step
Test duration:	48 h
Test parameter:	Avoidance behavior determined at the end of the test
Threshold value:	80%
Test medium:	Mixtures of OECD Artificial Soil and waste material
pH (control):	5.5-6.5
Moisture:	40-60% of the WHCmax
Temperature:	18-22°C
Light conditions:	16 h light (400-800 Lux), 8 h dark
Test vessels:	Bellaplast vessels, 11 x 15.5 x 6 cm
Volume / vessel:	500 g soil / soil-waste mixture
Validity criteria:	Mortality in the control ≤ 10% per vessel; distribution with same soil on both sides: 50±10% (see also Hund-Rinke and Wiechering 2001)
Reference chemical / Positive control:	Boric acid at 750 mg/kg soil (dry weight) should cause avoidance behavior

2.4.2 Terrestrial tests

In the Bacteria-test the control was quartz sand (with 50% to 75% of sand with particle size between 0,063 mm and 2 mm). The natural standard soil LUFA Soil 2.3 was used in the Plant test, which fulfilled the following conditions: organic carbon content ≤ 1.5%, pH between 5.0 and 7.5, and the fine fraction should comprise less than 20% of the soil dry weight. In the Earthworm Test the control soil was OECD Artificial Soil, consisting of 10% dry mass Sphagnum peat finely ground and with no visible plant remains (particle size < 1 mm), 20% of Kaolinite clay containing not less than 30% kaolinite and 69% industrial quartz sand (dominant fine sand with more than 50% to 75% of particle size 0.0563 mm to 0.2 mm).

2.5 Test design

All tests were performed following an Extended Limit Test design, i.e. with three dilutions of the tested waste eluate or solid waste. These concentrations differed between the two compartments as follows:

- Aquatic tests: control (0%), D8 (= 12.5%), D4 (= 25.0%), D2 (= 50.0%).
- Terrestrial tests: control (0%), D16 (= 6.25%), D8 (= 12.5%), D4 (= 25.0%).

Note that due to technical reasons (number of wells on a micro-well plate) the dilution steps differed from the rest in the genotoxicity tests: Control (0%), D12 (= 8.3%), D6 (= 16.7%), D3 (= 33.3.0%), D 1,5 (66.6%).

These dilutions were chosen in order to include the general limit concentration LID (= Lowest Ineffective Dilution) of 4 and 8 for aquatic and terrestrial tests, respectively. This approach is widely used in Germany for the assessment of contaminated land but has rarely been used in other countries (e.g. ISO 17616 (2008). The reason for the different dilutions in the aquatic and terrestrial tests is caused by the lower availability of contaminants in the solid test media.

2.6 Threshold (reference) values as effect criteria for the individual tests

Depending on the biological variability of each test system the effect criterion (i.e. which difference between a tested mixture and the respective control is considered as an effect) differs too (Tables 2-8). Originally, these criteria have been proposed for the evaluation of contaminated soil, partly in the test standard itself (e.g. ISO 17512-1 (2008a), partly in regulatory documents (e.g. Moser 2008) or, just as an example, in other international standards (e.g. ISO 17616 (ISO 2008b)). These threshold (or reference) values are used in order to decide whether a waste sample tested at a specific dilution has ecotoxic effects or not.

3. RESULTS

3.1 Aquatic tests

3.1.1 Umu genotoxicity test

In Table 9, the results of these genotoxicity tests are summarized. All tests were valid according to the ISO standard. In one test (No. 08 01 16) the induction rate could not be determined due to cytotoxicity. All tests were performed with (+S9) and without (-S9) metabolic activation.

3.1.2 Green algae growth test

The results of the Algae tests are given in Table 10. All tests were valid according to the ISO standard. Effects of up to 100% were found in all dilution steps in four samples (Nos. 06 03 16; 08 01 16; 19 08 14; 19 12 12. In addition, complete inhibition was found in the samples 10 01 17 and 19 12 05 in the two higher solutions (D2 and D4). No effects on Algae did occur in the samples 01 05 05, 17 01 07, 17 05 04 and 17 05 08. Regularly, dose-response relationships were observed.

3.1.3 Daphnia magna test

An overview of the results of tests with water fleas is given in Table 11. All tests were valid according to the ISO standard. The daphnids reacted most strongly in four samples (Nos. 08 01 16; 10 01 17; 12 01 17; 19 12 05). Very rarely – actually just once (No. 06 03 16) - a dose-response relationship was visible. In all other samples, no – or almost none (No. 19 12 07-A) – effects on daphnids did occur. No other test showed such a strong dichotomy: either a waste did strongly affect the test organisms or no effect at all was observed.

3.1.4 Summary of aquatic results

In Table 12 all aquatic results are summarized. In the genotoxicity tests no effects at all were observed. In contrast, out of 23 waste samples 13 of them were identified as ecotoxic in the Algae tests. The results of the daphnid tests were different – only five samples have to be classified as ecotoxic when using this test system.

TABLE 8: Overview on the effect criteria for the individual tests as given in the literature, to be used as threshold (or reference) values for the ecotoxicological hazard assessment of wastes. These dilution rates of waste in the culture medium and these biological effects are taken as reference in this study to classify waste as ecotoxic.

Test name and guideline	Ecotoxic if
Umu test (ISO 13829 (2000))	IR > 1.5 at dilution 25% (LID 4)
Algae test:(ISO 6341 (1996))	Effect > 25% at dilution 25% (LID 4)
Daphnia magna test (ISO 8692 (2004a))	Effect > 20% at dilution 25% (LID 4)
Arthrobacter globiformis test (ISO 18187 (2016))	Effect > 30% at dilution 12.5% (LID 8)
Plant growth test ISO 11269-2 (2008b))	Effect > 30% at dilution 12.5% (LID 8)
Earthworm Avoidance Test (ISO 17512-1 (2008a))	Effect > 80% at dilution 12.5% (LID 8)

TABLE 9: Induction rates of the umuC-Gen (without pH adjustment) in the genotoxicity test with waste eluates of 23 different waste materials. Effect criterion: IR ≥ 1.5. * No IR determined because of cytotoxicity. N.d. Not determined. Tests showing effects at dilution step 6 or higher are indicated as dark-shaded. S9: rat liver extract; used for metabolic activation of the bacteria.

	Waste code		Dilution steps [waste eluates]				LID_U-Value
			D12 [8.3%]	**D6** [16.7%]	**D3** [33.3%]	**D1.5** [66.6%]	
Induction rate	**01 05 05**	-S9	0.51	0.49	0.54	0.48	<1.5
		+S9	0.69	0.77	0.73	0.97	
	06 03 16	-S9	1.25	1.37	1.40	1.42	<1.5
		+S9	1.04	1.01	1.12	1.03	
	08 01 16	-S9	*	*	*	*	n.d.
		+S9	*	*	*	*	
	10 01 17	-S9	0.57	0.95	1.00	1.10	<1.5
		+S9	1.02	1.05	0.99	1.06	
	12 01 17	-S9	0.62	0.78	0.81	0.76	<1.5
		+S9	0.84	0.93	1.03	1.00	
	17 01 07	-S9	0.85	0.9	0.8	0.86	<1.5
		+S9	1.01	1.04	0.91	1.03	
	17 02 01	-S9	1.06	1.15	1.19	1.17	<1.5
		+S9	1.09	1.14	1.19	1.03	
	17 05 06	-S9	1.04	1.01	0.88	0.93	<1.5
		+S9	1.00	1.12	0.95	0.91	
	17 05 04	-S9	0.94	1.14	1.11	1.08	<1.5
		+S9	1.14	1.10	1.00	1.08	
	17 05 08	-S9	1.11	1.20	1.18	0.95	<1.5
		+S9	0.94	0.79	0.67	0.60	
	17 08 02	-S9	1.11	1.01	0.92	1.12	<1.5
		+S9	1.13	1.04	0.92	0.74	
	17 09 04-A	-S9	0.83	0.91	0.78	1.27	<1.5
		+S9	1.00	1.05	1.06	1.02	
	17 09 04-B	-S9	0.84	1.04	0.96	1.06	<1.5
		+S9	0.85	1.06	1.34	1.27	
	19 08 02	-S9	1.45	0.71	0.91	0.75	<1.5
		+S9	1.29	1.21	1.01	0.74	
	19 08 14	-S9	0.47	0.54	0.52	0.45	<1.5
		+S9	0.63	0.72	0.74	0.79	
	19 10 04	-S9	1.07	1.10	1.12	1.01	<1.5
		+S9	1.12	0.99	0.88	1.03	
	19 12 05	-S9	0.98	0.98	0.94	1.01	<1.5
		+S9	1.05	0.94	0.85	0.97	
	19 12 07	-S9	0.91	1.07	0.92	0.97	<1.5
		+S9	0.90	0.99	0.84	0.92	
	19 12 07-A	-S9	0.76	1.09	1.00	1.14	<1.5
		+S9	0.98	0.80	0.98	1.06	
	19 12 07-B	-S9	0.38	1.09	1.00	1.36	<1.5
		+S9	1.02	1.13	1.12	1.08	
	19 12 12	-S9	0.38	1.09	1.00	1.36	<1.5
		+S9	1.02	1.13	1.12	1.00	
	19 13 02	-S9	0.97	1.01	1.00	1.01	<1.5
		+S9	1.04	0.96	0.90	0.88	
	20 01 38	-S9	1.02	0.93	0.89	0.99	<1.5
		+S9	1.09	0.80	0.78	0.63	

TABLE 10: Inhibition (in % of the control) of the growth of *Pseudokirchneriella subcapitata* in the Algae test with waste eluates of different waste materials. Effect criterion: 25%. Tests showing effects at dilution step 8 or higher are indicated as dark-shaded.

	Waste code	Dilution steps [waste eluates]			LID_A-value
		D8 [12.5%]	**D4** [25%]	**D2** [50%]	
Growth inhibition [%]	**01 05 05**	-16	-15	-5	2
	06 03 16	82	>100	>100	> 8
	08 01 16	>100	>100	>100	> 8
	10 01 17	1	>100	?	8
	12 01 17	67	85	>100	> 8
	17 01 07	-1.4	1.6	1.0	2
	17 02 01	13	41	>100	8
	17 05 04	-1	-3	5	2
	17 05 06	0	9	53	4
	17 05 08	-4	-4	-2	2
	17 08 02	7	3	21	4
	17 09 04-A	39	44	52	> 8
	17 09 04-B	0	9	42	4
	19 08 02	5	18	71	4
	19 08 14	>100	>100	>100	> 8
	19 10 04	2	12	>100	4
	19 12 05	34	>100	>100	>8
	19 12 07	30	33	41	>8
	19 12 07-A	13	18	44	4
	19 12 07-B	18	41	76	8
	19 12 12	>100	>100	>100	> 8
	19 13 02	13	28	50	8
	20 01 38	31	44	85	> 8

3.2 Terrestrial tests

3.2.1 Arthrobacter globiformis test

In Table 13, the results of the tests with this bacterial test are summarized. In one test (No. 01 05 05) an additional pasteurization was performed because of the high microbial activity of this sample. In one other test (No. 10 01 17) no dose-response relationship was observed. All tests were valid according to the ISO standard. No waste type caused a 100% effect on the dehydrogenase activity of *A. globiformis*. However, in 10 samples (Nos. 01 05 05; 08 01 16; 10 01 17; 12 01 17; 17 02 01; 19 12 07; 19 12 07-A; 19 12 07-B; 19 12 12; 20 01 38) the dehydrogenase activity was lower than the control by > 30%. In contrast, in seven samples no effect was found in all dilution steps. Dose-response relationships were almost never observed. A clear increase of the dehydrogenase active did not occur. Despite the small amount of waste in these tests (0.6 g) these results confirm the robustness of this test. However, the small sample size may have had an influence on the strong differentiation of the test results: only in two tests and LID-value of 8 was observed – all others were either very toxic or not toxic

3.2.2 Higher plant test (B. napus)

In Table 14, the results of the tests with the plant *B. napus* test are summarized. All tests were valid according to the ISO standard. In three tests (Nos. 06 03 16; 08 01 16; 19 08 14) no seed germination (or at least no growth of the seedlings did occur) was observed. In addition, effects higher than 30% were observed in ten samples at all dilution steps (Nos. 01 05 05; 10 01 17; 17 02 01; 17 08 02; 17 09 04°; 17 09 04-B; 19 12 07; 19 12 07-A; 19 12 07-B; 20 01 38). In case effects did occur, they followed a dose-response-relationship. Only in eight tests no or low (i.e. <30%) effects were found. Very conspicuous is sample No. 17 05 06, (dredged material without contaminants) which caused a strong increase of growth; i.e. probably it contained nutrients.

3.2.3 Earthworm avoidance test

In Table 15, the results of the tests with the earthworm *E. fetida* are summarized. All tests were valid according to the ISO standard. Only in one test an avoidance behavior of 100% in all dilutions was observed (No. 08 01 16). An avoidance behavior of more than 80% did occur in six samples (Nos. 01 05 05; 06 03 16; 10 01 17; 17 02 01; 17 09 04-A; 20 01 38). No avoidance effect was visible in six tests (Nos. 17 05 04; 17 05 06; 19 08 02; 19 13 02). No dose-response relationship was observed in four samples (Nos. 17 05 08; 19 12 07, 19 12 07-B; 20 01 38). In some tests, several samples seemed to be attractive the earthworms (No. 19 08 02 and 19 13 02) – especially at higher dilution steps (D8 and D16).

TABLE 11: Immobilization [%] of 20 juvenile water fleas (per test vessel) in the *Daphnia*-test with waste eluates of 23 different waste materials. Effect criterion: 20%. Tests showing effects at dilution step 8 or higher are indicated as dark-shaded.

	Waste code	Dilution steps [waste eluates]			LID_D-value
		D8 [12.5%]	**D4** [25%]	**D2** [50%]	
Inhibition of mobility [%]	**01 05 05**	5	0	0	2
	06 03 16	5	45	100	8
	08 01 16	100	100	100	> 8
	10 01 17	100	100	100	> 8
	12 01 17	90	100	100	> 8
	17 01 07	0	0	5	2
	17 02 01	0	5	0	2
	17 05 04	0	0	0	2
	17 05 06	0	0	0	2
	17 05 08	0	20	0	2
	17 08 02	0	10	0	2
	17 09 04-A	0	0	5	2
	17 09 04-B	0	0	0	2
	19 08 02	0	5	0	2
	19 08 14	0	0	0	2
	19 10 04	0	0	0	2
	19 12 05	50	80	75	> 8
	19 12 07	0	0	0	2
	19 12 07-A	0	0	75	4
	19 12 07-B	5	5	5	2
	19 12 12	0	0	0	2
	19 13 02	0	0	0	2
	20 01 38	5	0	0	2

TABLE 12: Results of the aquatic tests (LID-values) with waste eluates. * No IR determined because of cytotoxicity.

Waste code	Umu-Test: LID_U (*S. choleraesius*)	Algae: LID_A (*P. subcapitata*)	Daphnia: LID_D (*D. magna*)
01 05 05	<1.5	2	2
06 03 16	<1.5	> 8	8
08 01 16	*	> 8	> 8
10 01 17	<1.5	8	> 8
12 01 17	<1.5	> 8	> 8
17 01 07	<1.5	2	2
17 02 01	<1.5	8	2
17 05 04	<1.5	2	2
17 05 06	<1.5	4	2
17 05 08	<1.5	2	2
17 08 02	<1.5	4	2
17 09 04-A	<1.5	> 8	2
17 09 04-B	<1.5	4	2
19 08 02	<1.5	4	2
19 08 14	<15	> 8	2
19 10 04	<1.5	4	2
19 12 05	<1.5	> 8	> 8
19 12 07	<1.5	> 8	2
19 12 07-A	<1.5	4	4
19 12 07-B	<1.5	8	2
19 12 12	<1.5	> 8	2
19 13 02	<1.5	8	2
20 01 38	<1.5	> 8	2

TABLE 13: Inhibition of the dehydrogenase activity of *A. globiformis* in the Bacteria contact test with 23 different waste materials; effect criterion 30%. Lightly-shaded cells: no dose-response relationship. * Additional pasteurization performed. N.d.: Not determined (light-shaded cells). Tests showing effects at dilution step 16 or higher are indicated as dark-shaded.

Inhibition of the dehydrogenase activity [%]	Waste code	Dilution steps			LID_B-value
		D16 [6.25% Waste]	**D8** [12.5% Waste]	**D4** [25% Waste]	
	01 05 05 *	43.5	55.3	60.6	> 16
	06 03 16	27.4	61.3	80.6	16
	08 01 16	83.5	91.6	96.1	> 16
	10 01 17	98.1	92.7	81.9	> 16
	12 01 17	30.7	43.6	79.6	> 16
	17 01 07	-1.9	16.6	34.5	8
	17 02 01	46.8	65.8	83.3	> 16
	17 05 04	3.4	-0.6	-4.6	4
	17 05 06	-3.5	-0.9	5.9	4
	17 05 08	-0.9	2.8	13.3	4
	17 08 02	1.3	-5.2	5.3	4
	17 09 04-A	0.8	44.9	n.d.	16
	17 09 04-B	33.3	42.6	n.d.	> 16
	19 08 02	10.2	8.4	37.2	8
	19 08 14	4.5	33.0	42.0	16
	19 10 04	25.3	33.2	75.1	16
	19 12 05	1.3	3.8	28.5	4
	19 12 07	58.4	82.1	85.8	> 16
	19 12 07-A	60.9	84.9	94.8	> 16
	19 12 07-B	75.8	93.9	96.1	> 16
	19 12 12	33.7	52.7	77.3	> 16
	19 13 02	8.0	10.7	13.4	4
	20 01 38	53.2	83.9	88.0	> 16

3.2.4 Summary of terrestrial results

In Table 16 all terrestrial results are summarized. No test failed. In the *Arthrobacter* test 15 wastes were identified as ecotoxic, in the plant tests 14 and in the earthworm tests just six.

3.3 Summary: classification of all test results (aquatic and terrestrial tests together)

In this chapter, the results of the aquatic and terrestrial tests are presented together (Table 17). Afterwards, the assessment principles proposed by Pandard and Römbke (2013) will be applied (e.g. using the same threshold values and limit concentrations) with one exception: instead of the luminescent bacteria test (ISO 11348-3 (2007a) the genotoxicity test (ISO 13829 (2000)) was used. However, the latter one did not show any effects. Therefore, in the following the classification will be performed without the bacterial tests in order to avoid a bias when comparing the aquatic and terrestrial effects. However, and referring to Table 13, it should be kept in mind that in the terrestrial bacterial tests 15 out of 23 wastes were classified as ecotoxic.

According to the tiered approach proposed by Pandard and Römbke (2013), the results of the aquatic tests are considered first. Out of the 23 wastes tested 13 breached the LID of 4. Algae reacted more sensitively since they were affected in all these 13 cases. Only five wastes were toxic for water flea. No waste did only affect the daphnids. The different sensitivity pattern of these two organisms is also shown by the fact that the Algae were not affected at all by five wastes (LID = 2) and showed small effects in tests with another five wastes (LID = 4). In contrast, the daphnids showed a strong yes/no pattern: in 17 tests, there was no effect (LID = 2), meaning that only one waste caused a small effect. In summary, in this sample of wastes (with one exception all of them had no mirror entries) 57% of them are ecotoxic.

In the terrestrial tests 14 out of 23 wastes are considered to be ecotoxic. Again, the sensitivity of the two species differs considerably: all of these 14 waste samples were toxic to plants, but only six of them affected the earthworms strongly. Eight wastes did not affect the plants and just one caused a small effect. The respective numbers for the earthworms are 13 and four. No waste was classified as ecotoxic only in the earthworm tests. Looking only at the outcome of the terrestrial tests 61% of all wastes did affect terrestrial organisms. According to the proposed scheme, only the ten wastes evaluated as non-ecotoxic in the aquatic tests are assessed in the second step, using the results of the terrestrial tests. Five

TABLE 14: Reduction of biomass (in comparison to the control in %) of *B. napus* (turnip) in the plant growth tests with 23 different waste materials; effect criterion 30%. No conspicuous observations were made. * No seeds did germinate at all. Tests showing effects at dilution step 16 or higher are indicated as dark-shaded.

Waste code	Dilution steps			LID_P-value
	D16 [6.25% Waste]	**D8** [12.5% Waste]	**D4** [25% Waste]	
01 05 05	85.5	84.4	82.4	> 16
06 03 16	*	*	*	> 16
08 01 16	*	*	*	> 16
10 01 17	53.4	79.2	93.3	> 16
12 01 17	-4.4	7.4	-3.2	4
17 01 07	-11.8	-15.9	12.4	4
17 02 01	54.0	69.8	89.8	> 16
17 05 04	-22.8	-21.3	-18.9	4
17 05 06	-46.0	-61.3	-65.7	4
17 05 08	9.8	13.9	11.5	4
17 08 02	36.1	54.6	63.7	> 16
17 09 04A	94.9	97.0	98.0	> 16
17 09 04B	68.7	72.2	82.5	> 16
19 08 02	-2.7	-0.6	-2.7	4
19 08 14	*	*	*	> 16
19 10 04	22.6	49.8	68.2	16
19 12 05	-1.5	5.5	7.2	4
19 12 07	76.5	82.1	87.9	> 16
19 12 07-A	72.5	78.6	81.0	> 16
19 12 07-B	63.4	81.2	80.5	> 16
19 12 12	21.7	21.6	45.8	8
19 13 02	-31.2	-3.6	28.8	4
20 01 38	63.0	70.6	87.9	> 16

Reduction of biomass compared to control in %]

out of the 10 wastes caused effects in the tests with soil organisms. Therefore, in total 18 wastes out of 23 (= 78%) are classified as hazardous following the concept of Pandard and Römbke (2013).

However, when comparing those nine cases in which either aquatic or terrestrial organisms reacted more sensitively it seems that sensitivity does not differ between both organism groups, since in five tests aquatic organisms were reacting stronger than their terrestrial counterparts – and in four tests this situation was just the other way around. Finally, the inclusion of the bacterial results should be briefly discussed. As mentioned earlier, the inclusion of the genotoxicity data (no effects at all) would not change the number of wastes classified as being hazardous from aquatic testing. Assuming that instead the luminescent Bacteria test would have been used either no change or an increase in ecotoxic wastes would happen. However, in the case of the terrestrial bacteria test we do have data (see Table 14): in 15 out of 23 tests with *A. globiformis* strong effects were found, but with two exceptions (Nos. 12 01 17 and 19 12 12) those were samples which already were identified as toxic in the plant or earthworm tests. In addition, both exceptions were also toxic to aquatic organisms, meaning that the overall results would not change.

3.4 Discussion of the toxicity of the wastes used in this project

Despite the fact that the quite high number of wastes tested here more data are needed in order to cover the full range of different waste types. This aim could either be reached by closing gaps regarding waste types, or by testing samples the same waste types as done here but coming from other sites in order to get an overview how much this property differs within one waste type.

The ecotoxicological test methods used here are robust, practical and reliable. Only one out of 24 samples (No. 11 01 10: highly condensed but still fluid galvanic sludge) could not be tested, partly because of its physico-chemical properties, partly because of its unknown human toxicology. Therefore, from a technical point of view the use of ecotoxicological test methods is recommended.

The results of such tests are neither correlated between them (i.e. each test has its own "effect profile") nor is the information gained redundant to information from other sources (e.g. chemistry) regarding the question whether a specific waste is classified as hazardous or not. Twenty wastes are mirror entries and can be hazardous or not depending on the concentration of hazardous substances. In contrast, in ecotoxicological tests almost 75% all test-

TABLE 15: Avoidance behavior (in comparison to the control in %) of the earthworm E. fetida in the earthworm avoidance tests with 23 different waste materials; effect criterion 80%. All tests were valid according to the ISO standard. Lightly-shaded cells: no dose-response relationship. Tests showing effects at dilution step 8 or higher are indicated as dark-shaded.

Avoidance behavior [%]	Waste code	Dilution steps			LID_R-value
		D16 [6.25% Waste]	**D8** [12.5% Waste]	**D4** [25% Waste]	
	01 05 05	88	92	100	> 16
	06 03 16	68	88	100	16
	08 01 16	100	100	100	> 16
	10 01 17	72	92	100	16
	12 01 17	14	68	88	8
	17 01 07	-2	40	88	8
	17 02 01	52	82	88	16
	17 05 04	0	4	32	4
	17 05 06	-24	40	20	4
	17 05 08	48	40	36	4
	17 08 02	10	52	56	4
	17 09 04-A	60	64	94	8
	17 09 04-B	-32	44	52	4
	19 08 02	-68	-68	-40	4
	19 08 14	16	28	56	4
	19 10 04	28	76	90	8
	19 12 05	-10	8	30	4
	19 12 07	20	66	52	4
	19 12 07-A	-34	-16	4	4
	19 12 07-B	52	-28	24	4
	19 12 12	22	36	46	4
	19 13 02	-60	-22	21	4
	20 01 38	44	84	78	16

ed wastes are classified as hazardous. So, it seems that this kind of testing is more sensitive in identifying wastes which could be ecotoxicologically hazardous.

Right now, it is impossible to say whether the results of this study are representative for the relationship between ecotoxicological testing and the classification of wastes according to the List of Wastes in general. However, further ecotoxicological tests with a broader range of wastes, trying to cover the range of the waste types and subtypes, are recommended in order to get a better understanding of the hazard properties of wastes. In case such studies will be performed a chemical characterization of the test samples is highly recommended. Assuming that such a data set will be available, the suitability and robustness of the different classification approaches could be assessed and recommendations for legal handling could be formulated.

4. DISCUSSION

4.1 Test performance, species selection and species sensitivity

4.1.1 Test substrate characterization

There is a need for an improved description of the sampling, handling (especially the pre-treatment, e.g. the particle size) and storage of waste samples before they are used in ecotoxicological tests. Without that kind of information comparability of results is hampered. In addition, the properties of the individual waste samples should be characterized as good as possible, both in terms of their physical appearance as well as their chemical composition. Data from general data bases (as used here) are not sufficient. Despite the fact that each waste sample by definition differs from each other such information could be used to understand better the reasons for ecotoxicity of waste samples.

4.1.2 Selection of the ecotoxicological test methods and species

Regarding the composition of the test battery it is recommended to follow all recommendations of Pandard and Römbke (2013). This includes the testing of waste eluates with the bacterial luminescent test (ISO 11348-3 (2007a) instead of the umu genotoxicity test (ISO 13829 (2000), as originally proposed after the ring test (Moser & Römbke 2009), for the following reasons:

- Genotoxicity is a very specific endpoint which seems to be rarely relevant for wastes, at least to my experience no sample tested in our lab did show any signs of genotoxicity;

TABLE 16: Results of the terrestrial tests (LID-values) with solid wastes.

Waste code	Arthrobacter Test: LID_B (*A. globiformis*)	Higher Plant Test: LID_P (*P. subcapitata*)	Earthworm Avoi-dance Test: LID_R (*E. fetida*)
01 05 05	> 16	> 16	> 16
06 03 16	16	> 16	16
08 01 16	> 16	> 16	> 16
10 01 17	> 16	> 16	16
12 01 17	> 16	4	8
17 01 07	8	4	8
17 02 01	> 16	> 16	16
17 05 04	4	4	4
17 05 06	4	4	4
17 05 08	4	4	4
17 08 02	4	> 16	4
17 09 04-A	16	> 16	8
17 09 04-B	> 16	> 16	4
19 08 02	8	4	4
19 08 14	16	> 16	4
19 10 04	16	16	8
19 12 05	4	4	4
19 12 07	> 16	> 16	4
19 12 07-A	> 16	> 16	4
19 12 07-B	> 16	> 16	4
19 12 12	> 16	8	4
19 13 02	4	4	4
20 01 38	> 16	> 16	16

- At the time of the European Waste Ringtest there was no real alternative for a standardized microbial test in soil available.

This situation has changed considerably: the Arthrobacter Test, originally developed for sedimenst, was modified successfully in order to perform it both in soils and wastes (e.g. Marques et al. 2018). Still, the genotoxicity test is an option for those wastes where there are hints that the waste to be tested may contain genotoxic components. Focusing on the aquatic compartment Weltens et al. (2012) proposed it as screening tests for the hazard classification of wastes in a similar test battery, i.e. the Algae growth inhibition test, the Daphnia immobilization test and, in addition, the fish larval mortality test. These authors discuss the luminescent Bacteria test as a fast alternative.

4.1.3 Sensitivity of the selected ecotoxicological tests

Looking at the outcome of this project it could be argued that it would be sufficient to use in the future only those tests which have been most sensitive. In detail, the overall number of wastes affecting organisms would not change if only the Algae and the Arthrobacter tests would have been performed here. However, this conclusion is premature because it is based on just 23 waste samples. Experiences from other regulatory areas, in particular the testing of chemicals, has shown that it is not possible to identify "the most sensitive species" because it simply does not exist (Cairns 1986).

When studying different wastes in the international ring test (Moser and Römbke 2009) or fly ashes (Römbke and Moser 2007) with the umu test rarely genotoxicity was found. However, Brackemann et al. (2000) report genotoxic reactions in an acid eluate prepared from stoker-fired furnace ash as well as in three wastes from chemical industry. Since genotoxicity is a very important endpoint the low number of data should not be taken as an excuse to disregard this test.

The ecological relevance and sensitivity of the Algae test is often considered as high (Deventer et al. 2004). For example, they reacted often more sensitively in eluates from different wastes (mainly ashes) than daphnids or luminescent Bacteria (Kaneko 1996; Lapa, 2002a; Lapa 2002b). Therefore, they are regularly proposed as part of an ecotoxicological test battery for wastes (e.g. Pandard et al. 2006; Moser and Römbke 2009). However, it is known that the two species recommended in the Algae tests could react differently when exposed to the same waste (Moser and Römbke 2009).

Tests with the water-flea *Daphnia magna* have often been used for waste testing, in particular with waste incineration ashes (e.g. Kaneko 1996; Triffault-Bouchet et al. 2003; LFU 2004; Pandard et al. 2006; Römbke and Moser 2007). Results from the international ringtest confirm the

TABLE 17: Classification of the individual tests using the threshold values and limit concentrations described above. Grey cells: Ecotoxicological effects higher than the test-specific threshold values did occur at the respective limit concentrations (LID aquatics = >4; LID terrestrial > 8). Black cells: ecotoxicologically hazardous according to the HP 14 property. Note that the bacterial tests were not included since the umu-test did not show any effects.

Waste code	Aquatic tests			Terrestrial tests			Overall Hazard evaluation
	LID_A	LID_D	Ecotox. LID > 4	LID_P	LID_R	Ecotox. LID > 8	
01 05 05	2	2		> 16	> 16		
06 03 16	> 8	8		> 16	16		
08 01 16	> 8	> 8		> 16	> 16		
10 01 17	8	> 8		> 16	16		
12 01 17	> 8	> 8		4	8		
17 01 07	2	2		4	8		
17 02 01	8	2		> 16	16		
17 05 04	2	2		4	4		
17 05 06	4	2		4	4		
17 05 08	2	2		4	4		
17 08 02	2	2		> 16	4		
17 09 04-A	> 8	2		> 16	8		
17 09 04-B	4	2		> 16	4		
19 08 02	4	2		4	4		
19 08 14	> 8	2		> 16	4		
19 10 04	4	2		16	8		
19 12 05	>8	> 8		4	4		
19 12 07	>8	2		> 16	4		
19 12 07-A	4	4		> 16	4		
19 12 07-B	8	2		> 16	4		
19 12 12	> 8	2		8	4		
19 13 02	8	2		4	4		
20 01 38	> 8	2		> 16	16		

high practicability and sensitivity of this test system. It was by far the most often performed test but at the same time the one with the lowest number of invalid data sets (Moser and Römbke 2009). In the light of these experiences the low sensitivity in this study is difficult to explain. It might be that water flea react mainly to heavy metals (Seco et al. 2003), which – by chance – were not so often occurring in the 23 wastes tested.

Despite the fact that the Arthrobacter test has been used for quite some time (mainly in sediments) it has only been standardized for soils quite recently. Therefore, the number of experiences in waste testing are limited, mainly with incineration ashes (Deventer et al. 2004; Römbke et al. 2009). Positive experiences in the international ring test (Moser and Römbke 2009) and in a recent interlaboratory comparison test (Marques et al. 2018). In particular its high practicability (short duration, low costs, high sensitivity) has increased its usage.

When testing the effects of contaminant soils on plants often the dicotyledonous species *Brassica rapa* (*or B. napus*) is reacting most sensitively (Wilke et al. 1998; Kalsch et al. 2006b). In addition, the test is very robust, meaning that it is a regular part of terrestrial ecotoxicological test batteries. In fact, while the number of plant test with wastes is still limited it is the test most often performed with solid waste samples. Again, different ashes are the best studied samples (Wong and Wong 1989; Deventer al. 2004; Römbke and Moser 2007). It could also be shown that ashes with different physico-chemcial properties do cause different effects on plants (Quilici et al. 2004).

The earthworm avoidance test has been developed and standardized about ten years ago (ISO 17512-1 (2007)), i.e. the amount of data regarding its use for waste testing is limited. However, when used in the international Ringtest it became clear that it is much more sensitive than the earthworm acute test (ISO 11268-1 (1993)), which was proposed earlier for this task (Moser and Römbke 2009). However, the results were often variable – an observation which is known from tests with contaminated soils (Hund-Rinke et al. 2003). This might be caused by the fact that the worms do not only react to toxic contaminants but also to physico-chemical properties (Natal-da-Luz et al. 2008). Kobeticova et al. (2010) confirm the suitability of the earthworm avoidance test for waste evaluation, also pointing out that other oligochaete species such as enchytraeid seem to be less sensitive.

Huguier et al. (2015) studied a wide range of (mainly) organic wastes in tests with various aquatic and terrestrial species (partly with more than one endpoint), trying to identify a suitable test battery. Plants (*Avena sativa*, *Brassia rapa*) and earthworms (*Eisenia fetida*), seem to be suitable for this specific group of wastes. In addition, the authors confirm the good comparability of results from avoidance and reproduction earthworm tests. This information supports the choice made here but cannot easily be transferred to wastes in general.

The information summarized in this chapter confirms that a battery of six test methods (plus, if needed, a genotoxicity test) selected by Pandard and Römbke (2013) is needed for the ecotoxicological characterization of wastes since they react sensitively to different stressors and their interactions.

4.2 Test design, threshold (reference) and limit values

4.2.1 Test design

Both an "Extended Limit Design" (i.e. testing three (or more) dilution steps with fixed ratios) as well as the EC-approach do allow an ecotoxicological classification of wastes.

In Germany, the LID-approach (= Lowest Ineffective Dilution) is widely used for the evaluation of waste waters or contaminated soils, partly because the effort needed is relatively low (e.g. only three dilution steps are needed) (e.g. DiBt 2008). In order to assess the ecotoxicological hazard of a waste sample it is necessary to define a limit concentration, usually given in percent of the overall tested amount (e.g. 12.5% = LID 8). These limit concentrations cannot be defined based on test results but must be set-up before testing, using the following criteria:

- The limit concentration should be practical when classifying wastes;
- They must be protective, i.e. hazardous wastes must be clearly identifiable.

So far, legally no limit concentration has been fixed. Unfortunately, only few proposals have been found in the literature, mainly addressing aquatic tests (e.g. Kostka-Rick 2004b; DIBt 2008). However, based on these hints and the experiences made in the European Ringtest on Wastes, a LID = 4 for the aquatic tests and a LID = 8 for the terrestrial tests was proposed as being acceptable (Moser & Römbke 2009). The LID-approach did work well for this testing and assessment program, but it has its limitations (e.g. it highly depends on the concentrations tested). In any case it was possible to differentiate ecotoxic and non-ecotoxic wastes. Beyond this yes/no-decision it is also possible to assess, how toxic the respective waste or the specific test organism is, since three dilution steps were used. Only results from the umu- test are difficult to be assessed, since genotoxicity seems to be less dose-dependent than other endpoints. In addition, this test reacts only to specific contaminants, making it less sensitive in general.

In ecotoxicology in general the ECx approach is more common since it allows a more detailed but also robust assessment of ecotoxicological effects – as long as the whole response curve (ideally from 0 to 100%) is covered. In order to improve the robustness of this classification the use of an ECx design (aiming on the calculation of an EC50 value) would be better. In such a case, more concentrations than just three as in the "Extended Limit Approach" have to be tested (e.g. up to eight). However, when doing so the number of replicates per concentration is lowered, meaning that the overall testing effort would not increase very much. Pandard and Römbke (2013), recommending an ECx-design (i.e. an EC_{50} as limit concentration), could show that such an approach is reliable and protective. First experiences show that the ecotoxicological characterization of wastes do not differ much between these two methods (Pandard and Römbke 2013). However, the number of such comparisons is still very low. Therefore, from a scientific point-of-view the determination of an EC50 is the better and more robust option, being in-line with other areas of ecotoxicology (e.g. the risk assessment of chemicals).

4.2.2 Threshold (reference) values

The threshold values for the individual tests (e.g. 20% effect on the main test endpoint such as immobility in the Daphnia-test) used here are based on ideas firstly published by Moser (2008). Partly they are already mentioned in the respective test standards, partly they are specified in scientific publications focusing on waste classification. Ideally, such threshold values should be based on statistical considerations, but the respective comparable data sets are not (yet) available. Therefore, experiences with soil tests have been used too. In fact, there is a grey zone between contaminated soils and at least some wastes (e.g. in this project: No. 19 13 02: Solid wastes from soil remediation other than those listed in 19 13 01. Soil material strongly contaminated by PAH and mineral oil, but also Pb, Zn, Cu, Cr and PCB).

Independently whether the LID- or the ECx-approach is used there is always a third level of decision-making in case a test battery is used: in how many tests have the threshold values to be breached? For example, in order to characterize a waste as being hazardous at least one test has to show an effect on one aquatic or one terrestrial species. Another possibility would be to require effects on one species from each of the three taxonomic groups independently in which medium they had been tested. At this point, again the proposal of Pandard and Römbke (2013) is followed, meaning that a tiered approach is used. One aquatic or one terrestrial test has to show effects higher than the respective threshold value in order to classify the tested waste as hazardous according to the HP 14 property.

There has also been no criterion fixed legally so far regarding the outcome of the whole test battery. Previously – and following a recommendation given in ISO 17616 (ISO 2008b) for the assessment of contaminated soils – it was assumed that a waste is classified as "ecotoxic" in case the threshold values have been breached in one out of six tests. Later on, Pandard and Römbke (2013) modified this strategy (which works well as long as the number of aquatic and terrestrial tests is equal) in a way that the process is divided into two parts:

1. Assessment based on aquatic biotests: If one of the LID values in the eluate tests (the IR value of > 1.5 in the umu-test would be handled like a LID-value > 4) is above the proposed threshold values of 4 or 8, respectively, the waste is classified as hazardous and the overall procedure is stopped.
2. Otherwise, solid waste tests are carried out and the assessment procedure is repeated. The waste is considered as non-hazardous if the results of all biotests are below or equal to the threshold values.

4.3 Comparison of different approaches regarding the ecotoxicological characterization of wastes

The chemical composition of the tested wastes is not known. Therefore, it is impossible to calculate whether the respective waste sample has to be classified as "ecotoxic" or not. Alternatively, the List of Waste could be used as a reference, but in that list, only the "absolute" entries can be used to assess the proposed dilutions, meaning that a classification of the waste samples tested here is also not possible.

From the results of the tests (Table 17) and the proposition of dilution and effect rates for ecotoxicity classification (Table 8), 18 wastes are classified as ecotoxic (17 mirror entries and one hazardous 01 05 05*), and four wastes are classified as non-ecotoxic (three mirror entries and one non-hazardous 19 08 02). In other words, one hazardous waste is classified as ecotoxic, one non-hazardous waste is classified as non-ecotoxic and 20 "mirror entries" are classified 17 times as ecotoxic and three times as non-ecotoxic. Right now, it is impossible to say whether the results of this study are representative for the relationship between ecotoxicological testing and the classification of wastes according to the List of Wastes. However, further ecotoxicological tests with a broader range of wastes, trying to cover the range of the waste types and subtypes, are recommended in order to get a better understanding of the hazard properties of wastes. In case such studies will be performed a chemical characterization of the test samples is highly recommended. Assuming that such a data set will be available, the suitability and robustness of the different classification approaches could be assessed and recommendations for legal handling formulated.

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