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Final report

HP 14 classification of mirror entries in the List of Wastes – elaboration of proposals for further developing the German ‘Recommendations for the ecotoxicological characterization of wastes’

by:

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German Environment Agency

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Abstract: HP 14 classification of mirror entries in the List of Wastes – elaboration of proposals for further developing the German 'Recommendations for the ecotoxicological characterization of wastes'

Waste from so-called mirror entries has to be categorized as hazardous or non-hazardous depending on its composition. Hazard properties (HP) are determined based on concentrations of the waste constituents or testing. For the hazard property HP 14 (ecotoxic), there are no specific requirements at EU level for the classification based on testing (bioassays). In Germany, recommendations for the ecotoxicological characterization of wastes were published by UBA in 2013. The objective of the present project was to develop proposals for updating and further developing the UBA recommendations. A literature search was performed to identify biotest-based strategies for the HP 14 classification of waste, approaches to sampling, sample pre-treatment and elution as well as relevant ecotoxicological test methods. The strategy proposed in the UBA recommendations for HP 14 classification of mirror entries was verified, and initial suggestions were made for its update and further development. The strategy was then reviewed based on sampling, sample preparation and ecotoxicity testing of 10 waste samples from mirror entries (flue-gas dust, soil and stones, fluff-light fractions and dust). Based on the results and experiences and the discussions with the project advisory group, the proposals for updating and further developing the UBA recommendations were further elaborated. They relate to sampling, sample pre-treatment, subsampling in the laboratory, elution, ecotoxicity testing, and minimum requirements for reporting. In addition, issues were identified for which there is a need for action at regulatory level and proposals were made for adjustments to the test guidelines for the biotests.

Kurzbeschreibung: Einstufung von Spiegeleinträgen im Abfallverzeichnis nach HP 14 – Erarbeitung von Vorschlägen für eine Weiterentwicklung der Handlungsempfehlung zur ökotoxikologischen Charakterisierung von Abfällen

Abfälle aus sog. Spiegeleinträgen müssen je nach Abfallzusammensetzung als gefahrenrelevant oder nicht gefahrenrelevant eingestuft werden. Dabei werden gefahrenrelevante Eigenschaften anhand der Konzentrationen der Abfallinhaltsstoffe oder anhand einer Prüfung ermittelt. Für das Gefährlichkeitsmerkmal HP 14 (ökotoxisch) gibt es auf EU-Ebene keine konkreten Vorgaben für eine Einstufung anhand von Prüfungen (Biotests). In Deutschland wurde 2013 eine Handlungsempfehlung des UBA zur ökotoxikologischen Charakterisierung von Abfällen veröffentlicht. Ziel des vorliegenden Projekts war es, Vorschläge für eine Aktualisierung und Weiterentwicklung dieser Handlungsempfehlung zu erarbeiten. Eine Literaturrecherche wurde durchgeführt, um Biotest-basierte Strategien für die HP 14-Einstufung von Abfällen, Herangehensweisen bei Probenahme, Probenvorbehandlung und Elution sowie relevante ökotoxikologische Testverfahren zu identifizieren. Die in der UBA-Handlungsempfehlung vorgeschlagene Strategie zur HP 14-Einstufung von Spiegeleinträgen wurde überprüft, und erste Vorschläge für eine Aktualisierung und Weiterentwicklung wurden gemacht. Die Teststrategie wurde anschließend anhand der Beprobung, Aufbereitung und ökotoxikologischen Untersuchung von 10 Abfallproben aus Spiegeleinträgen (Filterstaub, Boden und Steine, Shredderleichtfraktionen und Staub) überprüft. Aufbauend auf den Ergebnissen und Erfahrungen und unter Berücksichtigung der Diskussionen mit dem projektbezogenen Begleitkreis wurden die Vorschläge für eine Aktualisierung und Weiterentwicklung der UBA-Handlungsempfehlung weiter ausgearbeitet. Sie betreffen Probenahme, Probenvorbehandlung, Teilung von Proben im Labor, Elution, ökotoxikologische Testung und Mindestanforderungen an Berichte. Außerdem wurden Punkte identifiziert, für die auf regulatorischer Ebene Handlungsbedarf besteht, und es wurden Vorschläge für Anpassungen der Testrichtlinien für die Biotests gemacht.

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List of abbreviations

Abbreviation	Explanation
AFNOR	Association Française de Normalisation (official French body for standardisation)
APA	Agência Portuguesa de Ambiente (Portuguese Environment Agency)
AVV	Abfallverzeichnis-Verordnung (German list of wastes)
BAC	Benzalkonium chloride
BMNT	Österreichisches Bundesministerium für Nachhaltigkeit und Tourismus (Austrian Federal Ministry for Sustainability and Tourism)
CDF	Normal-cumulative distribution function
CEN	Comité Européen de Normalisation (European Committee for Standardisation)
CEN/TC	Technical Committee of CEN
CEN/TR	Technical Report of CEN
CI	Confidence interval
CLP	Classification, Labelling and Packaging
CS	Composite sample (sample comprising several random samples)
CV	Coefficient of variation
d_{05}	Nominal screen size of particles: screen hole diameter allowing 5% of sample weight to pass
d_{95}	Nominal screen size of particles: screen hole diameter allowing 95% of sample weight to pass
DIN	Deutsches Institut für Normung (German Institute of Standardisation)
DMSO	Dimethyl sulfoxide
DW	Dry weight
EC_X	Effect concentration (EC) with the effect strength of X%
EN	European standard
FW	Fresh weight
g	Correction factor for particle size distribution (CEN/TC 292)
G-value	Dilution level with the highest concentration of the waste or eluate, at which no ecotoxic effect on the test organisms is recorded
HP	Hazardous property
INERIS	Institut National de l'Environnement Industriel et des Risques (French National Institute for Industrial Environment and Risks)
IPA	Information Portal Waste Assessment
ISO	International Organisation for Standardisation

Abbreviation	Explanation
ISPRA	Istituto Superiore per la Protezione e la Ricerca Ambientale (Italian Institute of Environmental Protection and Research)
L/S	Liquid/solid
LAGA	Bund/Länder-Arbeitsgemeinschaft Abfall (working group on waste of the German federal states)
LC_X	Concentration leading to X% mortality
LID	Lowest ineffective dilution
LOEC	Lowest observed effect concentration (lowest test concentration at which significant effects are detected)
LUFA	Landwirtschaftliche Untersuchungs- und Forschungsanstalt (German agricultural research institute)
LS	Laboratory sample
M	Mass
Mg	Megagram (1 Mg = 1 t)
MITECO	Ministerio para la Transición Ecológica y el Reto Demográfico (Spanish Ministry for the Ecological Transition and Demographic Challenge)
M_{SAM}	Minimum sample mass (minimum sample size)
MWI	Municipal waste incineration
NOEC	No observed effect concentration (highest test concentration, at which no significant effects are detected)
OECD	Organisation for Economic Cooperation and Development
p	Fraction (proportion) of particles with a certain characteristic (the characteristic to be determined)
PAH	Polycyclic aromatic hydrocarbons
PE	Polyethylene
POP	Persistent organic pollutant
PP	Polypropylene
REACH	Registration, evaluation, authorisation and restriction of chemicals
RFU	Relative fluorescence units
RS	Random sample (individual sample, also called increment in publications of CEN/TC 292)
SAG	Collection of algal cultures at the University of Göttingen
SAM	Sample
SEPA	Scottish Environment Protection Agency
SNPA	Sistema Nazionale per la Protezione dell'Ambiente (Italian National System for the Protection of the Environment)

Abbreviation	Explanation
SP	Sample preparation
T1, T2, etc.	Test run 1, test run 2, etc.
TCS	Toxicity classification system
TS	Test sample
TU	Toxic unit
UBA	German Environment Agency
UNI	Ente Italiano di Normazione (Italian Standardisation Authority)
V	Volume
V_{SAM}	Minimum sample volume (minimum sample size)
Z2	Assignment value for soil analysis according to LAGA
ρ_B	Bulk density (kg/dm³)
ρ_P	Particle density of a solid (kg/dm³)
ρ_R	Raw density of a porous solid (kg/dm³)
σ	Standard deviation
σ^2	Variance

Glossary

Term	Explanation
Basic quantity (population)	Specific, spatially and/or temporally defined amount of material to be examined
Characteristic	Distinguishable property of particles from a basic quantity (population)
Composite sample	Sample obtained by combining and mixing individual samples from a basic quantity (population). From the composite sample, an estimate for the average value for a given characteristic is obtained
Estimated value	The result of an analysis of random samples is – as expected value – an estimate of the true value of a characteristic
Expected value	The value that the respective parameter is likely to have on average. Estimate of the true value of a characteristic
Field sample	Material sampled in the field from a basic quantity (including random, composite, and aggregate samples)
Laboratory sample	Sample or subsample provided to the laboratory, if necessary after sample pre-treatment
Particulate heterogeneity	Heterogeneity of the material that is due to the specific load of the characteristic(s) in single particles. It cannot be reduced by mixing. Homogenisation requires shredding/crushing
Random sample	Individual sample taken in a single sampling operation that is limited locally and temporally to one sampling point (also termed increment)
Representativeness	Qualitative measure of the extent to which the content of a characteristic in a sample corresponds to the content of the characteristic of the defined basic quantity (population). Can be described by the coefficient of variation (CV)
Sample preparation	Preparation of a test sample from the laboratory sample. May include drying, sieving, homogenising and/or division of the sample
Sample pre-treatment	Preparation of laboratory sample(s) from the field sample. Sample pre-treatment can include mixing, homogenising, division, drying, sorting, crushing/shredding, sieving and conserving of the sample
Sample processing	Preparation of the analytical, test or measurement samples. According to DIN 19747, sample processing usually includes drying and, if necessary, crushing/shredding of the sample
Test sample	Sample produced from the laboratory sample, from which material is taken for testing or analysis

Summary

The European List of Wastes (Decision 2000/532/EC, amended by Decision 2014/955/EC) was adopted into German law (Abfallverzeichnis-Verordnung, AVV) and contains a list of waste types. These are categorised into absolute hazardous entries, absolute non-hazardous entries and mirror entries. Mirror entries are waste types listed in pairs; their designation only differs with regard to the reference to hazardous substances contained in the waste. Depending on the specific case or waste composition, the respective waste has to be allocated to the hazardous or non-hazardous mirror entry. Hazardous properties are determined by calculation based on the concentrations of the waste constituents or based on testing. If a hazardous property of a waste has been assessed both based on the concentrations of hazardous substances and by testing, the results of the tests are crucial for the classification as hazardous or non-hazardous waste according to the current legal situation. For the hazard property (HP) 14 (ecotoxic), Regulation (EU) 2017/997 contains specific requirements for the calculation method (including concentration limits for classification based on the content of substances that are ozone-depleting or acutely and/or chronically hazardous to water organisms). However, at EU level there are no specific requirements for HP 14 classification based on testing, i.e. it is not specified which bioassays should be used and which results should lead to an HP 14 classification. Therefore, decisions on the acceptability and interpretation of the results of biological tests with waste samples are currently under the responsibility of the EU member states. In Germany, recommendations for the ecotoxicological characterization of wastes were developed (UBA 2013) based on extensive work on the assessment of the environmental risks of waste.

The objective of the present project was to elaborate proposals for updating and further developing these UBA recommendations and to identify open issues. To this end, various biotest-based approaches for HP 14 classification were first compared in a European context. The strategy proposed in the UBA recommendations for HP 14 classification of mirror entries was verified, and initial suggestions were made for its update and further development. The test strategy was then reviewed based on the sampling, sample preparation and ecotoxicological testing of 10 waste samples from mirror entries. Considering the results of the experimental work and the discussions with the project advisory group, proposals for an update and further development of the UBA recommendations were further elaborated.

Literature search and initial review of the testing strategy proposed in the UBA recommendations

A literature and internet search was performed to identify biotest-based strategies for HP 14 classification of waste, and approaches regarding sampling, sample pre-treatment and elution as well as relevant ecotoxicological test methods. Information on strategies for HP 14 classification was compiled for Austria, Belgium (Flanders), the Czech Republic, Denmark, Finland, France, Germany, Italy, Portugal, Serbia, Slovakia, Spain, Sweden, and the United Kingdom, based on a CEN/AFNOR survey and national guidance documents. In the different European countries, approaches for HP 14 classification of waste are very heterogeneous. There are differences regarding the criteria for using ecotoxicity tests, the specifications of the maximum particle size of the waste to be tested, the elution methods, specifications for pH adjustment prior to aquatic testing, the types of ecotoxicity tests used, test design, and limit concentrations for HP 14 classification. Similarly, strategies and methods proposed in scientific publications are diverse.

The approach proposed in the UBA recommendations was reviewed in the light of the results of the literature review and new guidelines. Over the last 10 years, several guidance documents regarding sampling were published at the European level (EN 14899, CEN/TR 15310-1 to -5). These are based on the 'Theory of Sampling' dating back to Pierre Gy, which, in addition to the LAGA guideline PN 98, has also been the basis for the approach proposed in the UBA

recommendations. With regard to the specifications on particle size and the elution of waste, the procedure suggested by UBA (2013) is in accordance with current European standards (EN 12457-2, EN 14735), which are also used in several other European countries, and which were used in several published studies on the ecotoxicity of waste. The recommendation of UBA (2013) to initially perform aquatic bioassays without pH adjustment and to use the result from these tests for HP 14 classification corresponds to the specifications provided in EN 14735. In comparison to other European countries, the test battery proposed in the UBA recommendations is one of the more comprehensive test batteries. With the exception of the emergence and root length test with cress, for which further experimental investigations would first need to be performed, no test systems with a high relevance for inclusion in the test battery were identified. Likewise, there was no reason to reduce the number of tests in the test battery or to change the test design (tests with at least 5 dilution levels of the waste or waste eluate to determine the EC_{50}). According to the UBA recommendations, a waste is classified as ecotoxic (HP 14), if an EC_{50} of ≤10% waste or eluate content is determined in at least one of the bioassays. Identical or similar procedures are also used in several other European countries and are described in several published studies on the ecotoxicity of waste.

Sampling, sample preparation and ecotoxicological testing

Based on suggestions of UBA, BMUV, the project advisory group and the project team, the following waste types (mirror entries) were selected: flue-gas dust (10 09 09*/10 09 10), soil and stones (17 05 03*/17 05 04) and fluff-light fraction and dust (19 10 03*/19 10 04). The aim was to analyse at least one sample of the hazardous and non-hazardous mirror entry for each waste type. However, no producer of 19 10 03* waste could be identified during the project. The following wastes were sampled and investigated in ecotoxicity tests:

- Flue-gas dust (10 09 09*) from iron and steel casting: aged material (storage period >4 weeks) and fresh material (storage period <4 weeks)
- Flue-gas dust (10 09 10) from iron and steel casting: material from two foundries (plants A and B)
- Soil and stones (17 05 03*): excavated geogenic material from an open-cast lignite mine and material from the side verges of a federal road
- Soil and stones (17 05 04): material from the side verges of a secondary road
- Fluff-light fraction and dust (19 10 04): material (sieved to <10 mm) from two plants (plant A: two batches, plant B: one batch)

The classification of the sampled wastes as hazardous or non-hazardous mirror entry was carried out by the waste owners and was not necessarily based on the HP 14 criterion.

During sampling and sample preparation, the requirements of LAGA guideline PN 98 and European standards (CEN/TR 15310, EN 14735) were met. In the aquatic ecotoxicity tests, aqueous eluates of the waste samples were used, which were prepared using a one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h. In the terrestrial ecotoxicity tests, solid waste samples (particle size <2 mm in the microbial test and <4 mm in the other tests) were used. Samples of the 10 wastes mentioned above were analysed in the following bioassays:

- Aquatic bioassays: acute *Daphnia* test (DIN EN ISO 6341), algal growth inhibition test in microtiter plates (DIN 38412-59) and luminescent bacteria test (DIN EN ISO 11348-2).

- Terrestrial bioassays: solid contact test with *Arthrobacter globiformis* (ISO 18187), growth inhibition test with *Brassica rapa* (ISO 11269-2) and avoidance test with earthworms (ISO 17512-1)

For the aquatic bioassays, up to three test runs were carried out. In the first test run for each waste sample, the eluates were tested at the following dilution levels: 50, 25, 12.5, 6.3 and 3.1% (all tests) and, additionally, 1.6, 0.8 and 0.4% (luminescent bacteria test). If effects >50% were recorded at all dilution levels, higher dilution levels were tested in the following test run for deriving an EC_{50}. The first test run for each waste sample was performed without pH adjustment. If toxicity was observed at dilution levels, where pH was outside the range tolerated by the test species, a further test run was performed with pH adjustment. In this test run, pH was only adjusted for dilution levels with pH values outside the tolerance range for the respective species. Parallel to each test run with adjusted pH, an additional test run without pH adjustment was carried out to evaluate reproducibility of the results. Further test repeats of the aquatic toxicity tests (also for eluates without toxic effects in the first test run) were performed to investigate reproducibility of the test results.

For the terrestrial tests, one test was carried out for each waste sample, with five dilution levels of the (solid) waste: 25, 12.5, 6.3, 3.1 and 1.6%. One waste sample was additionally analysed using a rapid test to determine potential nitrification and inhibition of nitrification by means of ammonium oxidation (DIN EN ISO 15685).

The results of the bioassays with the 10 waste samples showed that the aquatic tests are highly reproducible (an evaluation of reproducibility of the terrestrial tests was not part of the present project). In most cases, the algal and the *Daphnia* tests were more sensitive than the luminescent bacteria test. The terrestrial tests tended to be slightly less sensitive than the aquatic tests.

In 3 out of 4 cases, the waste samples assigned by the waste owner to the hazardous mirror entry were classified as hazardous by HP 14 based on the bioassays. The only exception was the material from the side verges of a federal road, which showed no toxicity in any of the bioassays. This lack of toxicity might be related to the high clay content of the waste sample.

Waste samples assigned by the waste owner to the non-hazardous mirror entry were classified as hazardous by HP 14 in 5 out of 6 cases based on the bioassay results. In 4 of these cases, the results obtained with more than one test method were below the limit concentration (EC50 ≤10% waste or eluate content). The high toxicity of the samples of fluff-light fraction and dust (19 10 04) was particularly remarkable.

Proposals for an update and further development of the UBA recommendations

Based on the results of the literature search and the experience gained during sampling, sample preparation, elution and ecotoxicity testing of the 10 waste samples, suggestions were made for updating and further developing the UBA recommendations. The discussions with the project advisory group were considered. The proposals for an update and further development of the UBA recommendations relate to sampling, sample pre-treatment, subsampling in the laboratory, elution, ecotoxicological testing, and minimum requirements for reports. In addition, issues were identified, for which there is a need for action at regulatory level, and proposals were made for adaptations of some of the test guidelines for the bioassays.

Sampling and sample pre-treatment

Regarding sampling, the UBA recommendations from 2013 already refer to a European standard being developed. Meanwhile, the technical reports CEN/TR 15310 are available. Concerning the fraction of particles with the characteristic to be determined, and the desired reliability of the

results, it is proposed that the UBA recommendations are adapted to the approach described in these European technical reports. Sampling for ecotoxicological evaluation of waste should be carefully planned in accordance with CEN/TR 15310-1. At least 16 probabilistic individual samples should be taken and combined to a composite sample. If the sample contains material (oversized particles) >4 mm, a decision needs to be taken using the minimum sample mass formula of CEN/TR 15310-1 as to whether the oversized particles should be discarded or crushed/shredded and included in the sample.

Subsampling in the laboratory

Basically, there are two approaches to obtain samples for performing individual bioassays: (a) dividing the laboratory sample to obtain a subsample (test sample), and (b) taking several (smaller) subsamples from the laboratory sample and combining them to a composite (test) sample. A simulation with random numbers was carried out using approximate distributions for parameter contents to estimate the effects of these two approaches on the variance of the characteristic's contents in the samples used for bioassays. Based on the simulation results and in analogy to the sampling procedure described above, it is recommended to take at least 16 individual samples from the carefully homogenised laboratory sample and to combine them into a composite test sample.

Elution

In line with EN 14735, UBA (2013) proposes to elute waste using the one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h according to EN 12457-2. Both the UBA recommendations and EN 14735 should specify whether or, more specifically, to what extent this method is suitable for eluting organic substances and poorly soluble inorganic substances from waste samples.

Biotests

For the aquatic and the terrestrial compartment, the test battery proposed in the UBA recommendations covers the taxonomic groups of plants, invertebrates and microorganisms, and the trophic levels of producers, consumers and destruents. Compared to other European countries, it is one of the more comprehensive test batteries.

For the 10 waste samples analysed, the aquatic bioassays tended to be more sensitive than the terrestrial ones. However, as the results of the UBA project PROSOIL have shown, aquatic tests are not in all cases protective for soil organisms. By additionally performing terrestrial biotests in cases where all results from aquatic tests are negative, toxic effects of poorly water-soluble waste constituents on soil organisms can be detected. Bioassays with terrestrial organisms should therefore remain part of the test battery.

In the solid contact test with *A. globiformis*, a high variability of results was frequently recorded, especially in tests with heterogeneous waste (fluff-light fractions). This variability was most likely caused by the very small amounts of waste used in this test. The rapid test to determine potential nitrification according to DIN EN ISO 15685 could be a possible alternative to the solid contact test with *A. globiformis*. To verify the suitability of this test, an adaptation of the method and further comparative experimental studies would be necessary.

Effects on terrestrial vertebrates should be covered by other hazard-relevant properties (HP 5: specific target organ toxicity, HP 6: acute toxicity, HP 10: toxic for reproduction). However, possible effects on fish are not covered by other hazard properties. If a test with the taxonomic group of fish is to be added to the bioassay battery for the HP 14 classification of wastes in mirror entries, it is proposed to check whether one of the available alternative methods is suitable (the fish egg test according to DIN EN ISO 15088, the fish embryo test according to

OECD test guideline 236, or the fish cell line test according to OECD test guideline 249). In this case, experimental studies and, possibly, methodological adaptations would be necessary.

The UBA recommendations specify that the aquatic and terrestrial bioassays should be carried out with at least five dilution levels of the waste or waste eluate to determine EC50 values. The project advisory group suggested that it should be examined whether limit tests could be an option. Limit tests could, for example, be performed with an eluate or waste content of 12.5%. In analogy to the environmental risk assessment for chemical substances, limit tests with waste or waste eluates should be used to verify the absence of ecotoxic effects. If a significant effect is found in a limit test, a test with five waste or eluate dilutions should be performed to determine the EC_{50}.

According to Decision 2000/532/EC, the results of testing are crucial for the classification of a waste, if the respective hazardous property has been assessed based on the concentrations of hazardous substances and based on testing. This opens the possibility of using biotests to exonerate a waste classified as ecotoxic using the calculation method. However, this option is only partly justified:

- When a waste is classified as HP 14 using the calculation method solely because of substances that are acutely hazardous to water organisms but lacks ecotoxicity in all bioassays of the test battery, it appears justified to exonerate this waste based on the test results. In this case, a low bioavailability could be the reason for the lack of ecotoxicity in the bioassays. Only in cases where the classification as acutely hazardous to water organisms is based on fish toxicity only, it should not be possible to exonerate the waste based on the results obtained with a bioassay battery that does not contain a fish test.
- When a waste has been classified as HP 14 using the calculation method due to substances with a long-term hazard to the aquatic environment, it does not appear justified to exonerate this waste based on acute effect concentrations determined with the current bioassay battery. To exonerate a waste, which – using the calculation method – has been classified as long-term hazardous to the aquatic environment based on the results of chronic ecotoxicity tests with individual waste constituents, results of chronic ecotoxicity tests with the waste eluate should be required.
- When a waste has been classified as HP 14 using the calculation method due to ozone-depleting substances, it is not justified to exonerate this waste using bioassays.

These last two points should be addressed at EU level: it should be specified in which cases the results of bioassays can prevail over the results obtained with the calculation method.

Currently, waste testing is only explicitly mentioned in the guideline for the solid contact test with *A. globiformis*. It is recommended to also include the testing of waste samples or eluates in the scope of the guidelines for the other tests of the UBA test battery and to add information on handling of waste samples or eluates.

In the UBA recommendations it should be specified that the pH should not be adjusted in the tests relevant for HP 14 classification, even if the test guideline states that a pH adjustment is possible or recommended, as is the case in the algal and luminescent bacteria test. In addition, it should be considered if for each biotest pH ranges should be specified, outside of which it does not make sense to perform the test, since the pH alone is likely to lead to severe toxicity.

According to ISO 11269-2, the growth inhibition test with *B. rapa* should be carried out with 12 dilution levels. However, to determine whether the EC50 is ≤ or > the limit concentration of 10% waste content, 5 dilution levels are sufficient.

Harmonisation between the different EU member states regarding the limit concentration would be desirable, also regarding the transboundary transport of waste.

Minimum requirements for reports

Following a suggestion of the project advisory group, an overview was compiled of key information that reports on sampling, sample preparation, storage, subsampling, elution and ecotoxicity testing should contain to enable the competent authority to evaluate the results. The corresponding specifications are given in detailed form in the standards and test guidelines for the relevant methods. Information on minimum requirements for reports was compiled in a tabular form, and could become an annex to the revised UBA recommendations.

Possibilities and limitations of ecotoxicity tests compared to the calculation method

For the calculation method for HP 14 classification, chemically analysed concentrations of those waste constituents are used that are classified as hazardous to the ozone layer (H420), acutely hazardous to the aquatic environment (H400) and/or long-term hazardous to the aquatic environment (H410-H413) in accordance with the CLP Regulation. Waste constituents classified as acutely or long-term hazardous to the aquatic environment are only considered, if their concentrations reach or exceed relatively high cut-off values (H400, H410: 1 g/kg waste wet weight; H411-H413: 10 g/kg waste wet weight). In the calculation method, only those substances are included, for which a harmonized or a notified classification ('self-classification') is available. When performing chemical analyses, which are the basis for the calculation method, the substances to be analysed are selected based on the available information on the composition of the waste. Other pollutants that may be present in the waste are not considered. In addition, substances present in concentrations below the detection limit of the used analytical method have no impact on HP 14 classification.

Based on the results of ecotoxicity tests, a statement can be made about the combined effects of all toxic substances in the waste that are bioavailable under test conditions. In contrast to the calculation method, this includes substances with concentrations below the cut-off values of the calculation method or below the detection limits of the analytical method, and substances that are not detected with the used analytical method. The bioassay results also reflect possible interactions between different waste constituents. In addition to acute toxicity to aquatic organisms, the test battery recommended by UBA (2013) also covers acute toxicity to terrestrial organisms. To evaluate chronic effects, the bioassay battery would need to be adapted. Potential hazards to the ozone layer cannot be detected with bioassays.

Overall, the calculation method and the use of bioassays for HP 14 classification of waste from mirror entries are therefore complementary approaches. A further development of the procedures for HP 14 classification of mirror entries at EU level would be desirable, considering the possibilities and limitations of the calculation method and biotests.

Zusammenfassung

Hintergrund und Ziele

Das europäische Abfallverzeichnis (Entscheidung 2000/532/EG, geändert durch Beschluss 2014/955/EG) wurde mit der Abfallverzeichnis-Verordnung (AVV) in deutsches Recht umgesetzt und enthält eine nicht abschließende Liste von Abfallarten. Diese sind in absolut gefahrenrelevante Einträge, absolut nicht gefahrenrelevante Einträge und Spiegeleinträge eingeteilt. Spiegeleinträge sind paarweise aufgeführte Abfallarten, deren Bezeichnung sich nur durch den Hinweis auf im Abfall enthaltende gefährliche Stoffe unterscheidet. Die betreffenden Abfälle müssen je nach konkreter Sachlage bzw. Abfallzusammensetzung dem gefahrenrelevanten oder nicht gefahrenrelevanten Spiegeleintrag zugeordnet werden. Dabei werden gefahrenrelevante Eigenschaften rechnerisch anhand der Konzentrationen der Abfallinhaltsstoffe oder anhand einer Prüfung ermittelt. Wenn eine gefahrenrelevante Eigenschaft eines Abfalls sowohl anhand der Konzentration gefährlicher Stoffe als auch mittels einer Prüfung bewertet wurde, sind nach derzeitiger Rechtslage die Ergebnisse der Prüfung ausschlaggebend für die Einstufung als gefährlicher oder nicht gefährlicher Abfall. Für das Gefährlichkeitsmerkmal HP 14 (ökotoxisch) enthält die Verordnung (EU) 2017/997 konkrete Vorgaben für die Berechnungsmethode (einschließlich Schwellenwerten für die Einstufung anhand des Gehalts an Stoffen, die die Ozonschicht schädigen oder akut bzw. chronisch wassergefährdend sind). Für eine HP 14-Einstufung anhand von Prüfungen gibt es auf EU-Ebene hingegen keine konkreten Vorgaben, d. h. es ist nicht spezifiziert, welche Biotests eingesetzt werden sollen und welche Ergebnisse zu einer HP 14-Einstufung führen sollen. Daher liegen Entscheidungen über die Annehmbarkeit und Auslegung der Ergebnisse biologischer Prüfungen mit Abfallproben derzeit in der Verantwortung der EU-Mitgliedstaaten. In Deutschland wurde zu diesem Zweck auf Basis umfangreicher Arbeiten zur Bewertung der Umweltrisiken von Abfällen eine Handlungsempfehlung zur ökotoxikologischen Charakterisierung von Abfällen erstellt (UBA 2013).

Ziel des vorliegenden Projekts war es, Vorschläge für eine Aktualisierung und Weiterentwicklung dieser UBA-Handlungsempfehlung zu erarbeiten und offene Punkte zu identifizieren. Dazu wurden zunächst verschiedene Biotest-basierte Ansätze für die HP 14-Einstufung im europäischen Kontext verglichen. Die in der UBA-Handlungsempfehlung vorgeschlagene Strategie zur HP 14-Einstufung von Spiegeleinträgen wurde überprüft und erste Vorschläge für eine Aktualisierung und Weiterentwicklung wurden gemacht. Die Teststrategie wurde anschließend anhand der Beprobung, Aufbereitung und ökotoxikologischen Untersuchung von 10 Abfallproben aus Spiegeleinträgen überprüft. Unter Berücksichtigung der Ergebnisse der experimentellen Arbeiten und der Diskussionen mit dem projektbezogenen Begleitkreis wurden die Vorschläge für eine Aktualisierung und Weiterentwicklung der UBA-Handlungsempfehlung weiter ausgearbeitet.

Literaturrecherche und erste Überprüfung der in der UBA-Handlungsempfehlung vorgeschlagenen Teststrategie

Eine Literatur- und Internetrecherche wurde durchgeführt, um Biotest-basierte Strategien für die HP 14-Einstufung von Abfällen, Herangehensweisen bei Probenahme, Probenvorbehandlung und Elution sowie relevante ökotoxikologische Testverfahren zu identifizieren. Informationen zu Strategien bei der HP 14-Einstufung wurden für Belgien (Flandern), Dänemark, Deutschland, Finnland, Frankreich, Großbritannien, Italien, Österreich, Portugal, Schweden, Serbien, die Slowakei, Spanien und Tschechien auf Basis einer CEN/AFNOR-Umfrage und nationaler Leitfäden zusammengestellt. Die Herangehensweisen bei der HP 14-Einstufung von Abfällen in den verschiedenen europäischen Ländern sind sehr heterogen. Die Unterschiede betreffen die

Kriterien für den Einsatz ökotoxikologischer Tests, Vorgaben für die maximale Korngröße des zu testenden Abfalls, die Elutionsverfahren, Vorgaben zur Einstellung des pH-Werts vor Durchführung der aquatischen Tests, die Art der eingesetzten ökotoxikologischen Testverfahren, das Testdesign und die Grenzkonzentrationen für die HP 14-Einstufung. Auch die in wissenschaftlichen Veröffentlichungen vorgeschlagenen Strategien und Methoden sind divers.

Die in der UBA-Handlungsempfehlung vorgeschlagene Vorgehensweise wurde vor dem Hintergrund der Ergebnisse der Recherche und neuer Richtlinien überprüft. Die Probenahme betreffend wurden in den letzten 10 Jahren auf europäischer Ebene etliche Richtlinien veröffentlicht (EN 14899, CEN/TR 15310-1 bis -5). Diese basieren auf der auf Pierre Gy zurückgehenden ‚Theory of Sampling', die neben der LAGA-Richtlinie PN 98 auch bereits die Grundlage für die in der UBA-Handlungsempfehlung vorgeschlagene Vorgehensweise ist. Hinsichtlich der Vorgaben zur Korngröße und zur Herstellung von Abfalleluaten ist die Vorgehensweise laut UBA (2013) in Übereinstimmung mit aktuellen europäischen Normen (EN 12457-2, EN 14735), nach denen auch in mehreren anderen europäischen Staaten verfahren wird und die in etlichen veröffentlichten Studien zur Ökotoxizität von Abfällen eingesetzt wurden. Die Empfehlung des UBA (2013), aquatische Biotests zunächst ohne pH-Einstellung durchzuführen und das Ergebnis aus dem Test ohne pH-Einstellung für die HP 14-Einstufung zu verwenden, entspricht den Vorgaben der EN 14735. Die in der UBA-Handlungsempfehlung vorgeschlagene Biotestbatterie gehört im Vergleich mit anderen europäischen Staaten zu den umfangreicheren Testbatterien. Abgesehen vom Auflauf- bzw. Wurzellängentests mit Kresse, für den jedoch weitere experimentelle Untersuchungen nötig wären, wurden zunächst keine weiteren Testsysteme mit einer hohen Relevanz für die Aufnahme in die Testbatterie identifiziert. Es gab keinen Anlass dazu, den Umfang der Biotestbatterie zu reduzieren oder das Testdesign (Tests mit mindestens 5 Verdünnungsstufen des Abfalls bzw. Abfalleluats zur Ermittlung der EC_{50}) zu ändern. Nach der UBA-Handlungsempfehlung ist ein Abfall als ökotoxisch (HP 14) einzustufen, wenn in mindestens einem der durchgeführten Biotests eine EC_{50} von ≤10% Abfall- bzw. Eluatanteil ermittelt wird. Diese oder eine ähnliche Vorgehensweise wird auch in mehreren anderen europäischen Staaten eingesetzt und ist in etlichen veröffentlichten Studien zur Ökotoxizität von Abfällen beschrieben.

Probenahme, Probenvorbereitung und ökotoxikologische Testung

Basierend auf Vorschlägen von UBA, BMUV, dem projektbezogenen Begleitkreis und den Projektnehmern wurden folgende Abfallarten (Spiegeleinträge) ausgewählt: Filterstaub (10 09 09*/10 09 10), Boden und Steine (17 05 03*/17 05 04) und Shredderleichtfraktionen und Staub (19 10 03*/19 10 04). Es wurde angestrebt, je Abfallart mindestens eine Probe des gefahrenrelevanten Spiegeleintrags und mindestens eine des nicht gefahrenrelevanten Spiegeleintrags zu untersuchen. Im Verlauf des Projekts konnte jedoch kein Erzeuger für Abfälle, die der Abfallart 19 10 03* zugeordnet wurden, identifiziert werden. Folgende Abfälle wurden beprobt und in ökotoxikologischen Tests untersucht:

- Filterstaub (10 09 09*) vom Gießen von Eisen und Stahl: Altmaterial (Lagerdauer >4 Wochen) und Frischmaterial (Lagerdauer <4 Wochen)
- Filterstaub (10 09 10) vom Gießen von Eisen und Stahl: Material aus zwei verschiedenen Gießereien (Anlagen A und B)
- Boden und Steine (17 05 03*): geogener Aushub aus einem Braunkohletagebau und Straßenbankett von einer Bundesstraße
- Boden und Steine (17 05 04): Straßenbankett von einer Nebenstraße

- Shredderleichtfraktionen und Staub (19 10 04): Material (Absiebungen <10 mm) aus zwei verschiedenen Anlagen (Anlage A: zwei Chargen, Anlage B: eine Charge)

Die Zuordnung der Abfälle zum gefahrenrelevanten bzw. nicht gefahrenrelevanten Spiegeleintrag erfolgte durch den Abfallbesitzer und beruhte nicht notwendigerweise auf dem Kriterium HP 14.

Bei der Probenahme und Probenvorbereitung wurden die Vorgaben der LAGA PN 98 und europäischer Normen (CEN/TR 15310, EN 14735) erfüllt. In den aquatischen Ökotoxizitätstests wurden wässrige Eluate der Abfallproben eingesetzt, die mittels einstufigem Schüttelverfahren mit einem Flüssigkeits-/Feststoffverhältnis von 10 L/kg Abfalltrockengewicht und einer Dauer von 24 h hergestellt wurden. In den terrestrischen Ökotoxizitätstests wurden die festen Abfallproben (Korngröße <2 mm im mikrobiellen Test und <4 mm in den anderen Tests) eingesetzt. Proben der o.g. 10 Abfälle wurden in folgenden Biotests untersucht:

- Aquatische Biotests: akuter Daphnientest (DIN EN ISO 6341), Algenwachstumshemmtest in der Mikrotiterplatte (DIN 38412-59) und Leuchtbakterientest (DIN EN ISO 11348-2)
- Terrestrische Biotests: Feststoffkontakttest mit *Arthrobacter globiformis* (ISO 18187), Wachstumshemmtest mit *Brassica rapa* (ISO 11269-2) und Vermeidungstest mit Regenwürmern (ISO 17512-1)

Für die aquatischen Biotests wurden bis zu 3 Testdurchläufe durchgeführt. Im ersten Testdurchlauf für jede Abfallprobe wurden die Eluate in folgenden Verdünnungsstufen geprüft: 50%, 25%, 12,5%, 6,3% und 3,1% (alle Tests) sowie zusätzlich 1,6%, 0,8% und 0,4% (Leuchtbakterientest). Wenn in allen Verdünnungsstufen Effekte >50% auftraten, wurden im folgenden Testdurchlauf höhere Verdünnungsstufen eingesetzt, um die EC_{50} berechnen zu können. Der erste Testdurchlauf für jede Abfallprobe wurde ohne pH-Anpassung durchgeführt. Wenn Toxizität in Verdünnungsstufen auftrat, deren pH außerhalb des vom Testorganismus tolerierten Bereichs lag, erfolgte ein weiterer Testdurchlauf mit pH-Anpassung. Der pH-Wert wurde dabei jeweils nur in den Verdünnungsstufen eingestellt, deren pH außerhalb des Toleranzbereichs für die betreffende Art lag. Parallel zu jedem Testdurchlauf mit angepassten pH-Werten wurde ein zusätzlicher Testdurchlauf ohne pH-Anpassung durchgeführt, um die Reprozierbarkeit der Ergebnisse zu untersuchen. Weitere Wiederholungen der aquatischen Toxizitätstests (auch für Eluate ohne toxische Effekte im ersten Testdurchlauf) dienten ebenfalls dazu, die Reproduzierbarkeit der Testergebnisse zu untersuchen.

Für die terrestrischen Tests wurde ein Test pro Abfallprobe durchgeführt, in dem jeweils fünf Verdünnungsstufen des Abfalls (als Feststoff) eingesetzt wurden: 25, 12,5, 6,3, 3,1 und 1,6%. Eine Abfallprobe wurde zusätzlich mit einem Schnelltest zur Bestimmung der potenziellen Nitrifizierung und Hemmung der Nitrifizierung mittels Ammoniumoxidation (DIN EN ISO 15685) untersucht.

Die Ergebnisse der durchgeführten Biotests mit den 10 Abfallproben zeigen, dass die aquatischen Tests sehr gut reproduzierbar sind (eine Untersuchung der Reproduzierbarkeit der terrestrischen Tests war im Rahmen des vorliegenden Projekts nicht vorgesehen). In den meisten Fällen waren Algen- und Daphnientests sensitiver als der Leuchtbakterientest. Die terrestrischen Testverfahren waren tendenziell etwas weniger empfindlich als die aquatischen.

Die vom Abfallbesitzer dem gefahrenrelevanten Spiegeleintrag zugeordneten Abfallproben wurden in 3 von 4 Fällen anhand der Biotests als gefährlich nach HP 14 eingestuft. Die einzige Ausnahme war das Straßenbankett (17 05 03*), das in allen eingesetzten Biotests keine

Toxizität zeigte. Hier könnte die fehlende Toxizität in den durchgeführten Biotests mit dem hohen Lehmgehalt der Abfallprobe zusammenhängen.

Abfallproben, die vom Abfallbesitzer dem nicht gefahrenrelevanten Spiegeleintrag zugeordnet worden waren, wurden in 5 von 6 Fällen anhand der Biotestergebnisse als gefährlich nach HP 14 eingestuft. In 4 dieser Fälle lagen dabei die Ergebnisse von mehr als einem Testverfahren unterhalb der Grenzkonzentration (EC_{50} ≤10% Abfall- bzw. Eluatanteil). Auffällig war v. a. die hohe Toxizität der untersuchten Absiebungen der Shredderleichtfraktionen (19 10 04).

Vorschläge für eine Aktualisierung und Weiterentwicklung der UBA-Handlungsempfehlung

Auf Basis der Ergebnisse der Literaturrecherche und der Erfahrungen, die bei der Probenahme, Probenvorbereitung, Elution und ökotoxikologischen Untersuchung der 10 Abfallproben gewonnen wurden, wurden Vorschläge für eine Aktualisierung und Weiterentwicklung der UBA-Handlungsempfehlung ausgearbeitet. Dabei wurden die Diskussionen mit dem projektbezogenen Begleitkreis berücksichtigt. Die Vorschläge für eine Aktualisierung und Weiterentwicklung der UBA-Handlungsempfehlung betreffen Probenahme, Probenvorbehandlung, Teilung von Proben im Labor, Elution, ökotoxikologische Testung und Mindestanforderungen an Berichte. Außerdem wurden Punkte identifiziert, für die auf regulatorischer Ebene Handlungsbedarf besteht, und es wurden Vorschläge für Anpassungen der Testrichtlinien für die Biotests gemacht.

Probenahme und Probenvorbehandlung

Die Probenahme betreffend wird in der UBA-Handlungsempfehlung von 2013 bereits auf einen in der Erarbeitung befindlichen europäischen Standard hingewiesen. Inzwischen liegen die technischen Berichte CEN/TR 15310 vor. Es wird vorgeschlagen, die Handlungsempfehlung im Hinblick auf die Merkmalswahrscheinlichkeit und die angestrebte Aussagesicherheit an die in diesen europäischen Regelwerken vorgeschlagene Vorgehensweise anzupassen. Der Gewinnung von Proben für die ökotoxikologische Untersuchung von Abfällen sollte eine sorgfältige Probenahmeplanung nach CEN/TR 15310-1 vorausgehen. Es sollten mindestens 16 probabilistischen Einzelproben entnommen und zu einer Mischprobe vereinigt werden. Enthält diese Probe Material (Überkorn) >4 mm, sollte unter Verwendung der Mindestprobenmasseformel der CEN/TR 15310-1 entschieden werden, ob das Überkorn verworfen oder zerkleinert und der Probe wieder zugeführt wird.

Teilung von Proben im Labor

Um Proben für die Durchführung einzelner Biotests zu gewinnen, gibt es grundsätzlich zwei Wege: (a) die Teilung (Verjüngung) der Laborprobe und (b) die Entnahme von Einzelproben aus der Laborprobe und anschließende Vereinigung zu einer Mischprobe. Um die Auswirkungen auf die Varianz von Merkmalsgehalten in Prüfproben abschätzen zu können, wurde eine Simulation mit Zufallszahlen unter Verwendung von Näherungsverteilungen für Parametergehalte durchgeführt. Auf Basis der Simulationsergebnisse und in Analogie zur Vorgehensweise bei der Probenahme wird empfohlen, mindestens 16 Einzelproben aus der sorgfältig homogenisierten Laborprobe zu entnehmen und diese zu einer Mischprobe zu vereinigen.

Elution

Vom UBA (2013) wird – in Übereinstimmung mit der Norm EN 14735 – die Elution von Abfällen mittels einstufigem Schüttelverfahren mit einem Flüssigkeits-/Feststoffverhältnis von 10 L/kg Abfalltrockengewicht und einer Dauer von 24 h nach DIN EN 12457-2 vorgeschlagen. Sowohl in der UBA-Handlungsempfehlung als auch in der EN 14735 sollte spezifiziert werden, ob bzw. in welchem Umfang sich dieses Verfahren dazu eignet, organische Schadstoffe und schwer lösliche anorganische Schadstoffe aus Abfallproben zu eluieren.

Biotests

Die in der UBA-Handlungsempfehlung vorgeschlagene Testbatterie deckt für das aquatische und das terrestrische Kompartiment jeweils die taxonomischen Gruppen Pflanzen, wirbellose Tiere und Mikroorganismen und die trophischen Ebenen Produzenten, Konsumenten und Destruenten ab. Im Vergleich mit anderen europäischen Staaten gehört sie zu den umfangreicheren Testbatterien.

Für die untersuchten 10 Abfallproben waren die aquatischen Biotests tendenziell sensitiver als die terrestrischen. Wie die Ergebnisse des UBA-Projekts PROSOIL gezeigt haben, sind aquatische Tests aber nicht in allen Fällen für Bodenorganismen protektiv. Durch die zusätzliche Durchführung terrestrischer Biotests bei Vorliegen von ausschließlich negativen Ergebnissen der aquatischen Tests können zudem ggf. toxische Effekte schwer wasserlöslicher Abfallinhaltsstoffe auf Bodenorganismen detektiert werden. Biotests mit terrestrischen Organismen sollten deshalb Teil der Testbatterie bleiben.

Im Feststoffkontakttest mit *A. globiformis* wurde häufig eine hohe Variabilität der Ergebnisse festgestellt, v. a. in Tests mit heterogenen Abfällen (Shredderleichtfraktionen). Die sehr kleinen Probenmengen, die in diesem Test eingesetzt werden, sind die wahrscheinlichste Ursache für die festgestellte Variabilität. Der Schnelltest zur Bestimmung der potenziellen Nitrifizierung nach Richtlinie DIN EN ISO 15685 könnte eine mögliche Alternative zum Feststoffkontakttest mit *A. globiformis* sein. Um die Eignung dieses Tests zu überprüfen, wären zunächst methodische Anpassungen und weitere vergleichende experimentelle Untersuchungen nötig.

Effekte auf terrestrische Wirbeltiere sollten durch andere gefahrenrelevante Eigenschaften abgedeckt sein (HP 5: spezifische Zielorgan-Toxizität, HP 6: akute Toxizität, HP 10: Reproduktionstoxizität). Mögliche Effekte auf Fische werden hingegen nicht durch andere gefahrenrelevante Eigenschaften abgedeckt. Wenn die Biotestbatterie für die HP 14-Einstufung von Abfällen in Spiegeleinträgen um einen Test mit der taxonomischen Gruppe der Fische ergänzt werden soll, wird vorgeschlagen, zu prüfen, ob sich eine der vorliegenden Alternativmethoden (der Fischeitest nach DIN EN ISO 15088, der Fischembryotest nach OECD-Testrichtlinie 236 oder der Fischzelllinientest nach OECD-Testrichtlinie 249) eignet. Hier wären zunächst experimentelle Untersuchungen und ggf. methodische Anpassungen nötig.

Die UBA-Handlungsempfehlung gibt vor, dass die aquatischen und terrestrischen Biotests mit mindestens fünf Verdünnungsstufen des zu prüfenden Abfalls bzw. Abfalleluats durchgeführt werden sollen, um EC_{50}-Werte zu ermitteln. Vom Begleitkreis wurde angeregt, zu prüfen, ob die Durchführung von Limit-Tests eine Option sein könnte. Limit-Tests könnten z.B. mit einem Eluate- bzw. Abfallanteil von 12,5% durchgeführt werden. Analog zur Umweltrisikoabschätzung für chemische Substanzen sollten Limit-Tests mit Abfällen bzw. Abfalleluaten dazu dienen, die Abwesenheit ökotoxischer Effekte zu belegen. Wenn in einem Limit-Test ein signifikanter Effekt auftritt, sollte ein Test mit fünf Abfall- bzw. Eluatverdünnungen durchgeführt werden, um die EC_{50} zu ermitteln.

Laut Entscheidung 2000/532/EG sind die Ergebnisse der Prüfung ausschlaggebend für die Einstufung eines Abfalls, wenn die betreffende gefahrenrelevante Eigenschaft anhand der Konzentration gefährlicher Stoffe und mittels einer Prüfung bewertet wurde. Aus dieser Vorgabe ergibt sich die Möglichkeit, einen Abfall, der mit der Berechnungsmethode als ökotoxisch eingestuft wurde, über Biotests zu entlasten. Diese Möglichkeit ist jedoch nur z. T. sinnvoll:

- Wenn ein Abfall mit der Berechnungsmethode ausschließlich wegen akut gewässergefährdender Inhaltsstoffe als HP 14 eingestuft wird, aber in keinem der Biotests der Testbatterie ökotoxisch ist, ist eine Entlastung dieses Abfalls auf Basis der Biotestergebnisse

sinnvoll. In diesem Fall könnte eine geringe Bioverfügbarkeit der Grund für die fehlende Ökotoxizität in den Biotests sein. Nur wenn die Einstufung als akut gewässergefährdend ausschließlich auf der Fischtoxizität basiert, sollte keine Entlastung anhand einer Biotestbatterie, die keinen Fischtest enthält, möglich sein.

- Wenn ein Abfall mit der Berechnungsmethode aufgrund chronisch gewässergefährdender Inhaltsstoffe als HP 14 eingestuft wurde, ist eine Entlastung anhand der mit der aktuellen Biotestbatterie ermittelten akuten Effektkonzentrationen nicht sinnvoll. Abfälle, die mit der Berechnungsmethode aufgrund der Ergebnisse chronischer Ökotoxizitätstests mit einzelnen Abfallinhaltsstoffen als chronisch gewässergefährdend eingestuft wurden, sollten nur mit Ergebnissen chronischer Ökotoxizitätstests mit dem Abfalleluat entlastet werden können.
- Auch wenn ein Abfall mit der Berechnungsmethode wegen Ozon schädigender Inhaltsstoffe als HP 14 eingestuft wurde, ist eine Entlastung mittels Biotests nicht sinnvoll.

Die beiden zuletzt genannten Punkte betreffend sollte auf EU-Ebene geregelt werden, in welchen Fällen eine HP 14-Einstufung nach der Berechnungsmethode durch die Ergebnisse welcher Biotests revidiert (entlastet) werden kann.

Die Abfalltestung wird zurzeit nur in der Richtlinie für den Feststoffkontakttest mit *A. globiformis* explizit erwähnt. Es wird empfohlen, die Testung von Abfallproben bzw. -eluaten auch in den Anwendungsbereich der Testrichtlinien für die anderen Tests der UBA-Testbatterie aufzunehmen und Hinweise zum methodischen Umgang mit Abfallproben bzw. -eluaten zu ergänzen.

In der UBA-Handlungsempfehlung sollte spezifiziert werden, dass der pH-Wert im ersten, für die HP 14-Einstufung relevanten Test nicht eingestellt werden soll, auch wenn laut Testrichtlinie eine pH-Einstellung möglich bzw. empfohlen ist, wie im Algen- und Leuchtbakterientest. Hier wäre außerdem zu überlegen, ob für die verschiedenen Tests pH-Bereiche angegeben werden sollten, außerhalb derer eine Testdurchführung nicht mehr sinnvoll ist, weil allein aufgrund des pH-Werts mit einer starken Toxizität zu rechnen ist.

Der Wachstumshemmtest mit *B. rapa* soll laut ISO 11269-2 mit 12 Verdünnungsstufen durchgeführt werden. Um zu ermitteln, ob die EC_{50} ≤ oder > der Grenzkonzentration von 10% Abfallanteil ist, reichen jedoch 5 Verdünnungsstufen aus.

Die Grenzkonzentration betreffend wäre eine Harmonisierung zwischen den verschiedenen EU-Mitgliedstaaten wünschenswert, auch in Hinblick auf den grenzüberschreitenden Transport von Abfällen.

Mindestanforderungen an Berichte

Auf Anregung des projektbezogenen Begleitkreises wurde zusammengestellt, welche Kerninformationen Berichte zu Probenahme, Probenaufbereitung, -lagerung, -teilung, Elution und Biotestung enthalten müssen, damit die zuständige Behörde die Ergebnisse bewerten kann. Entsprechende Vorgaben sind bereits in detaillierter Form in den Normen bzw. Testrichtlinien für die betreffenden Verfahren enthalten. Diese Informationen wurden in tabellarischer Form zusammengestellt. Informationen zu den Mindestanforderungen an Berichte könnten ein Anhang zur überarbeiteten UBA-Handlungsempfehlung werden.

Möglichkeiten und Grenzen ökotoxikologischer Tests im Vergleich zur Berechnungsmethode

In die Berechnungsmethode zur HP 14-Einstufung gehen chemisch-analytisch bestimmte Konzentrationen von laut CLP-Verordnung als Ozonschicht schädigend (H420), akut wassergefährdend (H400) und/oder chronisch wassergefährdend (H410-H413) eingestuften

Abfallinhaltsstoffen ein. Dabei werden als akut oder chronisch gewässergefährdend eingestufte Abfallinhaltsstoffe nur berücksichtigt, wenn ihre Konzentrationen relativ hohe Berücksichtigungsgrenzwerte (H400, H410: 1 g/kg Abfallfeuchtgewicht; H411-H413: 10 g/kg Abfallfeuchtgewicht) erreichen oder überschreiten. In die Berechnung gehen außerdem nur die Stoffe ein, die eine entsprechende harmonisierte Einstufung haben oder für die Selbsteinstufungen vorliegen. Bei den chemisch-analytischen Untersuchungen, die die Grundlage für die Berechnungsmethode sind, werden nur die Stoffe erfasst, nach denen – auf Grundlage der vorliegenden Informationen zur Abfallzusammensetzung – gesucht wird. Andere ggf. vorhandene Schadstoffe bleiben unberücksichtigt. Schadstoffe, die in Konzentrationen unterhalb der Nachweisgrenze der verwendeten analytischen Methode vorliegen, haben ebenfalls keine Auswirkung auf die HP 14-Einstufung.

Anhand von ökotoxikologischen Testverfahren kann eine Aussage über die kombinierten Effekte aller unter Testbedingungen bioverfügbaren toxischen Stoffe im Abfall getroffen werden. Im Unterschied zur Berechnungsmethode schließt dies Stoffe ein, deren Konzentrationen unterhalb der Berücksichtigungsgrenzwerte oder der chemisch-analytischen Nachweisgrenzen liegen oder die mit dem verwendeten chemisch-analytischen Verfahren nicht erfasst werden. In die Ergebnisse von Biotests gehen außerdem mögliche Wechselwirkungen zwischen den verschiedenen Abfallinhaltsstoffen ein. Mit der vom UBA (2013) empfohlenen Testbatterie wird zusätzlich zur akuten Toxizität für aquatische Organismen auch die akute Toxizität für terrestrische Organismen erfasst. Um chronische Effekte zu erfassen, müsste die Biotestbatterie angepasst werden. Eine Schädigung der Ozonschicht kann mit Biotests nicht erfasst werden.

Insgesamt sind die Berechnungsmethode und der Einsatz von Biotests zur HP 14-Einstufung von Abfällen aus Spiegeleinträgen also zwei sich ergänzende Ansätze. Es wäre wünschenswert, wenn die Vorgehensweise zur HP 14-Einstufung von Spiegeleinträgen auf EU-Ebene unter Berücksichtigung der Möglichkeiten und Grenzen von Berechnungsmethode und Biotests weiterentwickelt werden würde.

1 Background and objectives of the project

1.1 Regulatory background

According to the German Circular Economy Act (KrWG 2023)[1, 2], a waste is defined as substance, material, or object that its holder discards, intends to discard, or is required to discard. The European List of Wastes (Decision 2000/532/EC as amended by Decision 2014/955/EC; EC 2014, 2015), which was transposed into German law (Abfallverzeichnis-Verordnung; AVV 2020), contains a non-exhaustive list of waste types[3]. These are categorised into absolute hazardous and absolute non-hazardous entries, and mirror entries. Absolute hazardous waste types are considered hazardous without further assessment, absolute non-hazardous waste types are considered non-hazardous without further assessment[4] . Mirror entries are pairs of waste types indicated in the List of Wastes and in the AVV, which have a designation that only differs with regard to the presence or absence of a reference to hazardous substances contained in the waste[5] . Depending on the specific situation or waste composition, the waste in question has to be allocated to the hazardous or non-hazardous mirror entry (EU 2018, section 2.1.2). If a waste has one or more of the hazardous properties HP 1 to HP 15[6] or contains certain persistent organic pollutants (POPs) in concentrations above the specified limit values, it has to be classified as hazardous waste (EC 2015, EU 2018, AVV 2020, see also Figure 1). Absolute hazardous and mirror hazardous waste types are marked with asterisks (EU 2018, AVV 2020, § 3, paragraph 1).

In order to allocate a waste to a hazardous or non-hazardous mirror entry, sufficient information on the presence and content of hazardous substances in this waste has to be obtained, as specified in the 'Commission notice on technical guidance on the classification of waste' (EU 2018, section 2.1.2; see also Figure 1). This includes, e.g. information on the process by which the waste is generated and on input substances and intermediates of this process, information from the original producer of the substances/objects before they became waste (e.g. safety data sheets, product data sheets), as well as information from databases on waste analysis and chemical-analytical data for the waste. Hazardous properties of a waste can be determined either based on the concentrations of waste constituents or by testing (Commission Decision 2000/532/EC, EC 2015, see also AVV 2020, Annex to § 2, paragraph 1, section 2.2.2). The used test methods should be in accordance with Regulation (EC) 440/2008 (REACH test methods, EC 2019) or other internationally recognised test methods and guidelines. Analogous to the CLP Regulation ((EC) No 1272/2008, Article 7; EC 2021), animal tests should only be carried out, if there is no suitable alternative method. When a hazardous property of a waste has been assessed both based on the concentrations of waste constituents and by testing, the results of

[1] See also Waste Framework Directive (2008/98/EC, EC 2018, Article 3).

[2] In this report, we usually refer to the consolidated versions of Directives and other legislations that were current when the respective work package of the present project was completed.

[3] The 'Commission notice on technical guidance on the classification of waste' (2018/C 124/01, EU 2018) provides explanations on the correct interpretation and application of the relevant legislation on waste classification.

[4] In individual cases, a competent authority may consider a waste, which is listed as non-hazardous, as hazardous, and vice versa. In Germany, such deviating classifications have to be reported to the Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (AVV 2020, § 3).

[5] Example of an entry pair: 10 01 14* bottom ash, slag and boiler dust from co-incineration containing hazardous substances; 10 01 15 bottom ash, slag and boiler dust from co-incineration other than those mentioned in 10 01 14.

[6] HP 1: explosive, HP 2: oxidising, HP 3: flammable, HP 4: irritant – skin irritation and eye damage, HP 5: specific target organ toxicity (STOT)/aspiration toxicity, HP 6: acute toxicity, HP 7: carcinogenic, HP 8: corrosive, HP 9: infectious, HP 10: toxic for reproduction, HP 11: mutagenic, HP 12: release of an acute toxic gas, HP 13: sensitising, HP 14: ecotoxic, HP 15: waste capable of exhibiting a hazardous property listed above not directly displayed by the original waste.

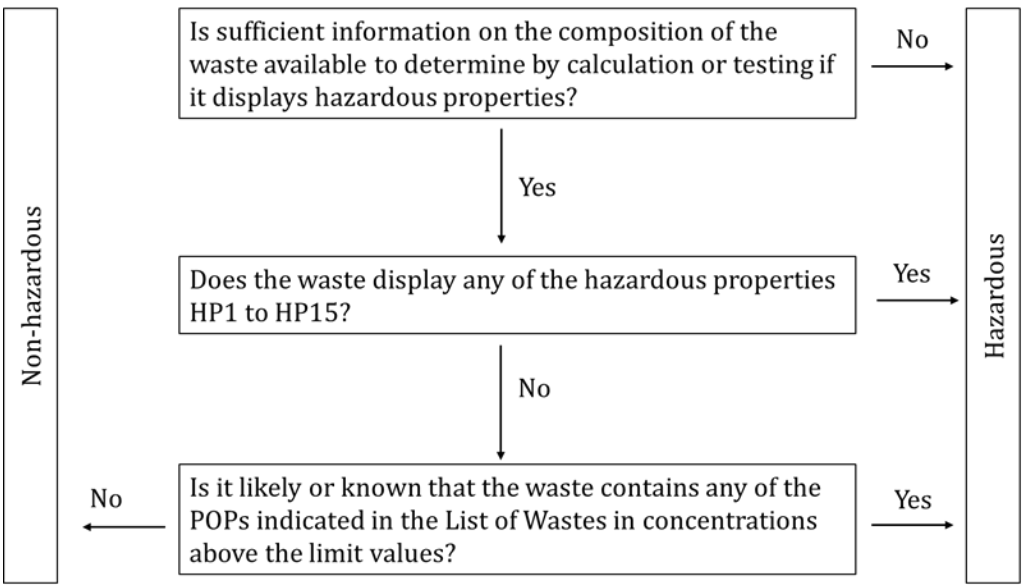
the test(s) are decisive for classification as hazardous or non-hazardous waste (EC 2015, AVV 2020).

For the hazardous property HP 14 ("ecotoxic: waste which presents or may present immediate or delayed risks for one or more sectors of the environment", Directive 2008/98/EC, EC 2018), it has been specified in Regulation (EU) 2017/997 (EU 2017, p. 4) how a waste shall be classified. The Regulation lays down rules on the calculation method, including concentration limits for classification based on the content of substances that are ozone-depleting (hazard statement H420) or acutely (H400) and/or chronically hazardous (H410-H413) to water organisms (see also section 6). These rules are harmonised with the CLP Regulation ((EC) 1272/2008, EC 2021). For an HP 14 classification based on testing, REACH test methods according to EC (2019; see above) or other internationally recognised methods shall be used. In addition, it is referred to the European List of Wastes (EC 2015) and to the CLP Regulation (Article 12, point b: consideration of a lack of bioavailability in the assessment). Regulation (EU) 2017/997 does not contain more specific requirements for HP 14 classification based on testing (biotests), i.e. it is not specified which biotests should be used and which limit concentration has to be reached for an HP 14 classification.

The 'Commission notice on technical guidance on the classification of waste' (EU 2018) provides guidance on sampling, elution and chemical analysis as well as on HP 14 classification using the calculation method, but not on biotests. It is noted that so far, there are no specific recommendations of the European Commission for an HP 14 classification based on biological testing. As a result, Member States must decide on the acceptability and interpretation of the results of biological tests with waste samples (case-by-case decisions). According to Annex 3.14 of the Commission notice, considerations on bioavailability should be taken into account as set out in Regulation (EU) 2017/997 (EU 2017; see above).

In some German federal states, implementation notes are available on the allocation of waste to the hazardous or non-hazardous mirror entry (IPA 2021, see also section 3.1.1).

Figure 1: Procedure of assigning a waste to a hazardous or non-hazardous mirror entry



Source: own illustration, ECT Oekotoxikologie GmbH, based on EU (2018), simplified

1.2 Objectives of the project

In the present project, proposals were elaborated for updating and further developing the 'Recommendations for the ecotoxicological characterization of wastes' of the German Environmental Agency (UBA 2013), and open issues were identified. The project focused on sections up to and including 6.1 ('Identification of ecotoxicological wastes in mirror…') of the UBA recommendations. Sections 6.2 ('Detailed ecotoxicological characterisation of waste') and 7 ('Ecotoxicological characterisation for assessing the risks of waste management scenarios') were beyond the scope of the project.

In a first step, different biotest-based approaches for HP 14 classification were compared in a European context, based on the results of a literature search (section 3). The strategy for HP 14 classification of waste from mirror entries proposed in the UBA recommendations was verified, and initial suggestions were made for its update and further development. The test strategy was then reviewed based on the sampling, sample preparation and ecotoxicological testing of 10 waste samples from mirror entries (section 4). Considering the results of the experimental work and the discussions with the project advisory group, the proposals for an update and further development of the UBA recommendations were then further developed (section 5). The proposals for revising the recommendations focussed on an (improved) technical implementation. Possibilities and limitations of the ecotoxicological test battery according to UBA (2013) in comparison to the calculation method were discussed (section 6). Gaps in the current legislation were identified as far as possible but could not be further addressed within the present research project.

2 Procedure for HP 14 classification of waste from mirror entries according to the current UBA recommendations

To assess the environmental risks of waste, extensive work was initiated in the last 15 years by the German Environment Agency (UBA; e.g. Moser & Römbke 2009, Römbke et al. 2009, Römbke & Ketelhut 2014). Based on this work, 'Recommendations for the ecotoxicological characterization of wastes' were developed (UBA 2013). These recommendations are not legally binding. In the following sections, the aspects of the UBA recommendations that are relevant for the present project are summarised.

2.1 Sampling

With regard to sampling, the UBA recommendations are based on the guideline PN 98 (LAGA 2004)[7] and on Pierre Gy's 'Theory of sampling' (Gy 1979, 1992, 2004a, b). The UBA recommendations aimed at combining the positive aspects of both approaches. In brief, they contain the following main suggestions regarding sampling:

- During sampling, at least 16 individual samples should be taken. These samples should be random samples. For each particle, the probability to become part of an individual sample should be the same.
- Sampling should ideally be performed from the particle mass flow falling from a conveyor belt or, if this is not possible, from a heap of waste. In the latter case, samples are taken from a flat layer generated using a wheel loader.
- The individual samples are combined to a composite sample, the field sample.

2.2 Sample pre-treatment

Sample pre-treatment includes all steps to prepare a laboratory sample from the field sample. Processes such as mixing, homogenising, sample division, sorting, crushing/shredding, drying and sieving can be used as part of sample pre-treatment (DIN 19747, 2009a). In the UBA recommendations (UBA 2013), the following processes are mentioned:

- As far as possible, the sample should not be crushed/shredded to avoid creating fresh surfaces.
- If the d_{95} of the material is >4 mm, there are two options. In case of a heterogeneous waste with a low mass content <4 mm, the material has to be crushed/shredded. However, it should not be finely ground. If the sample fraction <4 mm is sufficiently large, this fraction can be used for ecotoxicological testing, taking the mass fraction into account.
- If drying is required, the drying temperature should be <40°C.
- If interfering materials are removed from the sample, they have to be documented regarding their quantity and weight, as well as photographically.
- A sample division without prior reduction of particle size should be avoided.

The laboratory samples should be transported at a temperature of 4±2°C and should reach the laboratory within 48 h of sampling.

[7] The PN is available in an updated form, adapted to the current legal situation (LAGA 2019).

2.3 Sample preparation and processing, division of samples in the laboratory

Sample preparation in the laboratory usually includes homogenisation and division of the sample to obtain test samples. In individual cases, samples can also be crushed/shredded and sieved. As part of sample processing, processes such as drying and, if necessary, fine crushing are typically used (cf. DIN 19747, 2009a). However, for ecotoxicological testing, further crushing or shredding is generally not carried out.

In the UBA recommendation, it is stated that a further division of the sample may be necessary during sample preparation, because only a small sample mass may be required for an individual biotest. It is recommended to divide samples using a ripple splitter, or by coning and quartering. Since for the samples tested in the biotests, the representativeness regarding the evaluated properties is not known, parallel studies may provide information on the reliability of the results (see UBA 2013, section 5.2.4).

2.4 Elution

In section 5.2.5 of the UBA recommendations (UBA 2013), the production of waste eluates, aqueous extracts that are used to investigate the ecotoxicity of short-term water-eluable waste constituents, is described. Two elution procedures are mentioned:

a) A one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h according to DIN EN 12457-2 (2003a). This procedure is also recommended in DIN EN 14735 (2022). While according to DIN EN 14735 (section 11.2.1) 90±5 g waste dry weight should be used for each elution, UBA (2013) recommends using 100-200 g waste dry weight. If necessary, several parallel eluates should be produced and combined.

b) A column percolation method, e.g. according to DIN 19528 (2023a). However, it is noted that there is a lack of experience with regard to biotesting and limit concentrations.

2.5 Biotesting

According to UBA (2013, section 6.1), biotesting is necessary if the available information on waste composition and ecotoxicity of the individual waste constituents is not sufficient to classify the waste (mirror entry) as ecotoxic or not ecotoxic (Figure 2). In this case, three aquatic biotests are performed in a first step with the waste eluate, using organisms of different trophic levels and taxonomic groups: the luminescent bacteria test according to DIN EN ISO 11348-2 (2009[8]), the algal growth inhibition test according to DIN EN ISO 8692 (2012), and the acute *Daphnia* test according to DIN EN ISO 6341 (2013a) (see section 3.3.2). If the results of all aquatic tests are negative (i.e. if the EC_{50} values are >10% eluate), three terrestrial ecotoxicity tests are carried out: the solid contact test with *Arthrobacter globiformis* according to ISO 18187 (2016a), the seedling emergence and growth test with *Brassica rapa* according to ISO 11269-2 (2012a) and the avoidance test with earthworms according to ISO 17512-1 (2008a; see section 3.3.2).

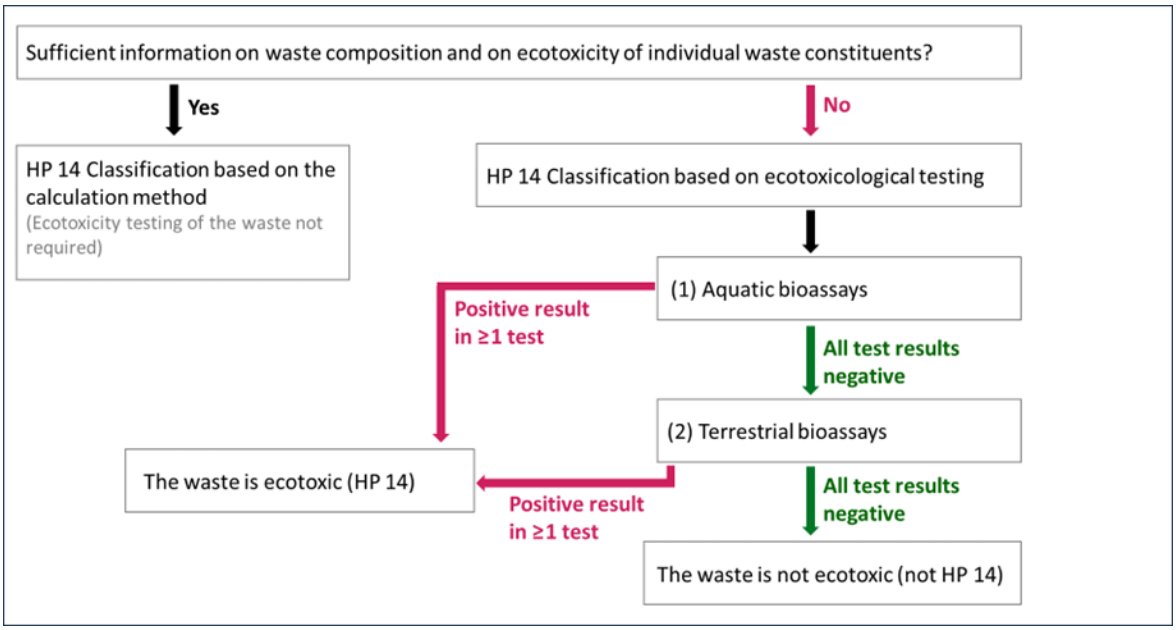
These six biotests were standardised years ago (see also section 2.6). The UBA recommendation allows for using other standardised test methods, for which sufficient experience with the testing of waste eluates or waste is available (UBA, 2013, section 6.1.3).

[8] Current version of the test guideline: DIN EN ISO 11348-2 (2023).

A waste is classified as ecotoxic (HP 14), if an EC_{50} of ≤10% waste or waste eluate is derived in at least one of the ecotoxicity tests.

The ecotoxicity tests shall be performed with at least five dilutions of the waste eluate or waste; limit tests are not foreseen in UBA (2013). In the aquatic toxicity tests, the pH of the eluate or eluate dilution shall not be adjusted. If toxic effects occur at dilution levels, where pH is outside the range tolerated by the test species, the test may be repeated with pH adjustment. However, results of tests with pH-adjustment are not relevant for HP 14 classification (UBA 2013, section 6.1.2).

Figure 2: Procedure for HP 14 classification of waste from mirror entries according to the UBA recommendations from 2013



Source: own illustration, ECT Oekotoxikologie GmbH, based on UBA (2013), simplified

2.6 Reproducibility of the results of the used biotests

The biotests according to ISO standards, which are mentioned in the UBA recommendations and used in the present project, have mostly been established for decades. In most cases, similar methods are also standardised as OECD guidelines for the testing of chemicals. Their often mandatory use is already regulated in other areas, such as the environmental risk assessment of chemicals, plant protection products, biocides and pharmaceuticals, and the assessment of water, wastewater and sludge samples.

Interlaboratory comparison studies and ring tests are performed as part of the standardisation process prior to publication of a standard method. In addition, a regular participation in ring tests is, for instance, required as part of the accreditation in accordance with ISO 17025 (DIN EN ISO/IEC 2018). Most ISO and OECD test guidelines include testing of a reference substance (positive control) with a known toxicity (determined during ring testing), either regularly (e.g. twice a year) or in parallel in each test run. Compliance with the target range (e.g. for the EC_{50} of the respective reference substance) is a prerequisite for the validity of the tests carried out with the substances or samples to be assessed.

For the testing of waste samples, an international ring test was carried out (Moser & Römbke 2009). It was organised by UBA, and 60 laboratories from 15 countries participated. In this ring

test, three waste types were tested: municipal waste incineration ash (MWI ash), soil contaminated with polycyclic aromatic hydrocarbons (PAHs), and waste wood mainly contaminated with copper-based wood preservatives. The basic biotest battery consisted of the algal growth inhibition test, the acute *Daphnia* test, the luminescent bacteria test, the acute earthworm test and the growth inhibition test with higher plants. Five additional aquatic and terrestrial test methods were used in some laboratories, including the avoidance test with earthworms and the solid contact test with *A. globiformis*.

3 Literature search and first verification of the test strategy proposed in the UBA recommendations

A literature and internet search was performed to identify strategies for HP 14 classification of waste using the calculation method and ecotoxicological testing (including guidance from other Member States), new test methods potentially suitable for the assessment of the toxicity of waste samples, and studies on the ecotoxicity of different waste types. Both, peer-reviewed scientific journals as well as so-called grey literature, such as reports, conference contributions or university theses were considered[9].

3.1 Strategies for HP 14 classification of waste

3.1.1 Strategies in different European states

In 2020, CEN/AFNOR conducted a survey on approaches to HP 14 classification of waste in different European countries (particularly EU Member States, but also candidate countries). Inter alia, it was asked, if the HP 14 classification is based on the calculation method and/or biotests, which limit concentrations are defined for biotests, if only aquatic or also terrestrial test methods are used, which elution method and test design are employed and if the pH of the eluate is adjusted (CEN 2020, 2021a). Based on this survey, reports of Sander et al. (2008) and Planchon et al. (2015) and an internet search, national guidance documents on HP 14 classification were identified for several European states[10].

The following evaluation is mainly based on identified national guidance documents for HP 14 classification and on the CEN/AFNOR survey. Information from the evaluated national guidance documents and CEN (2020, 2021a) was not always consistent. In such cases, information from the national guidance was used. In cases, where information in the main body and annex ('raw results') of CEN (2021a) differed, information from the annex was used. In the present project it was not verified whether the identified national guidance documents are legally binding.

Information on HP 14 classification was found for Belgium (Flanders), Denmark, Germany, Finland, France, Great Britain, Italy, Austria, Portugal, Sweden, Serbia, Slovakia, Spain, and the Czech Republic. The calculation method for HP 14 classification is used in almost all of these states, the only exception being Slovakia[11]. Biotests (ecotoxicological tests) are used in 11 of the 14 states (see Table 1). National guidance documents for HP 14 classification are available in 8 of the above-mentioned states, and the current versions of these documents were published in most cases within the last few years. In Belgium and Denmark, national guidance documents are in preparation (CEN 2021a).

[9] The search was carried out at the end of 2021/beginning of 2022.

[10] The national guidance documents mentioned in Sander et al. (2008) and Planchon et al. (2015) are now mostly available in updated versions.

[11] According to CEN (2021a), HP 14 classification in Slovakia is exclusively based on biotests. No justification for this approach is given in CEN (2021a).

Table 1: Overview of the use of the calculation method and biotests for HP 14 classification in different European (mostly EU) states and the availability of national guidance

State	Calculation method[a]	Ecotoxicological testing[a]	National guidance
Belgium (Flanders)	Yes[b]	Yes[b]	In preparation[b]
Denmark	Yes[b]	No[b]	In preparation[b]
Germany	Yes	Yes	UBA (2013)
Finland	Yes[b]	Yes[b]	Ministry of the Environment (2019)[b, c]
France	Yes	Yes	INERIS (2016)
UK	Yes[b]	Not recommended, very rarely used[f]	Natural Resources Wales, SEPA, Environment Agency (2021)
Italy	Yes[b]	Yes[b]	SNPA (2020)
Austria	Yes[b]	Yes[b]	BMNT (2018)
Portugal	Yes[b]	No[d]	APA (2020)
Sweden	Yes[b]	Yes[b]	No[b]
Serbia[e]	Yes[b]	No[b]	Implementation of CEN/TR 16110[b]
Slovakia	No[b]	Yes[b]	No[b]
Spain	Yes	Yes	MITECO (2021)
Czech Republic	Yes[b]	Yes[b]	No[b]

[a] If no other source is mentioned, the information is based on the national guidance indicated in column 4; [b] according to CEN (2021a); [c] guidance document presumably in Finnish language (not found through internet search); [d] according to Bandarra et al. (2021); [e] candidate country that participated in CEN/AFNOR survey; [f] the calculation method is preferred, because the waste composition is mostly known and animal testing and the testing of mixtures should be avoided (see also Table 2).

Specifications, in which cases an HP 14 classification should be based on the calculation method and in which cases ecotoxicity tests should be used, were identified for 6 countries (Table 2). In Germany, Finland, France, Austria and Spain, ecotoxicity tests are performed if a classification using the calculation method is not possible, because there is insufficient information on waste composition (UBA 2013, BMNT 2018, CEN 2021a, MITECO 2021). This is, for instance, the case if the waste contains unknown organic substances (MITECO 2021). According to the UBA recommendations, ecotoxicity tests are also necessary if waste constituents have not been classified under the hazardous substances legislation (UBA 2013, section 6.1.1). According to the implementation notes for allocation of waste to mirror entries harmonised between the German federal states Berlin and Brandenburg, an allocation should be made based on (a) available hazard classifications, (b) experiences made during the implementation process, and (c) chemical-analytical investigations. If "neither an argumentative nor an analytical" allocation is possible, test methods should be used (Ministerium für Landwirtschaft, Umwelt und Klima 2020, Senatsverwaltung für Umwelt, Verkehr und Klimaschutz 2020, p. 4 and 9).

If the available information on waste composition is sufficient, an HP 14 classification can be performed in Germany, Finland and France using the calculation method alone (UBA 2013, CEN 2021a). However, it is possible to additionally perform ecotoxicity tests. In Finland and Austria, biotests can be used, if it is suspected that the aquatic toxicity of the bioavailable substances differs from the result obtained with calculation method (BMNT 2018, CEN 2021a). In the Austrian guidance, it is stated that for waste that is hazardous to the aquatic environment according to the calculation method[12], biotests can be carried out to demonstrate the lack of bioavailability of the contaminants and to classify the waste as not hazardous to the aquatic environment. If a waste has been classified as not hazardous to the aquatic environment using the calculation method, no ecotoxicity tests are required (BMNT 2018).

In Belgium, waste can – according to the CEN/AFNOR survey – be classified using the calculation method, ecotoxicity tests or a bioavailability-based method[13] . However, it is not indicated when to use which method. In the UK, HP 14 classification is generally performed using the calculation method, due to reservations regarding biotesting (see Table 2 and Natural Resources Wales, SEPA, Environment Agency 2021, p. C50).

[12] In the Austrian guidance, no differentiation is made between waste classified with the calculation method as (a) acutely hazardous to the aquatic environment and (b) chronically hazardous to the aquatic environment (BMNT 2018, p. 8-10)

[13] No further information was found on this method, which was still under discussion at the time of the CEN/AFNOR survey (CEN 2021a, see Table 2.)

Table 2: Use of the calculation method and ecotoxicity tests for HP 14 classification in the European states, where ecotoxicity tests are used

State	Use of calculation method and ecotoxicity tests[a]	Remark	Reference
Belgium (Flanders)	Three alternative options: classification based on (1) calculation method, (2) ecotoxicity tests, (3) a method based on bioavailability	Method (3) is still under discussion; it is not indicated when to use which method	CEN (2021a)
Germany	If there is sufficient information on waste composition, HP 14 classification can be based on the calculation method, and no ecotoxicological tests need to be performed. If there is insufficient information on waste composition or if waste components are not classified under the hazardous substances legislation, ecotoxicological tests are required for HP 14 classification.	See flow chart in UBA (2013), based on Pandard & Römbke (2013)	UBA (2013)
Finland	An HP 14 classification can be made using the calculation method alone. Ecotoxicity tests can be performed if: (1) an HP 14 classification with the calculation method is not possible, because the chemical composition of the waste is not sufficiently known; or (2) it is suspected that the aquatic toxicity of the bioavailable substances differs from the result of the calculation method.	—	CEN (2021a)
France	The HP 14 classification can be based on the calculation method alone, if the waste composition is sufficiently known: the content of chemical-analytically identifiable organic and inorganic substances must be at least 90% (according to XP X30-489, AFNOR 2013). If this is not the case, classification is based on ecotoxicity tests. An HP 14 classification based on ecotoxicity tests alone (without carrying out the calculation method) is also possible.	No corresponding specifications in the national guidance (INERIS 2016)	CEN (2021a)
UK	HP 14 classification is generally based on the calculation method. It is assumed that the chemicals contained in waste are known in almost all cases. Ecotoxicological testing is not recommended, because (a) tests with vertebrates (fish), i.e. animal testing, should be avoided, and (b) the testing of mixtures is difficult and should be avoided. Ecotoxicological testing of 'water accommodation fractions' is not sufficient to evaluate waste (i.e. a mixture). Information on degradability ('rapid degradability') and bioaccumulation potential may also be necessary.	In the national guidance, aquatic ecotoxicity tests are mentioned, but these tests are not recommended (see left).	Natural Resources Wales, SEPA, Environment Agency (2021)
Italy	Not specified	No guidance beyond 2000/532/EC, 2008/98/EC and 2017/997	ISPRA (2018), SNPA (2020), CEN (2021a)

State	Use of calculation method and ecotoxicity tests[a]	Remark	Reference
Austria	(1) Performance of ecotoxicity tests, if an HP 14 classification using the calculation method (a) using already available data on waste constituents and (b) after extensive chemical-analytical analysis of the waste is not possible, e.g. since the waste contains unknown organic substances. (2) If a waste has been classified as hazardous by HP 14 using the calculation method, ecotoxicity tests can be carried out to demonstrate the lack of bioavailability of the contaminants and to classify the waste as not hazardous to the aquatic environment. If a waste has been classified as not hazardous to the aquatic environment using the calculation method, no ecotoxicity tests are necessary.	See flow chart in BMNT (2018)	BMNT (2018), CEN (2021a)
Sweden	Not specified	It is referred to Directive 2008/98/EC (Annex III) and the documents cited therein. No further guidance	CEN (2021a)
Spain	Performance of ecotoxicity tests, if an HP 14 classification with the calculation method is not possible, e.g. because information on waste composition is insufficient, and it is not possible to generate this information by chemical analyses.	—	MITECO (2021)

[a] In cases where information from the evaluated national guidance document and CEN (2021a) differs, the table is based on the national guidance. For Slovakia and the Czech Republic, CEN (2021a) does not contain any relevant information.

3.1.1.1 Sampling, sample pre-treatment, elution

In the UBA recommendations, it is pointed out that is a challenging task to collect representative waste samples. For heterogeneous waste, it is assumed that only a collection of waste-characterising samples is possible. These samples may not fully meet the requirements for representativeness (precision, reliability, and reproducibility; see also PN 98, LAGA 2019). Recommendations were elaborated for waste-characterising sampling for HP 14 classification, which are adapted to the requirements of biological investigations, and which aim at avoiding systematic errors. As far as possible, samples should be obtained from the mass flow falling from a conveyor belt across the entire cross-section of this mass flow at random times, or from a waste heap as specified in section 2.1(UBA 2013).

All other national guidance documents that were evaluated do not contain any specifications for sampling. This is probably due to the fact that further guidance has been developed at the EU level during the last 10 years. For instance, the 'Commission notice on technical guidance on the classification of waste' (EU 2018) refers to the standard EN 14899 (CEN 2005) and a series of technical reports (CEN/TR 15310-1 to -5, CEN 2006a-e). In detail, these are:

- EN 14899 (CEN 2005) and CEN/TR 15310-5 (CEN 2006e): preparation of a sampling plan
- CEN/TR 15310-1 (CEN 2006a): criteria for sampling under various conditions, sampling techniques
- CEN/TR 15310-2 (CEN 2006b): sampling techniques for different waste types
- CEN/TR 15310-3 (CEN 2006c): subsampling in the field
- CEN/TR 15310-4 (CEN 2006d): sample packaging, storage, preservation and transport

The development at European level (CEN Technical Committee 292) is based on the approach of Pierre Gy (Gy 1979, 1992, 2004a, b). In the Commission notice on technical guidance (EU 2018), it is mentioned that other procedures are acceptable if they produce similarly reliable results. LAGA PN 98 is mentioned in a footnote. For ecotoxicological studies, EN 14735 (CEN 2021b)[14] on the preparation of waste samples for ecotoxicity tests should also be considered.

The specifications regarding particle size of the waste to be eluted or used in terrestrial ecotoxicity tests range from <1 mm to <10 mm, with <4 mm according to EN 12457-2 (CEN 2002a)[15] being used in most countries (Table 3).

[14] German version: DIN EN 14735 (2022).

[15] German version: DIN EN 12457-2 (2003).

Table 3: Specifications regarding the particle size of the waste that is eluted or used in terrestrial ecotoxicity tests

Particle size	State	Note[a]	Reference
<1 mm	Italy	—	CEN (2021a)
	Sweden	—	CEN (2021a)
<2 mm	Germany	For microbial tests in soil	UBA (2013), CEN (2021a)
<4 mm	Belgium (Flanders)	EN 12457-2	CEN (2021a)
	Germany	EN 12457-2	UBA (2013), CEN (2021a)
	Finland	EN 12457-2	CEN (2021a)
	France	EN 12457-2	INERIS (2016), CEN (2021a)
	Slovakia	EN 12457-2	CEN (2021a)
	Spain	EN 12457-2, EN 12457-4	MITECO (2021)
<10 mm			
	Austria	EN 12457-4	CEN (2021a)
	Czech Republic	EN 12457-4	CEN (2021a)

[a] EN 12457-2 (CEN 2002a), German version: DIN EN 12457-2 (2003); EN 12457-4 (CEN 2002b), German version: DIN EN 12457-4 (2002b).

To produce eluates for aquatic ecotoxicity tests, a one-stage batch leaching procedure with a liquid to solid ratio (L/S) of 10 L/kg waste dry weight and a duration of 24 h is used in most states (see Table 4).

According to the CEN/AFNOR survey, a leaching procedure with a significantly higher liquid to solid ratio (up to 1,000,000 L/kg), a longer duration (28 d) and a pH of 5.5 is used in Sweden. This method was also used to produce eluates for chemical-analytical investigations (Stiernström et al. 2015 cited in Wahlström et al. 2016).

In addition to the one-stage batch leaching procedure (DIN EN 12457-2), a column percolation method (e.g. DIN 19528, 2023a) is mentioned in the UBA recommendations (UBA 2013, section 5.2.4) as an option. However, it is noted that experience with ecotoxicological tests with column eluates is lacking.

Concerning elution, it is referred to the OECD 'Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals' (OECD 2019) in Italy and Spain. In Italy, water-accommodated fractions of highly soluble metallic waste components are produced (100 mg/L, i.e. 10,000 L/kg; Pivato et al. 2020, CEN 2021a). The Spanish guidance (MITECO 2021; section 15.2.1.1) refers to the section 'Multi-component substances' of OECD (2019), but the practical implications with regard to elution are not clear.

Table 4: Specifications for leaching tests to produce eluates for aquatic ecotoxicity tests

Method	State	Remark	Reference
One-stage batch process, L/S = 10 L/kg, Duration: 24 h	Belgium (Flanders)	EN 12457-2, EN 14735	CEN (2021a)
	Germany	EN 12457-2, EN 14735. In genotoxicity tests, a solid phase extract of the aqueous eluate can be used.	UBA (2013), CEN (2021a)
	Finland	EN 12457-2, EN 14735	CEN (2021a)
	France	EN 12457-2, EN 14735 Eluates are filtered: (a) 100 µm; (b) additionally 0.45 µm for all test organisms except *Daphnia magna*	INERIS (2016), CEN (2021a)
	Austria	ÖNORM S 2117 (based on EN 14735, but particle size <10 mm according to EN 12457-4)[a]	BMNT (2018), CEN (2021a)
	Sweden	EN 12457-2	CEN (2021a)
	Slovakia	EN 12457-2, EN 14735	CEN (2021a)
	Spain	EN 12457-2, EN 12457-4	MITECO (2021)
	Czech Republic	EN 12457-4	CEN (2021a)
L/S = 1,000,000 L/kg (partly lower), duration: 28 days, pH 5.5 (partly lower)	Sweden	Method still in development, based on CLP Regulation	CEN (2021a)
Column percolation method	Germany	e.g. DIN 19528 (2023a). Optional, no experience with biotests with the eluates and limit concentrations for HP 14 classification	UBA (2013)
OECD Guidance document 23 (OECD 2019)	Italy	Production of water-accommodated fractions for highly soluble metallic components	CEN (2021a)
	Spain	It is referred to section 7.9 'Multi-component substances'[b]	MITECO (2021)

L/S: liquid to solid ratio. [a] Austrian Standards International (2018); [b] in addition to the one-stage batch procedure (EN 12457-2 and -4).

In Belgium (Flanders) and Italy, the pH of eluate is generally not adjusted (CEN 2021a). In most other countries, pH is adjusted or can be adjusted, if it deviates too much from the range tolerated by the test organism. In Germany, Finland, Sweden, and Slovakia, a first test is carried out without pH adjustment as specified in DIN EN 14735. When toxic effects occur at dilution levels, where pH is outside the range tolerated by the test species, a second test with adjusted pH may be performed to identify the cause of the toxicity (Table 5).

Table 5: Adjustment of the pH value of the eluate or the eluate dilutions

pH adjust-ment	Details	State	Reference
No	—	Belgium (Flanders)	CEN (2021a)
		Italy	CEN (2021a)
Yes	Adjustment of the pH of the eluate when it is below 5,5 or above 8.5	France	INERIS (2016), CEN (2021a)
	Adjustment of the pH of the eluate according to the corresponding ISO standards for the ecotoxicity tests	Czech Republic	CEN (2021a)
Yes	According to EN 14735: first test without pH adjustment. In case of toxic effects at dilution levels with pH values outside the range tolerated by the test species: a second test with adjusted pH may be performed to identify the cause of toxicity	Germany	UBA (2013)
		Finland	CEN (2021a)
		Sweden	CEN (2021a)
		Slovakia	CEN (2021a)
	Luminescent bacteria and *Daphnia* test: Adjustment of pH allowed, if pH of eluate below 6.0, or above 8.5 (luminescent bacteria) or 9.0 (*Daphnia*) Algal test: Adjustment of pH of the aqueous sample to 8.1, if pH of eluate below 6.0 or above 8.5	Austria	BMNT (2018)
In discussion	—	UK	CEN (2021a)

3.1.1.2 Ecotoxicity tests

In the following, an overview is given of the aquatic and terrestrial ecotoxicity tests and the limit concentrations for HP 14 classification in the different states. For France, an alternative test battery, which is being implemented, is listed in CEN (2021a) in addition to the current test battery (INERIS 2016). As the German test battery (UBA 2013), this alternative test battery is based on Pandard & Römbke (2013). For the Czech Republic, CEN (2021a) contains information on two different test batteries with different limit concentrations. The waste owner can decide which of the two test batteries is used. According to information from M. Svobodová (Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic, pers. comm., 11 March 2022) only one of these two test batteries can be used after a transitional period and the limit concentrations were modified[16].

In Belgium (Flanders), Germany, Finland, France, Italy, Austria, Sweden, Slovakia, Spain and the Czech Republic, aquatic toxicity tests are used for HP 14 classification of waste (UBA 2013, INERIS 2016, BMNT 2018, CEN 2021a, MITECO 2021, M. Svobodová, pers. comm., 11 March 2022). In most of these states, only short-term tests are carried out (Table 6). The algal growth inhibition test[17] and the acute *Daphnia* test are part of the test batteries in all these states, the

[16] The following tables contain information on the current test battery and the current limit concentrations in the Czech Republic at the time of CEN/AFNOR survey (CEN 2021a).

[17] Due to the short generation time of the algae, the algal growth inhibition test (test duration: 72 hours) covers several generations and is, therefore, classified as chronic test (EC 2018, ECHA 2023a). From this test, chronic effect concentrations (NOEC, EC_{10}, EC_{20}) can be derived. However, for HP 14 classification, only the EC_{50} is used in the countries mentioned above (see Table 9).

luminescent bacteria test is used in 7 out of the 10 states. Acute fish tests are only employed in Italy and Slovakia.

Chronic toxicity tests with aquatic organisms are used only in two states: a chronic toxicity test with *Ceriodaphnia dubia* in France (current test battery) and a reproductive test with *Daphnia magna* in Spain (Table 6). In Spain, chronic toxicity for aquatic organisms is only assessed, if the acute aquatic toxicity tests are negative, i.e. do not indicate toxicity (MITECO 2021).

Terrestrial ecotoxicity tests are used less frequently for HP 14 classification than aquatic tests: they are only used in 6 of the 10 states mentioned above (Table 7). In Germany and France, tests with terrestrial organisms are performed, when all tests with aquatic organisms are negative (UBA 2013, CEN 2021a). Tests with higher plants are carried out in Germany, France, Slovakia, Spain and the Czech Republic. Tests with the terrestrial microorganism *Arthrobacter globiformis* are used in Germany, France (alternative test battery) and Spain. The avoidance test with earthworms is part of the German test battery and is intended to replace the acute earthworm test in France (UBA 2013, INERIS 2016, CEN 2021a, MITECO 2021, M. Svobodová, pers. comm., 11 March 2022).

Table 6: Toxicity tests with aquatic organisms

Ecotoxicity test[a]	Belgium (Flanders)	Germany	Finland	France		Italy	Austria	Sweden	Slovakia	Spain	Czech Republic
				Current test battery	Alternative test battery[b]						
Inhibition of light emission of *Aliivibrio fischeri* (formerly *Vibrio fischeri*)	X	X	X	X	X	—	X	X	—	—	X
Growth inhibition test with green algae	X	X	X	X	X	X	X	X	X	X	X
Inhibition of mobility of *Daphnia magna*	X	X	X	X	X	X	X	X	X	X	X
Acute fish toxicity test	—	—	—		—	X	—	—[c]	X		—
Chronic toxicity test with C*eriodaphnia dubia*	—	—	—	X	—	—	—	—	—	—	—
Reproductive test *w*ith *Daphnia magna*	—	—	—	—	—	—	—	—	—	X	—
Reference	CEN (2021a)	UBA (2013)	CEN (2021a)	INERIS (2016), CEN (2021a)	CEN (2021a)	CEN (2021a)	BMNT (2018), CEN (2021a)	CEN (2021a)	CEN (2021a)	MITECO (2021)	M. Svobo-dová, pers. comm., 11/03/2022

[a] Information in the table is generally based on national guidance documents where available. For information from CEN (2021a): in some cases, information in the main body and annex ('raw results') of CEN (2021a) was not consistent. In these cases, information from the annex was used. [b] Current work on the implementation of the test battery proposed by Pandard & Römbke (2013). [c] According to the main part of CEN (2021a), the acute fish test is used in Sweden, but according to the annex, a test with the nematode *Caenorhabditis elegans* is used. Further information on the test with *C. elegans* is missing, e.g. it is not clear whether it is an acute or chronic test and whether it is carried out in water or soil. Therefore, this test was not included in the table.

Table 7: Toxicity tests with terrestrial organisms

Ecotoxicity test[a]	Belgium (Flanders)	Germany	Finland	France		Italy	Austria	Sweden[c]	Slovakia	Spain	Czech Republic
				Current test battery	Alternative test battery[b]						
Dehydrogenase activity of *Arthrobacter globiformis*	—	**X**	—	—	**X**	—	—	—	—	**X**	—
Root growth of *Lactuca sativa* in soil	—	—	—	—	—	—	—	—	(**X**)[c]	—	**X**
Seedling emergence and growth of higher plants	—	**X**	—	**X**	**X**	—	—	—		**X**	—
Acute test with earthworms	—	—	—	**X**	—	—	—	—	—	—	—
Avoidance test with earthworms	—	**X**	—	—	**X**	—	—	—	—	—	—
Reference	CEN (2021a)	UBA (2013)	CEN (2021a)	INERIS (2016), CEN (2021a)	CEN (2021a)	CEN (2021a)	BMNT (2018), CEN (2021a)	CEN (2021a)	CEN (2021a)	MITECO (2021)	M. Svobodová, pers. comm., 11/03/2022

[a] Information in the table is generally based on national guidance documents where available. For information from CEN (2021a): in some cases, information in the main body and annex ('raw results') of CEN (2021a) was not consistent. In these cases, information from the annex was used. [b] Current work on the implementation of the test battery proposed by Pandard & Römbke (2013). [c] According to CEN (2021a), a terrestrial plant test is carried out. However, there is no further information on this test.

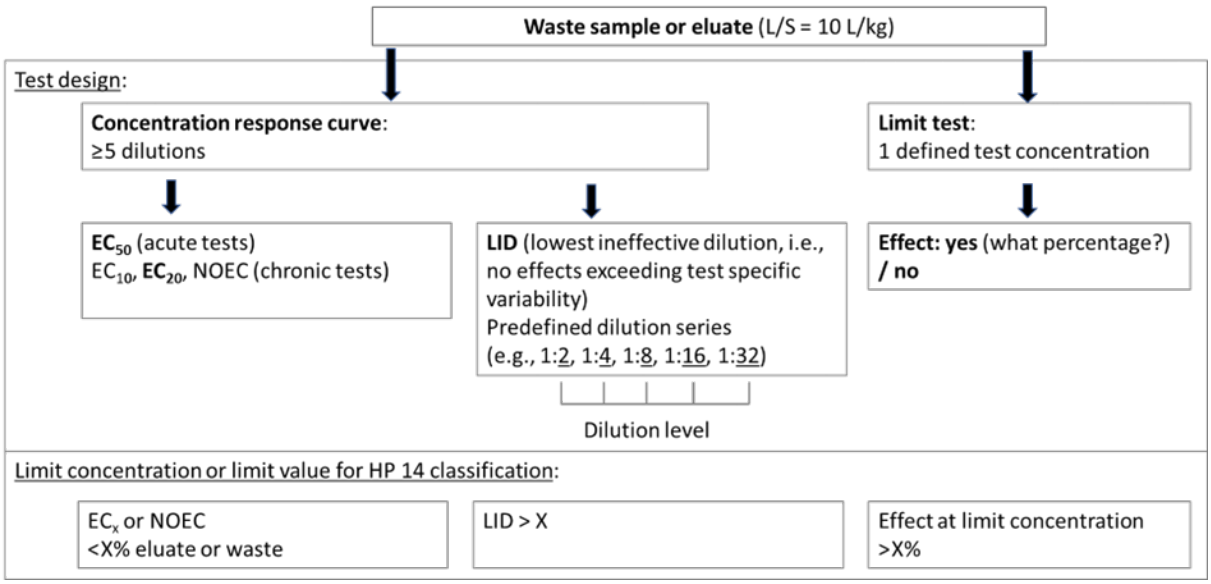
3.1.1.3 Test design and limit concentrations

The ecotoxicity tests used for HP 14 classification in the various countries differ in their test design (see overview in Figure 3 and Table 8). In most states, full concentration response curves with at least 5 dilution levels of the waste or waste eluate are generated. In acute ecotoxicity tests, an EC_{50} is derived (the concentration resulting in 50% effect on the relevant test endpoint), in chronic tests an EC_{20}[18] or a NOEC is determined (the highest test concentration, at which no significant effects on the test endpoints are detected; see Table 9).

In some states, ecotoxicity tests are carried out with a predefined dilution series, and the lowest ineffective dilution (LID) is derived[19].

In two states, limit tests are performed with only one specified test concentration[20] , partly with the option to perform an EC_X test, if an effect occurs in the limit test (see Table 8).

Figure 3: Overview of different test designs in ecotoxicity tests for HP 14 classification



Source: own illustration, ECT Oekotoxikologie GmbH

In several states, limit concentrations or limit values have been defined for short-term aquatic ecotoxicity tests, above or below which a tested waste is classified as ecotoxic (Figure 3). However, some of these differ considerably (cf. Table 9).

In most states, a waste is classified as HP 14 if the EC_{50} from a short-term aquatic test is < or ≤ an eluate content of 10% in the test medium or if the LID is >8 (i.e. >12.5% eluate content).

In the Czech Republic, limit tests are carried out with 10% eluate (100 mL eluate/L of test medium). A waste is classified as ecotoxic, if at least 50% inhibition occurs in the limit test. This

[18] In chronic ecotoxicity tests, EC_{10} or NOEC values are usually determined. However, an EC_{20} <1% eluate content is used as limit concentration for the chronic aquatic toxicity test in France (see Table 9).

[19] The LID is the lowest sample dilution that does not result in effects exceeding the test-specific variation (see e.g. DIN EN ISO 8692, 2012b, section 3.3). The extent of test-specific variation for the respective test is usually determined in pre-trials and then specified in the test guideline. For example, in the algal growth inhibition test according to DIN EN ISO 8692, the LID is the lowest dilution level with no or less than 5% inhibition of algal growth.

[20] With regard to the test procedure and evaluation, these limit tests differ from limit tests, which are performed for the environmental risk assessments of chemical substances. The latter are used to demonstrate the absence of ecotoxic effects (see section 5.6.2). For this purpose, a statistical evaluation of the test results is carried out. If no statistically significant effects of the substance on the test endpoint(s) are detected in a limit test, it can be concluded that the respective substance has no acute or chronic toxicity to the test organism. In limit tests with waste or waste eluate, the effect at the limit concentration is compared to a limit value, no statistical evaluation is carried out (see Figure 3).

limit value corresponds to the above-mentioned EC_{50} <10% eluate content. However, due to the use of a limit test and the resulting lack of a concentration-response curve, the uncertainty in the HP 14 classification is higher than for a classification based on a test with at least 5 concentration levels of the waste eluate[21].

In Austria and Spain, the limit concentrations for an HP 14 classification are much lower. In the following, the approach in these two countries is first described and then discussed.

According to the Austrian guidance (BMNT 2018), a very low eluate concentration is used in the limit test. For this purpose, the waste eluate produced with an L/S ratio of 10 (see section 3.1.1.1) is diluted by a factor of 1000. BMNT (2018) considers the elution as a 10-fold dilution of the waste, so that the total dilution related to the solid waste sample is 1:10,000. According to BMNT (2018), the resulting eluate dilution (0.1% eluate content) therefore contains a concentration of 100 mg of the solid waste sample per L of test medium. A limit test is performed with this eluate dilution. A waste is classified as HP 14, if an effect of at least 10, 20 or 25% is recorded in this test (depending on the test species and test guideline, BMNT 2018, cf. Table 9). If an effect occurs in the limit test and the option is used to perform an EC_X test (see Table 8), a waste is only classified as ecotoxic if the EC_{50} is ≤0.1% eluate content or ≤100 mg of the solid waste sample per L of test medium. According to BMNT (2018), the concentration used in the limit test and the limit concentration are based on the criteria for classification of substances as hazardous to the aquatic environment set out in the CLP Regulation (EC 2021, section 4.1.2.6, Table 4.1.0, point (b), (iii): chemical substances with EC_{50} and LC_{50} values[22] above 100 mg/L are not classified as hazardous to the aquatic environment.

According to the Spanish guidance (MITECO 2021), a waste is only classified as HP 14 (category: acute aquatic toxicity), if at least one EC_{50} is ≤ 1 mg waste fresh weight per L of test medium (see Table 9). This limit concentration is also based on the CLP Regulation: MITECO (2021) refers to a limit value for the classification of mixtures based on acute aquatic toxicity data (category acute 1, cf. EC 2021, section 4.1.3.3.3). When both acute and chronic ecotoxicity tests are carried out, a waste is not HP 14, if all NOEC values from chronic tests are >1 mg waste fresh weight per L of test medium, and all EC_{50} values from acute tests are >100 mg waste fresh weight per L of test medium. Thus, MITECO (2021) uses two different limit concentrations for acute ecotoxicity (see Table 9).

The legal requirements for HP 14 classification of waste refer to certain aspects of the CLP Regulation (EC 2021) and the REACH Regulation (EC 2022). Regarding the classification by means of testing, these are (a) the use of REACH test methods, (b) the avoidance of animal tests, and (c) the consideration of a lack of bioavailability in the assessment (cf. section 1.1). Regarding classification with the calculation method, the concentration limits and cut-off values for the concentrations of chemical substances contained in the waste that are ozone-depleting or acutely and/or chronically hazardous to water organisms are harmonised with the CLP Regulation (see sections 1.1 and 5.6.6).

In the approach according to the Austrian (BMNT 2018) and Spanish guidance (MITECO 2021), limit concentrations, which were defined for chemical substances or mixtures of chemical substances, are applied to the waste as a whole. However, waste is not considered as substance, mixture, preparation or article within the meaning of the CLP Regulation (EC 2021, Article 1)[23]

[21] See also footnote 20 and section 5.6.2.

[22] In the acute fish test, an LC_{50} is determined, an EC_{50} for the endpoint mortality.

[23] "Waste as defined in Directive 2006/12/EC of the European Parliament and of the Council of 5 April 2006 on waste is not a substance, mixture or article within the meaning of Article 2 of this Regulation" (Regulation (EC) 1272/2008, EC 2021, Article 1, paragraph 3).

and the REACH Regulation (EC 2022, Article 2)[24] (see also EU 2018, p. 10). Any ecotoxic substances contained in waste are generally embedded in a matrix (e.g. soil) that is not ecotoxic.

According to the Austrian guidance (BMNT 2018) biotests can be performed for a waste, which is hazardous to water organisms according to the calculation method, to prove the lack of bioavailability of the contaminants (see Table 2). If this option is used, most wastes are likely to be exonerated by the biotests that are performed with high dilutions of the waste eluate (see also section 5.6.6).

As mentioned in section 3.1.1.2, chronic toxicity tests with aquatic organisms for HP 14 classification are only performed in France and Spain. As limit concentration an EC_{20} <1% eluate content is used in France (current test battery; INERIS 2016).

According to the Spanish guidance (MITECO 2021), waste is classified as HP 14 (category: chronic aquatic toxic), if the NOEC determined in the performed chronic aquatic test is ≤1 mg/L[25] or if at least one of the EC_{50} values determined in the acute aquatic tests is ≤100 mg/L (both based on waste fresh weight per L of test medium, cf. Table 9). As discussed above, these limit concentrations are so low that waste will probably only be classified as HP 14 in very few cases.

In most countries where both aquatic and terrestrial biotests are used for HP 14 classification, the limit concentrations for both compartments are analogous: an EC_{50} < or ≤10% waste content in the test substrate, an LID >8 (Germany, France: current test battery, Slovakia) or >30% inhibition in a limit test with 10% waste content (Czech Republic; see Table 10). A limit concentration for chronic terrestrial ecotoxicity was only identified for France (current test battery: EC_{20} <1% waste content, INERIS 2016). In Spain, no limit concentrations for terrestrial testing have been defined.

In Belgium (Flanders), Germany, France, Austria, Spain and the Czech Republic, waste is classified as ecotoxic, if at least one biotest result is positive (UBA 2013, INERIS 2016, BMNT 2018, CEN 2021a, MITECO 2021). For Finland, Italy, Sweden and Slovakia information on the number of positive tests required for an HP 14 classification is lacking.

Summary

To sum up, the approaches to HP 14 classification of waste in different European states are very heterogeneous. Guidance documents on HP 14 classification are not available in all European countries (see also Beggio et al. 2021, Bishop & Hennebert 2021). The differences relate to the criteria for using ecotoxicological tests, the specifications for the maximum particle size of the tested waste, the elution methods, the type of ecotoxicity tests used, the test design and the limit concentrations for HP 14 classification. If possible, these issues should be harmonised at the EU level (see also Grenni et al. 2020), also with regard to the cross-border transport of waste.

[24] "Waste as defined in Directive 2006/12/EC of the European Parliament and of the Council is not a substance, preparation or article within the meaning of Article 3 of this Regulation" (Regulation (EC) 1907/2006, EC 2022, Article 2, paragraph 2).

[25] According to MITECO (2021), this limit concentration is based on the limit concentration of the CLP Regulation for the classification of mixtures based on chronic aquatic toxicity data (see EC 2021, section 4.1.3.3.4).

Table 8: Test design in ecotoxicity tests with aquatic and terrestrial organisms

Test design[a, b]	Belgium (Flanders)	Germany	Finland	France		Italy	Austria	Slovakia	Spain	Czech Republic
				Current test battery	Alternative test battery[c]					
EC_X (or NOEC)	—	**X**	**X**[d]	**X**	**X**	**X**	X if effects in the limit test[e]	**X**	**X**	—
LID	**X**	—	(X)[d]	(x)	—	—		—	—	—
Limit test (concentration tested)	—	—	—	—	—	—	**X (100 mg/L)**[f]	—	—	**X (100 mL/L; 100 g dw/kg dw)**
Reference	CEN (2021a)	UBA (2013)	CEN (2021a)	INERIS (2016), CEN (2021a)	CEN (2021a)	CEN (2021a)	BMNT (2018), CEN (2021a)	CEN (2021a)	MITECO (2021)	M. Svobodová, pers. comm., 11/03/2022

[a] In some cases, information in the main body and annex ('raw results') of CEN (2021a) was not consistent. In these cases, information from the annex was used. [b] CEN (2021a) does not contain any information on the used test design in Sweden. [c] Current work on the implementation of the test battery proposed by Pandard & Römbke (2013). [d] EC_X preferred. LID test, if the sample volume is not sufficient for a full dilution series to determine the EC_{50}. [e] If a significant effect is recorded in the limit test, an EC_{50} can be determined for the respective test organism. [f] Dilution of the eluate produced according to ÖNORM S 2117 by a factor of 1000, i.e. total dilution (based on the solid waste sample) is 1:10,000 according to BMNT (2018).

Table 9: Limit concentrations and limit values for ecotoxicity tests with aquatic organisms

	Belgium (Flanders)	Germany	Finland	France		Austria	Slovakia	Spain	Czech Republic
				Current test battery	Alternative test battery				
Elution with	L/S = 10								
Limit concentration[a] or limit value (% eluate content or dilution level of the eluate, unless otherwise specified)[b, c]	Acute toxicity: LID >8	Acute toxicity: EC_{50} ≤10%	Acute toxicity: EC_{50} <10% or LID >8	Acute toxicity: EC_{50} <10%, LID >8 Chronic toxicity: EC_{20} <1%	In discussion	Acute toxicity: Limit tests (100 mg/L): *A. fischeri*: ≥20% inhibition Green algae: ≥20% (ISO 8692, 2012b) or ≥25% (method according to Regulation (EU) 440/2008, Annex C.3, EC 2019) inhibition *D. magna*: ≥10% immobilisation EC_X tests: EC_{50} ≤100 mg/L (i.e. 0.1% eluate content)	Acute toxicity: EC_{50} ≤10%[d]	Acute toxicity: EC_{50} ≤1 mg/L*,[e] Chronic toxicity: NOEC ≤1 mg/L[e] or EC_{50} ≤100 mg/L[e] *But: A waste is not classified as HP 14 if all NOEC values >1 mg/L and all EC_{50} values >100 mg/L[f]	Acute toxicity: ≥50% inhibition in limit test with 10% eluate content
Reference	CEN (2021a)	UBA (2013)	CEN (2021a)	INERIS (2016), CEN (2021a)	CEN (2021a)	BMNT (2018), CEN (2021a)	CEN (2021a)	MITECO (2021)	M. Svobodová, pers. comm., 11/03/2022

LID: Lowest ineffective dilution. [a] The limit concentration is the effect concentration, at which the tested waste is classified as ecotoxic. [b] In some cases, information in the main body and annex ('raw results') of CEN (2021a) was not consistent. In these cases, information from the annex was used. [c] CEN (2021a) does not contain information on limit concentrations in Italy and Sweden. [d] Toxicity units (TU) ≥ 10 (TU = 100/EC_{50} [%]). [e] For solid waste, the L/S ratio of 10 L/kg in the elution must be taken into account, i.e. the test result has to be divided by 10. In addition, the water content of the original sample has to be considered (MITECO 2021). [f] Thus, two different limit concentrations for acute ecotoxicity are used according to MITECO (2021, p. 128).

Table 10: Limit concentrations and limit values for ecotoxicity tests with terrestrial organisms

	Germany	France		Slovakia	Spain	Czech Republic
		Current test battery	Alternative test battery			
Limit concentration[a, b] or limit value (% waste in the test substrate or dilution level of the waste)	EC_{50} ≤10%	Acute toxicity: EC_{50} <10%, LID >8 Chronic toxicity: EC_{20} <1%	In discussion	EC_{50} ≤10%[c]	No limit concentrations set	≥50% inhibition in the limit test with 10% waste in the test substrate
Reference	UBA (2013)	INERIS (2016), CEN (2021a)	CEN (2021a)	CEN (2021a)	MITECO (2021)	M. Svobodová, pers. comm., 11/03/2022

LID: Lowest ineffective dilution. [a] The limit concentration is the effect concentration, at which the tested waste is classified as ecotoxic. [b] In some cases, information in the main body and annex ('raw results') of CEN (2021a) was not consistent. In these cases, information from the annex was used. [c] Toxicity units (TU) ≥ 10 (TU = 100/EC_{50} [%]).

3.1.2 Suggestions made in scientific publications

In the scientific community, a consensus on an approach to HP 14 classification of waste is also lacking so far (see also Hennebert 2019, Bandarra et al. 2021, Beggio et al. 2021). As for the national guidance documents discussed in the previous section, the approaches and methods suggested in different publications are differing.

When selecting test organisms, the aim is often to cover the main trophic and taxonomic groups – with one exception: fish tests are rarely used for HP 14 classification. This is due to the facts that these tests are animal experiments, which are to be avoided (see section 1.1), and that a relatively high eluate volume is required for these tests (Pandard & Römbke 2013, Römbke et al. 2018).

Aquatic tests are used more frequently than terrestrial tests. This is partly justified by the fact that the classification according to the CLP Regulation is based exclusively on aquatic ecotoxicity tests (Wahlström et al. 2016, Römbke et al. 2018). However, terrestrial tests should be part of the HP 14 biotest battery to detect possible toxic effects of waste constituents with a low water solubility (Pandard & Römbke 2013, Planchon et al. 2015; see also section 5.6.2). The used aquatic and terrestrial biotests are discussed in section 3.2.

Some of the approaches suggested in publications have been incorporated into national guidance documents. For example, the German and the alternative French biotest battery are based on the proposal of Pandard & Römbke (2013).

The test strategy and test battery of Pandard & Römbke (2013) have been used by several other authors (e.g. Hennebert 2018, 2019, Pivato et al. 2020, Beggio et al. 2021), partly in a slightly modified form. With regard to pH adjustment prior to aquatic testing, Hennebert (2019) suggested that pH should not be adjusted in the eluate, but only in those dilutions that have been shown to be ecotoxic in a first test without pH adjustment. In this way, the precipitation of potentially toxic waste constituents can be minimised. In addition, Hennebert (2018, 2019) modified the limit concentrations for HP 14 classification for some of the tests proposed by Pandard & Römbke (2013). Based on the testing of 10 wastes that were classified as 'not HP 14', the maximum effect was determined in the 6 tests of the test battery mentioned above (2.25 to 15.8%, depending on the test). These values were used as a limit concentrations, either directly (Hennebert 2018) or as rounded values (5-15%, depending on the test; Hennebert 2019).

In most studies, it was suggested to classify a waste as HP 14, if at least one test result is positive (see overview of Römbke et al. 2018).

3.2 Studies on ecotoxicological test methods and results for waste assessment

3.2.1 Approach for the literature search and evaluation

The search for studies on ecotoxicological test methods and on results for waste assessment was performed within the literature collected by ECT during previous projects. In addition, a very broad search was carried out in the Web of Science by using the search string "waste ecotox* test*". This resulted in a total of 1,660 hits that were screened to identify relevant studies. Studies with liquid waste (e.g. landfill leachate) were excluded, because such waste is difficult to test in terrestrial bioassays. Studies with waste with a high organic matter content (e.g. slurry, sewage sludge and compost) were also excluded, as experience shows that these are problematic due to oxygen consumption in biotests. Focus was placed on work from Europe. Particular attention was paid to studies published since 2013 (i.e. since the publication of the UBA recommendations). In this way, about 80 publications were identified and evaluated. The extracted information was compiled in an Excel table. Since some of the publications contain identical test results, the table includes data from 67 different sources[26]. The structure of the Excel table is shown in Table 11. As most studies were published in English, the table was kept in English. It was made available to the UBA together with the present report.

The aim of the evaluation was, among other things, to obtain an overview of the following aspects:

- the types of waste tested so far using ecotoxicological methods,
- methods for sampling, sample pre-treatment and elution,
- the test methods and test batteries used,
- the applied assessment criteria for HP 14 classification.

The evaluation was also used to verify if the sample pre-treatment methods and the biotest battery recommended in UBA (2013) is suitable and up-to-date, and to make suggestions for possible modifications.

Table 11: Structure of the Excel table for evaluating the studies identified in the literature search on ecotoxicological test methods and results for waste assessment

Column	Explanation
Reference	Literature source (with reference to separate list)
Sample ID	Sample designation
Sample description	Description of the sample
Particle size	Particle size of the sample
Sample type	Type of sample, e.g. solid waste, eluate, leachate
EWC chapter	European Waste Catalogue: chapter
EWC code	European Waste Catalogue: waste code

[26] One of the studies was classified as confidential. It was evaluated, but the results were not included in this report.

Column	Explanation
EWC derived	Yes: waste code derived from sample description. No: waste code indicated in literature source
Sampling method	Method of waste sampling
Sample preparation methods	Method of sample production (e.g. elution method)
pH correction	Adjustment of pH before testing (yes/no)
pH original	Original pH of the sample
pH adjusted to	Sample pH adjusted to
Test organism	Tested species (e.g. *Daphnia magna*, *Eisenia fetida*, *Lemna minor*)
Compartment	Compartment (aquatic/terrestrial)
Group	Group of organisms (algae, microorganisms, plants, animals)
Measurement endpoint	Test endpoint (e.g. mortality, reproduction, growth)
Test duration	Test duration in days, hours or minutes
Test system	Test system (e.g. algal growth test, earthworm avoidance test)
Guideline/reference	Test guideline or reference for the test system
Assessment endpoint	Type of effect concentration (e.g. EC_{50}, G-value, LID, NOEC)
Endpoint unit	Unit of assessment endpoint (e.g. % waste content, g/kg, mg/L)
Endpoint value	Numerical value of the assessment endpoint
Assessment criterion	Assessment criterion (limit concentration, limit value) applied (e.g. LID >4 or NOEC <10% waste content)
Classification	Classification (not ecotoxic/ecotoxic)
Original classification	Originally used ecotoxicity classification (e.g. with refined gradation or points system)
Remarks	Remarks

3.2.2 Overview of the content of the Excel table and the used methods

The Excel table contains a total of about 3,500 rows, a row being defined as a unique combination of the information on the sample, elution method, pH adjustment, test organism, measurement endpoint and assessment endpoint contained in the respective columns. Approximately 60% of the rows contain data on aquatic and 40% on terrestrial test results from 58 and 39 literature sources, respectively. Approximately 600 samples from approximately 90 different waste types were tested, using 43 different test species, 20 aquatic and 23 terrestrial species (Table 12). The surprisingly high number of terrestrial test species is due to many different plant species that were used in the seedling emergence and growth test (ISO 11269-2, 2012a, and OECD 208, 2006a, see Table 12).

Water fleas (*D. magna*; 39 sources) were the most frequently tested species, followed by luminescent bacteria (mainly *Aliivibrio fischeri*; 35 sources) and unicellular green algae (*Desmodesmus subspicatus*, *Raphidocelis subcapitata* (formerly: *Pseudokirchneriella subcapitata*); 33 sources). In aquatic test batteries, algae and daphnids were most frequently combined (28 sources), partly with additional use of luminescent bacteria (22 sources) and (significantly less frequently) duckweed (*Lemna minor*); 7 sources). In the less commonly used terrestrial test batteries, the seedling emergence and seedling growth test with various plant species and the solid contact test with *A. globiformis* were often combined (8 literature sources), partly with additional use of the earthworm avoidance test with *Eisenia fetida* or *Eisenia andrei* (5 sources). Thus, the test battery recommended by UBA (2013) contains the most commonly used methods.

Regarding the used sampling method, only limited information is provided in the evaluated literature. In most studies, samples were eluted with the method recommended by UBA (2013): a one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h, mostly according to EN 12457-2 (19 sources) or DIN 38414-4 (10 sources). Particle size of the waste sample was often <4 mm (15 sources). However, in many studies, information on particle size is lacking.

The most frequently tested waste type were bottom ash and slag (mirror entry 19 01 11*/ 19 01 12) with about 80 different samples. The assessment endpoints used were NOEC/LOEC, EC/LC_X, toxic units (TU), and G and LID values. In about half of the evaluated studies, the tested waste samples were not classified as ecotoxic or not ecotoxic, since no assessment criterion (limit concentration or limit value) had been defined. In the remaining studies, various assessment criteria were used, most often an EC_X ≤10% (9 references), followed by LID values >4 or >8 (depending on the test system, 7 references), and the toxicity classification system (TCS) according to Persoone (1999, unpublished, described in Lapa et al. 2002; 5 references).

Table 12: Overview of aquatic test species and test systems used for waste testing based on the literature search

Test species	Test system	Test guidelines[a], test kits, references
Aliivibrio fischeri	Luminescent bacteria test	e.g. Blaise et al. (1994), DIN 38412-34, ISO 11348, Microtox
Brachionus calyciflorus	Chronic toxicity test	ISO 20666
Ceriodaphnia dubia	Chronic toxicity test	ISO 20665
	Effect on survival and reproduction	Ferrari & Férard (1996)
	Reproduction and survival	–
Chlamydomonas reinhardtii	Photosynthesis activity test	–
	Fluorescein diacetate test	Gilbert et al. (1992)
Corophium volutator	Acute toxicity test	ISO 16712
Danio rerio	Fish embryo toxicity test	Draft OECD proposal, modified according to Carlsson et al. (2009), DIN 38415-6
	Early life stage test	–
Daphnia magna	Acute test	e.g. Daphtoxkit, ISO 6341, OECD 202
	Reproduction test	EPA 600/4-91-002 (US EPA 1984)

Test species	Test system	Test guidelines[a], test kits, references
Desmodesmus subspicatus, *Raphidocelis subcapitata*	Algal growth inhibition test	e.g. Algaltoxkit, ISO 8692, OECD 201, Radetski et al. (1995)
Escherichia coli mutant	Microbial enzyme assay	ToxiChromopad, MetPAD/MetPLATE, Kwan (1995)
Lemna minor	Growth inhibition test	e.g. Devare & Bahadir (1994), ISO 20079, OECD 221
Leuciscus idus	Acute toxicity test	DIN 38412-31
Microorganisms	Inhibition of the dehydrogenase activity of activated sludge microorganisms	DIN 38412-3
	Oxygen consumption of microorganisms	–
	Respiration activity test	Offhaus (1965)
Nitocra spinipes	Acute test	SIS SS-02-81-06
	Larval development test	Based on Breitholtz & Bengtsson (2001), Breitholtz & Wollenberger (2003), Breitholtz et al. (2007)
	(Sub)chronic test	Breitholtz & Bengtsson (2001), Breitholtz et al. (2007)
Phaeodactylum tricornutum	Marine algal growth inhibition test	ISO 10253
Photobacterium phosphoreum	Luminescent bacteria test	e.g. DIN 38412-34, ISO 11348-3, Microtox
Pseudomonas putida	Growth inhibition test	DIN 38412-8, ISO 10712
Tetrahymena thermophila	Protozoan inhibition test	Protozoan-TOXKIT
Thamnocephalus platyurus	Survival tests	Thamnotoxkit, Centeno et al. (1995)
Xenopus laevis	Acute toxicity test	–

[a] Current versions of the mentioned test guidelines: DIN 38412-3 (2010), DIN 38412-8 (standard withdrawn, last version: 1991a), DIN 38412-31 (standard withdrawn, last version: 1989a), DIN 38412-34 (standard withdrawn, last version: 1997), DIN 38415-6 (standard withdrawn, last version: 2003), ISO 10253 (2016b), ISO 10712 (1995), ISO 11348-2 (2007a), ISO 11348-3 (2007b), ISO 16712 (2005a), ISO 20079 (2005b), ISO 20665 (2008c), ISO 20666 (2008b), ISO 6341 (2012c), ISO 8692 (2012b), OECD 201 (2011), OECD 202 (2004), OECD 221 (OECD 2006b), SIS 02 81 06 (1991).

Table 13: Overview of terrestrial test species and test systems used for waste testing based on the literature search

Test species	Test system	Test guidelines[a], test kits, references
Allium cepa	Toxicity test	Fiskesjö (1985, 1995)
	Plant root elongation test	Fiskesjö (1997)
Arthrobacter globiformis	Inhibition of dehydrogenase activity	e.g. DIN 38412-48, ISO 18187
Avena sativa, *Brassica camprestris* var. *chinensis*, *Brassica napus*, *Brassica oleracea*, *Brassica rapa*, *Hordeum vulgare*, *Lepidium* sp., *Lolium perenne*, *Lycopersicum esculentum*, *Pisum sativum*, *Raphanus sativus*, *Trifolium pratense*	Effects on emergence and early growth	ISO 11269-2, OECD 208
Caenorhabditis elegans	Effects on growth, fertility and reproduction	ISO 10872
Eisenia andrei, *Eisenia fetida*	Acute toxicity	ISO 11268-1
	Effects on behaviour	ISO 17512-1
	Effects on reproduction	ISO 11268-2
	Growth, sexual development, cocoon production and survival	–
Enchytraeus albidus, *Enchytraeus crypticus*	Avoidance test	–
	Reproduction test	ISO 16387
Folsomia candida	Feeding inhibition test	Domene et al. (2007), Domene (2007)
	Reproduction test	ISO 11267
H. vulgare, *Lactuca sativa*, *Sinapis alba*, *Triticum aestivum*	Inhibition of root growth	ISO 11269-1
L. sativa, *Lepidium sativum*, *T. aestivum*	Seed germination assay	AFNOR X31-201, modification of US-EPA 600/3-88-029 (US EPA 1988), Stephenson et al. (2000)
L. sativum	Germination test	Pinho et al. (2017)
	Growth test	–

Test species	Test system	Test guidelines[a], test kits, references
	Phytotoxicity test	Phytotoxkit, UNI 10780
	Root growth test	Neururer (1975), based on Devare & Bahadir (1994)
L. perenne	Toxicity test	Based on EPA/600/3-88/029 (US EPA 1988) and ASTM-E1963-09
Microorganisms	Dehydrogenase activity test	Shaw & Burns (2006)
	Abundance and activity of soil microflora using respiration curves	ISO 17155
S. alba	Growth inhibition test	STN 838303
	Root elongation toxicity test	CEMD (2003)
Trifolium repens	Germination test	–

[a] Current versions of the mentioned guidelines: AFNOR X31-201 48 (standard withdrawn, last version: 1982), ASTM E1963-09 (2014), DIN 38412-48 (standard withdrawn, last version: 2002), ISO 10872 (2020), ISO 11267 (2023), ISO 11269-1 (2012e), ISO 11268-1 (2012d), ISO 11268-2 (2023), ISO 16387 (2023), ISO 17155 (2012f), ISO 17512-1 (2008a), ISO 18187 (2016a), ISO 11269-2 (2012a), OECD 208 (2006a), STN 838303 (1999), UNI 10780 (1998).

3.2.3 Answering relevant questions based on the data

Based on the data identified in the literature search, the following questions relevant to the project were answered.

3.2.3.1 Are two microbial tests necessary?

The test battery according to UBA (2023) includes two microbial test methods: the (aquatic) luminescent bacteria test with *A. fischeri* according to ISO 11348-2 (2007a)[27] and the (terrestrial) solid contact test with *A. globiformis* according to ISO 18187. Especially regarding the recommended sequential use of the aquatic and terrestrial tests, the question arises if the use of the solid contact test in addition to the luminescent bacteria test and other terrestrial test methods yields additional information.

Comparison of the solid contact test with the luminescent bacteria test

In a first step, it was evaluated if the luminescent bacteria test and the solid contact test provide different information regarding the ecotoxicity of waste samples. Six publications were identified, in which the same samples were investigated using both tests. In these publications, a total of 77 samples from 32 different waste types were tested. Regarding the classification of the samples as (not) ecotoxic, 86 direct comparisons were possible (in some cases, both the EC_{50} and

[27] With an amendment from 2018.

the LID were determined). In 60 cases, the classification based on the luminescent bacteria test and the solid contact test was consistent: the respective samples were classified as ecotoxic in 29 cases, and as not ecotoxic in 31 cases. In 10 cases, the waste sample was ecotoxic in the luminescent bacteria test, but not ecotoxic in the solid contact test. In 16 cases, the sample was not ecotoxic in the luminescent bacteria test, while it showed ecotoxicity in the solid contact test. This was mainly the case for bottom ash and slag (19 01 11*/19 01 12) from the incineration of municipal waste (MWI ashes; see Römbke & Moser 2007). In four cases, the sample was not ecotoxic in all three aquatic tests (luminescent bacteria, *Daphnia*, algae), but it was ecotoxic in the solid contact test. Thus, it can be concluded that the solid contact test can provide valuable additional information compared to the luminescent bacteria test.

Comparison of the solid contact test with other terrestrial test methods

In a second step, it was examined if the solid contact test can provide additional information on the HP 14 classification of waste samples compared to other terrestrial test methods.

Deventer & Zipperle (2004) tested 24 samples from 13 different waste types. In addition to the solid contact test, the seedling emergence and seedling growth test according to OECD test guideline 208 was applied with oat (*Avena sativa*), cabbage (*Brassica oleracea*) and tomato (*Lycopersicum esculentum*). Initially, no differentiation was possible, since waste samples were ecotoxic in almost all cases (in 21 out of 23 cases in all tests). In one case, a sample was classified as ecotoxic only based on the solid contact test and the plant test with oat. However, the assessment was based on a very conservative assessment criterium: samples were only classified as ecotoxic, if the LID was >2. Application of an LID >8 as limit value, as commonly used in other studies and national guidelines (see section 3.1.1.3), results in a more differentiated picture. In this case, 15 samples would be ecotoxic in all test systems, 3 samples would be not ecotoxic or not clearly classifiable (a range is given for the LID, e.g. 2-10, presumably due to test repeats), and 3 samples would be ecotoxic in several but not all tests. Three samples would be classified as ecotoxic solely based on the solid contact test, making it the most sensitive test system.

Römbke & Moser (2007) investigated 12 different MWI ashes with the solid contact test, the seedling emergence and growth test (ISO 11269-2) with oat (*A. sativa*) and oilseed rape (*Brassica napus*), and the acute earthworm test (ISO 11268-1) with *Eisenia fetida*. An LID >8 was applied as limit value. One sample was ecotoxic in all tests, one in all tests except the acute earthworm test, two samples were ecotoxic in the solid contact test and the plant test with oat. Four samples were only ecotoxic in the solid contact test, another four samples were not ecotoxic in any of the tests. Thus, the solid contact test was the most sensitive test system, while the acute earthworm test and the growth inhibition test with oilseed rape were relatively insensitive.

Moser & Römbke (2009) tested 3 samples from 3 waste types (MWI ashes, soil contaminated with PAHs, waste wood mainly contaminated with copper-based wood preservatives) in an international ring test involving 60 laboratories from 15 countries. The following tests were carried out:

- Solid contact test (ISO 18187) with *A. globiformis*,
- Seedling emergence and growth test (ISO 11269-2) with oat (*A. sativa*) and turnip (*B. rapa*),
- Acute earthworm test (ISO 11268-1) with *E. fetida*,
- Earthworm avoidance test (ISO 17512-1) with *E. fetida*,

- Earthworm reproduction test (ISO 11268-2) with *E. fetida*,
- Enchytraeid reproduction test (ISO 16387) with *Enchytraeus* sp.,
- Collembolan reproduction test (ISO 11267) with *Folsomia candida*.

The geometric mean of the effect concentrations determined by the individual laboratories was reported for each test system and sample. Since the authors did not define an assessment criterion, an EC_{50} or LC_{50} <10% waste in the test substrate was used as limit concentration to compare the test systems (see section 3.1.1.3). Ash and soil were not ecotoxic in any test, while wood was ecotoxic in all tests except the acute earthworm test and the enchytraeid reproduction test, i.e. the latter tests showed a comparatively low sensitivity.

Römbke et al. (2010) examined 23 samples from 20 different waste types. In addition to the solid contact test, they used the seedling emergence and growth test (ISO 11269-2) with oilseed rape (*B. napus*) and the earthworm avoidance test (ISO 17512-1) with *E. fetida*. An LID >8 was used as limit value. Eleven samples were ecotoxic in all three tests, seven samples were not ecotoxic in any test. One sample was only ecotoxic in the solid contact test, one sample only in the earthworm avoidance test. Two samples were ecotoxic in the solid contact test and the plant test with *B. napus*. One sample was ecotoxic in the solid contact test and the earthworm avoidance test, one sample in the plant test and the earthworm avoidance test. Thus, the plant test did not provide any additional information compared to the other two test systems, since all samples could also have been classified as (not) ecotoxic based on the solid contact test and the earthworm avoidance test.

Hennebert (2018) tested 28 samples from 16 different waste types with the solid contact test, the seedling emergence and growth test (ISO 11269-2) with oat (*A. sativa*) and oilseed rape (*B. napus*) and the earthworm avoidance test (ISO 17512-1) with *E. fetida*. As assessment criterion, adapted specific limit concentrations for each test system were used (see section 3.1.2):

- Solid contact test: EC_{50} <2.25%,
- Seedling emergence and growth test with oat: EC_{50} <20.2%,
- Seedling emergence and growth test with oilseed rape: EC_{50} <13.7%,
- Earthworm avoidance test: EC_{50} <3.75%.

Seven samples were ecotoxic in all test systems, 10 samples were not ecotoxic in any test. Three samples only lacked toxicity in the solid contact test, while two samples only showed ecotoxicity in this test. Three samples were only ecotoxic in the seedling emergence and growth test with the two plant species, a further sample only in the plant test with oat. One sample was ecotoxic in the plant test with oat and in the earthworm avoidance test, one sample in the solid contact test and in the plant test with oat. Here, the solid contact test and the plant test with oat would have been sufficient to classify the samples as (not) ecotoxic.

A report of the Public Waste Agency of Flanders (OVAM 2018) describes the testing of eight samples from four different waste types using the solid contact test, the earthworm avoidance test (ISO 17512-1) with *E. fetida* and a growth inhibition test with *Lepidium* sp. based on OECD test guideline 208. As limit value, an LID >8 was used. Three samples were classified as not ecotoxic with all three test systems. Three samples were only ecotoxic in the earthworm avoidance test. One sample was ecotoxic in the solid contact test and the earthworm avoidance test, one sample in the plant growth test and the earthworm avoidance test. Thus, the

earthworm avoidance test would have been sufficient for classifying these samples as (not) ecotoxic.

Rebischung et al. (2018) tested a sample of cigarette butts with the seedling emergence and growth test (ISO 11269-2) with turnip (*B. rapa*) and lettuce (*Lactuca sativa*), the acute earthworm test (ISO 11268-1) and the earthworm avoidance test (ISO 17512-1), both with *E. fetida*. The applied assessment criterion was an EC_{10} or LC_{10} <10% waste content. No differentiation was possible, since the sample was ecotoxic in all tests.

Conclusion regarding the comparison of the solid contact test with the luminescent bacteria test and other terrestrial test methods

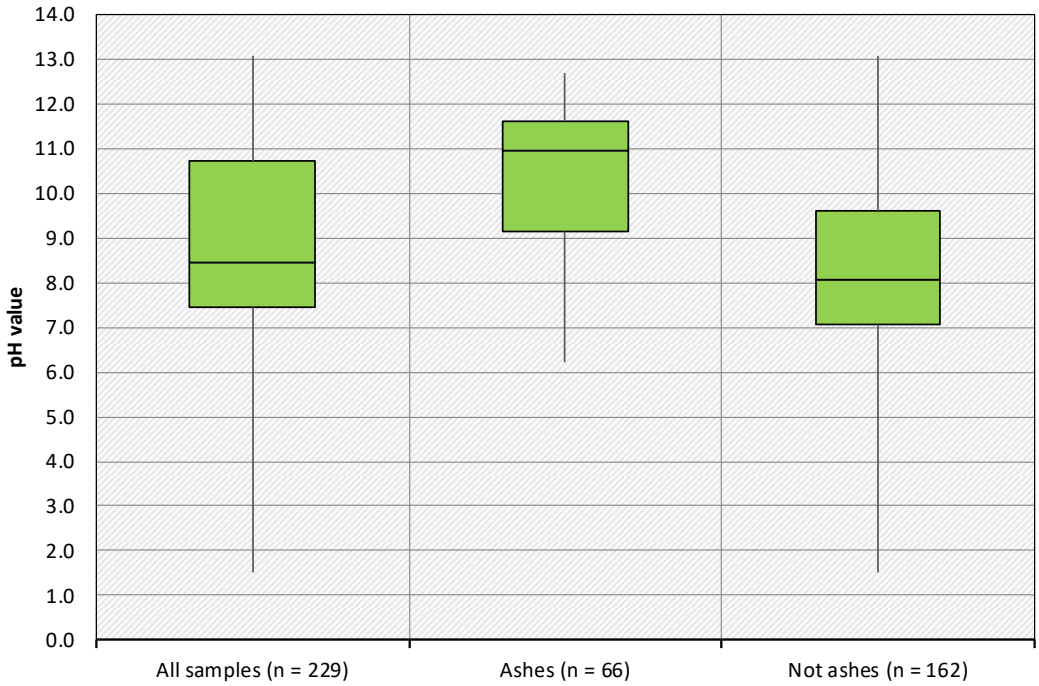
From the comparisons described above, it can be concluded that the solid contact test is a sensitive test system that can provide additional information on the ecotoxicity of waste samples compared to (a) the luminescent bacteria test and (b) other terrestrial test systems. It should, therefore, be part of the test battery used in the present project.

3.2.3.2 Impact of pH on HP 14 classification

With regard to the discussion on the impact of pH on HP 14 classification of waste and (differing) recommendations regarding the adjustment of the pH of waste eluates for biotesting (section 3.1.1.1), the pH range of waste samples tested in the identified literature was examined. Note that pH values ≤2 or ≥11.5 have an indicative value for classification of a waste as HP 4 (irritant) and HP 8 (corrosive; AVV 2020). Therefore, further tests for HP 14 would generally not be necessary in these cases.

For eluates obtained using a batch procedure with L/S = 10 L/kg and a duration of 24 h, pH values were partly outside the tolerance range of the tested species (>8.5), especially for ashes (10 01 01, 10 01 01 02, 10 01 14*/10 01 15, 10 01 16*/10 01 17, 19 01 11*/19 01 12, 19 01 13*/19 01 14). In some cases, pH was ≥11.5 (especially for eluates from ash samples), while pH values in a strongly acidic range were only present in a few cases (see Figure 4).

Figure 4: Box plots for pH distribution of waste eluates obtained using a batch procedure (L/S = 10 L/kg, duration: 24 h) (based on the literature search)



The boxes are bounded by the upper and lower quartiles; the line inside the box is the median; the whiskers extend to the minimum and maximum values. The sum of the sample numbers for ashes and other (not ash) waste samples does not correspond to the sum of all samples, because one sample could not be clearly assigned (waste type 19 01).
Source: own illustration, ECT Oekotoxikologie GmbH

Subsequently, it was evaluated in how many cases an adjustment of the pH led to a change of toxicity. Six studies were identified, in which the same samples were tested with and without pH adjustment (Table 14). A total of 12 different samples were tested, of which 10 originally had pH values ≥10 and two pH values <5. These were adjusted to the optimum range for the test organisms (pH 7.0–8.0). Overall, 18 results could be compared, 10 results of the luminescent bacteria test and 8 results of other aquatic test systems. An increase in toxicity was recorded only in one case (Bandarra et al. 2019). In 12 cases, pH adjustment led to a decrease in toxicity, and in the remaining 5 cases there was no significant change. For 6 of the eluates, the decrease in toxicity resulted in a change of the HP 14 classification from ecotoxic to not ecotoxic (Lapa et al. 2002), underlining the potentially strong influence of pH adjustment.

Table 14: Studies, in which the same sample was tested with and without pH adjustment, and effects on the ecotoxicity of the sample

Reference	Waste sample (waste code)	Elution or extraction method[a]	pH original	pH adjusted to	Test organism, test endpoint (test duration)	Effect concentration			Impact
							without pH adjustment	with pH adjustment	
Bandarra et al. 2019	Green liquor dregs (03 03 02)	EN 12457-2 (different L/S ratios)	10.4	7.0	*Lepidium sativum,* germination index (48 h)	EC_{50} (L/S ratio or L/kg)	124	>500	Increase in toxicity (ecotoxic)
					Aliivibrio fischeri, luminescence inhibition (30 min)		<10	<10	No change (not ecotoxic)
					Lemna minor, frond number (7 d)		>320	246	No change (ecotoxic)
					Daphnia magna, immobilisation (48 h)		130	36	Decrease in toxicity (ecotoxic)
Bernardo et al. 2010	Solid fraction of the residues from pyrolysis of a mixture of 30% (w/w) pine biomass, 30% (w/w) used tyres and 40% plastics (19 01 17*/19 01 18)	Extraction with dichloromethane, elution with $CaCl_2$solution (L/S 10 L/kg)	4.8	7.4	*A. fischeri,* luminescence inhibition (30 min)	EC_{50} (mg eluate/L)	2.4	3.6	No change (ecotoxic)
		Elution with $CaCl_2$solution (L/S 10 L/kg)	4.9	7.4			0.6	0.6	

Reference	Waste sample (waste code)	Elution or extraction method[a]	pH original	pH adjusted to	Test organism, test endpoint (test duration)	Effect concentration			Impact
							without pH adjustment	with pH adjustment	
Dias et al. 2017	Gasification bed char produced at 100% relative humidity and 850°C (19 01)	EN 12457-2 (L/S 10)	10	8.0	*A. fischeri*, luminescence inhibition (30 min)	EC_{50} (% eluate)	34.8	>99	Decrease in toxicity (not ecotoxic)
Lapa et al. 2002	Bottom ash from incineration plants (19 01 11*/19 01 12)	EN 12457-2 (L/S 10)	12.2	7.4	*Photobacterium phosphoreum*, luminescence inhibition (15 min)	EC_{50} (% eluate)	<1.0	77.7	Decrease in toxicity, not ecotoxic after adjustment
			11.4	7.4			9.3	>99.0	
			10.6	7.6			<1.0	75.3	
			12.0	7.4			<1.0	>99.0	
			12.5	7.4			<1.0	59.2	
			11.4	7.7			<1.0	>99.0	
Ferrari et al. 1999	Municipal solid waste incinerator bottom ash (19 01 11*/19 01 12)	XP X31-210 (L/S 10)	>11	8.0	*Raphidocelis subcapitata,* inhibition of growth (72 h)	EC_{50} (% eluate)	0.91	2.86	Decrease in toxicity (ecotoxic)
Mocová et al. 2019	Concrete waste, recycled concrete (17 01 01)	EN 12457-4 (L/S 10)	11.6	7.0	*D. magna*, Immobilisation (48 h)	EC_{50} (% eluate)	<6.25	<6.25	No change (ecotoxic)
					Desmodesmus subspicatus, growth inhibition (72 h)		50	100	Decrease in toxicity (not ecotoxic or unclear)
					L. minor, growth rate (7 d)		12.5	100	

Reference	Waste sample (waste code)	Elution or extraction method[a]	pH original	pH adjusted to	Test organism, test endpoint (test duration)	Effect concentration			Impact
							without pH adjustment	with pH adjustment	
					L. minor, chlorophyll content (7 d)		6.25	12.5	

[a] Current versions of the mentioned guidelines: XP X31-210 (standard withdrawn, last version: AFNOR 1998a), EN 12457-2 (CEN 2002a), EN 12457-4 (CEN 2002b).

3.2.3.3 Possibility of using the germination or root length test with cress (*Lepidium sativum*)

When checking if information on additional test methods relevant for waste testing has been published since the UBA recommendations were issued in 2013, the germination and root length tests with cress (*Lepidium sativum*) stood out. This test (with modifications) was used for waste testing in seven studies (Table 15).

Table 15: Use of the germination or root length test with cress (*Lepidium sativum*) for waste testing since 2013

Reference	Sample	Matrix	Endpoint	Test duration	Based on[a]
Bandarra et al. 2019	Eluate	Filter paper	Germination rate	48 h	Pinho et al. 2017
Bandarra et al. 2020	Eluate	Filter paper	Germination rate	48 h	Pinho et al. 2017
Barbale et al. 2021	Eluate	Filter paper	Germination rate, root length	72 h	UNI 10780
Kępys et al. 2021	Eluate	Filter paper	Germination rate, root length	72 h	Not specified
OVAM 2018	Solid waste	Standard soil	Biomass	4 d	OECD 208
Pinho et al. 2017	Eluate	Filter paper	Germination rate, root length	48 h	Not specified
Werle & Dudziak 2015	Eluate	Filter paper	Root length	24 h	Phytotoxkit

[a] Current versions of the mentioned test guidelines: OECD 208 (2006a), UNI 10780 (1998).

To assess the potential of this test for inclusion in a future terrestrial test battery, its sensitivity was evaluated in comparison to other test systems. Unfortunately, Werle & Dudziak (2015), Pinho et al. (2017), Bandarra et al. (2019, 2020) and Barbale et al. (2021) did not test the same samples with other terrestrial test systems. Kępys et al. (2021) only performed parallel tests with white mustard (*Sinapis alba*) using the same method. In all cases, results obtained with both test species were consistent. In the study of OVAM (2018), *Lepidium* sp. was not exposed to eluate-soaked filter paper (as used in all other studies) but to soil, in line with OECD test guideline 208. Biomass of germinated plants was evaluated after four days. Eight solid waste samples from four different waste types were investigated. In addition to the test with *Lepidium* sp., the earthworm avoidance test (ISO 17512-1) with *E. fetida* and the solid contact test with *A. globiformis* (ISO 10871) were carried out. An LID >8 was used as limit value. Three samples were classified as not ecotoxic in all three test systems, three samples were only ecotoxic in the earthworm avoidance test, one additionally in the solid contact test, and one additionally in the cress test. Therefore, the earthworm avoidance test would have been sufficient for the classification of these samples.

Thus, no reliable statement can be made on the sensitivity of the cress test in comparison to other terrestrial test systems. Nevertheless, testing with *L. sativum* could be an interesting alternative to the plant test with *B. rapa* due to the shorter test duration and the lower experimental effort. This should be further evaluated in future studies. With DIN EN 16086-2 (Soil improvers and growing media – determination of plant response – part 2: Petri dish test

using cress; DIN EN 2012), a European standard is available that could be adapted to waste testing, if necessary.

3.3 First verification of the strategy for HP 14 classification of mirror entries proposed in the UBA recommendations

According to the UBA recommendations, ecotoxicity tests should be used for HP 14 classification of waste from mirror entries, if a classification using the calculation method is not possible, since there is insufficient information on waste composition (UBA 2013, see also section 3.1.1). The possibility of exonerating a waste classified as HP 14 using the calculation method by means of ecotoxicity tests is not addressed in the UBA recommendations. This possibility exists because the results of biotests are decisive for classification (EC 2015). As discussed at the meeting with the project advisory group on 09 March 2022, this possibility is used by waste owners. It is problematic, if results of acute ecotoxicity tests (the current aquatic test battery mainly consists of acute tests; see sections 3.3.2 and 5.6.2) are used to exonerate waste, which is according to the calculation method chronically hazardous to water organisms (H410-H413).

In this context, it should be noted that according to the current version of the CLP Regulation (EG 2021) the classification of substances as long-term hazardous to the aquatic environment is based on the results of chronic aquatic tests. Only if no chronic aquatic toxicity data are available, the classification is based on acute aquatic toxicity data in combination with data on degradability and bioaccumulation potential as described in the first version of the CLP Regulation of 2008 (see ECHA 2017 and EG 2021).

If a waste has been classified as chronically hazardous to water organisms with the calculation method based on chronic biotests with individual waste constituents, results of chronic aquatic toxicity tests should be required to exonerate this waste. This issue should be addressed at the EU level. Specifically, there is a need to regulate in which cases a classification according to the calculation method can be revised (exonerated) by the results of which biotests. This issue is discussed in more detail in section 5.6.2.

3.3.1 Sampling and sample pre-treatment

In the 'Commission notice on technical guidance on the classification of waste' (EU 2018) sampling in accordance with the European standard EN 14899 and the technical reports CEN/TR 15310-1 to -5 is recommended. However, the UBA recommendations mainly refer to LAGA PN 98. According to EU (2018), sampling according to LAGA PN 98 is acceptable if it results in similarly reliable results (see section 3.1.1.1).

With regard to the specifications for particle size (<4 mm[28]) and the elution of waste (one-stage batch procedure, L/S = 10 L/kg, 24 h[29]), the procedure according to UBA (2013) is in accordance with current European standards (EN 12457-2, EN 14735), which are also used in several other European countries (section 3.1.1.1). However, it should be noted that the one-stage batch procedure (EN 12457-2) was developed to investigate mainly inorganic waste constituents. It is not designed to elute non-polar organic substances (see DIN EN 12457-2, 2003a). Eluates produced with this method contain short-term water-available constituents (UBA 2013)[30, 31]. As mentioned at the meeting with the project advisory group, this can lead, for example, to waste

[28] Microbial tests: <2 mm.

[29] Alternatively, the column percolation method can be used (see section 3.1.1.1).

[30] The same applies to the column percolation method.

[31] It is therefore pointed out that in the ecotoxicological characterisation for risk assessment of a planned recycling in an open system, methods should be used that reflect the conditions of this scenario (UBA 2013).

classified as chronically hazardous to water (H410) with the calculation method due to its zinc oxide content, being exonerated using bioassays, because the poorly soluble zinc oxide is not eluted with the one-stage batch procedure mentioned above.

3.3.2 Biotest battery

Compared to other European states, the biotest battery proposed in the UBA recommendations is one of the more extensive test batteries (see section 3.1.1.2). The recommended aquatic ecotoxicity tests are short-term tests. EC_{50} values are used for HP 14 classification. However, in the algal test (DIN EN ISO 8692, 2012), a chronic effect concentration (e.g. EC_{10} or NOEC) can also be derived. With the exception of the seedling emergence and growth test with higher plants, the recommended terrestrial tests are also short-term tests. Again, EC_{50} values are used for HP 14 classification. In some cases, chronic effect concentrations can be derived (see section 5.6.2). Regarding its sensitivity, the earthworm avoidance test is comparable to the chronic earthworm reproduction test (test duration: 56 days) according to ISO 11268-2 (ISO 2023a) or OECD 222 (OECD 2016a; see Scheffczyk et al. 2014). Thus, it is significantly more sensitive than the acute earthworm test (test duration: 14 days) according to ISO 11268-1 (ISO 2012d) or OECD 207 (OECD 1984).

It was verified if the tests mentioned in the UBA recommendations are up to date. The current versions of the test guidelines are indicated in Table 16. With the possible exception of the germination or root length test with cress[32] (see section 3.2.3.3), no further tests with a high relevance for inclusion in the test battery were identified. Conversely, there was no reason to reduce the size of the test battery for the ecotoxicological work carried out in the present project (see e.g. section 3.2.3.1). The used test procedures are discussed in more detail in section 5.6.2.

Table 16: Test battery suggested in the UBA recommendations (current versions of the test guidelines) with test specifications to derive EC_{50} values

Test	Test guideline	Exposure duration	Number of dilutions (plus control)	Replicates per dilution (control replicates)
Aquatic ecotoxicity tests (investigation of waste eluates)				
Inhibition of mobility of *Daphnia magna*	DIN EN ISO 6341 (2013a), corresponds to ISO 6341 (2012c)	48 h	5	4 (4)
Growth inhibition test with unicellular green algae (*Raphidocelis subcapitata*)[a]	DIN EN ISO 8692 (2012), corresponds to ISO 8692 (2012b)	72 h	5	3 (6)
Inhibition of light emission of *Aliivibrio fischeri* (formerly *Vibrio fischeri*)	DIN EN ISO 11348-2 (2009 and 2023)[b], corresponds to ISO 11348-2 (2007a)[c]	30 min	8	2 (2)

[32] Further experimental studies are needed to investigate whether the cress test could possibly replace the more complex test with terrestrial plants according to ISO 11269-2 (ISO 2012a).

Test	Test guideline	Exposure duration	Number of dilutions (plus control)	Replicates per dilution (control replicates)
Terrestrial ecotoxicity tests (investigation of waste samples)				
Inhibition of dehydrogenase activity of *Arthrobacter globiformis*	DIN EN ISO 18187 (2018), corresponds to ISO 18187 (2016a)	6 h	5	4 (4)
Effect on seedling emergence and growth of higher plants (*Brassica rapa*)	DIN EN ISO 11269-2 (2013b), corresponds to ISO 11269-2 (2012a)	14 d	12[d]	2 (6)
Avoidance test to determine effects on the behaviour of earthworms (*Eisenia fetida*)	DIN EN ISO 17512-1 (2020), corresponds to ISO 17512-1 (2008a)	48 h	5	5 (5)

[a] A draft standard for an algal growth test in microtiter plates was available at the start of the project (DIN 38412-59, 2021); this standard is now finalised (DIN 2022). [b] Update of the test guideline after the experimental work in the project has been completed. [c] With amendment ISO 11348-2:2007/Amd 1:2018. [c] To determine if the EC_{50} is ≤ or > the limit concentration of 10% waste, such a high number of dilutions is not necessary.

4 Sampling, sample preparation and ecotoxicological testing

In the present project, 10 waste samples from mirror entries should be evaluated using ecotoxicity tests to verify the biotest battery and – as far as possible – the test strategy for HP 14 classification suggested in the UBA recommendations.

4.1 Selection of waste types to be tested

It was aimed to investigate at least two samples per waste type, which should originate from different sources, ideally at least one sample from the hazardous and non-hazardous mirror entry, respectively. When selecting the waste samples to be tested, experience from previous projects (mainly Moser & Römbke 2009, Römbke et al. 2009, Römbke 2018) and suggestions of UBA, BMUV and the project advisory group were considered.

The waste types were selected considering the following aspects:

- The waste should be relevant in terms of annual volume and distribution in Germany and Europe (e.g. based on number and location of disposal facilities for the respective waste type).
- There should be access to the hazardous and non-hazardous mirror entry.
- There should be no concerns regarding technical problems during sampling.
- In the present project, chemical-analytical studies could not be performed. Therefore, waste types should be investigated, for which chemical-analytical data are available (ABANDA database) or could be made available by the waste suppliers (ideally for a period of 2-3 years).
- Waste types with a high content of organic matter (e.g. sewage sludge and compost) were excluded. They cannot be investigated in biotests, because their nutrient content and, thus, oxygen consumption is high.
- It is technically difficult to investigate liquid and aqueous waste (e.g. landfill leakage) in terrestrial tests (drying is not possible within 24 hours). Therefore, such waste was excluded.

Based on suggestions from UBA, BMUV, the project advisory group and the project team, four waste types (mirror entries) were selected at the project meeting on 10 February 2022: flue-gas dust (10 09 09*/10 09 10) from iron and steel casting, soil and stones (17 05 03*/17 05 04), fluff-light fraction and dust (19 10 03*/19 10 04), and wood (19 12 06*/19 12 07) from waste treatment plants. These suggestions were discussed at a meeting with project advisory group on 09 March 2022. The advisory group proposed to focus on waste that is classified as hazardous based on the criterion HP 14. However, to our knowledge, the corresponding data are not publicly available. With regard to wood (19 12 06*/19 12 07) from waste treatment plants, UBA, BMUV and the project advisory group had reservations due to possible overlaps with the Waste Wood Ordinance (AltholzV 2020). Therefore, this waste type was not investigated in the project. Table 17 gives an overview of the selected mirror entries. During the course of the project, the selection had to be partially modified (see section 4.2.7).

Table 17: Selected waste types (mirror entries)

Waste code	Waste type	Remark
10 09 09*/ 10 09 10	Flue-gas dust (from iron and steel casting)	Contains eluable heavy metals (possibly high concentrations).
17 05 03*/ 17 05 04	Soil and stones	Mass waste with considerable measurement uncertainty. Often hazardous according to the calculation method. For the project team, material from the side verges of roads (e.g. contaminated by tire abrasion) were of particular interest.
19 10 03*/ 19 10 04	Fluff-light fraction and dust	Mass waste, very heterogeneous composition, sample pre-treatment can be demanding. Various sources available (material is process specific).

4.1.1 Flue-gas dust (10 09 09*/10 09 10) from iron and steel casting

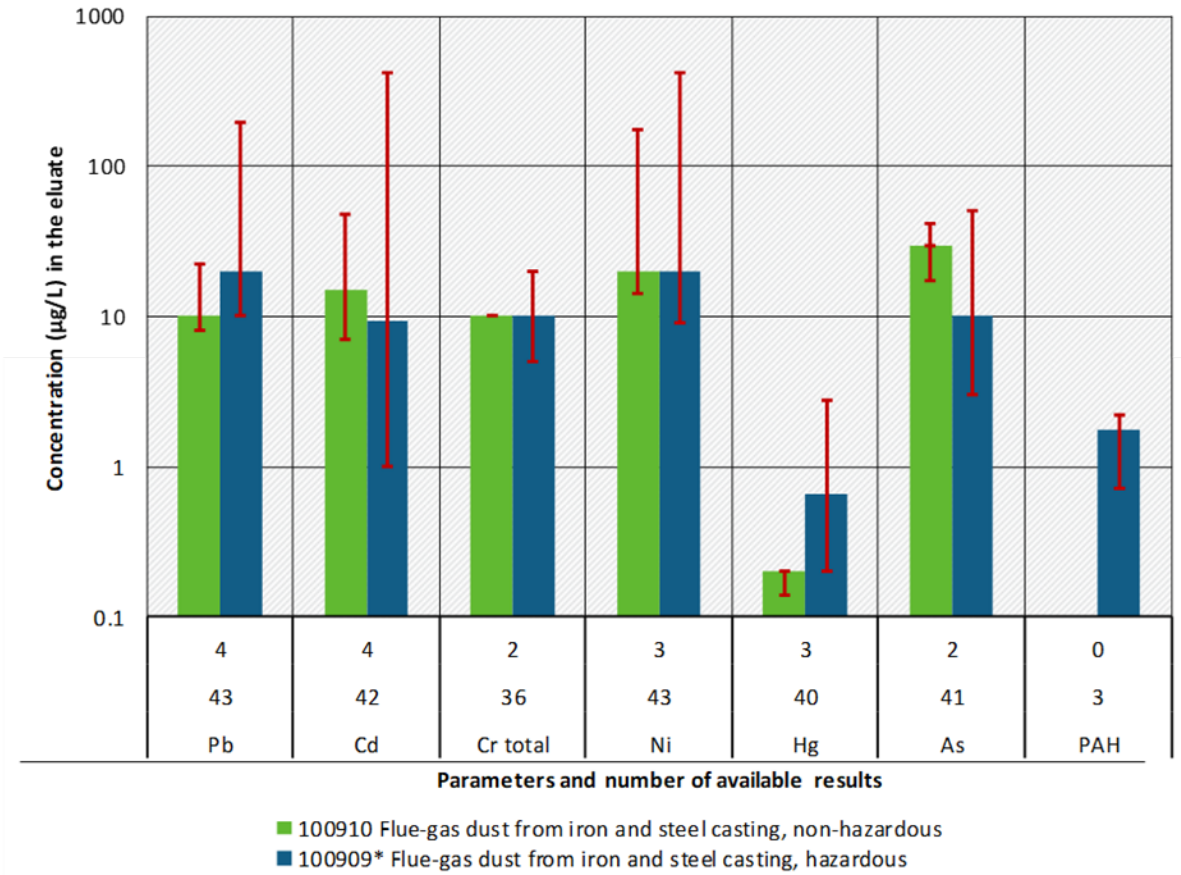
According to the Federal Statistical Office (Destatis 2022), a total of 62,400 t of flue-gas dust from iron and steel casting were delivered to waste disposal plants in 2019. The waste code 10 09 09* accounted for 12% (7.400 t). The material reached 24 different plants: seven landfills, five chemical-physical plants and twelve other plants. In the same year, 55,000 t of flue-gas dust with the waste code 10 09 10 were delivered to waste disposal plants, 34% of which (18,700 tons) left the facilities as waste for recycling.

Available analysis results for the waste codes 10 09 09*/10 09 10 were obtained in November 2021 from the ABANDA database, which was developed as an auxiliary tool for waste assessment. It collects, organises, and stores available information on the origin, fate and composition of waste. The results available in ABANDA for 10 09 09*/10 09 10 waste are summarized in Figure 5 and Figure 6; the type of presentation is explained below:

- Against the background of the relevant hazardous substances, the concentrations of lead (Pb), cadmium (Cd), total chromium (Cr), nickel (Ni), mercury (Hg), arsenic (As) and polycyclic aromatic hydrocarbons (PAHs) is shown for the hazardous and non-hazardous mirror entry.
- The number of available analyses is indicated in the legend of the x-axis (above: the number of analyses for the non-hazardous mirror entry, below the number of analyses for the hazardous mirror entry).
- The median values of the available data for the non-hazardous waste are shown as green bars, while the blue bars represent the median values for the hazardous waste.
- The fluctuation ranges shown in red cover the range from the 20th to the 80th percentile.
- The scaling of the y-axis is logarithmic for reasons of clarity.

The following figures first show the data for the eluate analyses and then those for the analyses of the solid waste.

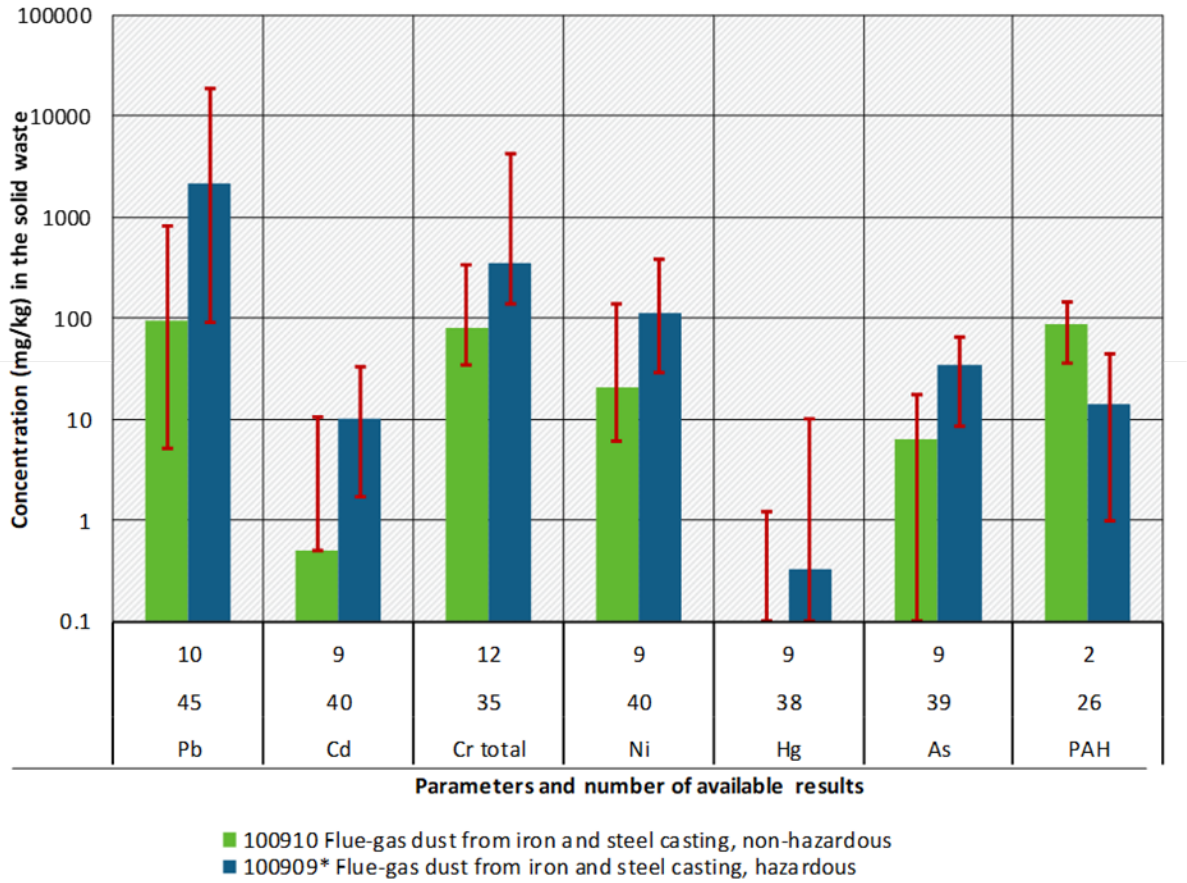
Figure 5: ABANDA data on analyses of eluates for flue-gas dust (10 09 09*/10 09 10) from iron and steel casting



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

Concerning the analysis data for the eluates, it is striking that only very few studies are available for the non-hazardous mirror entry. The concentrations in eluates in the hazardous and non-hazardous mirror entry are of a comparable order of magnitude.

Figure 6: ABANDA data on analyses of solid waste for flue-gas dust (10 09 09*/10 09 10)



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

With regard to the analyses of the solid waste, the classification as hazardous appears to be related to significantly higher levels of heavy metals, in particular, lead, while polycyclic aromatic hydrocarbons apparently play a minor role.

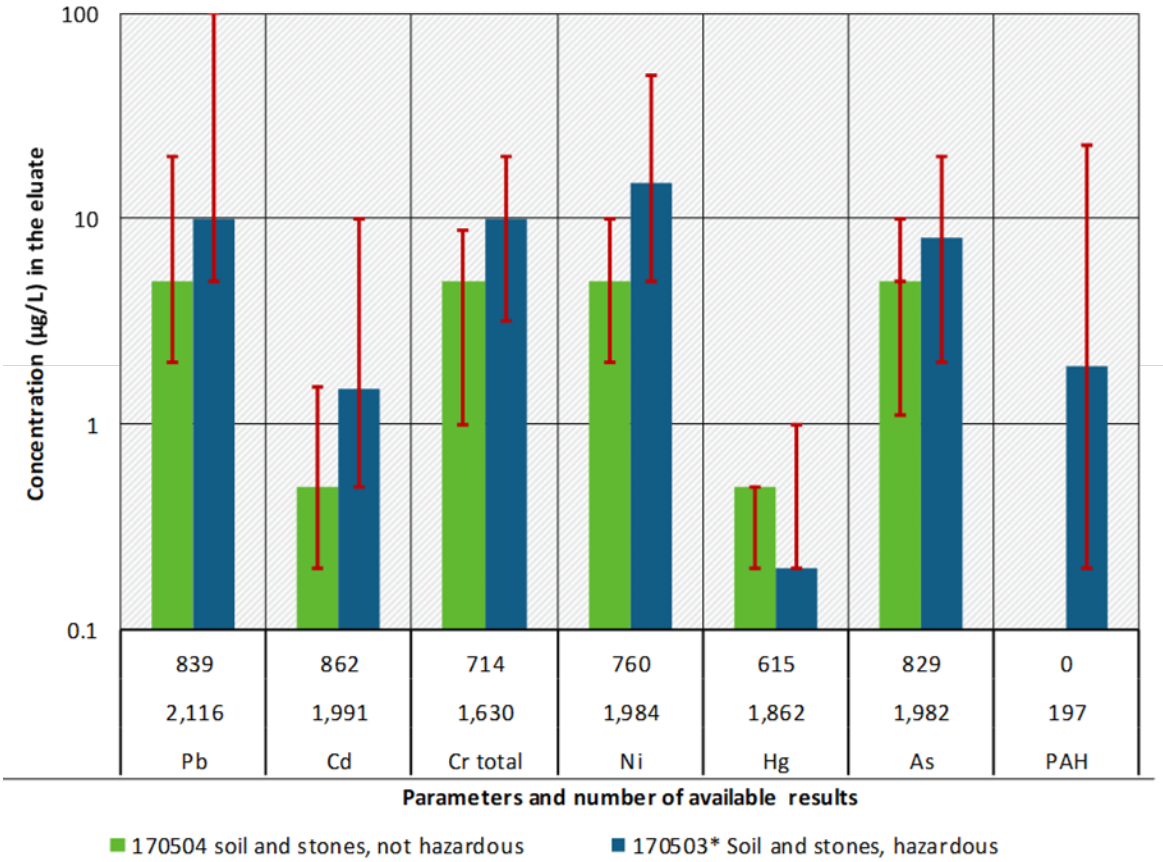
4.1.2 Soil and stones (17 05 03*/17 05 04)

There is a large number of treatment and processing plants for soil and stones. In the net balance (input – output) for all treatment plants (excluding surface mining sites[33]), the data for 2019 show a total flow of 20.4 million t of soil, of which more than 90% is deposited in landfills. Of the soil deposited in landfills, 6.5% (1.2 million t) is classified as hazardous.

Due to the high amounts of this waste, the ABANDA database contains many data (see Figure 7 and Figure 8).

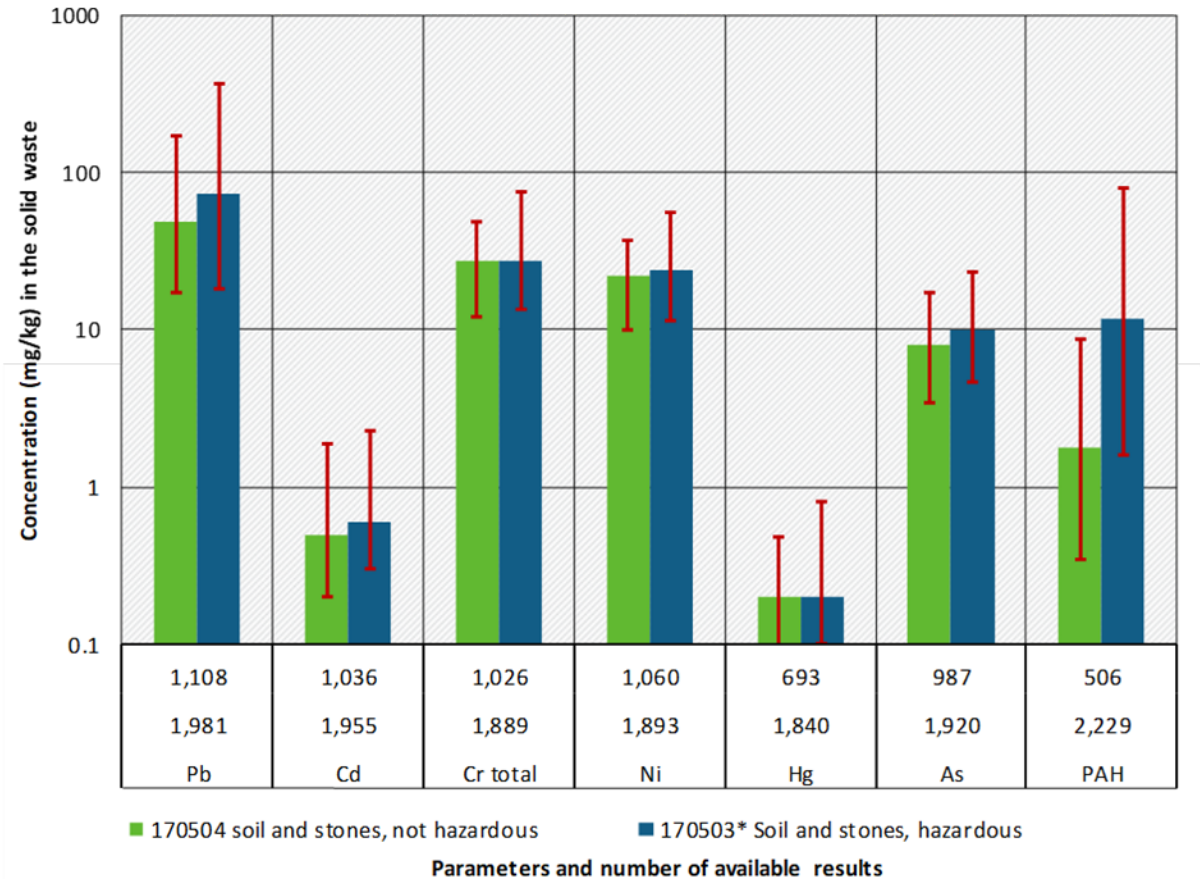
[33] Surface mining sites are open pits, from which raw materials (e.g. sand, gravel, lignite) are or were extracted (Statistisches Landesamt, Freistaat Sachsen, 2013).

Figure 7: ABANDA data on analyses of eluates for soil and stones (17 05 03*/17 05 04)



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

Figure 8: ABANDA data on analyses of solid waste for soil and stones (17 05 03/17 05 04)



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

The available analytical data for the solid waste show little differences in heavy metal concentrations between hazardous and non-hazardous soils. However, in hazardous soils the levels of PAHs are considerably higher.

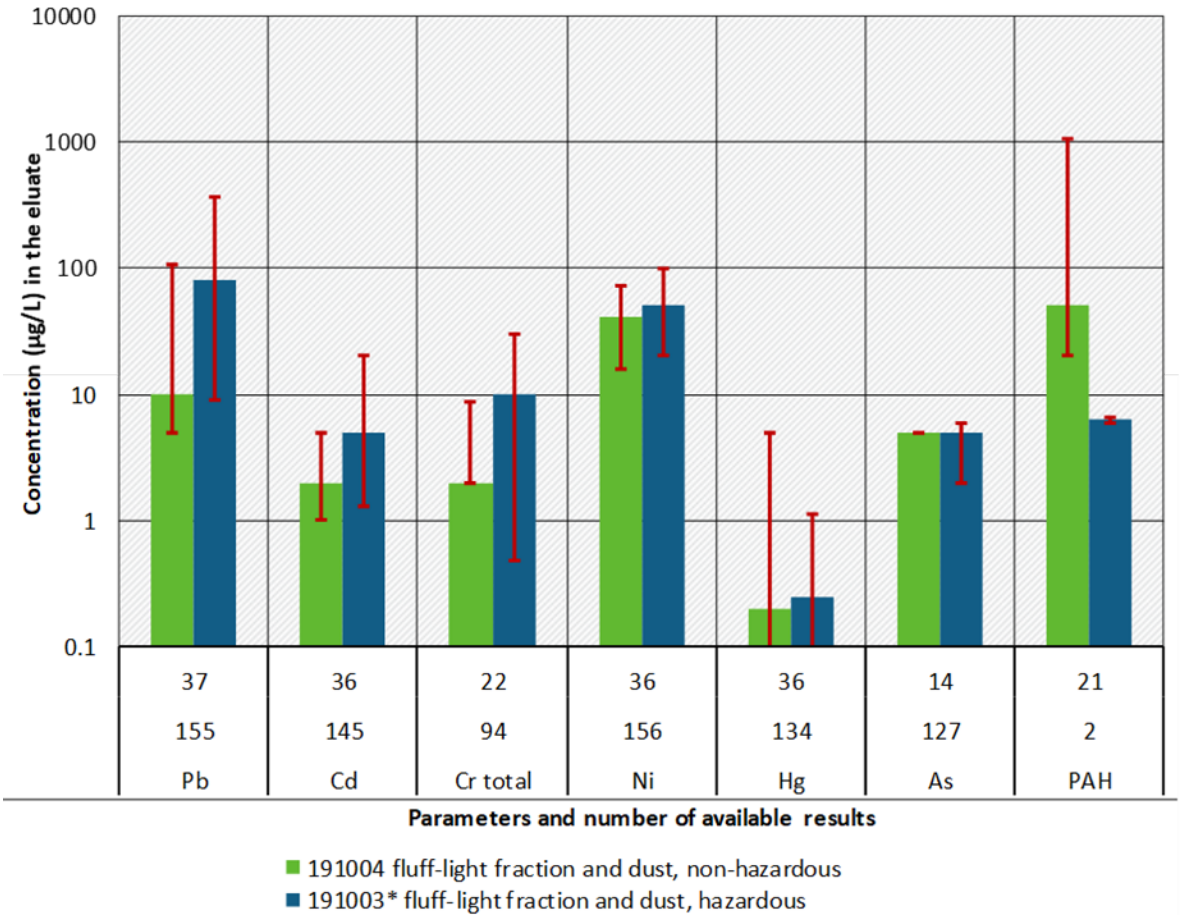
4.1.3 Fluff-light fractions and dust (19 10 03*/19 10 04)

Metal shredders are used for the mechanical processing of waste containing iron and aluminium and increasingly also for electrical waste. Fluff-light fractions are materials won by air separation[34] after shredding. Fluff-light fractions contain about 30% plastics and, additionally, rubber, wood, textiles, glass, and metals, such as those used in automotive engineering and electrical devices.

A reliable estimate of the volume of fluff-light fractions cannot be derived from the data obtained from the Federal Statistical Office. Expert estimates indicate that around 600,000 t of fluff-light fractions are generated annually, which pass through a wide variety of processing and recycling routes (Flamme 2019). Each year, around 15,000 t of fluff-light waste end up in waste incineration plants. Around 40% of this waste are classified as hazardous. Further 150,000 t per year, of which 23% are classified as hazardous, enter other facilities.

[34] Air separation is a mechanical separation process in which particles are sorted based on the ratio of mass force and flow resistance in a gas flow.

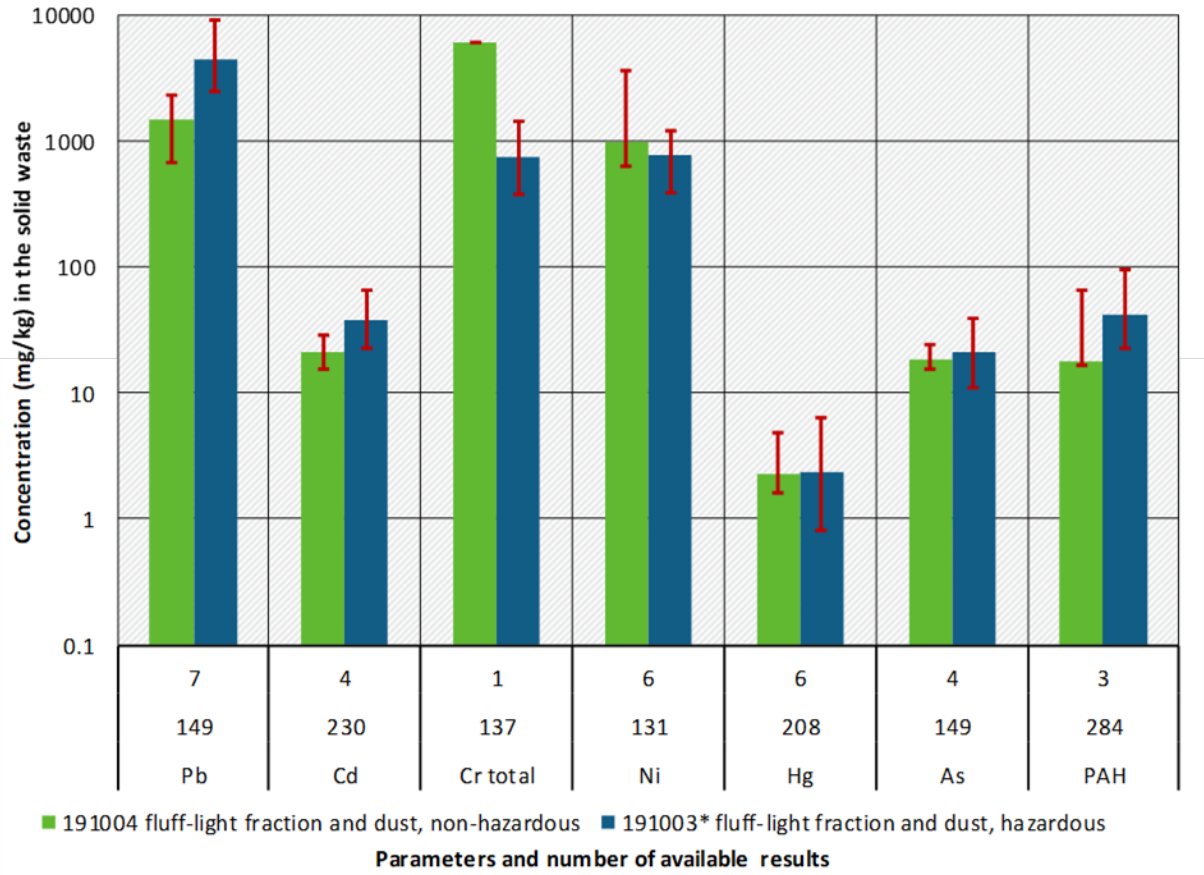
Figure 9: ABANDA data on analyses of eluates for fluff-light fractions and dust (19 10 03*/ 19 10 04)



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

The eluates show slightly increased concentrations of heavy metals for the waste classified as hazardous. However, measured concentration ranges in hazardous and non-hazardous wastes are overlapping. For PAHs, only 2 analyses are available for the hazardous waste (Figure 9).

Figure 10: ABANDA data on analyses of solid waste for fluff-light fractions and dust (19 10 03* /19 10 04)



Green bars: median values of the available data for the non-hazardous waste; blue bars: median values for the hazardous waste. Labelling of the x-axis, top: number of analyses for the non-hazardous mirror entry, below: number of analyses for the hazardous mirror entry. Red bars: fluctuation ranges (20th to 80th percentile).
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on ABANDA 11/21

Fluff-light fractions classified as hazardous are characterised by elevated PAH levels. Regarding heavy metal contents, the 80th percentiles for the elements lead, chromium and nickel in these fluff-light fractions (19 10 03*) exceed 1000 mg/kg. Results of chemical analyses of fluff-light fractions not classified as hazardous also reach this order of magnitude, but the number of available analyses is very low (see Figure 10).

4.2 Sampling and sample preparation

The present project aims to comply with European guidance on sampling (CEN/TR 15310, EN 14735) and with the requirements of LAGA guideline PN 98, which is mainly applied in Germany. With regard to sampling and sample pre-treatment, the aspects discussed below are essential for obtaining reproducible results (see also Ketelhut 2013).

The results of chemical-analytical measurements of the characteristic's content and of biotests are the basis for decisions regarding the further utilisation or treatment of waste. Therefore, test results must be sufficiently reliable. The measurement results obtained with the samples should therefore be reproducible within acceptable error limits.

Each measurement in heterogeneous materials requires the definition of the objective and the population. A population is the material that can be assumed with sufficient certainty as uniform regarding the waste code, its origin and its essential characteristics. A population can be

- a material flow,
- a heap, or
- another deposit

For waste with identical origin, identical waste code and identical batch, a certain uniformity can be assumed. This uniformity should be reproducibly reflected in the waste analyses. In case of doubts regarding uniformity, the waste material has to be separated into several populations (this must be justified in a comprehensive way). The maximum size of a population has to be determined by an expert in the sampling plan (EU 2018, EN 14899) against the background of the available information and framework conditions. According to CEN/TR 15310-1 (CEN 2006a), the sampling plan should cover the following technical objectives:

- Definition of the population
- Description of occurring variances
- Decision on a sampling strategy
- Scaling of the occurring heterogeneity
- Estimation of reliability and confidence interval

The basis of a reliable study is the collection of random samples. A random sample is a subset of the population that gives each particle of the population the same chance of becoming part of the sample (CEN 2006a). In the case of material flows, the individual samples must be randomly distributed in terms of time. In the case of stationary masses of waste, the individual samples must be randomly distributed in terms of space. For sampling to determine the mean content of characteristics and their effects, it is useful to distinguish between two types of heterogeneity (CEN 2006a):

1. temporal-spatial heterogeneity and
2. particulate heterogeneity.

4.2.1 Consideration of temporal-spatial heterogeneity

Temporal-spatial heterogeneity is a mixture heterogeneity. It describes fluctuations in the composition of a material mixture consisting of different constituents that are, however, identical or at least comparable regarding their composition. This can, e.g. be individual batches of a waste, which are comparable in terms of the constituents they contain but differ in the proportions of these constituents depending on the batch. In the case of technically generated material flows from continuously operating plants, the dosing of partial streams or short-term technical defects, for example, can have an impact on the waste composition. This temporal-spatial heterogeneity can affect the content of characteristics in random samples.

In relation to the population, this heterogeneity can be eliminated, for instance, by mixing a waste heap so that only a random difference in characteristics can be measured in sufficiently large composite samples. If an average content of characteristics shall be determined in a defined population, a sufficient number of random samples has to be taken.

4.2.2 Consideration of particulate heterogeneity

Particulate heterogeneity is the heterogeneity of the content of characteristics of the individual particles that form the population. It cannot be reduced by mixing, since the specific load of the characteristics is directly linked to the individual particles. If the sample contains the particle, its entire load (weight multiplied by content of the characteristics) is included in the measured value. The contents of characteristics of individual particles are generally not randomly distributed. For natural material, they are geologically or biologically defined. For synthetic materials, they are more or less defined depending on the specifics of the production process.

Since the particulate heterogeneity cannot be reduced by mixing, samples must always be sufficiently large. The load of characteristics, which is introduced into the sample by a single particle, should not significantly affect the sum of the loads of characteristics of all particles. In practice, it has proven useful to determine the sample size based on the load of characteristics of particles that are potentially carrying a certain characteristic.

The load of characteristics in a mixed sample is the sum of the loads of all particles in this sample. Therefore, the average weight of particles carrying this load relative to the average weight of all particles is important. For example, metals generally carry disproportionately high loads due to their high density. To ensure that the load of characteristics is not influenced by the random weight of individual particles with certain characteristics, a sample should always contain a sufficient number of particles with the relevant characteristics.

4.2.3 Preliminary considerations for sampling

The aim of sampling is to produce a random sample, i.e. to give each particle in the population an (as much as possible) identical chance of becoming part of the sample. Ideally, samples are taken from the falling material flow at the discharge point of a conveyor belt. The random samples must cover the entire period in which the population is produced. The sampling times should be scattered randomly over this period. A periodical timing is not recommended to avoid the risk of running parallel to any periodical timing of the system.

If it is not possible to take a sample from the falling material flow, the sample must be taken from a waste heap or other deposit. Since it is generally not known to what extent material segregation has taken place during the formation of the heap, it is recommended to avoid taking samples directly from the heap. The following options are available for obtaining random samples from stationary materials:

Deposits

- Mapping in a three-dimensional coordinate system,
- Determination of the coordinates for the individual samples from random numbers for each dimension.

Waste heaps

To counteract possible segregation due to the formation of heaps, it is recommended to work with wheel loaders and large masses. The wheel loader is used to collect the individual samples capturing the overall dimensions of the heap. The more heterogeneous the material is in terms of dimensions, the greater the risk of segregation and the more effort should be put into homogenisation.

To sample smaller waste heaps, a wheel loader can be used to convert the three-dimensional heap into a flat (close to two-dimensional) structure with a height, which allows samples to be

taken at random points using a suitable sampling device (e.g. sampling sleeves/sampling pipes). The heap is thoroughly mixed on a suitable surface using a wheel loader and then spread out by driving backwards with the shovel lowered to the target height. On the resulting area (e.g. 3 x 10 m), points can then be selected at random and sampled over the entire height of the carpet using suitably sized sampling sleeves/sampling pipes.

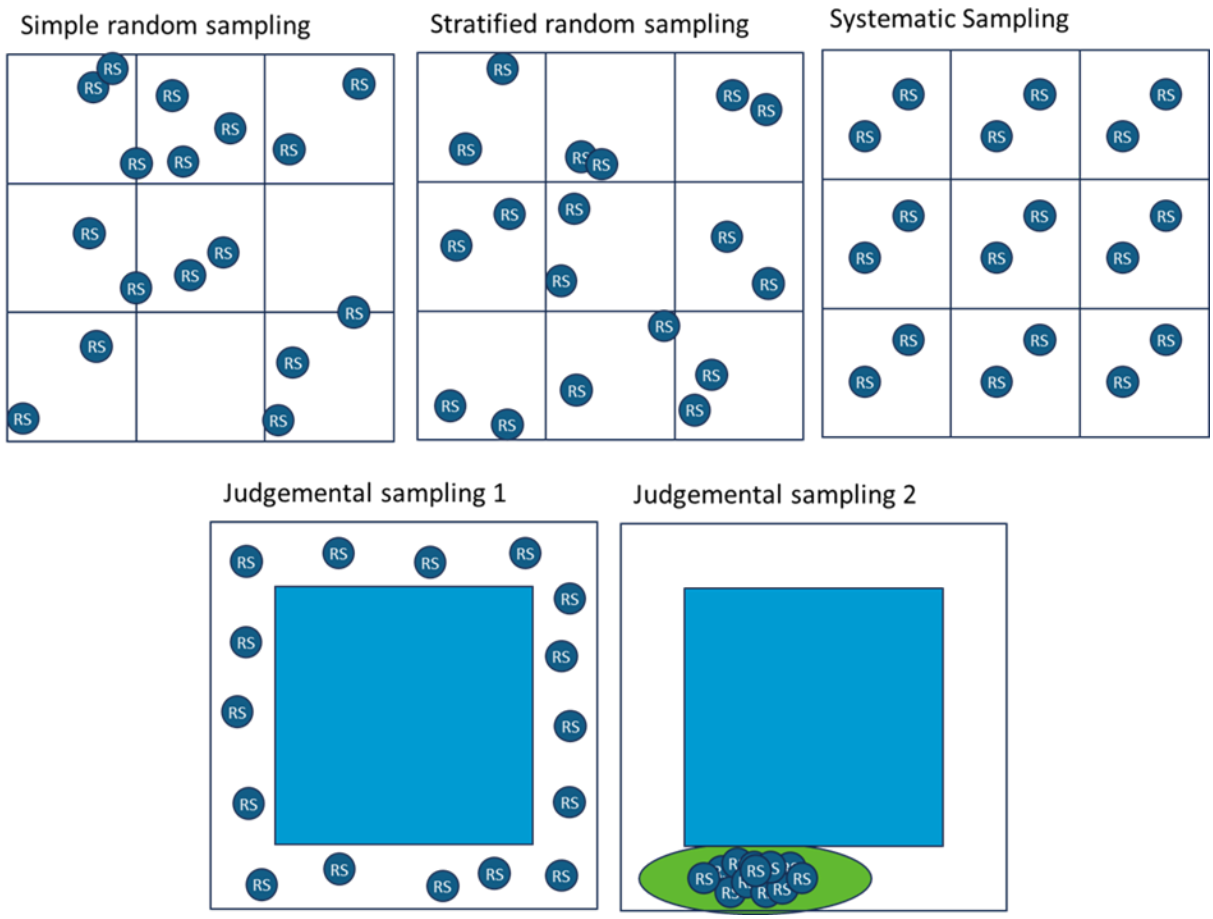
For larger waste heaps, sampling is more difficult. If it is possible to create vertical breaklines, a single sample of around 200 to 500 dm^3 can be obtained from a breakline by moving the shovel from a wheel loader from the bottom upwards. At least 16 samples obtained in this way are combined and mixed on a suitable surface. The mixed sample can then be spread out to obtain a flat structure and sampled using e.g. sampling sleeves/sampling pipes (again, at least 16 individual samples are taken). Alternatively, the mixed sample can also be transferred to a conveyor belt so that samples can be taken from the falling flow.

Which approach is ultimately chosen depends on the objective, the specific framework conditions and the technical possibilities for sampling. It is important to define and document the chosen sampling strategy. According to CEN/TR 15310-1, there are the sampling variants shown in Table 18 and Figure 11.

Table 18: Overview of sampling strategies according to CEN/TR 15310-1 (CEN 2006a)

Designation	Description
Simple random sample	Individual samples are completely randomly distributed across the population.
Stratified random sampling	Individual samples are randomly distributed in predefined temporal/spatial segments of the population.
Systematic sampling	Individual samples are evenly spaced across predefined temporal/spatial segments of the population.
Judgmental sampling (1)	Random sampling from a temporally/spatially restricted area.
Judgmental sampling (2)	Random sampling from a strongly temporally/spatially restricted area.

Figure 11: Sampling strategies according to CEN/TR 15310-1



RS: Random sample.

Source: own illustration, Ralf Ketelhut, Stoffstromdesign, based on CEN/TR 15310-1 (CEN 2006a)

From the presentation of the sampling strategies, it becomes clear that the requirement to give each particle an identical chance of becoming part of the sample is increasingly restricted. Depending on the framework conditions encountered, a decision on the sampling strategy must be taken and justified.

4.2.4 Preliminary considerations on the size and number of random samples

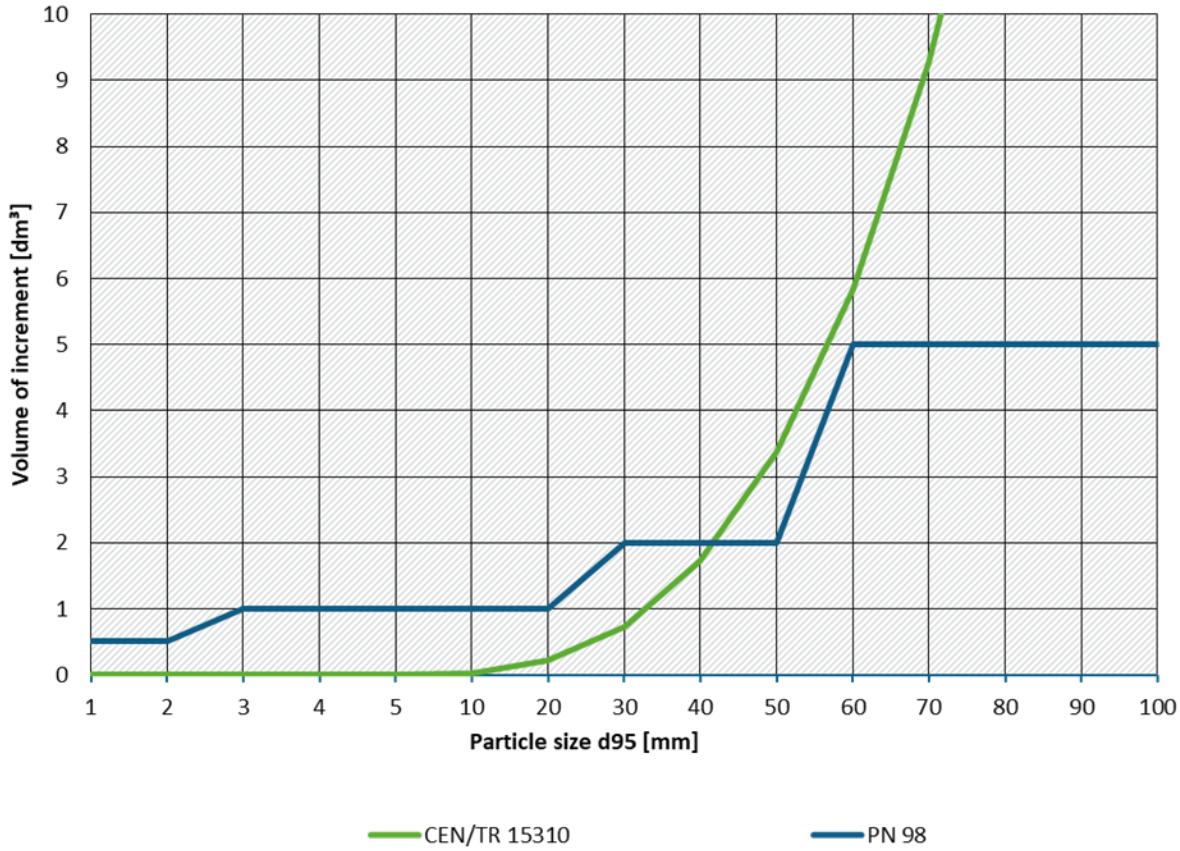
The size and number of random samples and their minimum sample mass are directly related to the particulate and temporal/spatial heterogeneity (CEN/TR 15310-1, Annex D). Regarding the collection of random samples, CEN/TR 15310-1 contains the following specifications:

- For particulate sizes d_{95} ≤3 mm, the sampling device should have a size of at least 10 mm in all spatial dimensions.
- For particle sizes d_{95} >3 mm, the sampling device should have at least 3 times the diameter of the largest particle.

According to CEN/TR 15310- 1, the minimum mass of a random sample (increment) is determined depending on the bulk density (ρ_B) and particle size (d_{95}) of the material. The minimum mass of a single sample should be at least 50 times higher than that of a spherical particle with the diameter of d_{95}.

According to LAGA PN 98, the minimum volume of a random sample is also based on the particle size of the material. However, the minimum volume is not determined directly by calculation, but based on a volume-proportional approach (LAGA 2019). Using the bulk density of the material, both approaches can be compared (Figure 12).

Figure 12: Comparison of the minimum volume of a random sample according to CEN/TR 15310-1 and LAGA PN 98



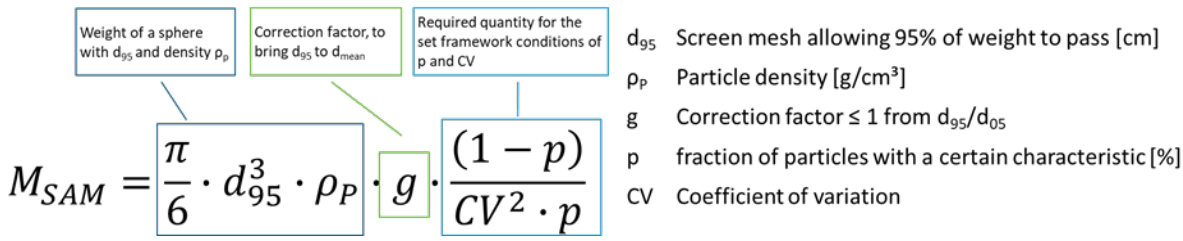
Source: own illustration, Ralf Ketelhut, Stoffstromdesign, based on CEN/TR 15310-1 (CEN 2006a) and LAGA PN 98 (2019)

Both approaches result in approximately comparable minimum volumes for particle sizes between 20 and 60 mm. For particle sizes <20 mm, the minimum volumes according to LAGA PN 98 are significantly higher than those according to CEN/TR 15310-1; for particle sizes >60 mm, the reverse is true. According to LAGA PN 98, particles with a size >120 mm should be analysed as individual samples or treated by sorting analysis in accordance with the recommendations of LfULG (2014).

4.2.5 Preliminary considerations regarding the sample mass

In the 'Theory of sampling' according to Pierre Gy, a desired coefficient of variation ('fundamental error') is defined. A minimum sample mass can be estimated using this coefficient of variation and information on particle dimensions, particle density, weight distribution and the assumed fraction of particles with certain characteristics (CEN/TR 15310-1; see Figure 13).

Figure 13: Formula for determining the minimum sample mass according to CEN/TR 15310-1



Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on CEN/TR 15310-1 (CEN 2006a)

The coefficient of variation defines the targeted reliability for the measurement. Generally, a coefficient of variation of 10% is used. The fraction of particles with a certain characteristic (p) indicates how the load of characteristics is distributed among the particles in the population. If the fraction of particles with a certain characteristic is not known, a value of 10% is commonly used. In the standard case that a characteristic carried by 10% of the particles shall be measured with a confidence level of ±20% (twice the standard deviation), the minimum number of particles required in a sample is 900 according to the formula given above. If a coefficient of variation of 5% (i.e. a statistical measurement error of ±10%) is desired, 1900 particles are required for an identical fraction of particles with a certain characteristic.

The estimated value for the mean sample mass (M_{SAM}) shown in the left part of the formula is based on the weight of a sphere with the same density as the particles in the sample. Since the d_{95} overestimates the mean particle dimension, the factor g is used to scale down the value of d_{95} to d_{mean}. The value for g can reach a maximum value of 1. An estimate for the minimum sample mass is derived from the estimated values for the number of particles required (against the background of the desired reliability) and the mean particle weight.

From the fact that the particle dimension is included in the formula to the third power, it is clear that particle size has a very large influence on the sample mass. For material with a particle density of 1 g/cm³ and a d_{95} of 1 cm, 118 g of sample mass is required according to the formula. When the material has a d_{95} of 2 cm, an almost eight-fold amount is required (942 g). When the material is crushed or sieved to a d_{95} of 0.4 cm, the minimum sample mass is only 15 g.

The particle dimension of centimetres (instead of millimetres) for the d_{95} used in the formula has the advantage that no conversion factor is required. The specification of the particle density in g/cm³ is identical to the value in kg/dm³ (i.e. kg/L or Mg/m³).

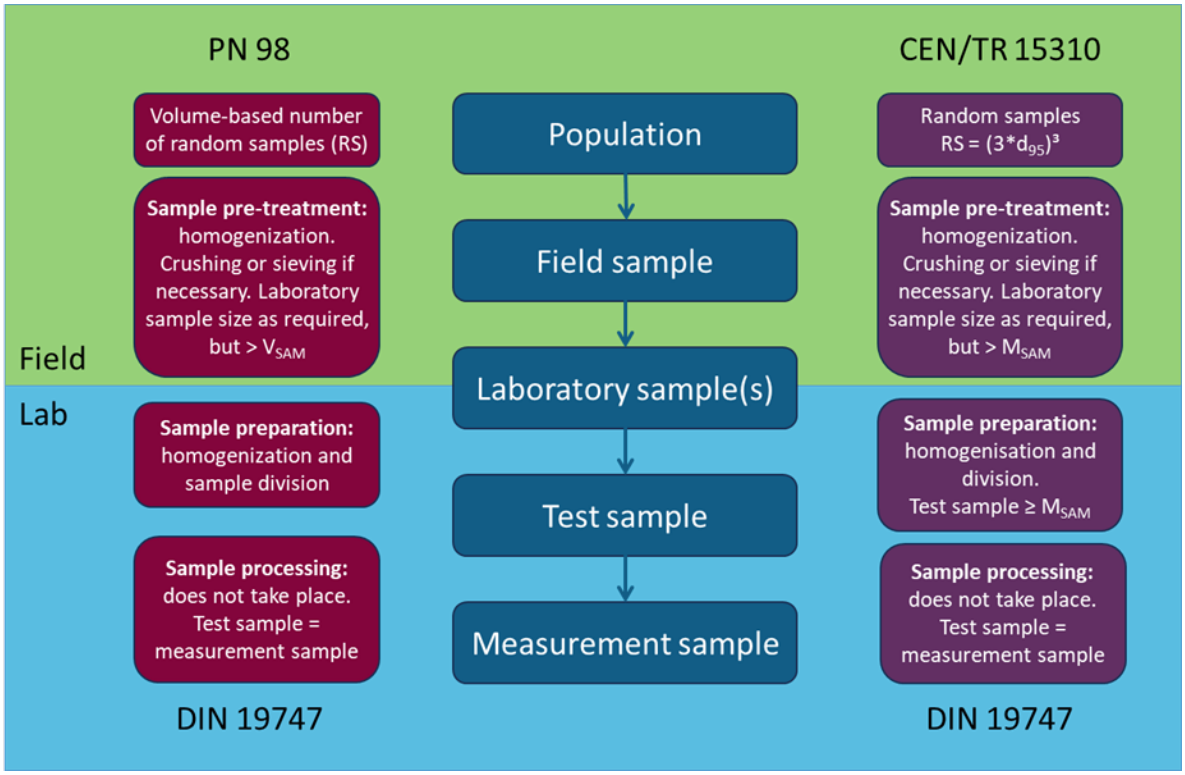
4.2.6 Preliminary considerations on sample pre-treatment, sample preparation and sample processing

The framework conditions for sample pre-treatment (i.e. the preparation of the laboratory sample(s) from the field sample), sample preparation (producing the test sample) and sample processing (producing the measurement sample) are specified by DIN 19747 (2009a) and PN 98 (LAGA 2019). Both guidelines contain a table with identical values for the minimum volume of the laboratory sample based on particle size. The larger the particles contained, the more sample mass is required (see above and Figure 12).

DIN 19747 (2009a) aims at achieving comparable, correct and reproducible results taking the properties of different waste types into account. Physico-chemical and biological analyses are explicitly addressed.

Figure 14 shows the procedure according to DIN 19747 in connection with LAGA PN 98 and CEN/TR 15310 and describes sample pre-treatment, sample preparation and sample processing for biological analyses.

Figure 14: Sample pre-treatment, preparation and processing for biological analyses



M_{SAM}: minimum sample mass, RS: random sample(s), V_{SAM}: minimum sample volume.
Source: own illustration, Ralf Ketelhut Stoffstromdesign, based on CEN/TR 15310-1 (CEN 2006a), DIN 19747 (DIN 2009a) and PN 98 (LAGA 2019)

Sample pre-treatment includes the mixing of random samples, removal of interfering materials where necessary, sieving to a particle size of ≤4 mm and, crushing/shredding of oversized particles.

In LAGA PN 98, it is generally assumed that the sample mass is larger than required for the analyses (including reserve samples). However, this is not the case for biological tests. For biotesting, the required sample mass depends on the used test systems. In most cases, it significantly exceeds the minimum sample size according to PN 98.

In DIN 19747 (2009a), it is also assumed that the mass of the laboratory sample is generally larger than the required mass of the test samples including the reserve samples. The authors of DIN 19747 are aware that sample splitting into the test sample leads to a stochastic error, but they hope to be able to control this error by homogenising (mixing) the samples before division. Here, information would be helpful on how far a sample can be divided without exceeding specified error limits.

There are clear indications that dividing the laboratory sample without prior crushing/ shredding can introduce large uncertainties into the test sample (Ketelhut 2013). The minimum sample mass according to CEN/TR 15310-1 can be helpful for a first estimation of the resulting error.

4.2.7 Sampling and sample pre-treatment for the selected waste types

During the project, the selection of waste types to be analysed (section 4.1) had to be slightly modified and sample pre-treatment had to be adapted. As mentioned in section 4.1, at least one sample of the hazardous and the non-hazardous mirror entry, respectively, should be analysed for each waste type. In this context, it should be pointed out that classification of a waste as hazardous or non-hazardous mirror entry is not necessarily based on the HP 14 criterion. The project team did not receive information on the background for classification of the sampled waste by the waste owners.

Table 19 provides an overview of the waste that was sampled and evaluated in ecotoxicological tests. In the following sections, sampling is briefly described. Details for the individual waste samples can be found in the sampling protocols.

Table 19: Waste sampled and evaluated in ecotoxicological tests during the project

Waste code	Waste type	Description of waste	Remark	Sampling
10 09 09*	Flue-gas dust	Flue-gas dust from iron and steel casting, batch 1	Aged material (storage period >4 weeks)	July 2022
		Flue-gas dust from iron and steel casting, batch 2	Fresh material (storage period <4 weeks)	July 2022
10 09 10		Flue-gas dust from iron and steel casting, plant A	—	June 2022
		Flue-gas dust from iron and steel casting, plant B	—	October 2022
17 05 03*	Soil and stones	Excavated geogenic material	Nickel-containing waste, open-cast lignite mine	June 2022
		Material from the side verges of roads	Federal road	October 2022
17 05 04			Secondary road	May 2022
19 10 04	Fluff-light fraction and dust	Fluff-light fraction and dust sieved to <10 mm	Plant A, batch 1	May 2022
			Plant A, batch 2	February 2023
			Plant B	February 2023

4.2.7.1 Flue-gas dust (10 09 09*/10 09 10) from iron and steel casting

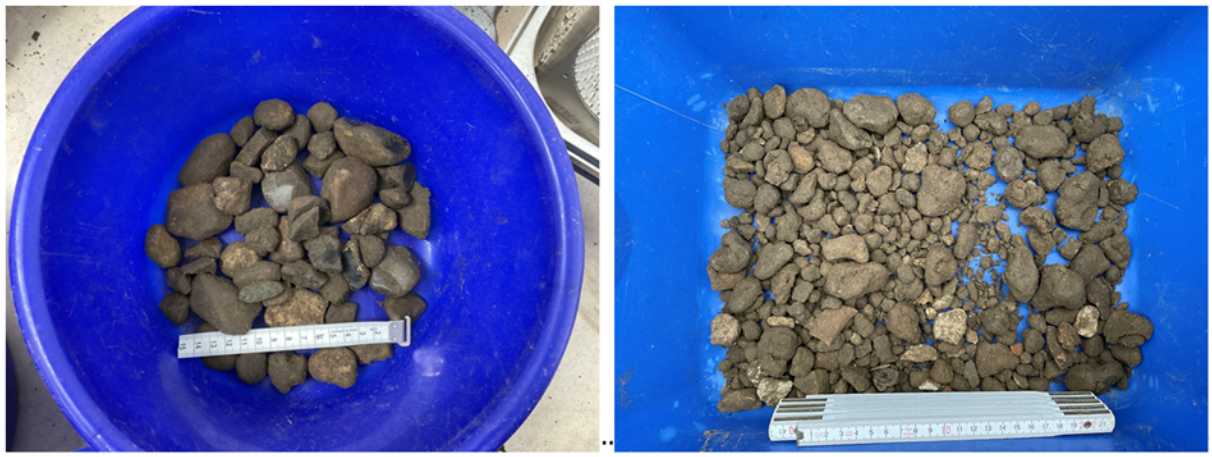
The evaluated flue-gas dusts (waste code 10 09 09*/10 09 10) from iron and steel casting were in big bags that had been filled continuously with trickling dust. For waste code 10 09 09*, it had originally been planned to examine waste from two production methods (grey cast iron and lamellar cast iron). As the planned separation of flue-gas dusts from these two production methods prior to sampling could not be realised, the following samples were taken instead:

- Aged material with a storage period >4 weeks (batch 1) and
- Fresh material with a storage period <4 weeks (batch 2).

Both samples contained a mixture of flue-gas dust from grey and lamellar cast iron. Due to separate storage locations, a clear distinction between aged and fresh material was possible. The

material was very fine-grained (d_{95} <1 mm). In individual samples with waste code 10 09 09*, some particles >4 mm were found that contained metallic components (Figure 15). If such particles became part of the sample analysed in the laboratory, they could have a significant influence on the test result.

Figure 15: Coarse particles from flue-gas dust (10 09 09*) from iron and steel casting



Coarse particles originating from approx. 5 kg of sampled material with waste code 10 09 09*
Source: own illustration, Ralf Ketelhut Stoffstromdesign

When sampling waste with the waste code 10 09 10 (i.e. the non-hazardous waste), material from two different foundries (plants A and B) was sampled as planned. Material from plant A originated from a shorter production period than material from plant B.

4.2.7.2 Soil and stones (17 05 03*/17 05 04)

The huge volumes of produced soil waste are assessed according to waste legislation on the one hand, and soil protection legislation on the other hand. Soil samples are generally characterised by a particle size spectrum exceeding a 4 mm particle size.

For this reason, sample preparation for the material from the side verges of a secondary road (17 05 04) was planned in such a way that oversized particles could be crushed with a jaw crusher. The crushing of a fraction of approx. 35% by weight of oversized particles (>4 mm) illustrates a fundamental dilemma when investigating heterogeneous material mixtures:

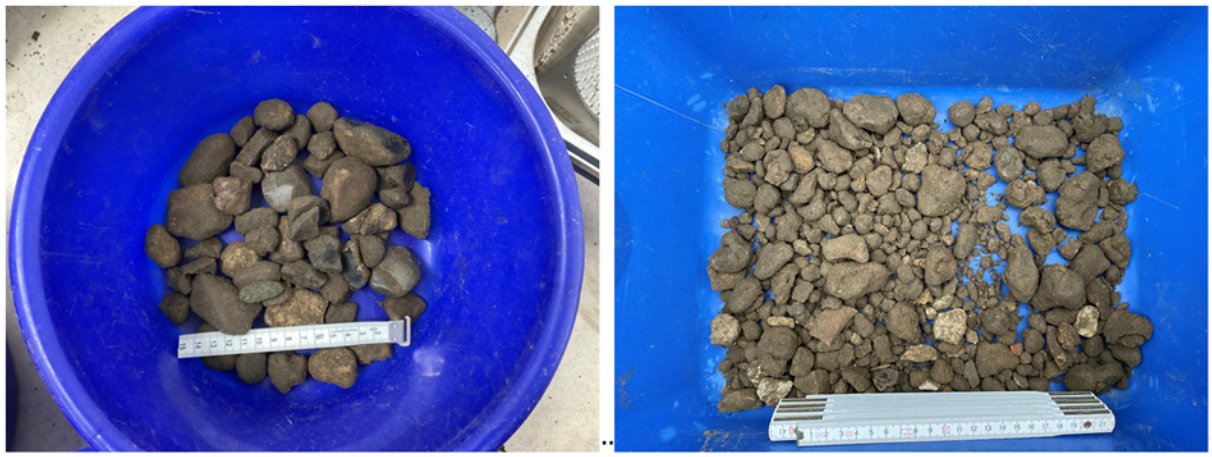
- In large particle size ranges, individual particles with a certain characteristic can introduce comparatively high loads of this characteristic into a sample.
- In sieve fractions with small particle numbers, this can lead to yes/no decisions regarding the presence of particles with a certain characteristic. This can result in the load of the respective characteristic being either over- or underestimated.
- Therefore, very large sample masses are required for a reproducible and reliable assessment of coarse fractions.

The second sampled waste from the mirror entry 'soil and stones' was excavated geogenic material from an open-cast lignite mine, which was of interest due to its low pH value in combination with the classification as 17 05 03*. The low content of oversized particles (approx. 5%) was favourable, because no jaw crusher was available on site.

The material from a federal road classified as 17 05 03* contained clay- or loam-like particles. Due to agglomeration, these particles could not be hand-sieved and remained in the fraction

with oversized particles. In the fraction >10 mm, the estimated clay/loam content was 84% by weight. This corresponds to a clay/loam content of approx. 15% in the total sample. The clay or loam was discarded with the oversized particles. For such material, pre-treatment with a jaw crusher is no option. It is not known to which extent particles potentially carrying a certain characteristic were removed with the clay/loam. According to the threshold values in the Federal Soil Protection and Contaminated Sites Ordinance, loamy and clayey soils contain higher concentrations of heavy metals than sandy soils (BBodSchV 2021, Annex 1, Table 1). Figure 16 illustrates the fractions of oversized particles in the sampled material from the side verges of roads.

Figure 16: Oversized particles in the processed samples from the side verges of roads (17 05 03*/17 05 04)



Large particles originating from 15 kg or 11 kg of sample material. Left: particles >20 mm from material from the side verges of a secondary road (17 05 04); right: particles >10 mm from material from the side verges of a federal road (17 05 03*). Source: own illustration, Ralf Ketelhut Stoffstromdesign

4.2.7.3 Fluff-light fraction and dust (19 10 03*/19 10 04)

Fluff-light fractions are an extreme example of a heterogeneous waste material that contains metals, glass and mineral components as well as wood, plastics and textiles.

In an earlier project for the UBA, fluff-light fractions with a d_{95} of 20 mm were analysed (see Römbke et al. 2010). To date, the processing of this material is more advanced. The waste fractions are separated by screening, and metals and other recyclable materials are largely removed by automatic sorting. During processing of the material, a waste sieved to a d_{95} of 10 mm is produced. This waste was sampled.

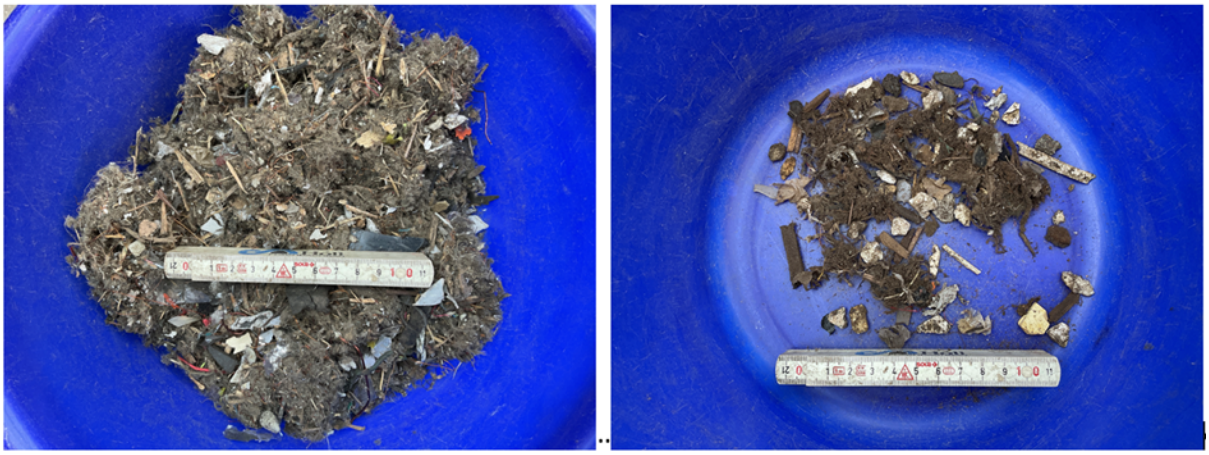
After having sampled fluff-light fraction and dust (19 10 04) in May 2022, an attempt was made to identify a source for fluff-light fraction and dust classified as hazardous (19 10 03*). However, the strong increase in energy prices due to the conflict in Ukraine had resulted in a significant reduction in the activities of the extremely energy-intensive shredder operations. Therefore, no 19 10 03* waste could be sampled. In consultation with the UBA, it was decided to analyse two further samples of fluff-light fraction and dust classified as non-hazardous (19 10 04), also in view of the high ecotoxicity of the first 19 10 04 sample (section 4.4.3). Samples were taken from the same plant as during the first sampling (plant A), and from a second plant (plant B). Sampling took place in February 2023. Again, the sieved fraction (<10 mm) was sampled. The objective was to clarify the following questions:

- Is the result of the initial analysis of the fluff-light fraction and dust (10 10 04) reproducible?

► Does material from another shredder plant have a similarly high toxicity?

The content of oversized particles (>4 mm) for plant A was approx. 15-20% by weight as during the first sampling. For plant B, it was similar (approx. 14% by weight). The oversized particles were very inhomogeneous, particularly due to metal particles and a high proportion of fluffy textile fibres. Crushing/shredding would have required cryogenic conditions. Figure 17 illustrates the heterogeneity of the analysed fluff-light fractions.

Figure 17: Fluff-light fraction and dust (19 10 04) from plant A (batch 2) and plant B



On the left: waste sample from plant A (batch 2); on the right: waste sample from plant B.
Source: own illustration, Ralf Ketelhut Stoffstromdesign

4.2.8 Elution of waste samples for testing with aquatic organisms

In the ecotoxicity tests with aquatic test organisms (luminescent bacteria, algae, daphnids), aqueous eluates of waste samples were used. The eluates were prepared according to DIN EN 12457-2 (2003a) and DIN EN 14735 (2022) in a one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h. Deionised water (conductivity <10 µS/cm) or ultrapure water (ISO 3696 grade 3 analytical reagent; conductivity ≤1 µS/cm; ISO 1987) was used as eluent[35]. The first three elutions were carried out with a waste dry weight of 50 g (17 05 04: material from the side verges of a secondary road, 19 10 04: fluff-light fraction and dust, plant A, batch 1, first elution) or 20 g (19 10 04: fluff-light fraction and dust, plant A, batch 1, second elution), all further elutions with a waste dry weight of 90±5 g. The waste wet weight corresponding to the above-mentioned dry weight was weighed into a 1-L wide-necked glass bottle, and the required amount of eluent was added. The vessel was capped and shaken 1-3 times overhead before being shaken at a speed of 10 rpm for 24±0.5 h at 20±2°C in an overhead shaker. Afterwards, suspended solids were allowed to settle for 15±5 min. The supernatant was decanted and filtered through a membrane filter (cellulose mixed ester, diameter: 47 mm, pore size: 0.45 µm) using a vacuum pump. If no separation of the liquid and solid phases had taken place after settling, an attempt was made to filter a subsample of the eluate. If a flow rate of at least 30 mL per cm^2 and h (cf. DIN EN 12457-2) was not achieved, the sample was centrifuged (30 min at 2000 g, at room temperature). Centrifugation was necessary for the flue-gas dust (10 09 10) from both plants, material from the side verges of both roads (17 03 03* and 17 05 04) and the fluff-light fractions and dust (19 10 04) from plant B. After filtration or centrifugation, conductivity and pH value were determined. Generally, one acute

[35] Deionised water was used for elution of the material from the side verges of a secondary road (17 05 04) and fluff-light fraction and dust (19 10 04, plant A, batch 1), ultrapure water (ISO 3696 grade 3) for the elution of all other waste samples.

Daphnia test, one algal growth inhibition test and one luminescent bacteria inhibition test were carried out with each eluate. Eluates were stored in the refrigerator for a maximum of 72 h as specified in DIN EN 14735 (2022).

4.3 Performance of the ecotoxicological tests

The selected waste types were analysed using the aquatic and terrestrial ecotoxicity tests listed in Table 16 (section 3.3.2), which the following modification. The algal growth inhibition test was carried out in 24-well microtiter plates based on DIN 38412-59 (draft, 2021) as proposed by the UBA at the 2nd project meeting [36].

4.3.1 Ecotoxicity tests with aquatic organisms

All waste samples were tested in the acute *Daphnia* test according to DIN EN ISO 6341 (2013a), the algal growth inhibition test in microtiter plates according to DIN 38412-59 (draft, 2021) and the luminescent bacteria test according to DIN EN ISO 11348-2 (2009), in 1–3 test runs.

4.3.1.1 General approach

For the aquatic tests, one eluate was first produced for each waste sample, and tested in a first test run at the following dilutions: 50%, 25%, 12.5%, 6.3% and 3.1% (all tests) as well as additionally 1.6%, 0.8% and 0.4% (luminescent bacteria test). If effects >50% occurred at all dilution levels, a second test run with higher dilution levels was performed to derive the EC_{50}. It was ensured that two dilution levels were tested both in the first and the second test run (e.g. 50, 25, 12.5, 6.3 and 3.1% in the first test run and 6.3, 3.1, 1.6, 0.8 and 0.4% in the second run).

The first test run for each waste sample was always performed without pH adjustment – as specified in UBA (2013) and DIN EN 14735 (2022) – even if pH in some or all dilution levels was outside the pH range specified in the respective test guideline as suitable for the test organisms (*Daphnia*: pH 6.0-9.0; algae and luminescent bacteria: pH 6.0-8.5). If toxicity occurred at dilution levels, where pH was outside the range tolerated by the test organism, a second test run with pH adjustment was performed. To minimise the influence of the pH adjustment and resulting changes in the dissociation of substances, precipitation reactions and complex formation, the approach suggested by Hennebert (2019) was used (section 3.1.2): pH was only adjusted in the dilution levels with pH values outside the tolerance range for the respective species. In these cases, pH was adjusted to the pH of the test medium. For pH adjustment, 1- or 10-molar hydrochloric acid or sodium hydroxide solution was used. Any precipitates that occurred were not removed, because they may contain potentially toxic elements (Hennebert 2019). Test runs with pH adjustment were carried out to investigate the cause of toxicity; they were not used for HP 14 classification (UBA 2013, DIN EN 14735).

Parallel to each test run with adjusted pH, an additional test run without pH adjustment was carried out to evaluate the reproducibility of the results. Further repetitions of the aquatic toxicity tests (also for eluates without toxic effects in the first test run) were used to investigate the reproducibility of the test results.

4.3.1.2 Acute *Daphnia* test

The acute *Daphnia* test was carried out according to DIN EN ISO 6341 (2013a; Table 20). Water fleas (*Daphnia magna*, age at test start <24 h) were exposed to five dilution levels of the eluate for 48 h. Reconstituted water according to DIN EN ISO 6341 was used as a control medium and

[36] This standard is now available in a finalised form (DIN 38412-59, published in December 2022). There are no significant differences between the draft according to which the algal tests were performed and the finalised version.

to prepare the dilutions of the eluate. Temperature, oxygen content and pH value were measured before placing the daphnids into the test vessels and at test end. After 24 and 48 h of exposure, mobility of the daphnids (number of immobile daphnids) was recorded.

The results of reference tests carried out every six months at ECT were used to verify sensitivity of *D. magna*. Sensitivity of the daphnids was confirmed by three tests (March 2022, September 2022, March 2023). The 24-h EC_{50} was 1.5 mg/L in the first two reference tests and 1.4 mg/L in the third test and, thus, in the range of 0.6-2.1 mg/L specified in DIN EN ISO 6341.

Table 20: Overview of the performance of acute toxicity tests with *Daphnia magna*

Test parameter	Specification
Test guideline	DIN EN ISO 6341 (2013a)
Test organism	*Daphnia magna* Straus, clone M10, neonates (<24 h old)
Test medium	Reconstituted water according to DIN EN ISO 6341
Test endpoint	Immobility
Exposure duration	48 h
Dilution levels	First test run: 3.1, 6.3, 12.5, 25 and 50% eluate; following test runs: lower dilution levels where required (see section 4.3.1.1)
Number of test organisms per test vessel	5
Number of replicates per dilution	4
Number of control replicates	4
Test vessels	50-mL Glass beakers, covered with watch glasses
Volume of test solution per test vessel	25 mL
Light-dark cycle	16 light/8 h dark
Temperature	20±2°C
Validity criteria	1) Immobility in controls ≤10% 2) 24-h EC_{50} for $K_2Cr_2O_7$ between 0.6 and 2.1 mg/L[a]

[a] According to DIN EN ISO 6341 (2013a), reference tests have to carried out within a period of one month before or after each test.

4.3.1.3 Algal growth inhibition test

As mentioned above, the algal growth inhibition test was carried out in 24-well microtiter plates according to the draft of DIN 38412-59 (2021). Algae (*Raphidocelis subcapitata*) were exposed to five eluate dilutions for 72 h and the inhibition of the average daily growth rate compared to the control was recorded (Table 21).

Four days before test start, a preculture with *R. subcapitata* (0.5 x 10^4 algal cells/mL) was prepared in Altenburger growth medium according to DIN 38412-59 (draft, 2021), so that the algae were in the exponential growth phase at test start. The algal cell count in the preculture was determined microscopically ≤4 h before the start of exposure. At test start, test solutions containing algae were prepared from the required volumes of eluate, 10-fold concentrated Altenburger growth medium, sterile deionised water and algal suspension (0.5 x 10^4 algal cells/mL). A control (Altenburger growth medium), a positive control ($K_2Cr_2O_7$: 0.8 mg/L) and a

blank (Altenburger growth medium without algae) were included in each microtiter plate. For the first test run with each eluate, an additional microtiter plate (the colour or fluorescence correction plate) was prepared with the same eluate dilutions but without algae to determine if the respective eluate led to a loss of fluorescence (due to its colouration or turbidity) or showed autofluorescence. The pH values of the test solutions were measured at test start and test end.

After 0, 24, 48 and 72 h of exposure, the contents of all wells of the test plate (i.e. the plate with algae) were homogenised by drawing up several times with a pipette. The test plate and the colour or fluorescence correction plate were shaken for 10 s in a fluorescence plate reader. After excitation at 430 nm, relative fluorescence units (RFUs) were measured from the top. An average value from nine individual measurements was determined for each well. For each test, a calibration curve with 0, 0.5, 2.5, 5, 25, 50 and 100 x 10^4 algal cells/mL was generated. Using the regression equation, measured fluorescence values were converted into cell counts/mL. The average daily growth rate was determined from the cell counts after 0 h and 72 h and used to derive the percentage of inhibition of the growth rate compared to the control.

When evaluating a test, a colour or fluorescence correction was taken into account, if a correlation was found between the eluate content and the fluorescence measurement values which, converted into cell numbers, would have corresponded to an increase[37] of 0.5 x 10^4 algal cells/mL[38] from the highest to the lowest waste dilution.

Table 21: Overview of the performance of algal growth inhibition tests with *Raphidocelis subcapitata*

Test parameter	Specification
Test guideline	DIN 38412-59 (draft, 2021)
Test organism	*Raphidocelis subcapitata* (SAG 61.81)
Test medium	Altenburger growth medium according to DIN 38412-59
Age of algal preculture at test start	4 days
Test endpoint	Growth rate
Exposure duration	72±1 h
Dilution levels	First test run: 3.1, 6.3, 12.5, 25 and 50% eluate; following test runs: lower dilution levels if required (section 4.3.1.1)
Cell density at the test start	approx. 0.5 x 10^4 cells/mL (according to DIN 38412-59: $5 \cdot 10^3$-1 x 10^4 cells/mL)
Number of replicates per dilution	3
Number of control replicates	3
Positive control	0.8 mg/L $K_2Cr_2O_7$
Test vessels	24-Well microtiter plates with 2-mL cavities
Volume of test solution per cavity	2 mL
Shaking frequency during exposure	100±5/min

[37] None of the evaluated waste eluates led to a loss of fluorescence due to its coloration or turbidity.

[38] This corresponds to the inoculated cell density.

Test parameter	Specification
Light regime	Permanent light
Light intensity	60-120 µmol/m^2 x s (≤10% fluctuation)
Temperature	23±2°C
Measurement of relative fluorescence units	0, 24, 48 and 72± 1 h after test start
Validity criteria	1) Exponential growth in controls: mean coefficient of variation of section-by-section growth rates (0-24, 24-48 and 48-72 h) ≤35% 2) Coefficient of variation of growth rates in controls after 72 h ≤7% 3) Average growth rate of controls after 72 h ≥1.2/d 4) Inhibition in positive control ($K_2Cr_2O_7$: 0.8 mg/L): 20-80%

4.3.1.4 Luminescent bacteria test

The luminescent bacteria test with the marine bacterium *A. fischeri* was carried out in accordance with DIN EN ISO 11348-2 (2009). Luminescent bacteria test kits (BioFix® or LUMISTOX, see Table 22) were used, which consist of liquid-dried luminescent bacteria suspension and reactivation solution. The test endpoint is the inhibition of bioluminescence after 30 min of exposure.

Conductivities of the eluates were measured and increased to 34±4 mS/cm by adding NaCl solution, and pH value and oxygen content were determined. The dilution levels to be tested were prepared by diluting the eluate with 2% NaCl solution, either directly in the glass cuvettes or (if measurement and adjustment of pH was necessary) in glass beakers. A positive control ($K_2Cr_2O_7$: 22.6 mg/L) was included in each test. The freeze-dried luminescent bacteria were reactivated according to the manufacturers' instructions. A luminometer was used to measure the bioluminescence at the start of exposure (0 min) and after 30 min of exposure.

Table 22: Overview of the performance of the luminescent bacteria tests

Test parameter	Specification
Test guideline	DIN EN ISO 11348-2 (2009)
Test organism	*Aliivibrio fischeri* (formerly *Vibrio fischeri*), liquid-dried bacteria
Test kits	Biofix® (Macherey Nagel), LUMISTOX (Hach)
Test medium	2% NaCl solution (pH 7.0±0.2)
Test endpoint	Inhibition of bioluminescence
Exposure duration	30 min
Dilution levels	First test run: 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25 and 50% eluate; following test runs: lower dilution levels where required (section 4.3.1.1**Fehler! Verweisquelle konnte nicht gefunden werden.**)[a]
Number of replicates per dilution	2
Number of control replicates	2
Positive control	$K_2Cr_2O_7$: 22.6 mg/L

Test parameter	Specification
Test vessels	Glass cuvettes
Volume of test solution per cuvette	1 mL
Temperature	15±1°C
Measurement of bioluminescence	At test start (0 min) and after 30 min
Validity criteria	1) f_{kt} Value[b] after 30 min between 0.6 and 1.3. 2) Deviation of measured bioluminescence of the control replicates from their mean value ≤3%. 3) For all dilution levels relevant to the determination of the EC_{50}: deviation of the measured bioluminescence of the replicates from their mean ≤3%. 4) For each batch of luminescent bacteria: 20-80% inhibition by 4.5 mg/L of 3,5-dichlorophenol, 25 mg/L of Zn (II) and 4 mg/L of Cr (VI)[c]. 5) In each test: 20-80% inhibition in the positive control (22.6 mg/L of $K_2Cr_2O_7$).

[a] In the luminescent bacteria tests with flue-gas dust (10 09 09*, batches 1 and 2), flue-gas dust (10 09 10, plant A, 2nd test run) and excavated geogenic material from an open-cast lignite mine (2nd and 3rd test run), the tested dilutions levels were by a factor of 2 lower (0.2-25% eluate), because dilution during testing had not been taken into account. [b] Correction factor for the fluctuation of the luminescence intensities measured in the control. [c] This validity criterion was verified by the manufacturers of the test kits.

4.3.2 Ecotoxicity tests with terrestrial organisms

The three terrestrial test methods – the solid contact test with *A. globiformis* according to ISO 18187 (2016a), the growth inhibition test with *B. rapa* according to ISO 11269-2 (2012a) and the avoidance test with earthworms according to ISO 17512-1 (2008a) – were carried out with the solid waste samples. For each waste sample, one test was performed with five dilution levels of the waste (25%, 12.5%, 6.3%, 3.1% and 1.6%).

One waste sample was additionally assessed with a rapid test for determination of potential nitrification and inhibition of nitrification by ammonium oxidation (test guideline DIN EN ISO 15685, 2021)[39].

4.3.2.1 Solid contact test with *Arthrobacter globiformis*

In the solid contact test according to ISO 18187 (2016a), the inhibition of dehydrogenase activity in the ubiquitous soil bacterium *A. globiformis* was evaluated (Table 23). For this purpose, waste samples were sieved to ≤2 mm in accordance with ISO 18187. The dilution levels to be tested were prepared by mixing the waste with quartz sand, nutrient solution according to ISO 18187 and *A. globiformis*, and incubated for 2 h at 30±1°C. Subsequently, the dye resazurin was added, which is transformed to resorufin due to the dehydrogenase activity of the bacteria. Resorufin can be detected fluorometrically. Depending on the bacterial activity, the colour of the samples and, thus, their fluorescence changes. By measuring fluorescence, an inhibition of bacterial growth as compared to the control can be quantified.

In the solid contact test, 5 dilution levels of the waste (see above), negative controls (in LUFA standard soil 2.2 and quartz sand), and the positive control benzalkonium chloride (BAC; 600 mg/kg waste dry weight; also in LUFA standard soil 2.2 and quartz sand) were tested. In

[39] Corresponds to ISO 15685 (ISO 2012g).

addition, two different blanks were included: (a) a blank to determine autofluorescence of the substrate (this is subtracted from fluorescence of the respective treatments), and (b) a blank to determine dehydrogenase activity in unpasteurised substrate to verify the efficiency of the deactivation step (pasteurisation) carried out before starting the measurement.

Table 23: Overview of the performance of the solid contact test with *Arthrobacter globiformis*

Test parameter	Specification
Test guideline	ISO 18187 (2016a)
Test organism	*Arthrobacter globiformis*
Test endpoint	Inhibition of activity of the enzyme dehydrogenase
Test substrate	Quartz sand
Exposure duration	2 h
Dilution levels	25, 12.5, 6.3, 3.1 and 1.6%
Number of replicates per dilution level	4
Number of control replicates	4
Test vessels	24-Well microtiter plates with lid
Substrate quantity per well	600±6 mg fresh weight (sieved to <2 mm)
Temperature (specified range)	30±1°C
Measurement of dehydrogenase activity	At test start (0 min) and after 15, 30, 45 and 60 min
Validity criteria	a) Mean fluorescence of the negative control increased at least fivefold between the first and last measurement. b) Inhibition in positive control between 30% and 80%. c) Coefficient of variation in negative control <15%.

4.3.2.2 Growth inhibition test with *Brassica rapa*

The growth inhibition test with higher plants was carried out according to ISO 11269-2 (2012a) with the following modification: according to the UBA recommendations, only one dicotyledonous species (*B. rapa*) was tested instead of a monocotyledonous and a dicotyledonous species (Table 24).

The light intensity was ≥200 µE x m^{-2} x s^{-1} and the relative humidity 30-70%. These conditions ensure good growth, as confirmed in previous studies. According to the dilution levels to be tested, the respective waste sample was mixed with control soil (LUFA standard soil 2.3) and added to plant pots. Then, 10 seeds of the test species were inserted in each pot. Seven days after the emergence of at least 50% of the plants in the controls, the number of seedlings was reduced to five representative plants per pot, and the test was continued for a further 7 days. On day 14, plants were harvested and shoot fresh weight was determined.

Table 24: Overview of the performance of the growth inhibition test with *Brassica rapa*

Test parameter	Specification
Test guideline	ISO 11269-2 (2012a)
Test organism	*Brassica rapa*
Test endpoint	Emergence, shoot fresh weight
Test substrate	LUFA standard soil 2.3
Exposure duration	14 days starting at 50% emergence in controls (= day 0)
Dilution levels	25, 12.5, 6.3, 3.1 and 1.6%
Number of replicates per dilution level	2
Number of control replicates	6
Test vessels	Planting pots (diameter: 11 cm)
Substrate quantity per test vessel	450 g dry weight
Temperature	23±3°C (a wider range is acceptable as long as the plants emerge and grow normally)
Measurement of emergence	Until day 14
Measurement of shoot fresh weight	On day 14
Validity criteria	a) Emergence in controls >70%. b) No visible phytotoxic effects and normal growth and morphology in controls. c) Survival of emerged seedlings in the controls >90%.

4.3.2.3 Avoidance test with earthworms

The avoidance tests with earthworms (*Eisenia fetida*) were performed according to ISO 17512-1 (2008a; Table 25). As test organisms, healthy, adult animals of the same age with an average weight between 300 and 600 mg were used. The waste samples were mixed with artificial soil (according to ISO 11268-2) to achieve a waste content between 1.6% and 25%. Artificial soil was used as control substrate.

The moistened substrates were filled into test vessels in such a way that one half of the vessels contained control substrate and the other half the respective waste dilution. For each vessel, ten worms were then placed on the boundary between the control substrate and the waste dilution. The test vessels were then incubated for 48 h (20±2°C, light-dark cycle: 16 light/8 h dark). Then, the worms in each half were counted for all vessels.

Table 25: Overview of the performance of the avoidance test with earthworms

Test parameter	Specification
Test guideline	ISO 17512-1 (2008a)
Test organism	*Eisenia fetida*
Test endpoint	Avoidance behaviour
Test substrate	Artificial soil according to ISO 11268-2

Test parameter	Specification
Exposure duration	48 h
Dilution levels	25, 12.5, 6.3, 3.1 and 1.6%
Number of replicates per dilution level	5
Number of control replicates	5
Test vessels	Bellaplast vessels (15.5 x 11 x 6 cm)
Amount of substrate per test vessel	500 g dry weight (250 g control soil, 250 g test soil)
Temperature	20±2°C
Measurement of avoidance behaviour	After 48 h
Validity criteria	a) Dead or missing worms: <10% in treatments. b) Average ratio of earthworms in both halves of the control replicates within the range of 60% to 40%.

4.3.2.4 Rapid test to determine potential nitrification

A sample of fluff-light fraction and dust from plant A (batch 2) was additionally tested with a rapid test for the determination of potential nitrification and inhibition of nitrification by ammonium oxidation (DIN ISO 15685, 2021). The reason for this was the relatively high variance of the results of the solid contact test with *A. globiformis* (see section 4.4). Due to the small amounts of samples used in the solid contact, inhomogeneities of waste samples can have a strong impact on the results. Therefore, a screening test was carried out to verify if the rapid test to determine potential nitrification is a possible alternative to the solid contact test.

The advantages of this test are the larger sample quantity (25 g vs. 0.6 g fresh weight in the solid contact test) and the higher ecological relevance: the test system does not only contain a single test species but the microorganisms that are naturally present in the soil. Since only a small residual amount of the waste sample was available, only two waste dilutions (6.3% and 25%) were tested, and the number of replicates was reduced to two (Table 26).

Table 26: Overview of the performance of the rapid test to determine potential nitrification

Test parameter	Specification
Test guideline	DIN ISO 15685 (2021)
Test organism	Natural microorganism community
Test endpoint	Inhibition of the nitrification rate
Test substrate	LUFA standard soil 2.3
Exposure duration	6 h (permanent shaking, 175 rpm)
Dilution levels	25 and 6.3% (usually 5 dilution levels)
Number of replicates per dilution level	2 (usually 4)
Number of control replicates	2 (usually 4)
Test vessels	250-mL Polyethylene bottles
Amount of substrate per test vessel	25 g (Fresh weight)

Test parameter	Specification
Temperature	25±2°C
Measurement of nitrite content	After 2 h and 6 h
Validity criteria	Not defined

4.3.3 Statistical evaluation

The results of the aquatic and terrestrial ecotoxicity tests were evaluated with the programme ToxRat Professional 3.3.0. For all tests, the EC_{50} was determined, the effect concentration that is relevant for HP 14 classification. For tests where either below or above 50% effect were recorded at all dilution levels, the EC_{50} is indicated as > the lowest tested dilution or < the highest tested dilution, respectively. For aquatic ecotoxicity tests with pH adjustment, no EC_{50} values were determined. As mentioned in section 4.3.1.1, these tests are not relevant for HP 14 classification. Furthermore, concentration-response relationships in these tests were often non-monotonous, since pH was usually only adjusted in individual dilution levels.

For quantal data (acute *Daphnia* test, avoidance test with earthworms), the EC_{50} values and their 95% confidence intervals (CI) were determined with linear regression analyses (probit, Weibull or logit analysis with linear maximum likelihood regression). For metric data (algal growth inhibition test, luminescent bacteria test, solid contact test with *A. globiformis*, growth inhibition test with *B. rapa*) non-linear regression methods (3-parameters normal-cumulative distribution function (CDF), Weibull- or logit-CDF) were first used. In cases, where no good curve fit was achieved with these methods, the above-mentioned linear regression methods were used. The quality of the curve fit was assessed visually and based on the results of the F-test and the $\chi 2$ goodness of fit test.

In the present project, the derived effect concentrations were reported with three significant digits to enable a reliable comparison with the limit concentration.

4.4 Results of ecotoxicological tests

In the following sections, the results of the ecotoxicological tests for the different waste types are presented (sections 4.4.1 to 4.4.3). Then, an overview of all results is given (section 4.4.4) and the results are discussed in comparison to literature data (section 4.5).

4.4.1 Flue-gas dust (10 09 09*/10 09 10) from iron and steel casting

For this waste type, two samples were tested for the hazardous and the non-hazardous mirror entry, respectively.

4.4.1.1 Flue-gas dust (10 09 09*) from iron and steel casting

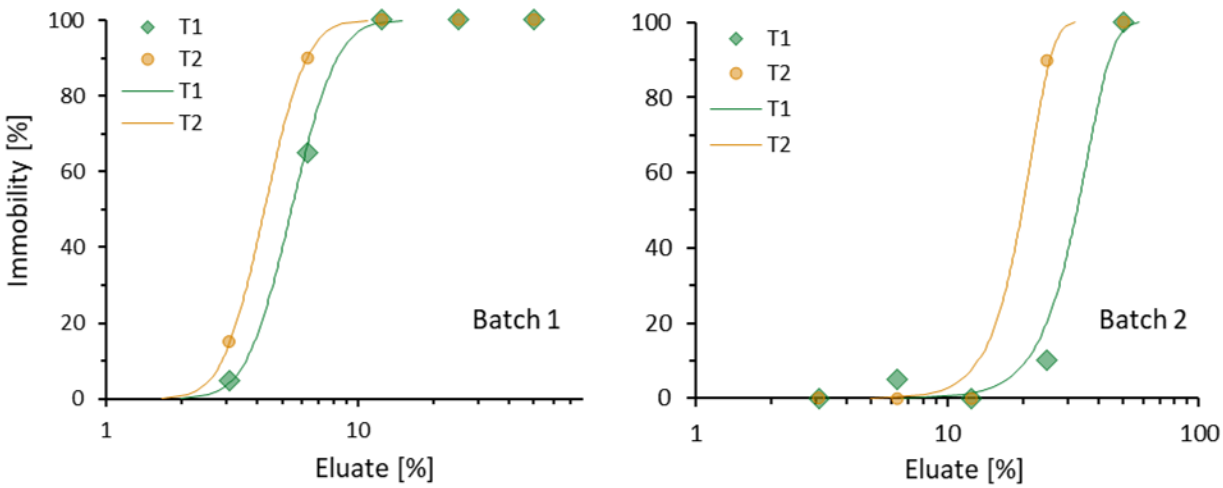
Flue-gas dust from iron and steel casting classified as hazardous by the waste owner (10 09 09*) was sampled in July 2022. Two batches were investigated: aged material with a storage period >4 weeks (batch 1) and fresh material with a storage period <4 weeks (batch 2).

4.4.1.1.1 Toxicity of flue-gas dust (10 09 09*) to aquatic organisms

4.4.1.1.1.1 Acute Daphnia test

In the acute *Daphnia* test, both batches of flue-gas dust (10 09 09*) were evaluated in two test runs, which showed comparable results (Figure 18). For the aged material (batch 1), EC_{50} values of 5.45% (CI: 4.57-6.47%) and 4.26% (CI: 3.59-5.03%) were determined. The fresh material (batch 2) proved to be significantly less toxic to *D. magna*. In both test runs, the EC_{50} values exceeded the limit concentration: 32.8% (CI: not determinable [n.d.]) and 19.8% (CI: 17.7-22.0%).

Figure 18: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *D. magna*. Immobility after 48 h depending on eluate content for batches 1 and 2



Batch 1: aged material, batch 2: fresh material. T1, T2: Test runs 1 and 2. Regression: probit analysis with linear maximum likelihood regression (batch 1), Weibull analysis with linear maximum likelihood regression (batch 2). In all presented tests, immobility in the control was 0%.
Source: own illustration, ECT Oekotoxikologie GmbH

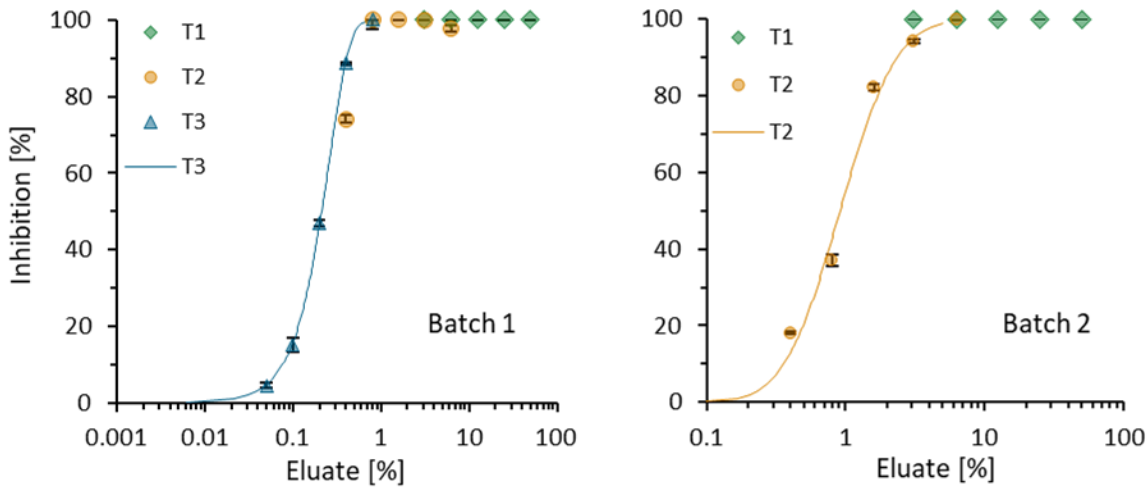
4.4.1.1.1.2 Algal growth inhibition test

In the algal growth inhibition test, the aged material (batch 1) of the flue-gas dust (10 09 09*) was analysed in three test runs (Figure 19). In the first test run, algal growth was completely inhibited in all dilution levels (3.1-50% eluate). In the second test run (0.4-6.3% eluate), growth

was also inhibited strongly (≥74%). In the third test run, the inhibition of the growth rate was between 5% (0.05% eluate) and 100% (0.8% eluate), an EC_{50} of 0.201% eluate (CI: 0.200-0.203%) was determined.

The fresh material (batch 2) also led to a complete inhibition of algal growth in all dilution levels (3.1-50% eluate) in the first test run (Figure 19). In the second test run, inhibitions between 18% (0.4% eluate) and 100% (6.3% eluate) were determined. The resulting EC_{50} (0.913% eluate; CI: 0.907-0.919%) was higher than the EC_{50} for the aged material.

Figure 19: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content for batches 1 and 2

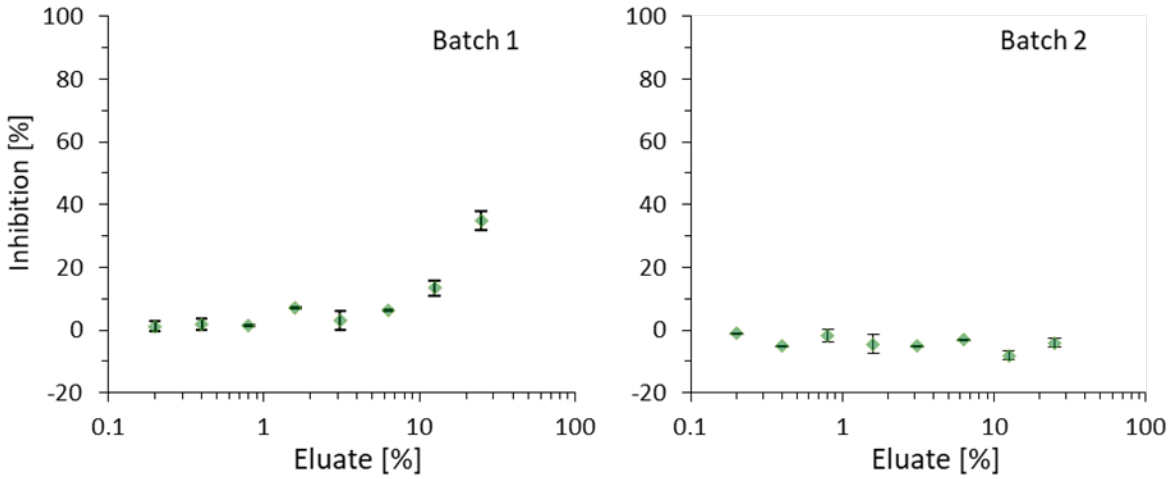


Inhibition of growth rates: mean values with standard deviations. Batch 1: aged material, batch 2: fresh material. T1, T2, T3: Test runs 1, 2 and 3. Regression: Weibull analysis with linear maximum likelihood regression (batch 1), probit analysis with linear maximum likelihood regression (batch 2).
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.1.1.3 Luminescent bacteria test

Both batches of flue-gas dust (10 09 09*) were analysed in the luminescent bacteria test in one test run. The aged material led to a maximum of 35% inhibition of bioluminescence in the tested dilution levels (0.4-25% eluate). The fresh material showed no toxicity towards *A. fischeri* (Figure 20). The EC_{50} values for both batches were thus >25% eluate.

Figure 20: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *A. fischeri*. Inhibition of bioluminescence after 30 min depending on eluate content for batches 1 and 2



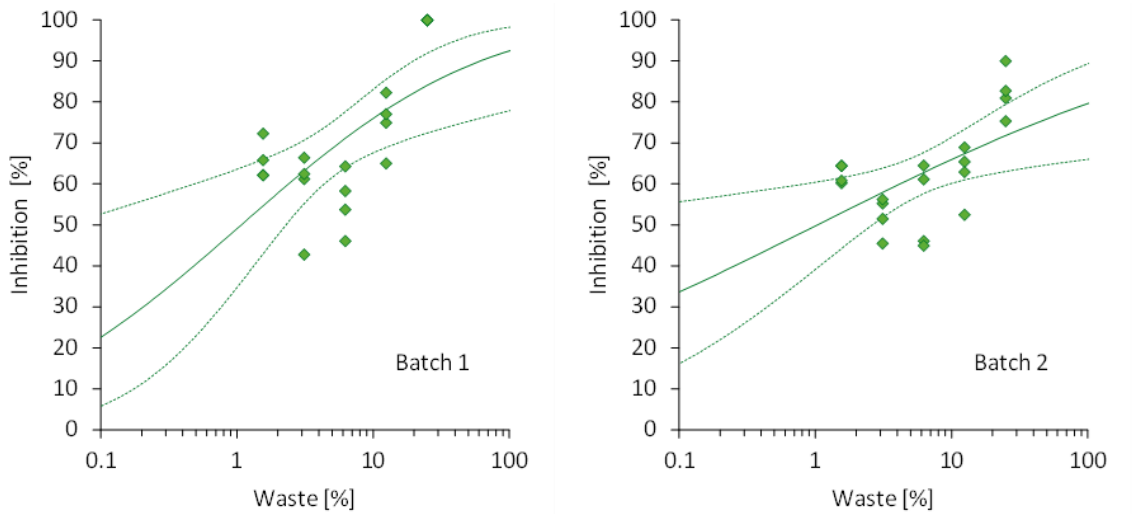
Inhibition of bioluminescence: mean values with standard deviations. Batch 1: aged material, batch 2: fresh material. T1, T2 and T3: Test runs 1, 2 and 3. No regression (<50% effect in all tested dilution levels).
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.1.2 Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to terrestrial organisms

4.4.1.1.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test with *A. globiformis*, results for the two batches of the flue-gas dust (10 09 09*) were comparable. A clear but non-monotonous concentration-response relationship was observed (Figure 21). For the aged material (batch 1), an EC_{50} value of 1.08% (CI: 0.056-2.29%) was determined, for the fresh material (batch 2) an EC_{50} of 1.03% (CI: 0.005-2.61%). For both batches, the EC_{50} values were thus below the limit concentration.

Figure 21: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *A. globiformis*. Inhibition of dehydrogenase activity depending on waste content for batches 1 and 2

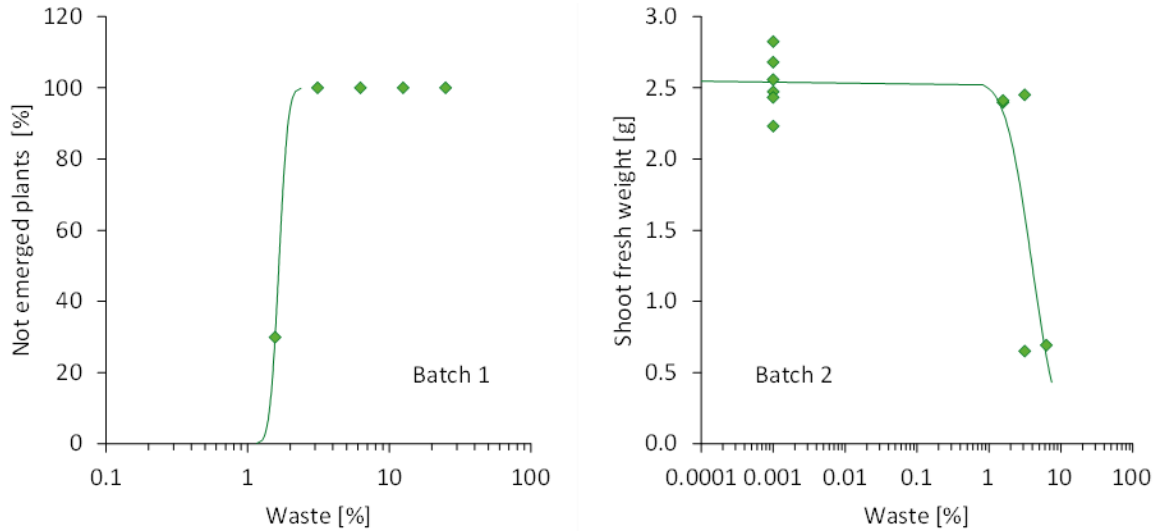


Batch 1: aged material, batch 2: fresh material. Regression (with 95% CI): Probit analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.1.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, results obtained for the two batches of the flue-gas dust (10 09 09*) differed (Figure 22). However, there was a clear effect on emergence in both tests. For the aged material (batch 1), no EC_{50} value could be determined for shoot fresh weight, since only plants at the highest dilution level (1.56% waste) had emerged. Here, the effect on shoot fresh weight was <50%. The EC_{50} value for emergence was 1.66% (CI: n.d.). For the fresh material (batch 2), an EC_{50} of 3.93% (CI: n.d.) was derived for shoot fresh weight, and an EC_{50} of 3.86% (CI: 1.06–11.3%) for emergence. For both batches, the EC_{50} values were thus below the limit concentration of 10%. For batch 2, a chronic effect value (EC_{10}) of 1.67% (CI: 0.550-5.09%) could additionally be determined for shoot fresh weight.

Figure 22: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *B. rapa*. Plants not emerged or shoot fresh weight after 14 d depending on waste content for batches 1 and 2



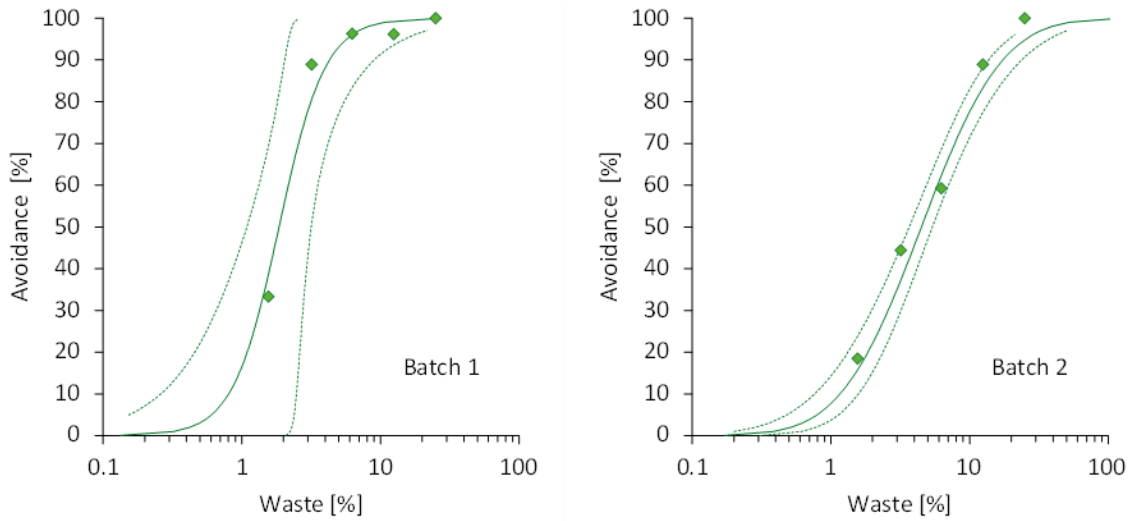
Shoot fresh weight: mean value per surviving plant and pot. Batch 1: aged material, batch 2: fresh material. Regression: probit analysis with linear maximum likelihood regression (batch 1), 3-parameter normal-cumulative distribution function (batch 2).

Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.1.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida*, results for the two batches of flue-gas dust (10 09 09*) differed with regard to the concentration-response relationship (Figure 23). For the aged material (batch 1), an EC_{50} of 1.86% (CI: 1.09–3.17%) was determined, for the fresh material (batch 2) a slightly higher EC_{50} of 4.49% (CI: 3.67–5.39%). For both batches, the EC_{50} values were thus below the limit concentration of 10%.

Figure 23: Toxicity of flue-gas dust (10 09 09*) from iron and steel casting to *E. fetida*. Avoidance after 48 h depending on waste content for batches 1 and 2



Avoidance: mean value of five replicates. Batch 1: aged material, batch 2: fresh material. Regression (with 95% CI): logit analysis with linear maximum likelihood regression (batch 1), probit analysis with linear maximum likelihood regression (batch 2).
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.2 Flue-gas dust (10 09 10) from iron and steel casting

For the non-hazardous flue-gas dust (10 09 10) from iron and steel casting, material from two different foundries was examined (plant A was sampled in June 2022, plant B in October 2022).

4.4.1.2.1 Toxicity of flue-gas dust (10 09 10) from iron and steel casting to aquatic organisms

4.4.1.2.1.1 Acute Daphnia test

For the flue-gas dust (10 09 10) from plant A, the two lowest tested dilution levels had pH values outside the suitable range for *D. magna* (pH 6.0-9.0) specified in DIN EN ISO 6341 (2013a): pH was 9.1 at an eluate content of 25%, and 9.3 and 9.6[40] at an eluate content of 50%. Therefore, eluates were first evaluated in a test run without pH adjustment, and then in two parallel test runs (a) without and (b) with pH adjustment (see section 4.3.1.1). The pH values of the two above-mentioned dilution levels were adjusted to pH 8.4-8.5.

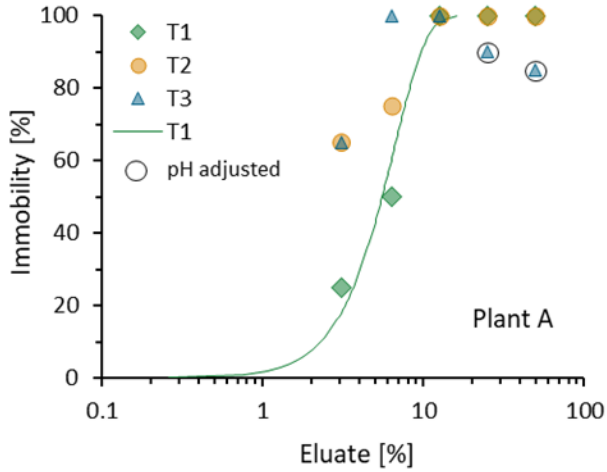
Similar toxicities were recorded in the two test runs without pH adjustment and in the not pH-adjusted dilution levels of the third test run. However, the effects were slightly lower in the first test run (EC_{50}: 5.53%, CI: 4.12-6.76%) than in the second (EC_{50} <3.1%) and third[41] (Figure 24). The pH adjustment in the dilution levels with 25 and 50% eluate led to a slight reduction in toxicity.

Flue-gas dust (10 09 10) from plant B had no effect on daphnids in both test runs (EC_{50} >50% eluate).

[40] First test run with the first waste eluate: pH 9.6; second test run with the second waste eluate: pH 9.3.

[41] No EC_{50} was determined for tests with pH-adjustment (see section 4.3.1).

Figure 24: Toxicity of flue-gas dust (10 09 10) from iron and steel casting from plant A to *D. magna*. Immobility after 48 h depending on eluate content



T1-T3: Test runs 1-3. T3 with pH adjustment in the dilution levels with 25 and 50% eluate. Regression: Weibull analysis with linear maximum likelihood regression. No immobility in controls (T1[42] -T3).
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.2.1.2 Algal growth inhibition test

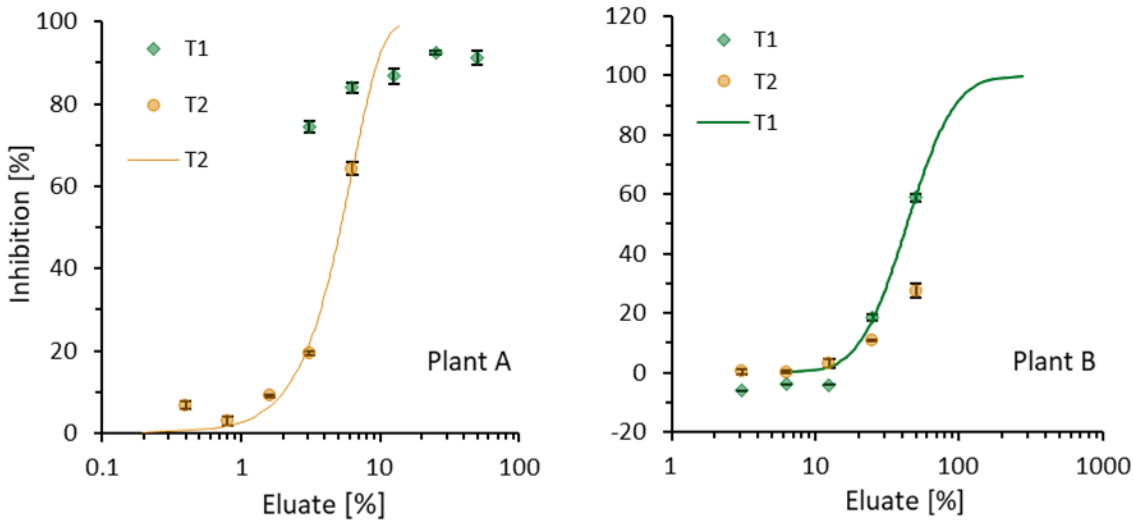
In the first test run[43], flue-gas dust (10 09 10) from plant A led to a >50% inhibition of algal growth rate at all dilution levels (EC_{50} <3.1% eluate content). In the second test run with higher dilutions, inhibition of growth rate was between 3% (0.8% eluate) and 64% (6.3% eluate). An EC_{50} of 5.21% (CI: 5.10-5.32%) was determined. The two dilution levels that were tested in both test runs (3.1 and 6.3% eluate) were less toxic in the second test run than in the first (see Figure 25).

In the first and second test run with flue-gas dust (10 09 10) from plant B, maximum inhibitions of growth rate were 59% and 28% (Figure 25). EC_{50} values of 43.5% (CI: 43.1-43.9%) and >50% were determined.

[42] First test run: only 15 control animals.

[43] In the first test run with flue-gas dust (10 09 10) from iron and steel casting from plant B and the material from the side verges of a federal road (17 05 03*), the same positive control was used. The $K_2Cr_2O_7$ concentration in this positive control was 8.0 mg/L instead of 0.8 mg/L. Therefore, inhibition of the growth rate (100%) was above the required range (20-80%). As the required sensitivity of the algae to $K_2Cr_2O_7$ (0.8 mg/L) was reached in all other test with inhibitions of 20-80%, results from the first test run were used.

Figure 25: Toxicity of flue-gas dust (10 09 10) from iron and steel casting from plants A and B to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content

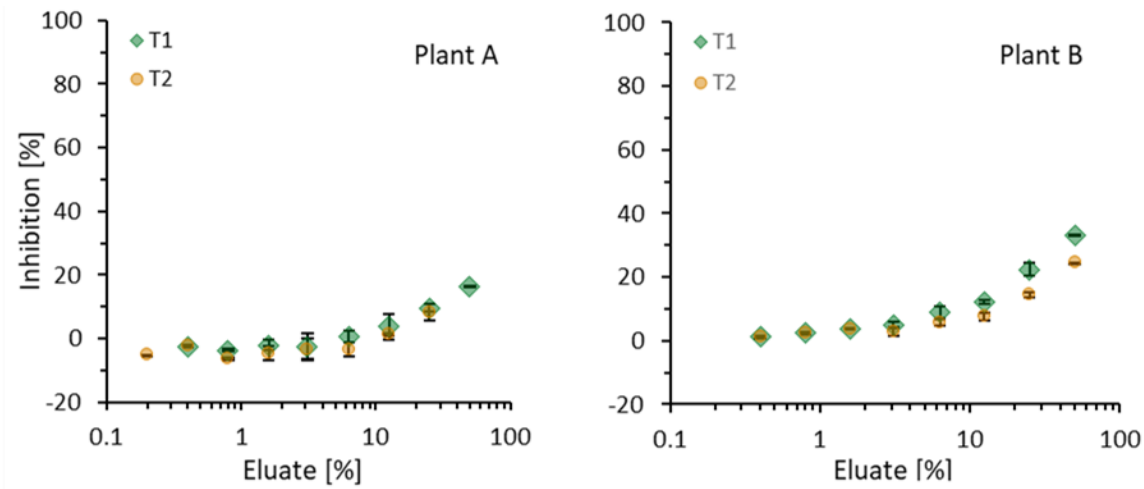


Inhibition of growth rate: mean values with standard deviations. T1 and T2: test runs 1 and 2. Regression: Weibull analysis with linear maximum likelihood regression (plant A), probit analysis with linear maximum likelihood regression (plant B).
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.2.1.3 4.4.1.2.1.3 Luminescent bacteria test

Flue-gas dust (10 09 10) from plants A and B was analysed in two test runs each in the luminescent bacteria test. Both wastes only led to a slight inhibition of bioluminescence (<20%, Figure 26). Hence, the derived EC_{50} values are above the highest dilution level tested (>25% for plant A, 2nd test run, >50% for the other tests). For both plants, the results from the two test runs are consistent.

Figure 26: Toxicity of flue-gas dust (10 09 10) from iron and steel casting from plants A and B to *A. fischeri*. Inhibition of bioluminescence after 30 min depending on eluate content



Inhibition of bioluminescence: mean values with standard deviations. T1 and T2: test runs 1 and 2.
Source: own illustration, ECT Oekotoxikologie GmbH

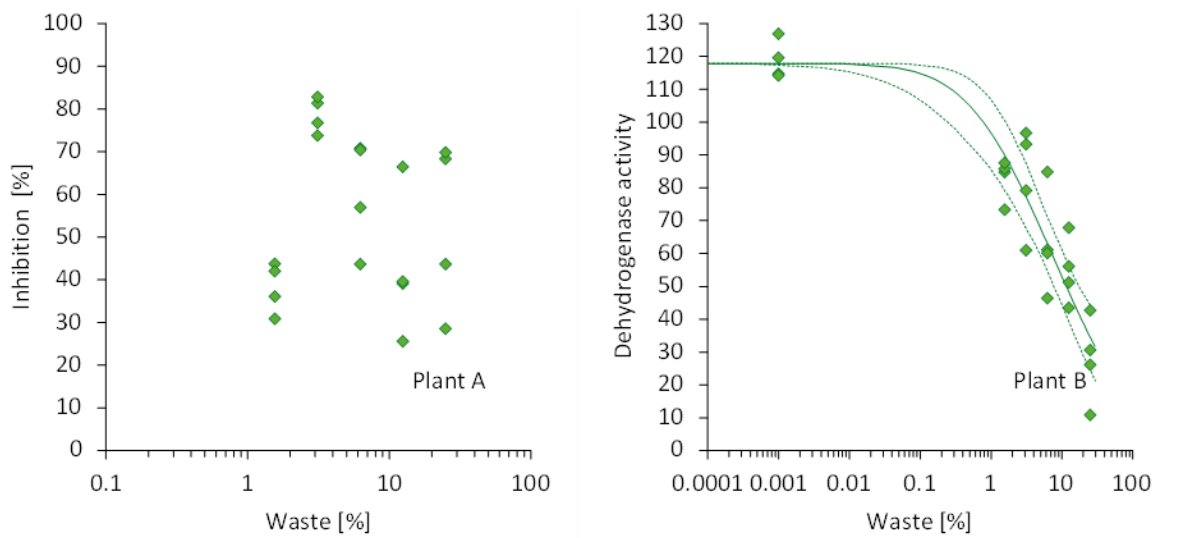
4.4.1.2.2 Toxicity of flue-gas dust (10 09 10) from iron and steel casting to terrestrial organisms

4.4.1.2.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test with *A. globiformis*, the results obtained with samples of the flue-gas dust (10 09 10) from both plants differed (Figure 27). For material from plant A, the concentration-response relationship was not monotonous, so that no EC_{50} value could be calculated. However, as inhibitions >50% were observed in several dilution levels, an ecotoxicity of the sample is assumed.

For material from plant B, there was a clear concentration-response relationship and an EC_{50} value of 7.56% (CI: 5.10–11.1%) was determined, which is below the limit concentration of 10%. However, this test run did not formally fulfil the validity criteria due to the lack of effect of the reference substance in the LUFA 2.2 soil. In contrast, a clear effect of the reference substance was observed in the quartz sand. For this reason and due to the fact that the available sample material was already more than 2 months old when the test had been evaluated, the test was not repeated.

Figure 27: Toxicity of flue-gas dust (10 09 10) from iron and steel casting to *A. globiformis*. (Inhibition of) dehydrogenase activity depending on waste content for plants A and B



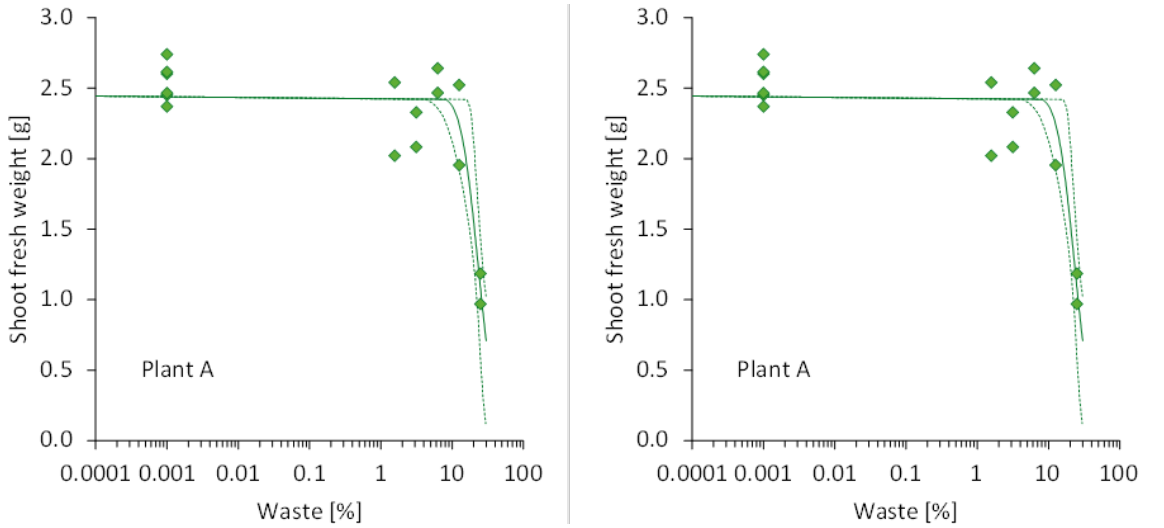
Regression (with 95% CI): no regression, because the concentration-response relationship is not monotonous (plant A), 3-parameter normal-cumulative distribution function (plant B).

Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.2.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, results obtained with flue-gas dust (10 09 10) from both plants differed slightly (Figure 28). For material from plant A, an EC_{50} value of 23.4% (CI: 20.0–27.4%) was determined, for material from plant B an EC_{50} >25%. Thus, EC_{50} values for both plants were above the limit concentration. For material from plant A, a chronic effect concentration (EC_{10}) of 13.2% (CI: 8.50-20.4%) could be determined additionally. For plant B, shoot fresh weight was reduced by more than 10% at all dilution levels, but there was no monotonous concentration-response relationship, so that no reliable EC_{10} could be determined.

Figure 28: Toxicity of flue-gas dust (10 09 10) from iron and steel casting to *B. rapa*. Shoot fresh weight after 14 d depending on waste content for plants A and B



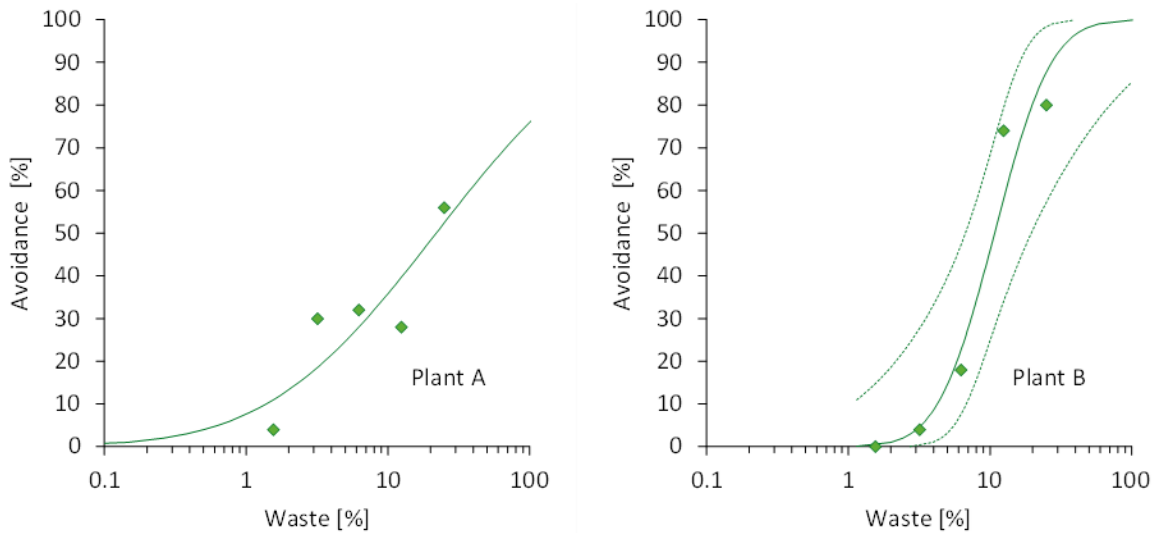
Shoot fresh weight: mean value per surviving plant and pot. Regression (with 95% CI): 3-parameter normal-cumulative distribution function.

Source: own illustration, ECT Oekotoxikologie GmbH

4.4.1.2.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida*, results obtained for flue-gas dust (10 09 10) from both plants also differed (Figure 29). For plant A, there was a clear concentration-response relationship. An EC_{50} of 21.9% (CI: n.d.) was calculated. However, as the concentration-response relationship was not statistically significant, this value is considered less robust. For material from plant B, an EC_{50} value of 10.8% (CI: 6.70–19.5%) was determined. Hence, the EC_{50} values for both plants were above the limit concentration (for plant B just above this limit value).

Figure 29: Toxicity of flue-gas dust (10 09 10) from iron and steel casting to *E. fetida*. Avoidance after 48 h depending on waste content for plants A and B



Avoidance [%]: mean values of five replicates. Regression: probit analysis with linear maximum likelihood regression (plant B, with 95% CI).

Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2 Soil and stones (17 05 03*/17 05 04)

Three waste samples from the mirror entry soil and stones (17 05 03*/17 05 04) were evaluated: excavated geogenic material from an open-cast lignite mine (17 05 03*; sampled in June 2022) and two samples from the side verges of roads, one classified as hazardous (17 05 03*; sampled in October 2022) and one as non-hazardous (17 05 04; sampled in May 2022) by the waste owner.

4.4.2.1 Excavated geogenic material from an open-cast lignite mine (17 05 03*)

4.4.2.1.1 Toxicity of excavated geogenic material (17 05 03*) to aquatic organisms

For the excavated geogenic material, three test runs were performed for all aquatic tests, including one test run (test run 3) with pH adjustment.

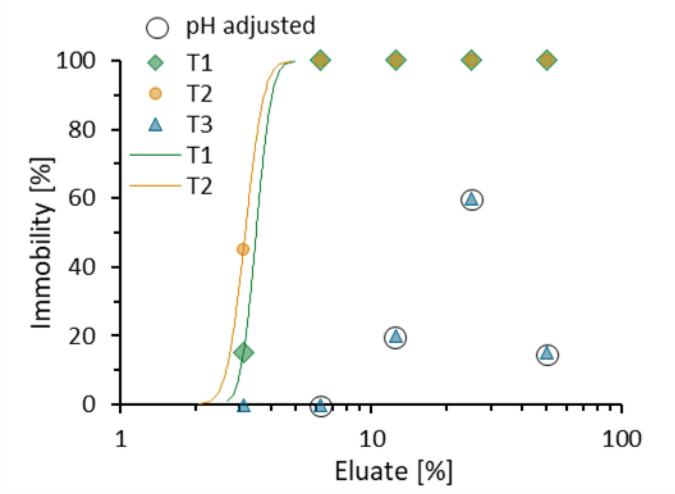
4.4.2.1.1.1 Acute Daphnia test

The lowest four dilution levels (6.3-50%) of the eluate from excavated geogenic material (17 05 03*) had pH values outside the tolerable range for *D. magna* specified in DIN EN ISO 6341 (2013a). At an eluate content of 6.3%, pH was 4.7 and 4.8[44], and at an eluate content of 50% it was 2.9. In the two test runs without pH adjustment, all daphnids were immobile at eluate contents of 6.3–50% (Figure 30). EC_{50} values of 3.49% (CI: n.b.) and 3.15% (CI: n.b.) were determined.

In the third test run, the pH values of the four dilution levels mentioned above were adjusted to the pH of the test medium. Neutralisation led to the formation of an orange-brown precipitate (presumably iron hydroxides) and the formation of two phases: a clear phase in the upper part of the test vessels and an orange-brown phase containing flocs of the precipitate in the lower part. With increasing eluate content, flocculation increased, and the two phases separated to a greater extent. At the same time, a reduction of the oxygen content was recorded. At the highest eluate concentration (50%), the O_2 content was 4.0 mg/L (control: 9.9 mg/L). At the end of exposure, oxygen concentrations in all dilution levels had increased to control level (9.6-9.8 mg/L). The pH adjustment significantly reduced the toxicity of the excavated geogenic material (17 05 03*; see Figure 30). The very strong reduction of the toxicity at an eluate content of 50% is particularly striking. Here, the clear separation between the lower phase containing flocs of precipitate and the clear upper phase has possibly led to the daphnids being less affected by the precipitates.

[44] First test run with the first waste eluate: pH 4.7; second test run with the second waste eluate: pH 4.8.

Figure 30: Toxicity of excavated geogenic material (17 05 03*) to *D. magna*. Immobility after 48 h depending on eluate content



T1-T3: Test runs 1-3; T3 with pH adjustment in the dilution levels with 6.3-50% eluate. Regression: probit analysis with linear maximum likelihood regression. 0% immobility in controls (T1[45] -T3).
Source: own illustration, ECT Oekotoxikologie GmbH

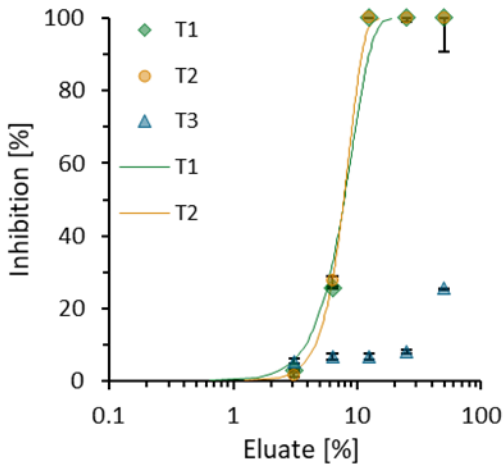
4.4.2.1.1.2 Algal growth inhibition test

In the algal growth inhibition test, the pH at an eluate content of 6.3% was at the lower limit of the tolerable pH range of 6.0-8.5 specified in DIN 38412-59 (2021). At eluate concentrations of 12.5-50%, pH values were ≤3.7. In the two test runs without pH adjustment, algal growth was completely inhibited in the three dilution levels with the highest eluate content (12.5-50% eluate; Figure 31). An EC_{50} of 7.85% (CI: 6.66-9.08%) was determined for the first test run, and an EC_{50} of 7.77% (CI: 7.56-7.99%) for the second test run.

In the third test run, pH values in the dilution levels with an eluate content of 6.3-50% were adjusted. As in the *Daphnia* test, orange-brown precipitates formed. These precipitates led to a loss of fluorescence that would have resulted in negative cell counts for the measurement at test start (0 h). Since the loss of fluorescence only affected the measurement at test start, the fluorescence correction plate was not taken into account when evaluating the test. Instead, the cell counts of the 0 h were set to the value of the inoculated cell titre (0.5 x 10^4 cells/ml). As can be seen in Figure 31, pH adjustment led to a strong reduction of the algal toxicity of the excavated geogenic material.

[45] The first test run only contained 15 control animals.

Figure 31: Toxicity of excavated geogenic material (17 05 03*) to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content



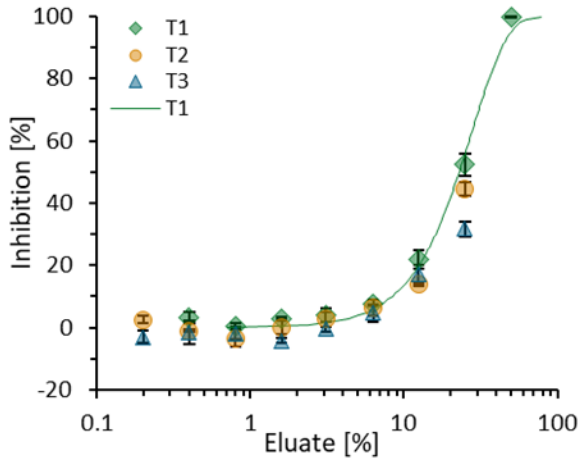
Inhibition of growth rate: mean values with standard deviations. T1–T3: Test runs 1-3; T3 with pH adjustment in dilution levels with 6.3-50% eluate. Regression: Weibull analyses with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.1.1.3 Luminescent bacteria test

In the luminescent bacteria test, only the dilution level with the lowest eluate content (0.4%) had a pH >6.0. The pH values at an eluate content of 0.8-50% were in the acidic range (pH 5.4 at 0.8% eluate, pH 2.7 at 50% eluate). The two test runs carried out without pH adjustment showed very similar results. An EC_{50} of 22.9% (CI: 20.7-25.3%) was determined in the first test run and an EC_{50} >25% eluate in the second (Figure 32).

Although the derived EC_{50} values were above the limit concentration of 10% eluate, a third test run with pH adjustment was performed. Differing from the procedure described in section 4.3.1.1, the pH of the eluate was adjusted, because – due to the lack of buffer capacity of the test medium used in the luminescent bacteria test (2% NaCl solution) – the pH in 7 of 8 dilution levels was clearly in the acidic range. As a result of neutralisation, green-brownish flocs formed in the eluate and the O_2 content fell to 0.4 mg/L. The eluate was stirred for 30 min before preparing the dilutions. This led in an increase of the O_2 content to 5.2 mg/L; the colour of the flocs changed to orange. Adjustment of pH led to a reduction in toxicity in the lowest tested dilution level (25% eluate). In all other dilution levels (0.2-12.5%), no clear effect was observed (Figure 32).

Figure 32: Toxicity of excavated geogenic material (17 05 03*) to *A. fischeri*. Inhibition of bioluminescence after 30 min depending on eluate content



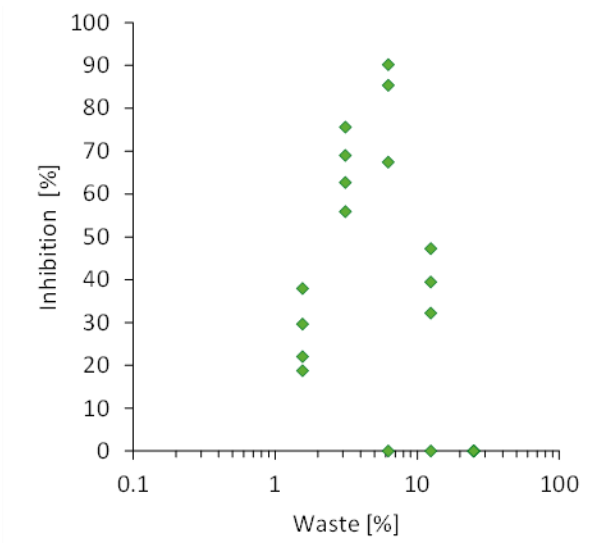
Inhibition of bioluminescence: mean values with standard deviation. T1, T2 and T3: test runs 1, 2 and 3. Regression: Weibull analysis with linear maximum likelihood regression. In T2 and T3, dilution levels of 0.2–25% eluate were tested (instead of 0.4-50%), because dilution during testing had not been considered. In T3, pH of the eluate was adjusted.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.1.2 Toxicity of excavated geogenic material (17 05 03*) to terrestrial organisms

4.4.2.1.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test with *A. globiformis*, excavated geogenic material (17 05 03*) led to an inverted U-shaped concentration-response relationship (Figure 33). While inhibition of up to 65% was observed at higher dilution levels, dehydrogenase activity was increased at the lowest dilution level (25% waste content). Possibly, bacterial growth was promoted by the iron content of the sample. No EC_{50} value could be calculated. Based on the lack of effect at the lowest dilution level, the EC_{50} was assumed to be >25%.

Figure 33: Toxicity of excavated geogenic material (17 05 03*) to *A. globiformis*. Inhibition of dehydrogenase activity depending on waste content

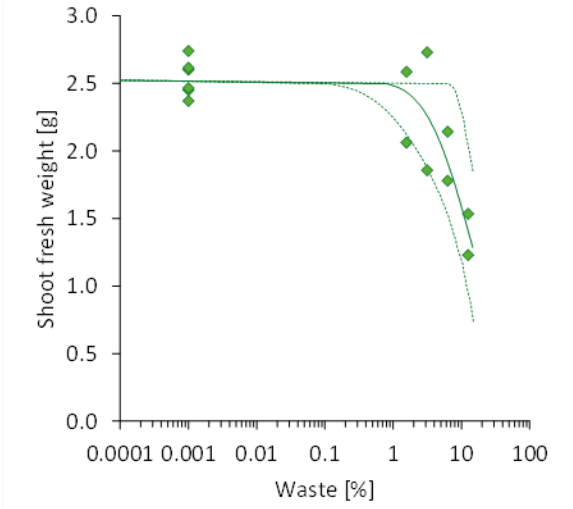


No regression due to increase of dehydrogenase activity at the lowest dilution level.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.1.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, the excavated geogenic material (17 05 03*) led to a clear concentration-response relationship (Figure 34). An EC_{50} of 15.1% (CI: 8.46-26.8%) was determined for shoot fresh weight. This value was slightly above the lowest dilution level at which plants had emerged (12.5% waste). Therefore, the EC_{50} is given as >12.5%. Additionally, a chronic effect concentration (EC_{10}) of 3.05% (CI: 1.08-8.60%) was derived.

Figure 34: Toxicity of excavated geogenic material (17 05 03*) to *B. rapa*. Shoot fresh weight after 14 d depending on waste content

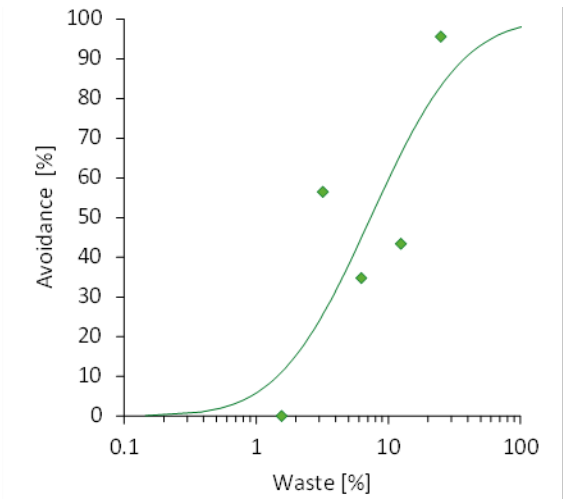


Shoot fresh weight: mean value per surviving plant and pot. Regression (with 95% CI): 3-parameter normal-cumulative distribution function.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.1.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida* and excavated geogenic material (17 05 03*), the concentration-response relationship was statistically not significant (Figure 35). Therefore, the calculated EC_{50} value of 7.36% (CI: n.d.) is not very robust.

Figure 35: Toxicity of excavated geogenic material (17 05 03*) to *E. fetida*. Avoidance after 48 h depending on waste content



Avoidance [%]: Mean values of five replicates. Regression: probit analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.2 Material from the side verges of a federal road (17 05 03*)

4.4.2.2.1 Toxicity of material from the side verges of a federal road (17 05 03*) to aquatic organisms

For the material from the side verges of a federal road (17 05 03*) classified as hazardous by the waste owner, two test runs were performed for all aquatic tests.

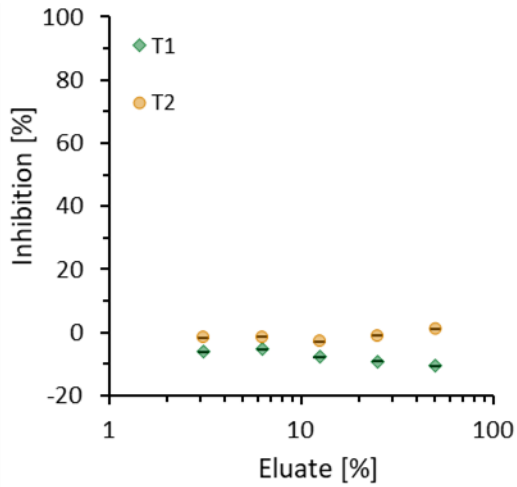
4.4.2.2.1.1 Acute Daphnia test

In both test runs, the material from the side verges of a federal road (17 05 03*) was not acutely toxic to *D. magna* (EC_{50} >50% eluate). Immobility in the controls was 0% in both tests.

4.4.2.2.1.2 Algal growth inhibition test

Due to the autofluorescence of material from the side verges of a federal road (17 05 03*), a fluorescence correction was taken into account when analysing the two algal growth inhibition tests. In both test runs, the eluates were non-toxic to the algae[46]: EC_{50} values were above 50% eluate. As can be seen in Figure 36, there was partly a slight increase in the growth of *R. subcapitata*.

Figure 36: Toxicity of material from the side verges of a federal road (17 05 03*) to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content



Inhibition of growth rate: Mean values with standard deviations. T1, T2: test runs 1 and 2.
Source: own illustration, ECT Oekotoxikologie GmbH

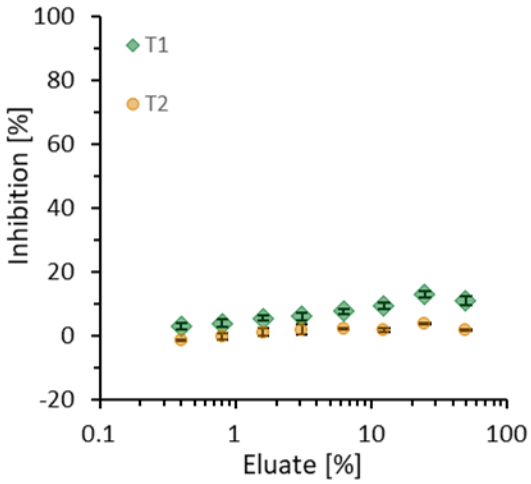
4.4.2.2.1.3 Luminescent bacteria test

EC_{50} values >50% eluate were determined in the luminescent bacteria test in both test runs. The observed inhibition of bioluminescence was less than 20% (Figure 37)[47].

[46] In the first test run with flue-gas dust (10 09 10) from iron and steel casting from plant B and the material from the side verges of a federal road (17 05 03*), the same positive control was used. The $K_2Cr_2O_7$ concentration in this positive control was 8.0 mg/L instead of 0.8 mg/L. Therefore, inhibition of the growth rate (100%) was above the required range (20-80%). As the required sensitivity of the algae to $K_2Cr_2O_7$ (0.8 mg/L) was reached in all other test with inhibitions of 20-80%, results from the first test run were used.

[47] The f_{kt} value (correction factor for variation in the control) after 30 min in test run 1 was 1.32 and, thus, outside the range specified in DIN EN ISO 11348-2 (2009: 0.6-1.3). Therefore, the test run is formally not valid. However, all measured f_{kt} values >1.3 occurred when using the same batch of luminescent bacteria. The increased f_{kt} values did not have a significant influence on the test result.

Figure 37: Toxicity of material from the side verges of a federal road (17 05 03*) to *A. fischeri*. Inhibition of bioluminescence after 30 min depending on eluate content



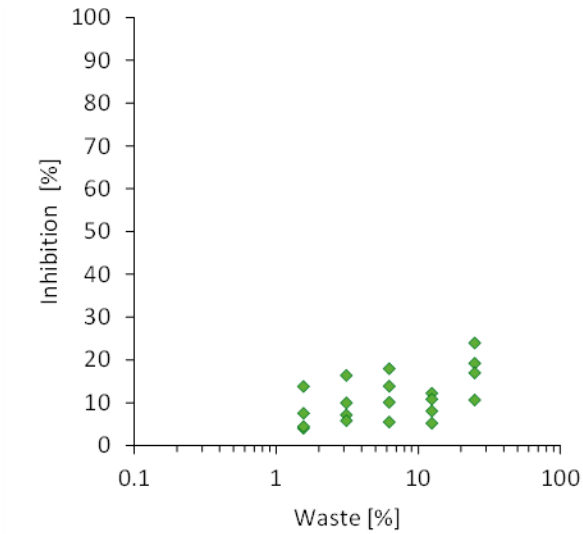
Inhibition of bioluminescence: mean values with standard deviations. T1, T2: test runs 1 and 2.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.2.2 Toxicity of material from the side verges of a federal road (17 05 03*) to terrestrial organisms

4.4.2.2.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test with *A. globiformis*, the evaluation of material from the side verges of a federal road classified as hazardous by the waste owner (17 05 03*) showed no clear effect up to the lowest dilution level (Figure 38). The EC_{50} was therefore >25% and thus above the limit concentration. Due to a lack of effect of the reference substance in the LUFA 2.2 soil, this test was formally not valid. However, a clear effect of the reference substance was observed in quartz sand. For this reason and since the available sample material was already over two months old after the test was analysed, the test was not repeated.

Figure 38: Toxicity of material from the side verges of a federal road (17 05 03*) to *A. globiformis*. Inhibition of dehydrogenase activity depending on waste content

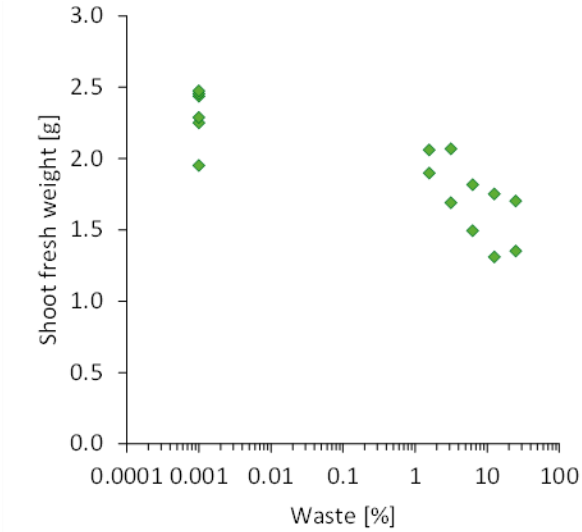


No regression, since <50% effect up to the lowest dilution level.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.2.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, analysis of the material from the side verges of a federal road (17 05 03*) showed a weak concentration-response relationship (Figure 39). Up to the lowest dilution level, effects were clearly below 50%, so that the EC_{50} was >25% and thus above the limit concentration. A chronic effect concentration (EC_{10}) of 0.445% (CI: 0.020–9.82%) could be calculated but was extrapolated below the highest tested dilution.

Figure 39: Toxicity of material from the side verges of a federal road (17 05 03*) to *B. rapa*. Shoot fresh weight after 14 d depending on waste content



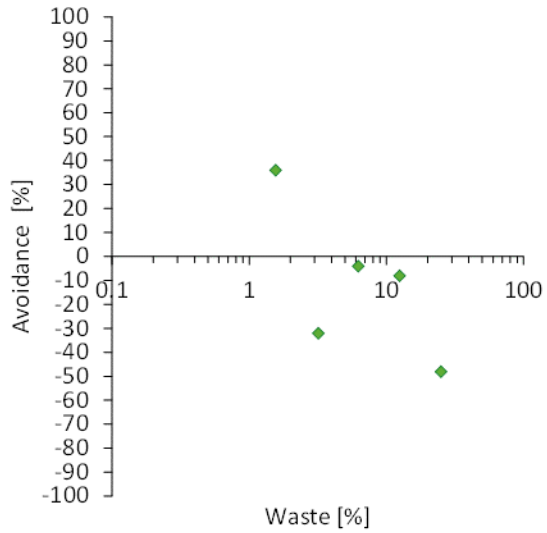
Shoot fresh weight: mean value per surviving plant and pot. No regression, due to <50% effect up to the lowest dilution level.

Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.2.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida*, material from the side verges of a federal road (17 05 03*) showed an almost inverse concentration-effect relationship (Figure 40). While avoidance of the test substrate was observed at the highest dilution level, the lower dilution levels attracted the earthworms, resulting in an EC_{50} >25%. Given that the sample was a natural soil, a preference of the earthworms for the sample over the OECD artificial soil (in the absence of contaminants perceptible to earthworms) is plausible.

Figure 40: Toxicity of material from the side verges of a federal road (17 05 03*) to *E. fetida*. Avoidance after 48 h depending on waste content



Avoidance [%]: Mean values of five replicates. No regression, due to attracting effect of the waste at lower dilution levels.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.3 Material from the side verges of a secondary road (17 05 04)

4.4.2.3.1 Toxicity of material from the side verges of a secondary road (17 05 04) to aquatic organisms

For the material from the side verges of a secondary road (17 05 04) classified by the waste owner as non-hazardous, only one test run was performed in the three aquatic tests due to the consistent results in all test systems (no toxicity, see below).

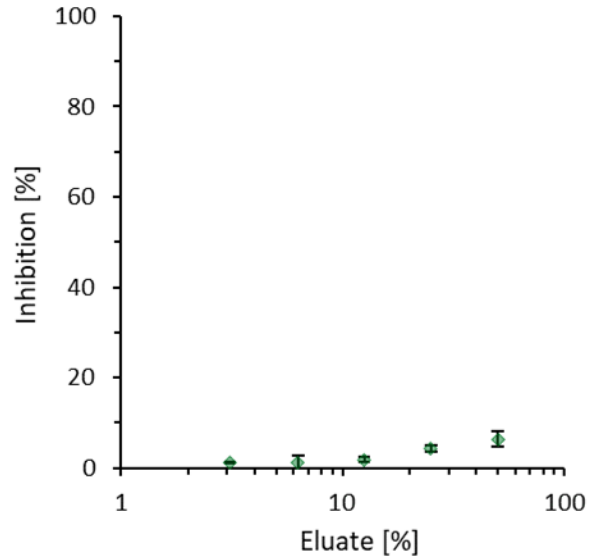
4.4.2.3.1.1 Acute Daphnia test

In the acute *Daphnia* test, immobility in the control and in all five dilution levels (3.1-50%) of the eluate of material from the side verges of a secondary road (17 05 04) was 0%, i.e. no toxicity was detected (EC_{50} >50%).

4.4.2.3.1.2 Algal growth inhibition test

Due to autofluorescence of the eluate of the material from the side verges of a secondary road (17 05 04), a fluorescence correction was included when evaluating the algal growth inhibition test. The eluate only led to a slight inhibition of algal growth in the two lowest dilutions (Figure 41; EC_{50} >50%).

Figure 41: Toxicity of material from the side verges of a secondary road (17 05 04) to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content

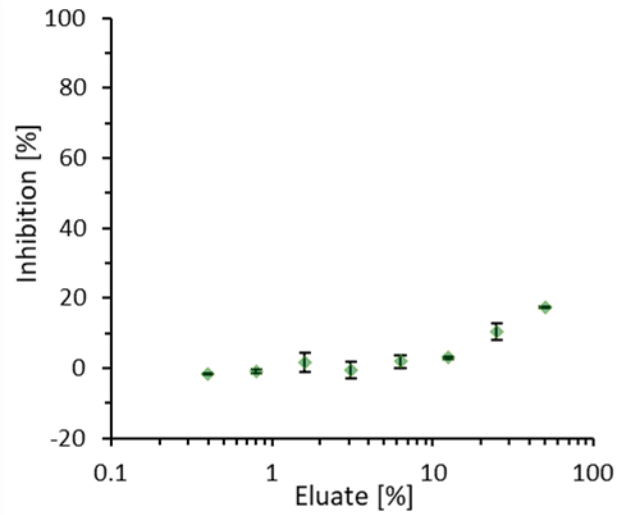


Inhibition of growth rate: mean values with standard deviations.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.3.1.3 Luminescent bacteria test

In the luminescent bacteria test, eluate of the material from the side verges of a secondary road (17 05 04) had also only minor effects in the lowest dilution levels. The inhibition of bioluminescence was below 20% (EC_{50} >50%; Figure 42).

Figure 42: Toxicity of material from the side verges of a secondary road (17 05 04) to *A. fischeri*. Inhibition of bioluminescence after 30 min depending on eluate content



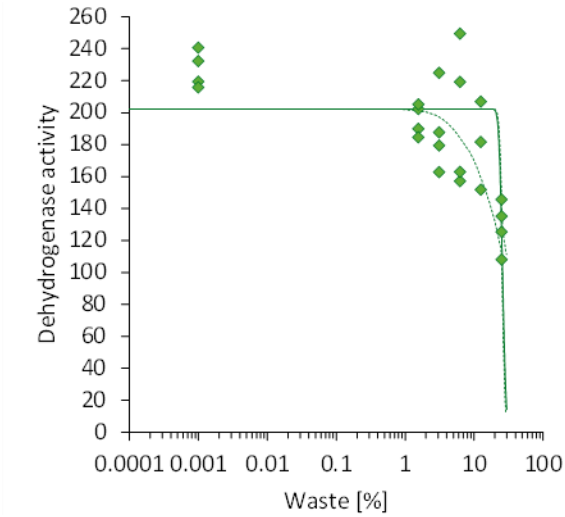
Inhibition of bioluminescence: mean values with standard deviations.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.3.2 Toxicity of material from the side verges of a secondary road (17 05 04) to terrestrial organisms

4.4.2.3.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test, material from the side verges of a secondary road (17 05 04) showed a clear effect only at the lowest dilution level (Figure 43). A (slightly extrapolated) EC_{50} of 25.9% (CI: 25.5–31.6%) was derived.

Figure 43: Toxicity of material from the side verges of a secondary road (17 05 04) to *A. globiformis*. Dehydrogenase activity depending on waste content

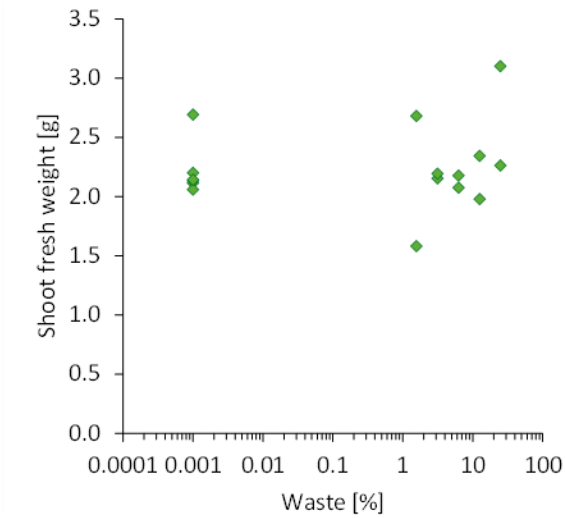


Regression (with 95% CI): 3-parameter normal-cumulative distribution function.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.3.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, material from the side verges of a secondary road (17 05 04) had no inhibitory effect. At the lowest dilution level, there was a slight increase in shoot fresh weight compared to the control (Figure 44). The EC_{50} value was >25%.

Figure 44: Toxicity of material from the side verges of a secondary road (17 05 04) to *B. rapa*. Shoot fresh weight after 14 d depending on waste content

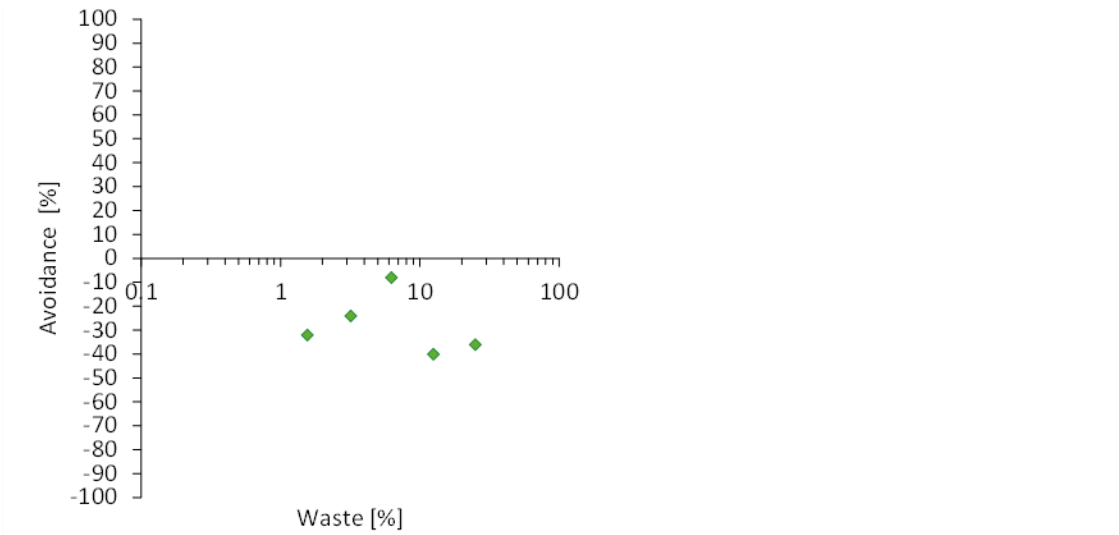


Shoot fresh weight: mean value per surviving plant and pot. No regression, as effect up to the lowest dilution level <50%.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.2.3.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida*, material from the side verges of a secondary road (17 05 04) had no effect, or a tendency towards an attracting effect (Figure 45). This had partly also been observed for material from the side verges of a federal road (17 05 03*) classified as hazardous by the waste owner (see section 4.4.2.2.2.3). It can be explained by the fact that the sample was a natural soil, which can be preferred over the OECD artificial soil in the absence of contaminants perceptible to earthworms. An EC_{50} of >25% was derived.

Figure 45: Toxicity of material from the side verges of a secondary road (17 05 04) to *E. fetida*. Avoidance after 48 h depending on waste content



Avoidance [%]: mean values of five replicates. No regression due to attracting effect of all dilution levels.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.3 Fluff-light fraction and dust (19 10 03*/19 10 04)

4.4.3.1 Fluff-light fraction and dust (19 10 03*)

As mentioned in section 4.2.7.3, no sample of fluff-light fraction and dust (19 10 03*) classified as hazardous was obtained. In consultation with the UBA, it was decided to examine additional samples of fluff-light fraction and dust (19 10 04) classified as non-hazardous by the waste owner.

4.4.3.2 Fluff-light fraction and dust (19 10 04, sieved to <10 mm)

Three samples were evaluated for fluff-light fraction and dust (19 10 04) classified as non-hazardous by the waste owners. Plant A was sampled in May 2022 (batch 1) and February 2023 (batch 2), plant B in February 2023. In all cases, samples were taken from material sieved to <10 mm.

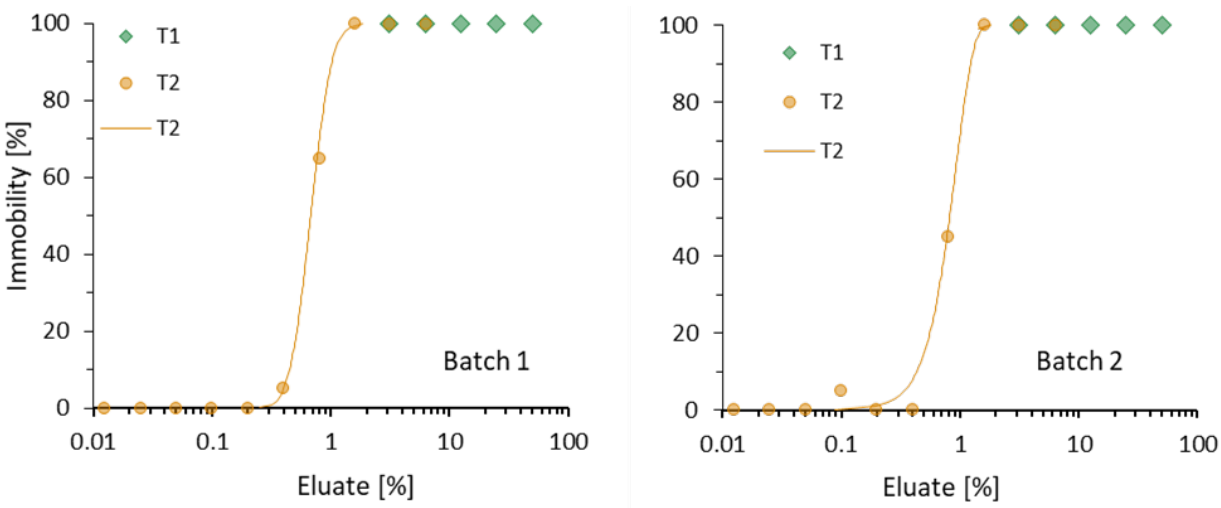
4.4.3.2.1 Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) to aquatic organisms

4.4.3.2.1.1 Acute Daphnia test

At test start, pH values for both batches of fluff-light fraction and dust (19 10 04) from plant A were between pH 9 and pH 10 in the two lowest dilutions (25 and 50% eluate), i.e. outside the suitable pH range for *D. magna* (pH 6.0-9.0) specified in DIN EN ISO 6341 (2013a). The first test run was carried out without pH adjustment. For both batches, all daphnids in the five dilution

levels tested (3.1-50% eluate) were already immobile after 24 h, i.e. half of the exposure time. The EC_{50} values are therefore <3.1% eluate (Figure 46). Due to the high toxicity, the second test run for both batches was performed with 10 dilution levels each: 6.25-0.0125% eluate. Because of the high dilutions, all pH values were within the tolerance range of *D. magna* and no test run with pH adjustment was necessary. Very similar EC_{50} values were determined for both batches, which were clearly below the limit concentration: 0.678% (CI: 0.571-0.804%) for batch 1 and 0.818% for batch 2 (CI: 0.150-2.07%).

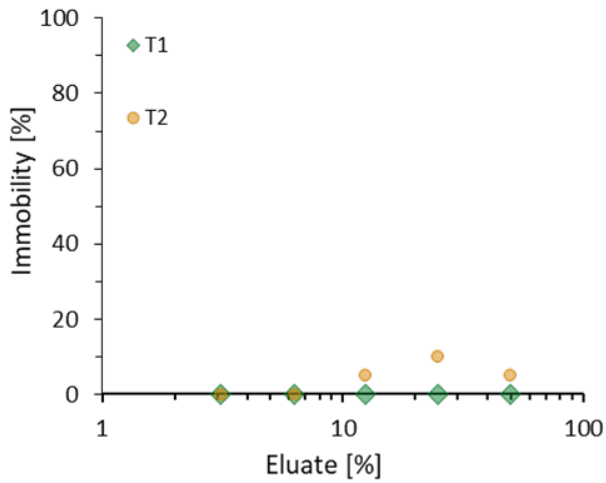
Figure 46: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *D. magna*. Immobility after 48 h depending on the eluate content for batches 1 and 2



Mean values. T1, T2: test runs 1 and 2, T2 with 10 dilution levels. Regression: probit analysis with linear maximum likelihood regression (batch 1), Weibull analysis with linear maximum likelihood regression (batch 2). Immobility in the control was 5% in the first test run for batch 1 and 0% in all other test runs.
Source: own illustration, ECT Oekotoxikologie GmbH

Fluff-light fraction and dust (19 10 04) from plant B was not toxic to *D. magna* in two test runs (Figure 47; EC_{50} >50% eluate).

Figure 47: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant B to *D. magna*. Immobility after 48 h depending on eluate content

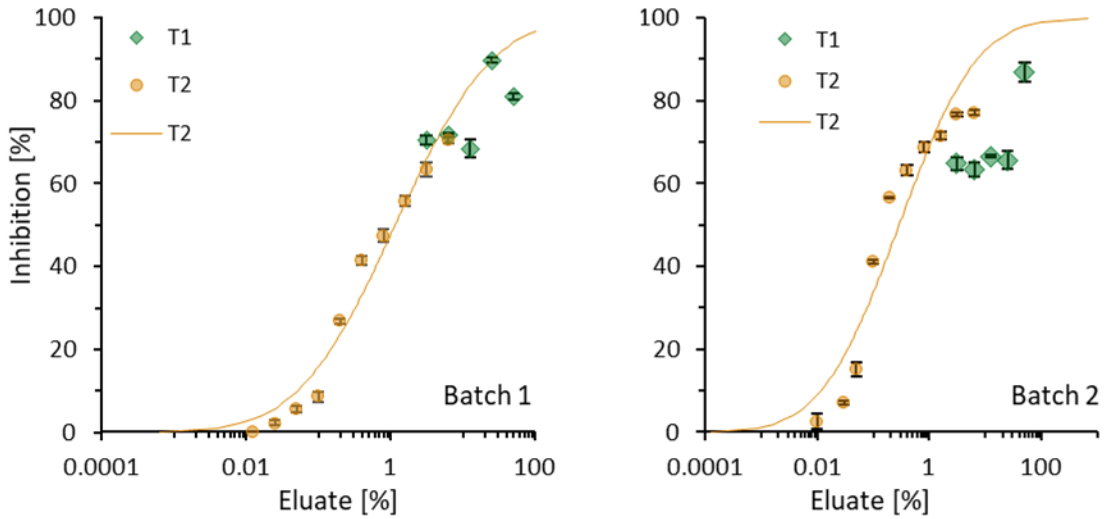


Mean values. T1, T2: test runs 1 and 2. Immobility in the control was 0% in both test runs.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.3.2.1.2 Algal growth inhibition test

In the algal growth inhibition test, both batches of fluff-light fraction and dust (19 10 04) from plant A were also analysed in two test runs. When evaluating the first test run (dilution levels: 3.1-50% eluate), a fluorescence correction was taken into account due to the autofluorescence of the eluates. Batch 1 caused a 68-90% and batch 2 a 63-87% inhibition of algal growth (Figure 48). Thus, EC_{50} values were <3.1% for both batches. Due to the high toxicity, the second test run for each batch was performed with 10 dilution levels each (6.25-0.0125% eluate). Due to the higher dilutions, no fluorescence correction had to be considered in the evaluation. Batch 1[48] led to a 0-70% inhibition of the growth rate, batch 2 to a 3-77% inhibition (Figure 48). The EC_{50} values were 1.16% (CI: 1.13-1.19%) for batch 1, and 0.287% (CI: 0.282-0.293%) for batch 2.

Figure 48: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content for batches 1 and 2

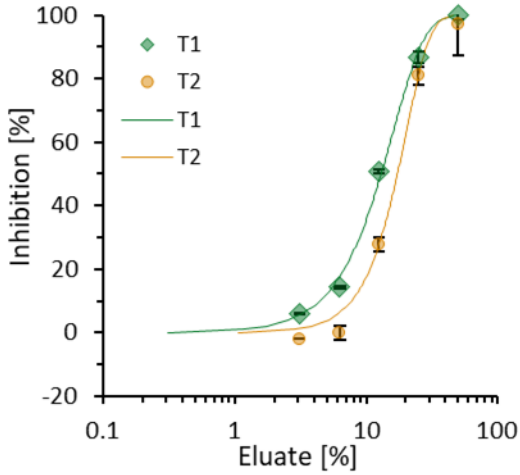


Inhibition of growth rate: mean values with standard deviations. T1–T3: test runs 1-3. Regression: Weibull analysis with linear maximum likelihood regression (batch 1), probit analysis with linear maximum likelihood regression (batch 2). Source: own illustration, ECT Oekotoxikologie GmbH

Fluff-light fraction and dust (19 10 04) from plant B was evaluated in the algal growth inhibition test in two test runs with dilution levels of 3.1-50% eluate. Due to the autofluorescence of the eluate, a fluorescence correction was considered in both test runs. The concentration response curves cover the complete range of growth inhibition in both test runs (Figure 49). For the first test run an EC_{50} of 13.0% (CI: 12.2-13.7%) was derived, for the second test run an EC_{50} of 17.3% (CI: 15.5-19.0%).

[48] In the second test run for batch 1 of fluff-light fraction and dust (19 10 04), the $K_2Cr_2O_7$ concentration in the positive control was 0.375 mg/L instead of 0.8 mg/L due to a dilution error. The inhibition of the growth rate was 7.5%, i.e. below the required range (20-80%). Since the required sensitivity of the algae to $K_2Cr_2O_7$ (0.8 mg/L) was demonstrated in all other test runs by an inhibition of 20-80%, the results from the second test run were used.

Figure 49: Toxicity of fluff-light fraction and dust from plant B (19 10 04, sieved to <10 mm) to *R. subcapitata*. Inhibition of growth rate after 72 h depending on eluate content



Inhibition of growth rate: mean values with standard deviation. T1, T2: test runs 1 and 2. Regression: Weibull analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

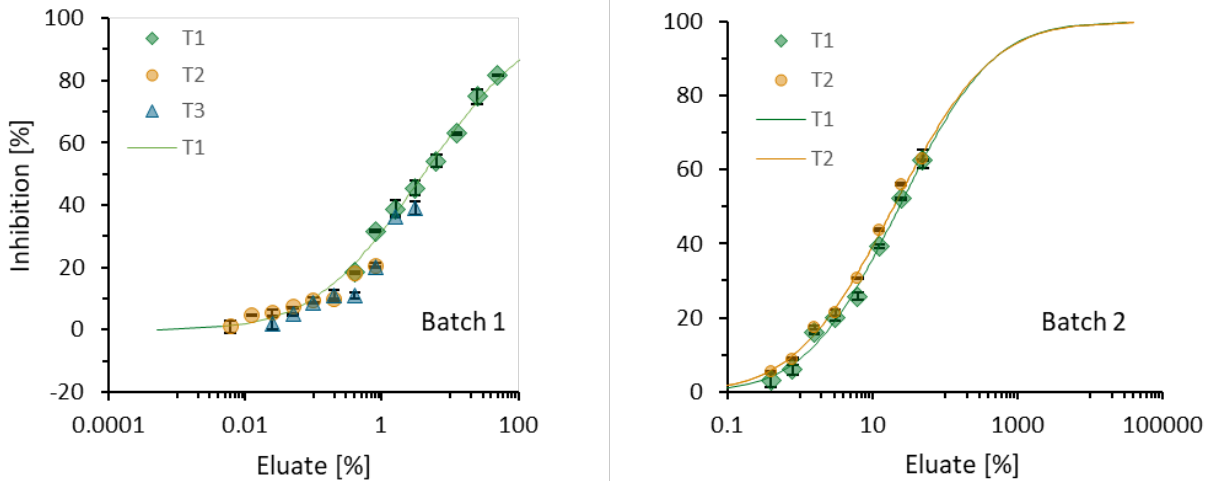
4.4.3.2.1.3 Luminescent bacteria test

Batch 1 of fluff-light fraction and dust (19 10 04) from plant A was analysed in three test runs. In the first test run, pH values for 25 and 50% eluate (pH 8.8 and 9.2, respectively) were outside the suitable pH range for *A. fischeri* (pH 6.0-8.5) specified in DIN EN ISO 11348-2 (2009). Exposure to the eluate of fluff-light fraction and dust led to inhibitions of bioluminescence ranging from 18% (0.4% eluate) to 82% (50% eluate; see Figure 50). An EC_{50} of 4.08% (CI: 3.46-4.80%) was derived. In the two further test runs, higher dilutions were tested as in the tests with daphnids and algae to further evaluate the lower range of the concentration-response curve. However, the EC_{50} values determined in these test runs were above the highest dilution levels used (>0.8% and >3.1%). The three concentration-response curves were consistent (Figure 50), i.e. the results of the first test run were supported by the second and third test run. Due to the higher dilutions in test runs 2 and 3, no pH adjustment was necessary.

Batch 2 of fluff-light fraction and dust (19 10 04) from plant A showed a lower toxicity to the luminescent bacteria than batch 1. In both test runs[49], inhibitions of bioluminescence ranged from ≤5% to 63% (Figure 50). The determined EC_{50} values of 23.5% (test run 1; CI: 20.1-27.9%) and 19.7% (test run 2; CI: 17.3-22.7%) were above the limit concentration.

[49] In both test runs, the f_{kt} value (correction factor for variation in the control) after 30 min was 1.4 and, thus, outside the range specified in DIN EN ISO 11348-2 (2009: 0.6-1.3). Therefore, both test runs with batch 2 of fluff-light fraction and dust were formally not valid. However, all measured f_{kt} values >1.3 occurred when using the same batch of luminescent bacteria. The increased f_{kt} values did not have a significant influence on the test result.

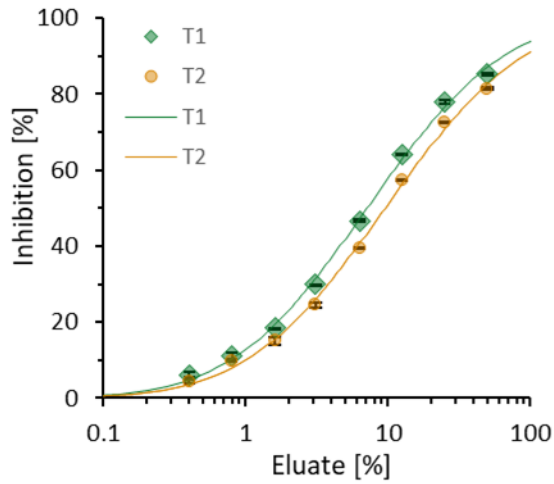
Figure 50: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *A. fischeri*: inhibition of bioluminescence after 30 min depending on the eluate content for batches 1 and 2



Inhibition of bioluminescence: mean values with standard deviation. T1, T2, T3: test runs 1, 2 and 3. Regression: probit analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

For fluff-light fraction and dust (19 10 04) from plant B, two test runs of the luminescent bacteria test were carried out, which showed comparable inhibitions of bioluminescence (Figure 51). Both EC_{50} values, 7.11% (CI: 6.59-7.68%) in test run 1[50] and 9.52% (8.76-10.4%) in test run 2, are below the limit concentration.

Figure 51: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant B to *A. fischeri*: inhibition of bioluminescence after 30 min depending on eluate content



Inhibition of bioluminescence: mean values with standard deviations. T1, T2: test runs 1 and 2. Regression: probit analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

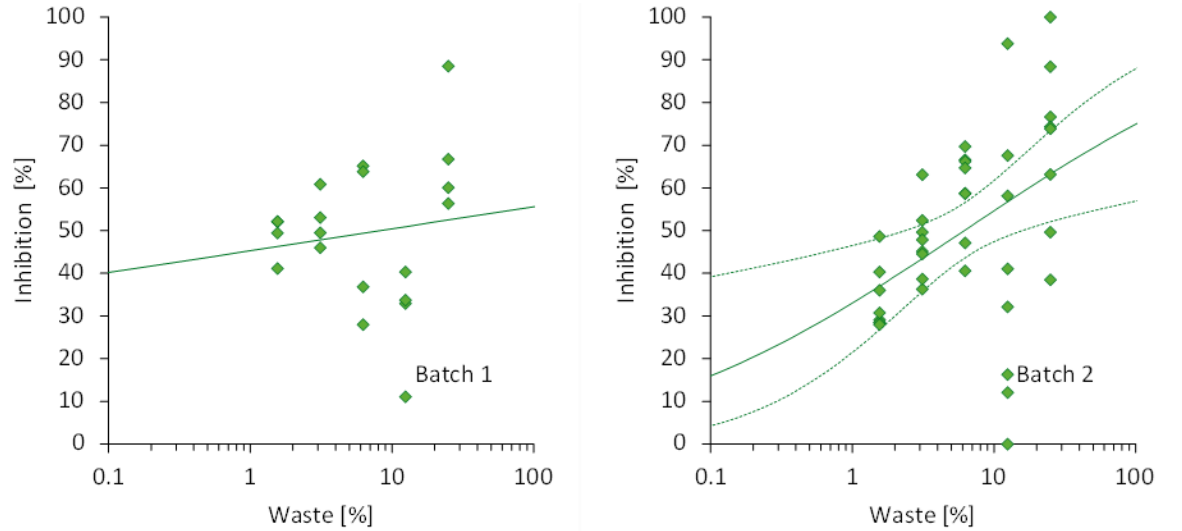
[50] Test run 1 is not formally valid, as the f_{kt} value after 30 min was 1.6, i.e. outside the range (0.6-1.3) specified by DIN EN ISO 11348-2 (2009). However, the very similar results of the two test runs show that this had no significant influence on the test result.

4.4.3.2.2 Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) to terrestrial organisms

4.4.3.2.2.1 Solid contact test with Arthrobacter globiformis

In the solid contact test with *A. globiformis*, results obtained with the two batches of the fluff-light fraction and dust (19 10 04) from plant A were similar (Figure 52). Due to the high variability of the measurement results in previous tests, the replicate number was increased from four to eight in the test with batch 2. For batch 1, an EC_{50} value of 8.20% (CI: n.d.) was calculated, but the concentration-response relationship was not monotonous. For batch 2, a clear concentration-response relationship was recorded; an EC_{50} of 6.16% (CI: 2.36-15.7%) was determined. For both batches, EC_{50} values are thus below the limit concentration.

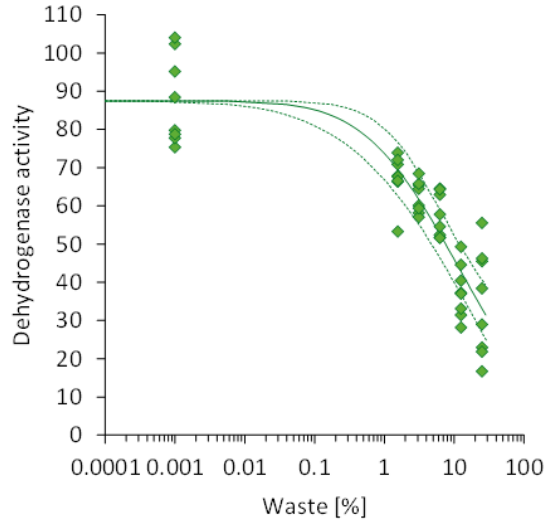
Figure 52: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *A. globiformis*. Inhibition of dehydrogenase activity depending on waste content for batches 1 and 2



Regression: probit analysis with linear maximum likelihood regression (batch 2 with 95% CI).
Source: own illustration, ECT Oekotoxikologie GmbH

The solid contact test with fluff-light fraction and dust (19 10 04) from plant B was also carried out with an increased replicate number (8 replicates). The test result shows a clear concentration-effect relationship (Figure 53). An EC_{50} of 11.7% (CI: 7.65-19.2%) was determined, which is just above the limit concentration.

Figure 53: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) to *A. globiformis*. Dehydrogenase activity depending on waste content for plant B

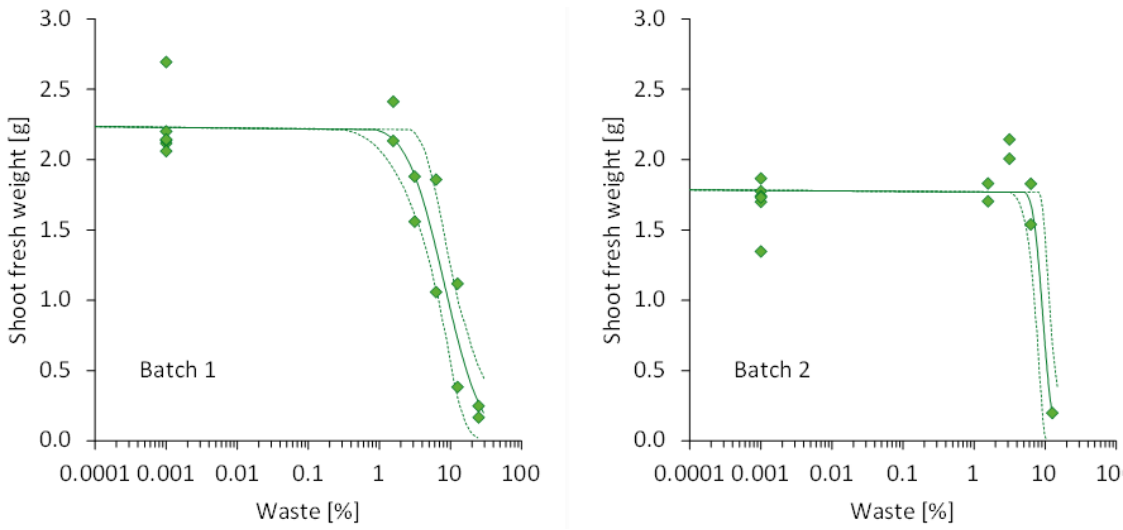


Regression (with 95% CI): 3-parameter normal-cumulative distribution function.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.3.2.2.2 Growth inhibition test with Brassica rapa

In the growth inhibition test with *B. rapa*, the two batches of fluff-light fraction and dust (19 10 04) from plant A led to different concentration-effect relationships: monotonic for batch 1, non-monotonic for batch 2, with a strong increase in the effect starting at the second-lowest dilution level (Figure 54). An EC_{50} of 8.20% (CI: 5.79-11.6%) was determined for batch 1. For batch 2, an EC_{50} of 9.25% (CI: 7.05-12.1%) was calculated for shoot fresh weight and a slightly lower EC_{50} for emergence (7.59%; CI: 6.25-9.20%). The EC_{50} values for both batches were thus below the limit concentration. Chronic effect concentrations (EC_{10} values) were also derived: 2.42% (CI: 1.08-5.46%) for batch 1, and 6.74% (CI: 4.54-10.0%) for batch 2.

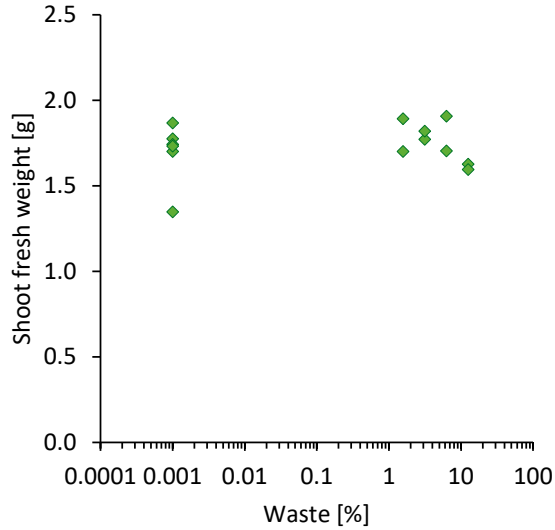
Figure 54: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *B. rapa*. Shoot fresh weight after 14 d depending on waste content for batches 1 and 2



Shoot fresh weight: mean value per surviving plant and pot. Regression (with 95% CI): 3-parameter normal-cumulative distribution function.
Source: own illustration, ECT Oekotoxikologie GmbH

The analysis of fluff-light fraction and dust (19 10 04) from plant B showed no concentration-effect relationship for shoot fresh weight (Figure 55), but there was a clear effect on emergence. No reliable EC_{50} could be determined for shoot fresh weight, because no effect >50% on this endpoint was observed at the lowest dilution level at which plants were emerging (12.5% waste). Therefore, the EC_{50} can only be indicated as >12.5%. For emergence an EC_{50} of 13.5% (CI: n.b.) was calculated. However, since the concentration-response relationship was not statistically significant, this value is considered less robust. The EC_{50} values were above the limit concentration. An EC_{10} of 12.6% (CI: n.b.) was derived, which is, however, subject to the same limitations as the EC_{50} for emergence.

Figure 55: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant B to *B. rapa*. Shoot fresh weight after 14 d depending on waste content

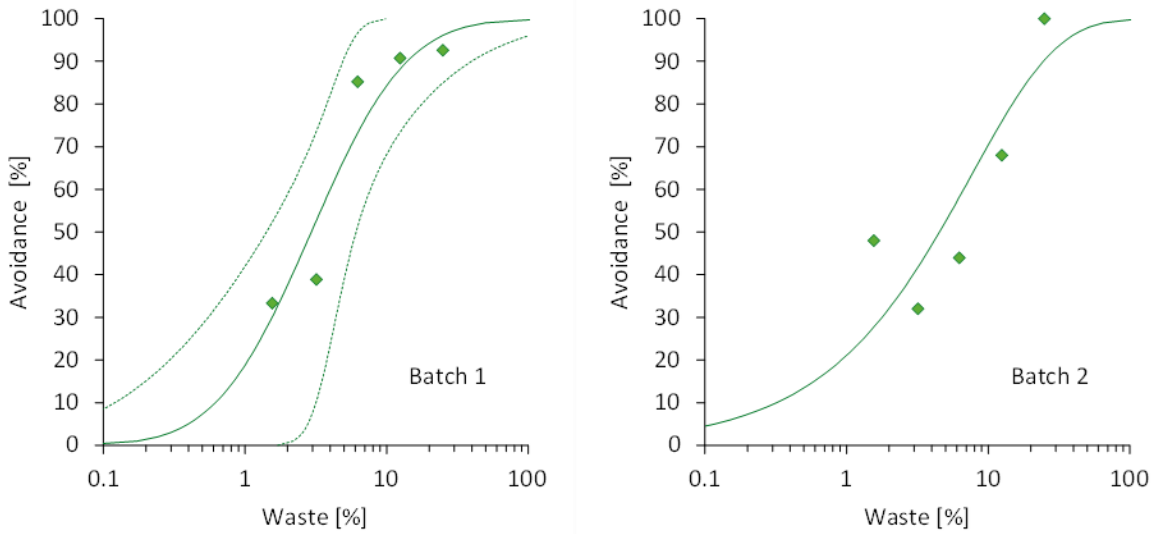


Shoot fresh weight: mean fresh weight per surviving plant and pot. No regression due to <50% effect up to the lowest dilution level, in which plants had emerged.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.3.2.2.3 Avoidance test with earthworms

In the avoidance test with *E. fetida*, the results for the two batches of fluff-light fraction and dust (19 10 04) from plant A were similar (with clear concentration-effect relationships; Figure 56). An EC_{50} of 2.94% (CI: 1.42-6.08%) was determined for batch 1. A slightly higher EC_{50} value of 4.53% (CI: n.b.) was calculated for batch 2. However, this value must be considered less robust, because the concentration-response relationship is not statistically significant. The EC_{50} values for both batches were below the limit concentration.

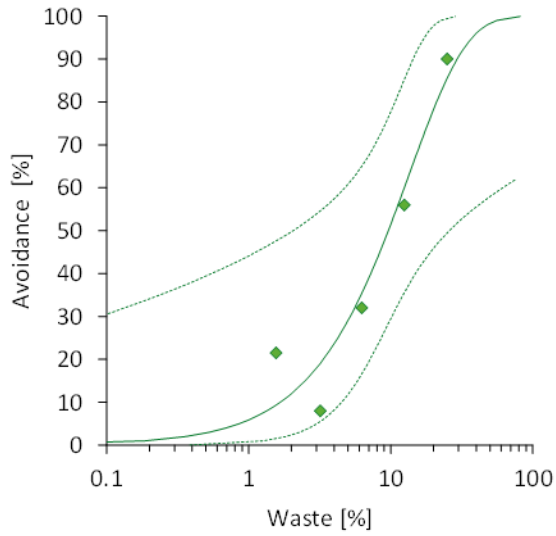
Figure 56: Toxicity of the fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A to *E. fetida*. Avoidance (%) after 48 h depending on waste content for batches 1 and 2



Avoidance [%]: mean values of five replicates. Regression: Weibull analysis with linear maximum likelihood regression (batch 1; with 95% CI), probit analysis with linear maximum likelihood regression (batch 2).
Source: own illustration, ECT Oekotoxikologie GmbH

Evaluation of fluff-light fraction and dust (19 10 04) from plant B showed a clear concentration-response relationship (Figure 57). An EC_{50} of 9.61% (CI: 2.05–25.9%) was derived, which was thus just below the limit concentration.

Figure 57: Toxicity of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant B to *E. fetida*. Avoidance (%) after 48 h depending on waste content

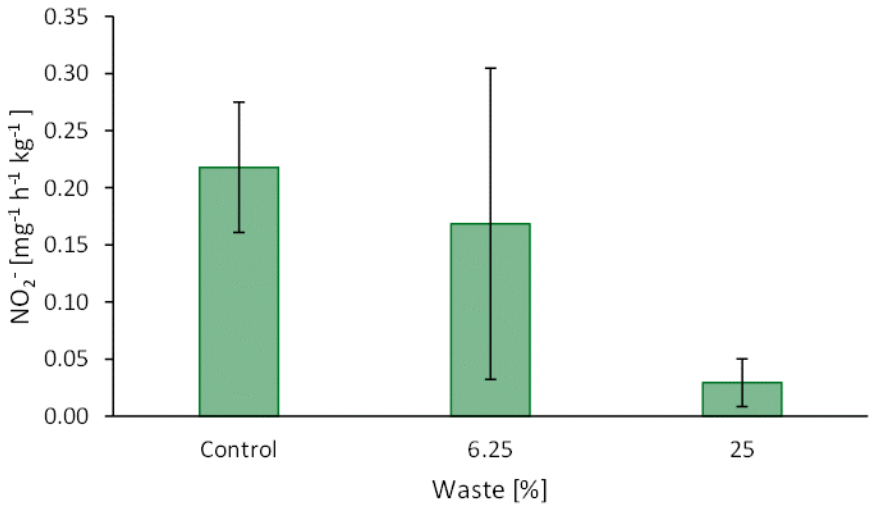


Avoidance [%]: mean values of five replicates. Regression (with 95% CI): Weibull analysis with linear maximum likelihood regression.
Source: own illustration, ECT Oekotoxikologie GmbH

4.4.3.2.2.4 Rapid test to determine potential nitrification

The result of the rapid test to determine potential nitrification showed clear effects at the dilution level with 25% waste content (Figure 58). This test was therefore similarly sensitive as the solid contact test, in which an EC_{50} of 6.16% had been determined for this sample (see above).

Figure 58: Effect of fluff-light fraction and dust (19 10 04, sieved to <10 mm) from plant A on nitrification rate after 6 h depending on waste content for batch 2



Source: own illustration, ECT Oekotoxikologie GmbH.

A possible problem when performing this test with mixtures of control soils and solid waste samples may be that the test organisms are the microorganisms naturally present in the soil. A mixture of the biologically active control soil with up to 25% potentially sterile waste may lead to a reduction in the total abundance of the microorganisms in the respective mixture. Hence, a reduction in the nitrification rate may not (only) be caused by a toxic effect of the waste sample. According to DIN ISO 15685 (2021), eluates are tested for biosolids (e.g. sewage sludge), while soil material (e.g. waste code 17 05 03*/17 05 04) from the field is to be tested in mixtures. As an alternative to testing eluates, a part of the control soil (depending on the waste type) could be mixed with a sterile material (e.g. quartz sand). In addition, control soil and test mixtures could be microbially inoculated using a standard procedure that needs to be defined to ensure comparable conditions in the control soil and in all test mixtures. In any case, further studies on soil microorganism testing and test sensitivity compared to the solid contact test are needed prior to recommending an alternative test for HP 14 classification of waste.

4.4.4 Summary of the results of the ecotoxicity tests

The results of the ecotoxicity tests show that the aquatic tests are highly reproducible (an investigation of the reproducibility of the terrestrial tests was not foreseen in this project). In most cases, the luminescent bacteria test was less sensitive than the algal and *Daphnia* tests (see Table 27).

The terrestrial tests tended to be slightly less sensitive than the aquatic tests. Only in one case (10 09 10: flue-gas dust from iron and steel casting from plant B), solely an effect concentration determined in a terrestrial test (the *Arthrobacter* test) was below the limit concentration. However, as discussed in section 4.4.1.2.2.1, this test was formally not valid due to a lack of effect of the reference substance in the LUFA 2.2 soil.

Waste samples assigned by the waste owner to the hazardous mirror entry were classified as hazardous by HP 14 based on the bioassay results in 3 out of 4 cases. The only exception was the material from the side verges of a federal road (17 05 03*), which showed no toxicity in all biotests used. As discussed during the 4th project meeting, an increased PAH content could have been relevant for its classification as hazardous waste. In addition, the high clay content could have led to a reduced bioavailability of toxic waste constituents.

Waste samples assigned by the waste owner to the non-hazardous mirror entry were classified as hazardous by HP 14 in 5 out of 6 cases based on the bioassay results. In 4 of these cases, the results obtained with more than one test method were below the limit concentration. The high ecotoxicity of the samples of fluff-light fraction and dust (19 10 04, sieved to <10 mm) was particularly remarkable.

Table 27: Overview of the results of the ecotoxicological tests

Waste code and type	Specification	Aquatic tests: EC_{50} (% eluate)								Terrestrial tests: EC_{50} (% waste)		
		Daphnids		Algae			Luminescent bacteria			*Arthrobacter*	Plants	Earthworms
10 09 09* Flue-gas dust from iron and steel casting	Batch 1	5.45	4.26	<3.1	<0.4	**0.201**	>25			1.08	1.66	1.86
	Batch 2	32.8	19.8	<3.1	**0.913**		>25			1.03	3.93	4.49
10 09 10 Flue-gas dust from iron and steel casting	Plant A	5.53	**<3.1**	5.21	**<3.1**		>50	>25		>25	23.4	21.9
	Plant B	>50	>50	43.5	>50		>50			**7.56**	>25	10.8
17 05 03* Soil and stones 17 05 03*	Excavated geogenic material	3.49	**3.15**	7.85	7.77		22.9	>25		>25	15.1	7.36
	Material from side verges of federal road	>50	>50	>50	>50		>50	>50		>25	>25	>25
17 05 04 Soil and stones	Material from side verges of secondary road	>50		>50			>50			>25	>25	>25
19 10 04 Fluff-light fraction and dust (material <10 mm)	Plant A, batch 1	<3.1	**0.678**	<3.1	1.16		4.08	>0.8	>1.6	8.20	8.20	2.94
	Plant A, batch 2	<3.1	0.818	<3.1	**0.287**		23.5	19.7		6.16	7.59	4.53
	Plant B	>50	>50	13.0	17.3		**7.11**	9.52		11.7	13.5	9.61

Only the results of tests without pH adjustment are indicated in the table. For waste samples that were ecotoxic in at least one test, the lowest EC_{50} is highlighted (bold). If two defined (i.e. not unbounded) EC_{50} values were determined for terrestrial plants (shoot fresh weight and emergence), the lower of these two values is indicated.

4.4.5 Derivation of chronic effect concentrations

For the algal growth inhibition test with *R. subcapitata* (DIN EN 38412) and the growth inhibition test with *B. rapa* (ISO 11269-2), chronic effect concentrations (EC_{10} values) were determined in addition to the acute effect concentration (EC_{50}) that have to be determined according to the UBA recommendations. In addition, the acute-to-chronic ratio (EC_{50}/EC_{10}) was calculated (Table 28). In the algal growth inhibition test, the EC_{10} was in most cases by a factor of 2 to 3 lower than the EC_{50}. However, for one waste sample, fluff-light fraction and dust from plant A, the difference was significantly higher (factor 22.6 or 24.7). In the growth inhibition test with *B. rapa*, the EC_{10} was by a factor of 1.37–4.95 lower than the EC_{50}. Note that an EC_{10} could not be determined for all waste samples.

Table 28: Chronic effect concentrations (EC_{10}) in the algal growth inhibition test with *R. subcapitata*, and in the growth inhibition test with *B. rapa* for the test endpoint shoot fresh weight

Waste code and type	Specification	Algal growth inhibition test			Growth inhibition test with *B. rapa*		
		EC_{10} (% eluate)	Confidence interval (% eluate)	EC_{50}/EC_{10}	EC_{10} (% waste)	Confidence interval (% waste)	EC_{50}/EC_{10}
10 09 09* Flue-gas dust from iron and steel casting	Batch 1	0.0897	0.0883-0.0911	2.24	<1.56[c]	—	—
	Batch 2	0.358[a]	0.353-0.362	2.55	1.67	0.550-5.09	2.35
10 09 10 Flue-gas dust from iron and steel casting	Plant A	2.01	1.89-2.14	2.59	13.2	8.50-20.4	1.77
	Plant B	20.2 23.7	19.8-20.6 22.4-24.9	2.15	n.d.[d]	—	—
17 05 03* Soil and stones	Excavated geogenic material	3.81 4.58	2.60-4.78 4.34-4.80	2.06 1.70	3.05	1.08-8.60	4.95
	Material from side verges of federal road	> 50[b] > 50[b]	— —	— —	0.445[e]	0.020-9.82	—
17 05 04 Soil and stones	Material from side verges of secondary road	>50[b]	—	—	>25[b]	—	—
19 10 04 Fluff-light fraction and dust (sieved to <10 mm)	Plant A, batch 1	0.0513	0.0485-0.0542	22.6	2.42	1.08-5.46	3.39
	Plant A, batch 2	0.0116	0.0111-0.0121	24.7	6.74	4.54-10.0	1.37
	Plant B	4.42 7.73	3.74-5.06 5.60-9.45	2.94 2.20	12.6	n.d.	—

n.d. = Not definable; [a] extrapolated value (lowest inhibition: 18%); [b] <10% inhibition at all evaluated dilution levels; [c] plants had only emerged in the control and in the highest dilution level; [d] >10% inhibition at all dilution levels, but no monotonous concentration-response relationship; [e] extrapolated value (lowest inhibition: 14%).

4.5 Discussion of the results of the ecotoxicological tests

In the following, the results of the ecotoxicological tests are discussed in comparison to data identified in the literature search (section 3.2).

4.5.1 Flue-gas dust (10 09 09*/10 09 10) from iron and steel casting

Pandard et al. (2006) analysed a furnace dust from the casting of iron (waste code: 10 09 09*/ 10 09 10) using the tests indicated in Table 29. The eluates used in the aquatic tests were prepared according to DIN EN 12457-2 (particle size <4 mm), their pH was adjusted to 5.5–8.5. For the terrestrial tests, waste dilutions were prepared from OECD artificial soil and the solid waste sample.

Table 29: Overview of the tests used by Pandard et al. (2006)

Test organism	Acute/ chronic	Endpoint	Effect concentration	Test duration	Test guideline[a]
Aquatic tests					
Raphidocelis subcapitata	Chronic	Growth rate	EC_{20}	72 h	NF T90-375
Daphnia magna	Acute	Immobility	EC_{50}	48 h	EN ISO 6341
Ceriodaphnia dubia	Chronic	Reproduction	EC_{20}	7 d	NF T90-376
Aliivibrio fischeri	Acute	Luminescence inhibition	EC_{50}	30 min	ISO 11348-3
Terrestrial tests					
Lactuca sativa	Acute	Emergence, biomass	EC_{50}	14 d	ISO 11269-2
Eisenia fetida	Acute	Mortality	LC_{50}	14 d	ISO 11268-1

[a] Current versions of the mentioned test guidelines: ISO 6341 (2012c), ISO 11268-1 (2012d), ISO 11269-2 (2012a), ISO 11348-3 (2007c), NF T 90-375 (standard withdrawn, last version: AFNOR 1998b), NF T90-376 (standard withdrawn, last version: 2000).

The authors do not indicate the specific effect concentrations that were derived but present the tested waste samples in a matrix based on the relative sensitivity of the used tests. The furnace dust from the casting of iron had a high toxicity in the aquatic tests (EC_{20} and EC_{50} values were clearly below 1% eluate), while the terrestrial tests were less sensitive (EC_{50} and LC_{50} values were >1% waste). According to their relative sensitivity, the tests were sorted as follows: *R. subcapitata* > *C. dubia* > *A. fischeri* > *D. magna* > *E. fetida* > *L. sativa*.

Hence, the sample was found to be ecotoxic as was also the case for the samples of this waste type evaluated in the present project. In addition, a higher sensitivity of the algal test as compared to the *Daphnia* and luminescent bacteria test was recorded.

4.5.2 Soil and stones (17 05 03*/17 05 04)

In contrast to the matrices of most other waste types, soil represents a natural habitat for a variety of organisms. The ecotoxicological assessment of potentially contaminated soils already has a history of around 30 years. It is also relevant in other regulatory areas, particularly in soil

and nature conservation and for the assessment of contaminated sites. In this context, the studies of Hund-Rinke et al. (2002), Jensen & Mesman (2006) and Römbke et al. (2006) should be mentioned. These activities have found their way into international standardisation (ISO 2019a, b). To date, there are more than 50 test standards from ISO/TC 190/SC 4 (Biological characterisation of soils) for the assessment of soil quality. Most of these can also be used for the assessment of waste. Accordingly, there is a large number of publications on this topic. Due to the diversity of the origin and the contamination of this waste type, it is almost impossible to compare the results of the present project with literature results, especially since no studies were identified in which geogenic excavations or material from the side verges of roads were explicitly analysed. For this reason, the individual studies on this waste type are not presented here. Information on the studies can be found in the Excel table (see section 3.2).

4.5.3 Fluff-light fraction and dust (19 10 03*/19 10 04)

Deventer & Zipperle (2004) analysed a waste sample assigned to the waste type 19 10 04. This sample was inhomogeneous and contained metals, plastics, and other material. The limit values for lead, copper, mercury, and total heavy metal in the solid material were exceeded. According to the provisional implementation instructions of the German federal state Baden-Württemberg (UVM 2002), the sample was therefore classified as waste requiring special supervision. It was evaluated using the tests indicated in Table 30. Eluates used in the aquatic tests were prepared based on DIN 38414-4 (1984)[51]. For the terrestrial tests, dilution levels were prepared from LUFA standard soil and the solid waste sample. The sample showed a low toxicity in the algal growth test (G-value 10) and the acute *Daphnia* test (G-value 2), intermediate toxicity in the luminescent bacteria test (G-value 16), the solid contact test (G-value 10-100) and the growth inhibition tests with higher plants (G-value >32). This led to a classification into toxicity class 2 (G-value >10-100; Table 30). The limit concentration (G-value >10) corresponds to the limit concentration of ≤10% eluate or waste content used in the present project.

Table 30: Overview of the biotests used by Deventer & Zipperle (2004) and the G-values determined with fluff-light fraction and dust (19 10 04)

Test organism	Endpoint	Test duration	Test guideline[a]	Effect concentration
Aquatic tests				
Desmodesmus subspicatus	Growth rate	72 h	DIN 38412-33	G-value = 10
Daphnia magna	Immobility	48 h	DIN 38412-30	G-value = 2
Aliivibrio fischeri	Luminescence inhibition	30 min	ISO 11348-2	G-value = 16
Terrestrial tests				
Arthrobacter globiformis	Dehydrogenase activity	2 h	DIN 38412-48	G-value = 10-100
Avena sativa, Brassica oleracea, Lycopersicum esculentum	Biomass	14-21 d	OECD 208	G-value > 32

[a] Current versions of the mentioned test guidelines: DIN 38412-30 (1989b), DIN 38412-33 (1991), DIN 38412-48 (standard withdrawn, last version: 2002), ISO 11348-2 (2007a), OECD 208 (2006a).

[51] This standard has been withdrawn.

Garcia Geronasso (2010) investigated a heterogeneous mixture of shredded light particles (including dust from hoover cleaning and shredded mixed metal scrap from recycling plants) that was assigned to the waste type 19 10 04. According to chemical analyses, the mixture contained relatively high concentrations of lead, cadmium, cyanide, mercury, copper, zinc and arsenic. It consisted of approximately 86% light particles, 13% metal compounds and 1% minerals.

The author performed reproduction and feeding tests with springtails (collembolans) of the species *Folsomia candida*, the former according to ISO 11267[52] (test endpoint: number of juveniles after 28 d), the latter according to a method developed by Domene et al. (2007) (test endpoint: feeding inhibition after 48 h). For these tests, dilution levels were prepared from OECD artificial soil and the solid waste sample. For the feeding tests, artificial soil was prepared without peat, and the pH value of the mixtures was adjusted to pH 6.0±0.5.

In the reproduction test, the waste sample proved to be harmful to the population development and survival of the collembolans. The EC_{20} and EC_{50} were 4.82% and 14.1% waste content, respectively, the NOEC was 3.10% and the LC_{50} was 9.37%. In contrast, no significant difference was found between the control and the tested waste concentrations with regard to the feeding behaviour (NOEC ≥50% waste content).

Römbke et al. (2010) and Höss & Römbke (2019) analysed fluff-light fractions from a widely integrated shredder plant[53] assigned to waste code 19 10 04 with the tests listed in Table 31. Eluates for the aquatic tests were prepared according to DIN EN 12457-2. For the terrestrial tests, dilution levels of the solid waste were prepared with OECD artificial soil (avoidance test with earthworms), LUFA standard soils 2.3 (plant test) or 2.2 (nematode test), or quartz sand (bacterial contact test). The HP 14 criterion was considered fulfilled, if LID was >4 in aquatic tests, or >8 in terrestrial tests. Strong effects (LID = 16) were found in all terrestrial tests with the exception of the nematode test (LID = 0), but not in the two aquatic tests (LID ≤4; Table 31).

Table 31: Overview of the tests used by Römbke et al. (2010) and Höss & Römbke (2019) and the LID values determined with fluff-light fraction and dust (19 10 04)

Test organism	Endpoint	Test duration	Test method[a]	Effect concentration
Aquatic tests				
Raphidocelis subcapitata	Growth	72 h	ISO 8692	LID = 4
Daphnia magna	Immobility	24 h	ISO 6341	LID = 2
Terrestrial tests				
Arthrobacter globiformis	Dehydrogenase activity	2 h	ISO 10871	LID = 16
Brassica napus	Biomass	14-21 d	ISO 11269-2	LID = 16
Eisenia fetida	Avoidance behaviour	48 h	ISO 17512-1	LID = 16
Caenorhabditis elegans	Reproduction	96 h	ISO 10872	LID = 0

[a] Current versions of the mentioned test guidelines: ISO 6341 (2012c), ISO 8692 (2012b), ISO 10872 (2020), ISO 11269-2 (2012a), ISO 17512-1 (2008a), ISO 18187 (2016a).

[52] Current version of the test guideline: ISO 11267 (2023c).

[53] A widely integrated shredder plant has extensive processing facilities behind the shredder and recovers significantly more material than shredder plants of previous designs.

Deprez et al. (2012) and Weltens et al. (2014) analysed an unspecified 'shredder fluff' (waste code 19 10 03*) using the aquatic tests listed in Table 32. Organic extracts of the waste were prepared using acetone or an acetone/hexane (1+1) mixture. The effect concentrations were expressed as gram equivalents of the original sample per litre (geq/L) to relate the measured toxicity directly to the amount of waste and to compare the toxicity of different waste materials. A limit value of 5 geq/L (or >50% effect in the limit test with algae at 10 geq/L) was defined for the organic extracts. A high toxicity was recorded in all tests (EC_{50} <5 geq/L or 93% inhibition in the limit test with 10 geq/L; LC_{50} = 6.1 geq/L; see Table 32).

Table 32: Overview of the tests used by Deprez et al. (2012) and Weltens et al. (2014) and the effect concentrations derived for fluff-light fraction and dust (19 10 03*)

Test organism	Endpoint	Test duration	Test method[a]	Effect concentration
Raphidocelis subcapitata	Growth rate	72 h	OECD 201	93% inhibition in the limit test with 10 g_{eq}/L
Daphnia magna	Immobility	48 h	OECD 202	EC_{50}: 2.14 g_{eq}/L
Aliivibrio fischeri	Luminescence inhibition	30 min	ISO 11348-3	EC_{50}: 1.98 g_{eq}/L
Danio rerio	Mortality	48 h	Fish egg test	LC_{50}: 6.1 g_{eq}/L

[a] Current versions of the mentioned test guidelines: ISO 11348-3 (2007b), OECD 201 (2011), OECD 202 (2004).

The Public Waste Agency of Flanders (OVAM 2018) investigated a 'shredder fluff' (waste code 19 10 04) containing various types of coarse materials (rubber, plastic, metal, wires, cables, etc.) using the test systems indicated in Table 33. For the aquatic tests, an eluate was prepared with a one-stage batch procedure (L/S: 10 L/kg, 24 h). For the terrestrial test, dilution levels were prepared from the solid waste sample and OECD artificial soil. The waste showed no ecotoxicity in the aquatic tests (LID value ≤4 or EC_{50} >10% eluate). However, in the avoidance test with earthworms, a toxic effect was observed (LID value >8, Table 33).

Table 33: Overview of the tests used by OVAM (2018) and the effect concentrations determined for fluff-light fraction and dust (19 10 04)

Test organism	Endpoint	Test duration	Test guideline[a]	Effect concentration
Aquatic tests				
Raphidocelis subcapitata	Growth	72 h	OECD 201	EC_{50} = 69% LID ≤ 4
Daphnia magna	Immobility	48 h	OECD 202	EC_{50} > 100% LID ≤ 4
Aliivibrio fischeri	Luminescence inhibition	30 min	ISO 11348-3	EC_{50} > 45% LID ≤ 4
Terrestrial test				
Eisenia fetida	Avoidance behaviour	48 h	ISO 17512-1	LID > 8

[a] Current versions of the mentioned test guidelines: ISO 11348-3 (2007b), ISO 17512-1 (2008a), OECD 201 (2011), OECD 202 (2004).

In summary, five different waste samples assigned to the waste types fluff-light fraction and dust (19 10 03*/19 10 04) were evaluated in the ecotoxicological studies identified in the literature search. The methods used for sample preparation and testing were in part comparable to methods used in the present project. Due to the different sources of the waste sample (on which more detailed information was mostly lacking) and, partly, also due to methodological differences, the results are not directly comparable to the results obtained in the present project. However, as in the present project, some samples that were assigned to waste code 19 10 04 also proved to be ecotoxic in earlier studies.

5 Proposals for an update and further development of the UBA recommendations

Based on the literature search (section 3) and the experience gained during sampling, sample preparation, elution and ecotoxicological testing of the 10 waste samples, suggestions were made for updating and further developing the UBA recommendations. The discussions with the project advisory group at the meetings on 09 March 2022 and 02 March 2023 and the expert workshop on 29 August 2023 were considered. The proposals for an update and further development of the UBA recommendations relate to sampling, sample pre-treatment, subsampling in the laboratory, elution, ecotoxicological testing, and minimum requirements for reports.

In addition, issues were identified for which there is a need for action at regulatory level. Some suggestions are made for modifications of the test guidelines for the bioassays.

5.1 Sampling and sample pre-treatment

With regard to sampling, the 'Commission notice on technical guidance on the classification of waste' (EU 2018) refers to the technical reports CEN/TR 15310-1 to -5, but allows other approaches, such as LAGA guideline PN 98 (LAGA 2019) that is mainly applied in Germany, if they lead to similarly reliable results (see sections 3.1.1.1 and 4.2)[54].

The UBA recommendations from 2013 already refer to a CEN/TC 292 standard being developed; aspects that appeared important were integrated. The idea of defining a laboratory sample based on the number of particles contained in the sample corresponds to the specifications that are now detailed in the technical report CEN/TR 15310-1.

Analogous to the general recommendations of CEN TC 292 on the application of the minimum sample mass formula and specifically to the CEN/TR 15310 report series, it is proposed to adapt the UBA recommendations to the approach of CEN TC 292 regarding the fraction of particles with the characteristic(s) to be determined and the desired reliability of the results.

If no further information is available, it is assumed that 10% of the particles in the population contain the effective characteristic. In terms of reliability, a coefficient of variation (CV) of 10% is assumed. This means that the characteristics' content in the laboratory sample does not deviate by more than 20% (within the twofold standard deviation of approx. 95%) from the "true value" of 10% in the population. These assumptions shall ensure that a sufficient reliability is achieved and that the required effort remains manageable.

Due to the possibilities resulting from the CEN/TR 15310 reports to classify a sample qualitatively, it is recommended to perform sampling and sample pre-treatment according to CEN/TR 15310 and to compare the results with the specifications of LAGA PN 98. Generally, the sample masses required for biological analyses clearly exceed the minimum sample masses according to the guidance. Hence, it is crucial to recognise how far a laboratory sample can be subdivided into a test sample without significantly reducing the reliability of the results. While the guideline DIN 19747 (2009a) does not contain any specific recommendation, the CEN/TR 15310 reports provide guidance.

The following procedure is recommended for obtaining samples for the biological analysis of waste.

[54] The procedure described in the LAGA guideline PN 98 is now also available as DIN standard: DIN 19698-1 (DIN 2014), which is very similar to the PN 98.

Careful planning of sampling according to CEN/TR 15310

As part of the planning of sampling, which is to be carried out by an expert in consultation with the waste owner and, if necessary, the customer, the basic quantity (population) should be determined, and the objective of the investigations should be defined. This includes a verification of the specific framework conditions at the sampling site as well as a collection of information on the origin of the waste and its temporal and spatial heterogeneity. Possibly, information such as the d_{95}, bulk density, particle density and details for estimating the distribution of characteristics among the particles can already be obtained here.

Based on this information, a sampling approach is selected, and the sampling procedure is determined. The required minimum sample mass depends on the particle dimensions. The minimum sample mass is determined using the values specified by the expert. The number of individual samples is selected based on a comparison of the mass for the individual samples and the minimum sample size. However, a composite sample should comprise at least 16 individual samples.

For the field sample, care must be taken to ensure that after separation of interfering materials and oversized particles (>4 mm), the remaining sample mass is sufficient to perform the desired investigations.

Collection of at least 16 probabilistic individual samples

Based on the procedure defined in the sampling plan, at least 16 individual samples are taken from the population. From a probabilistic point of view, sampling from the falling material flow is ideal. Alternatively, samples can also be taken from a heap or from a flat structure prepared using a wheel loader. The type of sampling must be categorised in accordance with CEN/TR 15310 and justified. It has to be documented which tools are used.

Combination of individual samples into a mixed sample (field sample)

All individual samples (random samples) are combined into a mixed sample. Any reduction of the sample size from the field sample to the laboratory sample should be avoided to provide sufficient test material for biological investigations.

Joint sampling for biotests and chemical analyses

At the expert workshop, the question was raised as to whether a joint sampling can be performed to obtain samples for both, chemical analyses and bioassays. The objective of sampling is always to produce a subset of a population, which is as representative as possible, from which the characteristics of a population can be determined with sufficient certainty. The only difference between sampling for bioassays and sampling for chemical analyses is that a larger sample mass is required for bioassays. In principle, joint sampling for biotests and chemical analyses is therefore possible. Either parallel samples can be taken for biological and chemical analyses, or a sufficiently large sample can be taken and divided in the field to obtain samples for the biological and the chemical-analytical laboratory.

5.2 Sample pre-treatment to obtain a laboratory sample from the field sample

As part of the sample pre-treatment, the recommendation should remain to perform a sieve analysis using round-hole sieves. The generation of a simple sieve curve using hand sieving requires a manageable amount of effort, but provides valuable information on particle size distribution and allows to verify the assumed d_{95} using the original sample.

If the sample contains material >4 mm, an appropriate approach needs to be selected. According to the recommendations from 2013, this material should be crushed/shredded, if possible, and returned to the sample. However, it should be avoided to create too many new surfaces that would influence the elution behaviour of the sample. The material should not be finely ground. For small mass fractions >4 mm, it has been recommended in UBA (2013; section 5.2.4) to discard the oversized particles if necessary.

It is suggested to use the minimum sample mass formula according to CEN/TR 15310-1 for selecting the appropriate approach. The oversized particles (>4 mm) are a subsample of the laboratory sample, which a lower particle size limit that can be estimated as d_{05} = 4 mm. For this subsample, a minimum sample mass can be determined.

Example 1

For a soil sample with the estimated input values d_{95} = 20 mm, bulk density ρ_B = 0.92 kg/dm³, particle density ρ_P = 1.8 kg/dm³, a field sample with a mass of 8 kg is taken. During sample pre-treatment, 2,834 g (35.4 mass%) of oversized particles (>4 mm) are obtained. The sieve curve shows that the d_{95} is not 20 mm but 25 mm.

According to the sieve curve, d_{95} is set at 25 mm, and the sieve whole diameter of 4 mm is used as d_{05}. Particle density (ρ_P = 1.8 kg/dm³), the desired coefficient of variation (CV = 10%) and the fraction of particles with the characteristic to be determined (p = 10%) are retained.

For the selected example, the granulometric correction factor g is 0.25 and the minimum sample mass (M_{SAM}) is 3,313 g. The mass of oversized particles (2,834 g, see above) is below the minimum sample mass. Therefore, the subsample probably contains less than 900 particles. It does not meet the desired confidence level of CV = 10% (CV is 10.8%).

In this particular case, the mass of oversized particles is relatively high. The minimum sample mass of 3,313 g is almost reached, so that the CV in the oversized particle subsample (10.8%) does not differ substantially from the target CV (10%). Crushing/shredding is recommended.

If the mass of oversized particles was only 283 g (5.2 mass%), this would correspond to approx. 77 particles. In such a subsample, a fraction of particles with the characteristic to be determined of 10% can only be represented with a CV of 34%. Within the twofold standard deviation, this subsample can therefore have a characteristic's content between 3.2 and 16.8%. It is unlikely that this subsample randomly meets the "true characteristic's content" of 10%. Since the large particles can carry high characteristic's loads, it is in this case recommended to discard the oversized particles.

If the mass of oversized particles accrued during sieving is greater than the minimum sample mass for the subsample (see example 1), the oversized particles can be carefully crushed/shredded to a d_{95} of approx. 4 mm and returned to the sample. Care should be taken to avoid fine grinding that would create many fresh surfaces, which may influence elution

behaviour. For soil samples, it is recommended to use a jaw crusher with a gap width set to 4 mm, and to crush several times. For cutting mills, appropriate sieves should be selected.

If the mass of oversized particles is below the recommended minimum sample mass, the oversized particles should be discarded, since this subsample does not meet the minimum sample mass requirements. The separated particles >4 mm should still be weighed and documented photographically. A mass balance should be drawn up and documented for sample pre-treatment.

Irrespective of whether the resulting laboratory sample contains oversized particles crushed to <4 mm or not, the minimum sample mass must be recalculated for this sample in accordance with CEN/TC 15310-1 using the new input values resulting from sample pre-treatment, i.e. d_{95} = 4 mm and a modified bulk density, where applicable. The resulting value is the minimum sample mass for the test sample.

Example 2

The d_{95} is set to the sieve whole diameter of 4 mm. If required, an estimated value of 0.5 mm or 1 mm is used as d_{05}. In both cases, g = 0.25 is assumed. The particle density of ρ_P = 1.8 kg/dm³, the desired coefficient of variation (CV = 10%) and the fraction of particles with the characteristic to be determined (p = 10%) are retained unless there are reasons for a change.

For the selected example, the minimum sample mass (M_{SAM}) for the laboratory sample <4 mm is 13.6 g. This value indicates how large a subsample of the laboratory sample needs to be to represent a fraction of particles with the characteristic to be determined of p = 10% with a CV of 10%. It should be noted that this CV is part of the sample preparation. It must be added quadratically to the CV determined for sampling.

If a sample division is necessary to obtain samples for several laboratories, this must be shown in the mass balance. Ripple splitters, or coning and quartering are recommended for sample division. Any reserves of sample material have to be documented.

For storage or transport, the laboratory sample must be packaged. Good experience has been made with transferring the sample material into robust, light-tight PE bags, which are transported in PP drums. This allows cooling with ice packs without humidifying the sample.

The project advisory group expressed the view that it would be desirable to harmonise the guidance for sampling and sample preparation for soils and waste. In the area of soil protection, the German 'Mantelverordnung' (MVO 2021[55]) came into force on 01 August 2023. According to the MVO, the particle size of the soil to be eluted should be <2 mm. While a reduction of the particle size (to <2 mm instead of <4 mm) would further reduce particulate heterogeneity of the samples, it would at the same time also reduce representativeness of the sample for the population. For a d_{95} of 2 mm, the minimum sample mass of the measurement sample would be 3.4 g. If the sample mass used for the bioassays remains unchanged, reproducibility of the tests could be expected to improve. However, sample preparation would be more complex. Harmonisation with the MVO could also potentially prevent harmonisation between the different EU Member States. Currently, a particle size <4 mm is used for biotests with waste in several other European countries (see section 3.1.1.1).

The project advisory group expressed the wish to standardise the preparation of samples in the laboratory for biotests and chemical analyses. While joint sampling for bioassays and chemical

[55] Regulation on the introduction of a substitute construction materials regulation, on the revision of the federal soil protection and contaminated sites regulation and on the amendment of the landfill ordinance and the commercial waste ordinance.

analyses is possible as discussed above, technical requirements prevent joint sample processing in the laboratory, as described in the following.

In ecotoxicological studies with waste, the effect of bioavailable waste constituents on aquatic and terrestrial organisms is determined. Terrestrial tests are generally carried out with material sieved to <4 mm (or <2 mm for microbial bioassays with soil organisms). For the aquatic tests, aqueous eluates are used. The used elution procedure is explicitly designed to elute short-term water-available constituents, i.e. not all potentially toxic substances.

By contrast, it is the aim of chemical-analytical studies to determine the total content of certain waste constituents, possibly after extraction of these elements or compounds from the waste or after digestion of the entire sample (e.g. aqua regia digestion). To ensure that particulate heterogeneity does not cause a systematic error when dividing the sample, a carefully planned procedure of (a) reduction of the particle size and (b) sample division is necessary. Generally, test samples are finely ground to a particle size <250 µm.

5.3 Sample transport and sample storage

The transport of waste samples should be as short as possible so that no changes in the sample properties occur. The transport time should be considered as part of the storage time. It should be less than 48 h, and temperature during transport should be low. In the present project, the transport time was always less than 24 h. An attempt was made to keep the sample material at a low temperature using up to 20 commercially available cooling packs. However, the question arises whether a temperature of, e.g. 4°C is really required for transporting a waste that had been stored for a prolonged period at ambient conditions at its point of origin, also in view of the fact that a transport at a low temperature is more expensive.

Only a minor addition is therefore proposed to the recommendations for sample transport and storage. It is assumed that the sample material is chemically and physically stable for 48 h at ambient temperature (and protected from light). If there are indications that this is not the case, the laboratory sample should be transported at a temperature of 4±2°C.

The samples should not be stored at 4±2°C for longer than two months. If longer storage is necessary, an accompanying physical, chemical or biological analysis of waste-specific parameters should be carried out to determine any possible change in the waste samples during storage.

5.4 Sample division in the laboratory

In the UBA recommendations, a minimum particle number of 20,000 particles is recommended for the mass fraction above the 20th percentile, i.e. for the sample mass that remains when 20% fine particles are separated. Without reducing the particle size, the particle number should not be below this value. In practice, however, samples are divided to produce test samples for the individual bioassays without knowing the particle numbers contained in the test samples and usually without reducing the particle size.

Obtaining a test sample from a sufficiently large laboratory sample is basically equivalent to taking a sample from the laboratory sample. In DIN 19747 (2009a), various methods for obtaining the test sample are specified, such as use of a ripple splitter, a rotating sample splitter or a rotating tube splitter.

To achieve a high degree of homogeneity of the subsamples, DIN 19747 recommends the use of the cross-riffling method. In a systematic investigation of the variances occurring during sample preparation (Ketelhut 2013), large division errors occurred when obtaining test samples (100 g)

by repeated division of mixed construction waste, even for waste fractions with high proportions of the characteristic to be determined (>20%). The characteristic's content of the test sample can deviate by up to 60–80% from the characteristic's content of the laboratory sample. In a comparison of the division methods cross-riffling, fractional shovelling, ripple splitters, and coning and quartering, the use of ripple splitters, and coning and quartering were recommended (Ketelhut 2013).

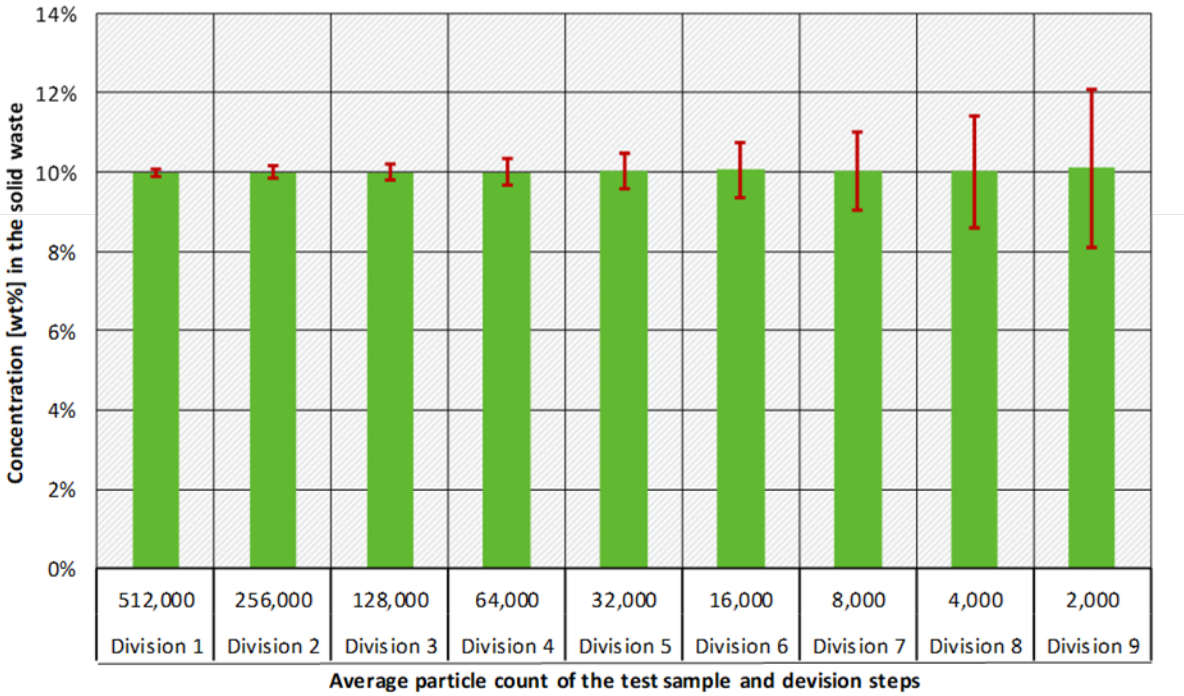
There are basically two ways to obtain a test sample:

- Division/splitting of the laboratory sample
- Obtaining a composite sample by taking individual samples from the laboratory sample

5.4.1 Computer simulation of both methods for obtaining a test sample

To estimate the effects on the variance of the content of a characteristic in test samples, a simulation with random numbers was performed using approximate distributions for parameter contents. A characteristic was selected that is carried by 10% of the particles. All particles have identical dimensions and do not differ in weight. The results of the simulations are shown in Figure 59 and Figure 60.

Figure 59: Simulation of the variances occurring when obtaining test samples by repeated division of the sample in halves



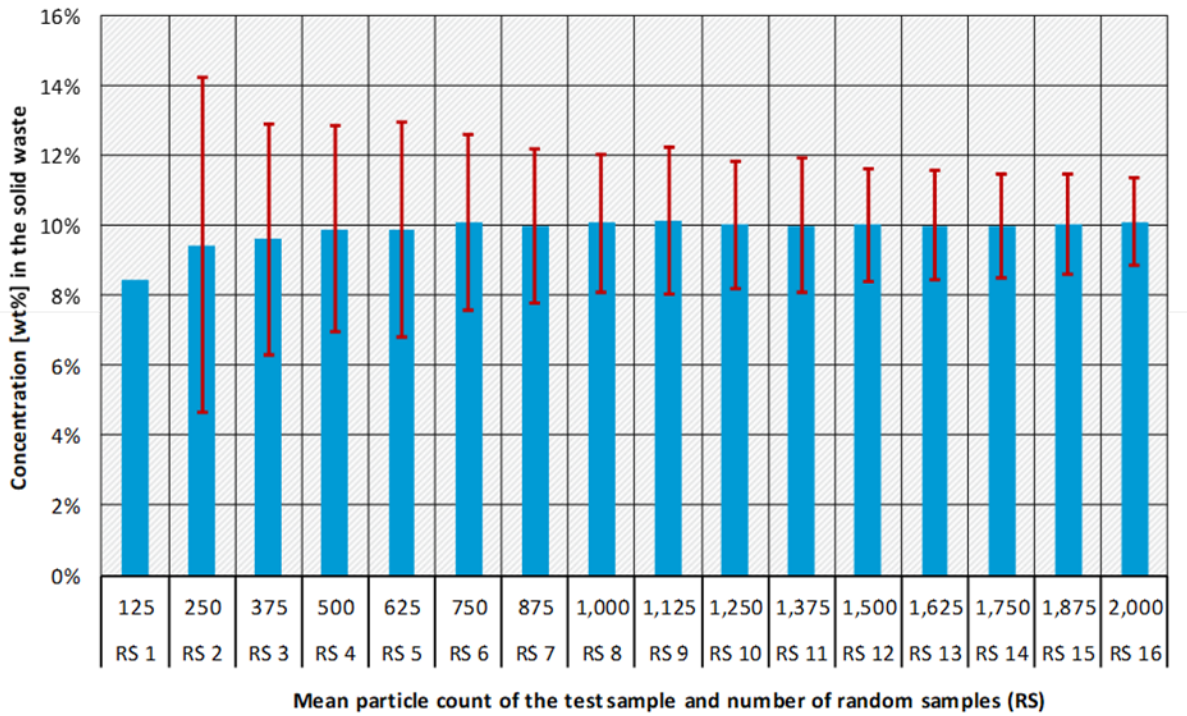
Simulated production of test samples from a laboratory sample with 1,024,000 particles for a fraction of particles with the characteristic to be determined (p) = 10%. The bar height is the expected value from 100 simulations, the antennae show twice the standard deviation.
Source: own illustration, Ralf Ketelhut Stoffstromdesign

The bar height represents the mean value of the content of a characteristic in the 100 test samples obtained by division. Irrespective of the division step, the mean value of all samples is very close to the real fraction of particles with the characteristic to be determined of p = 10%. The variance of the obtained results increases with each division step. If a sample of 1,024,000 particles is divided nine times to a target size of 2,000 particles, the characteristic's content of

the test sample is – within the twofold standard deviation – between 8 and 12%. The method of the (mathematically optimal) division into halves therefore leads to a coefficient of variation of 10%.

Alternatively, a test sample can also be obtained in such a way that 16 individual samples of 125 particles are taken from the population of the homogenised laboratory sample and combined into a composite sample.

Figure 60: Simulation of the variances occurring when obtaining test samples by taking random samples from the laboratory sample



Simulated production of test samples by taking random samples (RS) of 125 particles from the laboratory sample with a fraction of particles with the characteristic to be determined (p) = 10%. The bar height is the expected value obtained from the sum of the random samples, the antennas show twice the standard deviation.
Source: own illustration, Ralf Ketelhut Stoffstromdesign

The result of this second simulation shows that with an increasing number of random samples the expected mean value stabilises in the direction of the expected value for the characteristic's content and variance decreases. Under the conditions of the simulation, obtaining a test sample from a laboratory sample by taking 16 random samples and combining them into a composite sample leads to a coefficient of variation of 6%. Hence, this method appears to be more favourable than obtaining test samples by repeated division of the sample in halves.

Based on the simulation results and in analogy to the sampling procedure, it is recommended to obtain test samples from a sufficient number of random samples taken from the carefully homogenised laboratory sample. We recommend a number of ≥16 random samples, so that the size of the individual sample is ≤ one sixteenth of the mass of the test sample.

If possible, the required amount of test sample should be larger than the minimum sample mass of the laboratory sample determined during sample pretreatment.

Example 3

In the example described for sample pretreatment (example 2), the minimum sample mass is 13.6 g. If only 8 g of sample mass is used for a bioassay, this value is below the minimum sample mass. A test sample of 8 g contains only approx. 59% of the minimum sample mass and thus only approx. 531 particles. With such a test sample, a fraction of particles with the characteristic to be determined (p) of 10% can be represented with a coefficient of variation (CV) of 13%.

To determine the coefficient of variation for representing the fraction of particles with a certain characteristic in the test sample, the coefficients of variation for sampling (CV_{SAM}) and sample preparation (CV_{SP}) have to be aggregated. This is done by quadratic addition. The fraction of particles with a certain characteristic in the test sample therefore has a CV_{TS} of

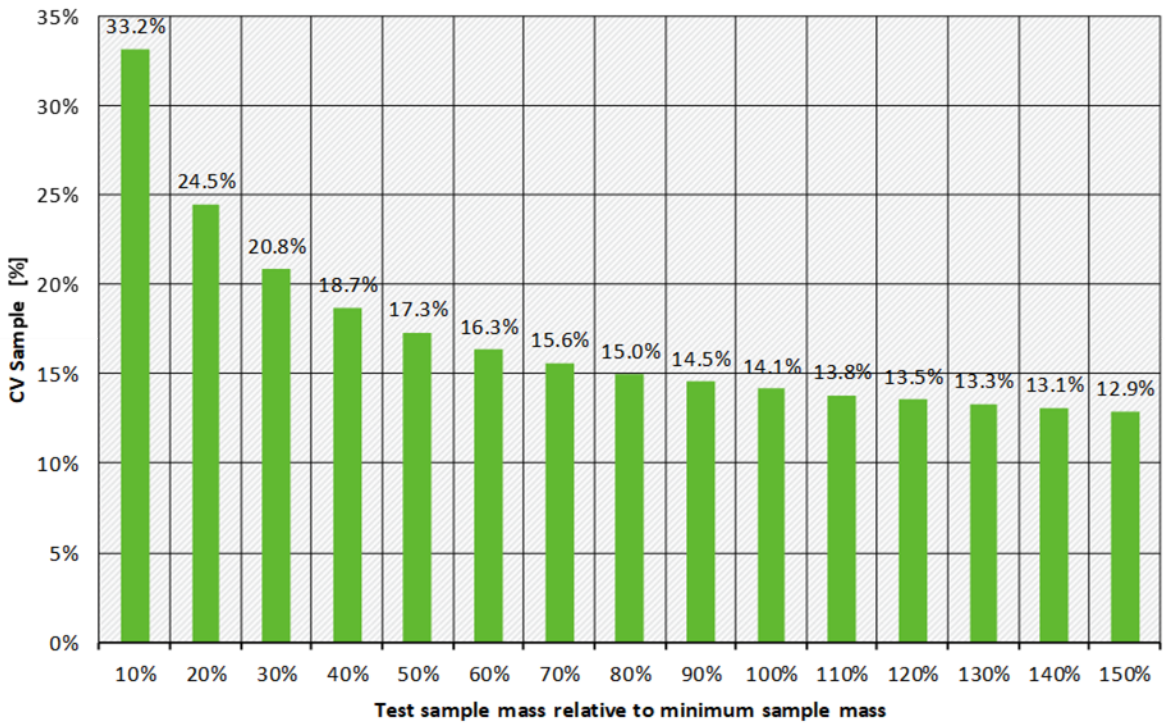
$$CV_{TS} = \sqrt{CV_{SAM}^2 + CV_{SP}^2} = \sqrt{0.01 + 0.017} \approx 16.4\%$$

It can deviate by approx. 33% from the assumed true value of 10% within the twofold standard deviation.

Due to the quadratic addition, the CV for the test sample cannot be lower than the CV for sampling (10%). If the minimum sample mass is used as the test sample, the total CV is 14.1% (see also Figure 14).

Since test samples are produced randomly, it is possible that a test sample of 8 g can show fractions of particles with the characteristic to be determined of 6.7% and 13.3% within the twofold standard deviation. It is therefore not surprising if measurement results from such test samples differ by a factor of 2.

Figure 61: Coefficient of variation (CV) of the content of a characteristic in a test sample derived by quadratic aggregation of the coefficients of variation for sampling and sample preparation



Assumptions: fraction of particles with a certain characteristic of p = 10%, CV for sampling = 10%
Source: own illustration, Ralf Ketelhut Stoffstromdesign

For very small test samples, the coefficient of variation can be large and the measurement uncertainty high. Exceeding the minimum sample mass has limited advantages for the precision of the measurement, given that the measurement uncertainty is limited by the CV for sampling.

5.5 Elution

As described in the UBA recommendations (UBA 2013, section 5.2.4), a leaching procedure is used to produce an aqueous extract for assessing the ecotoxicity of water-eluable waste components. In the first paragraph of section 5.2.4, it is noted that the elution method needs to be adapted to the analysis of waste samples containing organic pollutants. In the following, the elution of waste using a one-stage batch procedure with a liquid to solid ratio of 10 L/kg waste dry weight and a duration of 24 h according to DIN EN 12457-2 (2003a) is recommended. The elution method described in DIN EN 14735 is also based on this standard. DIN EN 12457-2 was developed to examine "mainly inorganic" waste constituents (see section 1 of this standard and Berger et al. 2013). This point is not addressed in DIN EN 14735. Both in the UBA recommendations and in DIN EN 14735, it should be specified whether or to what extent the one-stage batch procedure according to DIN EN 12457-2 is suitable to elute organic pollutants and poorly soluble inorganic pollutants (e.g. zinc oxide, see section 3.3.1)[56].

The project advisory group stated that a harmonisation of the guidance for sampling and sample preparation for soils and waste would be desirable (see section 5.2). In the field of soil protection, eluates are – according to the MVO (2021) – produced with an L/S ratio of 2 L/kg, either with a batch procedure according to DIN 19529 (DIN 2023b) or with a column percolation method according to DIN 19528 (DIN 2023a). For the percolation method (DIN 19528), it is noted in UBA (2013) that experience is lacking regarding biotesting of the obtained eluates and possible limit concentrations for HP 14 classification (Table 34). An adaptation of the elution method has implications for the results of the ecotoxicological tests performed with the eluates. If the elution method used is to be adapted or modified, comparative experimental investigations are necessary. Depending on the results of these studies, the limit concentrations for aquatic biotests might also need to be adapted.

The batch procedure according to DIN EN 12457-2 suggested in the UBA recommendation is also used in several other European countries (see section 3.1.1.1). A harmonisation with the MVO (2021) could therefore prevent a harmonisation of the elution procedures between the different EU Member States.

Table 34: Comparison of the recommended method for elution of waste samples with the recommended methods for the elution of soil samples according to MVO (2021)

	Method for elution of waste samples	Methods for elution of soil samples according to MVO (2021)	
Standard	DIN EN 12457-2 (2003a); see also DIN EN 14735 (2022)	DIN 19529 (2023b)	DIN 19528 (2023a)
Type of procedure	Batch procedure	Batch procedure	Percolation method
Liquid to solid ratio	10	2	2

[56] This issue was investigated for the batch procedure according to DIN 19529 (DIN 2023b) (Kalbe 2020, 2021).

	Method for elution of waste samples	Methods for elution of soil samples according to MVO (2021)	
Duration	24 h	24 h	—
Remark	Recommended procedure according to UBA (2013)	Not mentioned in UBA (2013)	Mentioned in UBA (2013), but experience on biotests with eluates and possible limit concentrations for HP 14 classification is lacking

5.6 Biotesting

5.6.1 General approach and test strategy

According to section 6.1.1 of the UBA recommendations, the calculation method should be used for HP 14 classification if sufficient data are available on waste composition and on the ecotoxicity of the individual waste constituents. However, in this case there is also the option of classifying the waste by means of biotests. According to Commission Decision 2000/432/EC (EC 2015; see also AVV 2020), hazardous properties of waste can be determined either based on the concentrations of the waste constituents as set out in Annex III to the Waste Framework Directive (2008/98/EC, EC 2018) or based on testing (see section 1.1). This should be mentioned in the UBA recommendations.

If the available data on waste composition and ecotoxicity of the individual waste constituents are not sufficient to classify the waste regarding its ecotoxicity, the HP 14 classification should – according to UBA (2013) – be based on biotests. This approach is conclusive and should be maintained.

When performing biotests, UBA (2013) recommends a stepwise approach: aquatic ecotoxicity tests are carried out first, and terrestrial ecotoxicity tests are only carried out if the results of all aquatic tests are negative. This approach makes sense and should be maintained, since (1) one positive test result is sufficient for an HP 14 classification, (2) the aquatic tests tend to be more sensitive than the terrestrial tests, and (3) the effort required to perform the terrestrial tests (especially for the growth inhibition test with *B. rapa*) is higher than for the aquatic tests.

5.6.2 Biotest battery: type and scope of tests

The test battery proposed in the recommendations (see also section 3.3.2) consists of three aquatic and three terrestrial toxicity tests, which are also mentioned in DIN EN 14735 (2022). The tests each cover (a) the taxonomic groups plants, invertebrates and microorganisms, and (b) the trophic levels producers, consumers and destruents. Thus, basic requirements for a test battery are met (Traas & van Leeuwen 2007, Römbke et al. 2018).

Necessity of terrestrial biotests

The test battery suggested by UBA (2013) is one of the more comprehensive test batteries compared to other European countries. For instance, only aquatic tests are used in many countries (section 3.1.1.2). The results of the present project show that the aquatic biotests tend to be more sensitive than the terrestrial ones. Overall, only one waste sample, flue-gas dust (10 09 10) from the casting of iron and steel from plant B, proved to be ecotoxic exclusively in a terrestrial test (section 4.4.4). Yet, this test was a solid contact test with *A. globiformis* that was formally not valid (see section 4.4.1.2.2.1). However, the evaluation of the data identified in the

literature search also showed that the solid contact test sometimes reacts more sensitively than aquatic tests (section 3.2.3.1).

In the aquatic tests of the biotest battery, eluates are used that contain short-term water-available constituents of the respective waste (section 5.5). Soil organisms are exposed to waste constituents in a different way than aquatic organisms: in addition to exposure via pore water, the uptake of soil particles is also relevant, especially for terrestrial invertebrates. Therefore, the bioavailability of pollutants and the resulting toxicity is different for soil organisms than for aquatic organisms. By additionally performing terrestrial bioassays in the presence of exclusively negative results of the aquatic tests potential toxic effects of poorly water-soluble waste constituents on soil organisms can be detected. For this reason, Pandard & Römbke (2013) and Planchon et al. (2015) recommended that terrestrial tests should be part of a bioassay battery for the HP 14 classification of waste (see section 3.1.2).

The need to use terrestrial bioassays in addition to aquatic tests was also a key result of the UBA project PROSOIL ('Protection of soil organisms: development of toxicity criteria for soil organisms in the framework of classification of substances and PBT assessment'; Scholz-Starke et al. 2022). In 2020, the EU Commission announced in its 'Chemical strategy for sustainability' that it would evaluate the feasibility of including terrestrial toxicity criteria in the CLP Regulation. In the project PROSOIL, the assumption was analysed that classifications according to the CLP Regulation, which are based on aquatic ecotoxicity data only, are conservative enough to cover possible hazards for soil organisms and thus ensure adequate protection of these organisms. Toxicity thresholds for soil organisms were determined using various statistical methods and compared with the aquatic classifications according to CLP. Scholz-Starke et al. (2022) showed that, depending on the statistical approach chosen to derive toxicity thresholds for soil organisms, 10-30% of all substances in the project database were not covered by the aquatic classifications according to CLP. The protection of soil organisms based on the aquatic toxicity data alone is therefore not sufficient. Thus, a bioassay battery for HP 14 classification of waste from mirror entries should include not only aquatic but also terrestrial test methods.

Type and scope of terrestrial biotests

In the solid contact test with *A. globiformis*, a high variability of the results was frequently observed, especially in tests with fluff-light fraction and dust. Doubling the number of replicates in some of the tests did not lead to any fundamental improvement. The very small sample quantities (only 0.6 g fresh weight per replicate) used in the solid contact test are the most likely cause of the observed variability. With heterogeneous waste such as fluff-light fraction and dust, individual particles with a high toxic load, which may or may not be present in the sample, have a strong effect on the test result.

The rapid test for determination of potential nitrification according to DIN EN ISO 15685 (2021) could be a possible alternative to the solid contact test with *A. globiformis*. In this microbial test, larger sample quantities are used. Suitability of this test was evaluated in a screening test with a waste sample (see sections 4.3.2.4 and 4.4.3.2.2.4). To further investigate whether the rapid test for determination of potential nitrification is more suitable for the examination of (heterogeneous) waste samples than the solid contact test with *A. globiformis*, methodological adaptations and further comparative experimental studies would be necessary (see section 4.4.3.2.2.4).

The test battery according to UBA (2013) does not include any terrestrial vertebrates. However, possible effects of waste on terrestrial vertebrates should be covered by other hazard

properties, in particular HP 5 (specific target organ toxicity), HP 6 (acute toxicity) and HP 10 (reproductive toxicity)[57].

Type and scope of aquatic biotests

Likewise, the test battery according to UBA (2013) does not include aquatic vertebrates. Possible effects on fish are not covered by other hazard-relevant properties. In analogy to the CLP Regulation (EC 2021), animal tests for the classification of waste from mirror entries may only be carried out if there is no suitable alternative method (see section 1.1).

Various alternative methods have been developed to replace acute fish tests. Embryos and larvae of vertebrates that are not yet able to feed independently do not fall under the scope of the German Animal Welfare Act (TierSchG 2023) and Directive 2010/63/EU on the protection of animals used for scientific purposes (EU 2019). Therefore, experiments with fish embryos and early fish larvae that still feed on the yolk sac are categorised as alternative methods. In the fish egg test with the zebrafish (*Danio rerio*) according to DIN EN ISO 15088 (2009b), exposure begins shortly after fertilisation and ends after 48 h, before the fish hatch. In Germany, this test replaces the acute fish test in accordance with the German Wastewater Charges Act (Bundesgesetzblatt 2005). A test with early life stages of the zebrafish was also developed for chemical testing, the fish embryo test according to OECD test guideline 236 (2013). In this test, exposure also begins shortly after fertilisation, but ends after 96 h, after hatching, but before the start of exogenous feeding[58]. Other alternative methods such as tests with fish cell lines have been developed and, in some cases, also standardised (e.g. with the RTgill W1 cell line; Fischer et al. 2019, OECD test guideline 249, 2021). However, as part of the legally required environmental risk assessments for chemical substances, there is currently no legally accepted alternative method for acute fish tests (see e.g. Katsiadaki et al. 2021, Belanger et al. 2023).

If a test with the taxonomic group of fishes is to be added to the biotest battery for the HP 14 classification of waste in mirror entries, it is proposed to examine whether the fish egg test according to DIN EN ISO 15088, the fish embryo test according to OECD test guideline 236 or the fish cell line test according to OECD test guideline 249 is suitable. Experimental investigations and, if necessary, methodological adjustments would be necessary.

In the present project it was shown that the algal growth inhibition test in microtiter plates (DIN 38412-59) is very suitable for the analysis of waste eluates. Therefore, we suggest that it should be mentioned in the UBA recommendations as an alternative to the algal test according to ISO 8692 or DIN EN ISO 8692.

Test design

The UBA recommendations clearly state that the aquatic and terrestrial bioassays should be carried out with at least five dilution levels of the waste or waste eluate for determining EC_{50} values (section 5.1.3). Limit tests are not foreseen. The project advisory group suggested to examine whether limit tests could be an option. If so, specific requirements for limit tests should be defined that could be included in the recommendations.

In the context of environmental risk assessments for chemical substances, limit tests are performed with a single substance concentration to demonstrate the absence of ecotoxic effects. The substance concentration used in such a limit test corresponds to the maximum substance concentration to be used in the respective test type. In acute tests with aquatic organisms, it is 100 mg/L (e.g. DIN EN ISO 6341 (2013a) and OECD test guidelines 201 (2011) and 202 (2004)),

[57] A detailed analysis of this issue is beyond the scope of this project.

[58] There are various terminologies for the early life stages of fish. Depending on the terminology, the embryonic phase ends with hatching or with the start of exogenous nutrition (see e.g. Blaxter 1988).

in chronic tests with aquatic organisms it is 10 mg/L (e.g. OECD test guideline 211 (2012)). In acute and chronic tests with terrestrial organisms, the limit concentration is 1000 mg/kg soil dry weight (e.g. OECD test guidelines 207, 222 and 232, OECD 1984, 2016a, b)[59]. For ensuring a sufficient statistical reliability, the number of replicates used in limit tests (both in the control and in the limit concentration) is generally higher than in tests with several substance concentrations. If no statistically significant effect on the test endpoint(s) is detected in a limit test, it can be concluded that the respective substance is not acutely or chronically toxic to the test organism. In case that a significant effect occurs in the limit test, a test with several (usually five) substance concentrations has to be carried out to determine the acute or chronic effect concentration.

If limit tests are carried out with waste or waste eluates, the used dilution level must be above 10% eluate or waste content to allow an HP 14 classification. We suggest performing the test with an eluate or waste content of 12.5%. Some of the guidelines for the tests recommended according to UBA (2013) contain specifications for the number of replicates to be used in limit tests. In cases where such specifications are lacking in the relevant guidelines, we propose doubling the number of replicates compared to testing with (at least) five waste or eluate dilutions (see Table 35).

In analogy to the environmental risk assessment for chemical substances, limit tests with waste or waste eluates should be used to demonstrate the absence of ecotoxic effects. If a significant effect occurs in a limit test, a test with (at least) five waste or eluate dilutions should be performed to determine the EC_{50}.

Table 35: Number of replicates in the biotests recommended by UBA (2013) for tests with ≥5 dilution levels and for limit tests

Test (test guideline)	Number of replicates per dilution level (number of control replicates) for tests with ≥5 waste or eluate dilutions	Number of replicates per dilution level (number of control replicates) in the limit test with 12.5% waste or eluate content	Explanation
Acute *Daphnia* test (ISO 6341, DIN EN ISO 6341)	4 (4)	4 (4)	Specifications according to DIN EN ISO 6341 (2013a)
Algal growth inhibition test (ISO 8692, DIN EN ISO 8692)	3 (6)	6 (6)	Specifications according to ISO 8692 (2012b)
Algal growth inhibition test in microtiter plates (DIN 38412-59)	3 (3)	6 (6)	No specifications according to DIN 38412-59 (2022); doubling the number of replicates is suggested
Luminescent bacteria test (ISO 11348-2, DIN EN ISO 11348-2)	2 (2)	4 (4)	No specifications according to DIN EN ISO 11348-2 (2009);

[59] If the water solubility of the substance to be tested is less than 100 mg/L or 10 mg/L, limit tests are performed with the maximum concentration that is water-soluble under test conditions.

Test (test guideline)	Number of replicates per dilution level (number of control replicates) for tests with ≥5 waste or eluate dilutions	Number of replicates per dilution level (number of control replicates) in the limit test with 12.5% waste or eluate content	Explanation
			doubling the number of replicates is suggested
Solid contact test with *Arthrobacter globiformis* (ISO 18187, DIN EN ISO 18187)	4 (4)	8 (8)	No specifications according to DIN EN ISO 18187 (2018); doubling the number of replicates is suggested
Growth inhibition test with *Brassica rapa* (ISO 11269-2, DIN EN ISO 11269-2)	2 (6)	4 (4)	Specifications according to ISO 11269-2 (2012a)
Avoidance test with earthworms (ISO 17512-1, DIN EN ISO 17512-1)	5 (5)	10 (10)	No specifications according to ISO 17512-1 (2008a); doubling the number of replicates is suggested

Necessity of chronic biotests, possibility to exonerate a waste based on biotest results

With the current test battery according to the UBA recommendations only acute effect concentrations (EC_{50} values) are determined. In the present project, it was discussed whether the test battery should also include chronic ecotoxicity tests. In chronic tests, test organisms are exposed throughout their entire life cycle or throughout at least one sensitive developmental stage, and EC_{10} or NOEC values are determined (ECHA 2008, 2023a, EC 2018).

The three recommended aquatic bioassays are short-term tests. However, due to the short generation time of the algae, the algal test with its exposure duration of 72 hours is covering several generations. Therefore, it is classified as chronic test (ECHA 2023a, EC 2018). Accordingly, this test can also be used to derive chronic effect concentrations (see also section 4.4.5). For this purpose, additional dilution levels of the waste eluate may need to be tested. The two other aquatic tests (*Daphnia* test and luminescent bacteria test) are acute tests (Table 36). To derive chronic effect concentrations for daphnids and aquatic microorganisms, tests with longer exposure times such as the daphnid test according to ISO 10706 (2000) and the activated sludge respiration inhibition test (OECD test guideline 209, 2010) would have to be used.

With an exposure duration of 14 days, the growth inhibition test with the terrestrial plant *B. rapa* is clearly the longest test in the test battery recommended by the UBA (2013). Depending on the regulatory framework, it is categorised as an acute or chronic test (under REACH as a chronic test, ECHA 2023b). In this test, EC_{10} or NOEC values can also be determined (section 4.4.5). The avoidance test with earthworms is – despite its test duration of only 48 h – similarly sensitive as the (chronic) earthworm reproduction test (test duration: 56 days) and significantly more sensitive than the acute earthworm test (Scheffczyk et al. 2014, Römbke et al. 2018). For determining chronic effect concentrations, the test design would need to be adapted. An increased number of earthworms per test vessel would be most important (according to ISO 17512-1 or DIN EN ISO 17512-1, ten earthworms are used per test vessel). The solid contact test with *A. globiformis* is an acute test. To determine chronic effect concentrations for soil

microorganisms, longer-term tests such as nitrogen or carbon transformation tests (OECD test guidelines 216 and 217, OECD 2000a, b) would have to be used (Table 36).

Table 36: Classification of the biotests recommended by UBA (2013) as acute or chronic tests and possibility to derive chronic effect concentrations in these tests

Test (test guideline)	Exposure duration	Classification of the test as acute or chronic	Possibility to derive chronic effect concentrations
Aquatic ecotoxicity tests			
Acute *Daphnia* test (ISO 6341, DIN EN ISO 6341)	48 h	Acute	No
Algal growth inhibition test (ISO 8692, DIN EN ISO 8692, alternatively: DIN 38412-59)	72 h	Acute and chronic	Chronic effect concentrations (e.g. EC_{10}) can be determined. Additional dilution levels may have to be tested for this, but no further adjustments to the test design would be necessary
Luminescent bacteria test (ISO 11348-2, DIN EN ISO 11348-2)	30 min	Acute	No
Terrestrial ecotoxicity tests			
Solid contact test with *Arthrobacter globiformis* (ISO 18187, DIN EN ISO 18187)	6 h	Acute	No
Growth inhibition test with *Brassica rapa* (ISO 11269-2, DIN EN ISO 11269-2)	14 d	Acute to chronic (depending on the regulatory framework)	Chronic effect concentrations (e.g. EC_{10}) can be determined. Additional dilution levels may have to be tested for this, but no further adjustments to the test design would be necessary
Avoidance test with earthworms (ISO 17512-1, DIN EN ISO 17512-1)	48 h	Acute, but similarly sensitive as the reproduction test with earthworms	The test design would have to be adapted to determine chronic effect concentrations (e.g. EC_{10}), especially increasing the number of earthworms per test vessel

In most other European countries, only acute bioassays are used for HP 14 classification of waste from mirror entries (see section 3.1.1.2). However, in view of the possibility of using biotests to exonerate a waste classified as ecotoxic based on the calculation method, this approach is problematic, as detailed below.

According to Commission Decision 2000/532/EC (EC 2015) and AVV (2020), the results of testing are decisive for classifying a waste as hazardous or non-hazardous, if the respective hazard property has been assessed both based on the concentrations of hazardous substances and by means of testing (section 1.1). Thus, it is possible to use biotests to exonerate a waste that has been classified as hazardous by HP 14 using the calculation method. However, this option only makes sense in one of three cases:

- If a waste is classified as HP 14 using the calculation method exclusively due to substances that are acutely hazardous to the aquatic environment, but is not ecotoxic in any of the six bioassays of the test battery, it makes sense to exonerate this waste based on the bioassay

results (i.e. acute effect concentrations above the limit concentration of 10% waste eluate or waste). In this case, a low bioavailability could be the reason for the lack of ecotoxicity in the bioassays. Bioavailability should be taken into account in the HP 14 classification (EU 2018, Annex 3.14, see also section 1.1). However, as discussed above, a fish test is lacking in the test battery according to UBA (2013). If the classification as acutely hazardous to the aquatic environment is solely based on fish toxicity, it should not be possible exonerate the waste using a bioassay battery that does not contain a fish test.

- If a waste is classified as HP 14 using the calculation method due to substances that are long-term hazardous to the aquatic environment (H410, H411 or H412), it does not make sense to exonerate this waste based on the acute effect concentrations determined with the current biotest battery. A waste classified as long-term hazardous to the aquatic environment using the calculation method (based on chronic bioassays with individual waste constituents) should only be exonerated based on the results of chronic ecotoxicity tests with the waste eluate.
- If a waste is classified as HP 14 using the calculation method due substances that are hazardous to the ozone layer (H420), it does not make sense to exonerate this waste based on biotests.

With regard to the last two points mentioned, there is a need for action at EU level. Specifically, there is a need to regulate in which cases a classification according to the calculation method can be revised (exonerated) by the results of which bioassays.

5.6.3 Scope of the test guidelines for the biotests

Currently, waste testing is only explicitly mentioned in the guideline for the solid contact test with *A. globiformis* (DIN EN ISO 18187). In the guideline for the growth inhibition test with *B. rapa*, the testing of waste materials (e.g. dredged material, sludge from municipal sewage treatment plants, composed material or manure) is mentioned (see Table 37). In the test guidelines for the avoidance test with earthworms and the three aquatic tests, the testing of waste or waste eluates is not mentioned in the scope, although all six tests of the biotest battery have been used for waste testing since years. It is suggested that the testing of waste samples or waste eluates is explicitly included in the scope of the test guidelines for the growth inhibition test with *B. rapa*, the avoidance test with earthworms and the three aquatic tests. The guidelines should also include information on the handling of waste samples and waste eluates.

Table 37: Scope of the test guidelines for the aquatic and terrestrial biotests with regard to the testing of waste samples

Test (guideline)	Scope
Aquatic ecotoxicity tests	
Acute *Daphnia* test (ISO 6341, DIN EN ISO 6341)	Investigation of chemical substances, industrial and municipal wastewater, aqueous extracts and leachates, eluates and pore water of freshwater sediments, freshwater (surface water and groundwater)
Algal growth inhibition test (ISO 8692, DIN EN ISO 8692)	Investigation of individual chemical substances, mixtures of substances and wastewater
Algal growth inhibition test in microtiter plates (DIN 38412-59)	Investigation of individual chemical substances, industrial and municipal wastewater, aqueous extracts and leachates, eluates of soils and

Test (guideline)	Scope
	freshwater sediments, pore water of freshwater sediments, freshwater (surface water and groundwater)
Luminescent bacteria test (ISO 11348-2, DIN EN ISO 11348-2)	Investigation of individual chemical substances, wastewater, aqueous extracts and leachate, eluates of sediments, pore water, freshwater (surface water and groundwater), brackish and marine water
Terrestrial ecotoxicity tests	
Solid contact test with *Arthrobacter globiformis* (ISO 18187, DIN EN ISO 18187)	Assessment of water-soluble and solid-bonded non-volatile impurities in natural samples, such as soils and wastes, testing of chemical substances
Growth inhibition test with *Brassica rapa* (ISO 11269-2, DIN EN ISO 11269-2)	Assessment of unknown soils, locally collected soils from industrial, agricultural and other sites, waste material (e.g. dredged material, sludge from municipal sewage treatment plants, composite material or manure)
Avoidance test with earthworms (ISO 17512-1, DIN EN ISO 17512-1)	Assessment of the habitat function of soils and the influence of chemicals and other pollutants on the behaviour of earthworms

5.6.4 Aquatic biotests: technical details

Overall, the technical performance of the three aquatic biotests with the selected waste samples proved to be unproblematic. Individual test runs with different eluates from subsamples of a waste sample yielded reproducible results.

Adjustment of the pH value

In the UBA recommendations, it is clearly stated that waste eluates should be analysed without adjusting the pH value. If toxic effects occur at dilution levels, where pH is outside the range tolerated by the test organisms, a second test with adjusted pH may be performed to identify the cause of the toxicity. However, the result of this second test is not relevant for HP 14 classification (sections 5.2.6 and 6.1.2). This procedure complies with the requirements of DIN EN 14735 (2022). An analogous approach is recommended in the acute *Daphnia* test (DIN EN ISO 6341). However, in the guidelines for the algal test (ISO 8692 and DIN 38412-59) the possibility of a pH adjustment is mentioned, and in the guideline for the luminescent bacteria test (DIN EN ISO 11348-2), it is recommended to adjust the pH value. Here, it should be specified in the UBA recommendations that the pH should not be adjusted in the first test run relevant for HP 14 classification, even if a pH adjustment is possible or recommended according to the test guideline. For waste testing, an adaptation of the guidelines for the algal (ISO 8692, DIN 38412-59) and luminescent bacteria test (DIN EN ISO 11348-2) to the specifications of DIN EN 14735 would be desirable.

According to DIN EN ISO 6341, pH values of 6.0-9.0 are suitable for daphnids; for algae and luminescent bacteria pH 6.0-8.5 is specified as suitable (DIN38412-59, DIN EN ISO 11348-2). If the pH values of the dilution levels of a waste eluate are clearly outside the above-mentioned ranges, it does not make sense to perform the test. It should be considered whether pH ranges should be specified for the different test organisms, outside of which it is no longer useful to perform the respective test, because pH alone is likely to result in high toxicity.

If a second test run is carried out with an adjusted pH, the pH adjustment procedure should be documented (see section 5.7). The pH in the individual dilution levels depends on the test

medium used and its buffer capacity (UBA 2013, Hennebert 2019). Due to the buffer capacity of the test medium, the pH in individual dilution levels may be within the tolerance range of the test organism, although the pH of the eluate is outside this range. Therefore, a pH adjustment may not be necessary in all dilution levels[60]. In the present project, pH values were only adjusted in those dilution levels, where pH was outside the tolerance range for the respective species (section 4.3.1.1) as suggested by Hennebert (2019). Thus, the influence of pH adjustment and associated changes in the dissociation of substances, precipitation reactions and complex formation was minimised. However, this procedure is associated with additional work. For the luminescent bacteria test, the volume of the prepared dilutions had to be increased to allow pH measurement and adjustment[61]. We would propose that the UBA recommendations should refer to the possibility of adjustment the pH in the individual dilution levels. However, given that biotests with adjusted pH are not relevant for HP 14 classification, this point has a low priority for section 6.1 of the recommendations, which describes the identification of hazardous waste in mirror entries[62].

When adjusting the pH, precipitations can occur. Neither the UBA recommendations nor DIN EN 14735 nor the test guidelines for the algal growth inhibition test (ISO 8692, DIN 38412-59) and the luminescent bacteria test (DIN EN ISO 11348-2) contain guidance on how to deal with such precipitations. The guideline for the acute *Daphnia* test (DIN EN ISO 6341) specifies – with reference to ISO 5667-16 (1998) – that precipitates should be removed. However, this recommendation is no longer included in the current ISO 5667-16 (2017). Instead, it is now stated that pH should not be adjusted if this leads to a change in the test result or to physico-chemical changes such as precipitations. Specifications on how to deal with precipitations occurring during pH adjustment could be included in the UBA recommendations. However, this point has a low priority, because tests with adjusted pH are not relevant for HP 14 classification.

Further technical details

According to the guideline for the acute *Daphnia* test (DIN EN ISO 6341), a reference test has to be available for each waste test, which was performed not more than one month before or after the respective waste test. The required frequency of reference tests appears to be very high and could be reduced during a revision of the test guideline. Two reference tests per year, such as required e.g. by OECD test guideline 202 for the acute *Daphnia* test (OECD 2004), appear to be sufficient.

According to DIN EN ISO 6341, there should preferably be least three test concentrations (here: dilution levels of the waste eluate) with a percentage of 10-90% of immobile daphnids for calculating an EC_{50}. In the tests performed with waste eluates, the dilution factor was 2 (eluate content: 50, 25, 12.5% etc.) and thus below the dilution factor of 2.2 recommended in DIN EN ISO 6341 for steep concentration-response curves. Yet, in most tests there were less than three dilution levels with 10-90% of immobile *Daphnia*. Nevertheless, it was possible to derive EC_{50} values (see section 4.4). Regarding this point, a revision of the test guideline would be desirable. A note regarding this issue could also be included in the UBA recommendations.

The luminescent bacteria test (DIN EN ISO 11348-2) with the marine bacterium *A. fischeri* is carried out at a sodium chloride content of 20 g/L. According to DIN EN ISO 11348-2, the salt

[60] This point is particularly relevant for the algal growth inhibition test in microtiter plates (DIN 38412-59), since the used medium contains a strong phosphate buffer.

[61] Furthermore, these are not the final test solutions. These are only prepared after the first luminescence measurement by adding 0.5 ml of test solution to 0.5 ml of luminescent bacteria suspension.

[62] It could be more relevant for sections 6.2 ('Detailed ecotoxicological characterisation of wastes') and 7 ('Ecotoxicological characterisation for assessing the risks of waste management scenarios'), which are beyond the scope of the project.

content should be measured, and the amount of sodium chloride adjusted accordingly, if samples with a "high salt concentration" are tested. Here, a specification would be desirable in the test guideline at which salinity or conductivity of the sample the amount of sodium chloride should be adjusted.

5.6.5 Terrestrial biotests: technical details

Generally, the technical performance of the terrestrial bioassays with the selected waste samples proved to be unproblematic. The relatively high variability of the results of the solid contact test with A. *globiformis* (ISO 18187) has already been discussed in section 5.6.2.

In this context, it should be noted that with regard to waste sampling ISO 18187 refers to the guideline EN 14735 (CEN 2021b), among others. However, at the same time a maximum storage time of two weeks at 4±2°C is specified. This is in direct contradiction to EN 14735, where a storage period of less than two months and/or a storage temperature of 4±2°C is considered appropriate to maintain the properties of the waste samples. In the present project, the specifications of EN 14735 were applied to the solid contact test. It is not plausible why the storage period should be shorter for the solid contact test; this should be adapted or justified in future versions of the guideline. Furthermore, ISO 18187 stipulates that the positive control should be tested not only with LUFA standard soil 2.2 but also with the respective control substrate (quartz sand in the case of waste samples). However, no acceptance criteria are given for this additional positive control. These criteria should be defined and amended.

Based on the experience gained in the experimental work, there is no need for methodological adaptations for the avoidance test with earthworms[63].

According to ISO 11269-2, the growth inhibition test with *B. rapa* should be carried out with 12 dilution levels. As already mentioned in section 3.3.2, such a high number of dilution levels is not necessary to determine whether the EC_{50} is ≤ or > the limit concentration of 10% waste content. A test with 5 dilution levels of the waste (25, 12.5, 6.3, 3.1 and 1.6%) as in the present project is completely sufficient. It is therefore proposed to adapt the test guideline during its review and revision, i.e. to reduce the number of required dilution levels for the application area of waste testing that should be amended (section 5.6.3). A higher number of dilution levels may be necessary if a chronic effect concentration (e.g. an EC_{10}) shall be determined (sections 4.4.5 and 5.6.2). In addition, it should be pointed out in section 5.1.3 of the UBA recommendations that it is sufficient to use five waste dilutions in the growth inhibition test with *B. rapa*.

5.6.6 HP 14 classification based on biotest results

Limit concentration

Based on Pandard & Römbke (2013), it is proposed in the UBA recommendations to categorise a waste as ecotoxic if the EC_{50} is ≤10% waste or eluate content. The same limit concentration is also used in France, Finland, and Slovakia. The limit concentrations used in the Czech Republic (≥50% inhibition in the limit test with 10% eluate content) and Belgium (Flanders: LID >8) are in a similar order of magnitude. In contrast, very different (significantly less stringent) limit concentrations apply in Austria and Spain (see section 3.1.1.3).

In Austria, the eluate produced with an L/S ratio of 10 is diluted by a factor of 1000 (BMNT 2018). The resulting eluate dilution (0.1% eluate) is designated as concentration of 100 mg of the solid waste sample per litre of eluate (BMNT 2018) and is used in limit tests. A waste is classified as HP 14 if effects ≥20% (luminescent bacteria test; algal test according to ISO 8692),

[63] Adjustments are only required if EC_{10} values shall be determined (see section 5.6.2).

≥25% (algal test according to Regulation (EU) 440/2008, Annex C.3, EC 2019) or ≥10% (*Daphnia* test) occur in the limit test, or if the EC_{50} is ≤0.1% eluate (100 mg of solid waste sample per L) in the EC_X test that shall be performed if effects are detected in the limit test.

As already mentioned in section 3.1.1.2, BMNT (2018) justifies this approach with guidance of the CLP Regulation for chemical substances (EC 2021). Limit values defined for chemical substances are applied to waste as a whole, although (a) waste is not considered as substance, mixture or article within the meaning of REACH (EC 2022) and the CLP Regulation (EC 2021), and (b) any ecotoxic substances contained in waste are embedded in a matrix (e.g. soil) (see section 3.1.1.2,).

If the Austrian limit concentration for EC_X tests (0.1% eluate) was applied to the results of the bioassays carried out in the present project, none of the tested waste samples would be classified as HP 14 (ecotoxic) (see Table 38).

Table 38: Comparison of the classification according to the UBA Recommendations (2013) and the classification according to the Austrian guidance (BMNT 2018)

Waste code and type	Waste sample: specification	Classification according to UBA (2013)	Classification according to BMNT (2018)	Lowest EC_{50} (most sensitive test organism)
10 09 09* Flue-gas dust from iron and steel casting	Batch 1	HP 14	not HP 14	0.201% Eluate (algae)
	Batch 2	HP 14	not HP 14	0.913% Eluate (algae)
10 09 10 Flue-gas dust from iron and steel casting	Plant A	HP 14	not HP 14	<3.1% Eluate (algae, *Daphnia*)
	Plant B	HP 14	not HP 14	7.56% Waste (*Arthrobacter*)
17 05 03* Soil and stones	Excavated geogenic material	HP 14	not HP 14	3.15% Eluate (*Daphnia*)
	Material from the side verges of federal road	not HP 14	not HP 14	No toxicity in any of the tests
17 05 04 Soil and stones	Material from the side verges of secondary road	not HP 14	not HP 14	No toxicity in any of the tests
19 10 04 Fluff-light fraction and dust (sieved to <10 mm)	Plant A, batch 1	HP 14	not HP 14	0.678% Eluate (*Daphnia*)
	Plant A, batch 2	HP 14	not HP 14	0.287% Eluate (algae)
	Plant B	HP 14	not HP 14	7.11% Eluate (luminescent bacteria)

Concerning the limit concentration, a harmonisation between the different EU Member States would be desirable, also regarding transboundary transport of waste. In view of the objective of Directive 2008/98/EC (EC 2018, Article 1) to protect the environment by preventing or reducing adverse effects of the generation and management of waste, the selected limit concentration should ensure that waste, which has a harmful effect on aquatic or terrestrial organisms, is classified as HP 14.

Procedure for HP 14 categorisation

According to the UBA recommendations, a waste is classified as ecotoxic (HP 14) if at least one bioassay result is positive. Analogous to the procedure in the environmental risk assessment of chemical substances (e.g. ECHA 2008), the most sensitive test result is thus decisive. This approach is consistent. It is also used in several other European countries (section 3.1.1.3). Here too, a harmonisation at EU level would be desirable.

5.7 Minimum requirements for reports

As suggested by the project advisory group, it was compiled which key information reports on sampling, sample preparation, storage, sample division, elution and biotesting must contain to enable the competent authority to evaluate the results. The corresponding specifications are given in relatively detailed form in the test guidelines and other guidance for the relevant methods. These requirements were compiled in a tabular form (see Annexes A.1 to A.4).

The specifications for sampling (Annex A.1) and sample pre-treatment (Annex A.2) are based on EN 14899 (CEN 2005), CEN/TR 15310-1 (2006a), PN 98 (LAGA 2019) and DIN 19747 (2009a).

The specifications for elution (Annex A.3) are based on DIN EN standards 12457-2 (2003a) and 14735 (2022), those for biotests (Annex A.4) are based on the standards for the corresponding biotests: ISO guidelines 11269-2 (2013b), 17512-1 (2020) and 18187 (2016a) as well as DIN EN ISO 6341 (2013a), DIN EN ISO 11348-2 (2023) and DIN 38412-59 (2022).

Tables with the minimum requirements for reports on sampling, sample pretreatment, elution and biotests could become an annex to the UBA recommendations.

6 Possibilities and limitations of ecotoxicological tests compared to the calculation method

An HP 14 classification can be based on the calculation method or on bioassays (see section 1.1). The calculation method for HP 14 classification is based on chemically analysed concentrations of waste constituents classified as hazardous to the ozone layer (H420), acutely hazardous to the aquatic environment (H400) and/or long-term hazardous to the aquatic environment (H410-H413) according to the CLP Regulation (Table 39). Waste is classified as ecotoxic if the threshold value for at least one of the four criteria listed in Table 39 is reached or exceeded.

For the calculation method, harmonised classifications of the waste constituents are relevant. If there is no harmonised classification for a waste constituent, the waste owner should refer to available self-classifications (notified classifications). Here, information on self-classifications given in the C&L inventory and safety data sheets, which are available to the company that generated the waste, are particularly important (EU 2018).

Waste constituents classified as acutely or long-term hazardous to the aquatic environment are only considered in the calculation method if their concentrations reach or exceed the cut-off values indicated in Table 39 (EU 2018). Both the concentration limits for HP 14 classification and the cut-off values are based on the concentration of the respective substance in waste fresh weight (EU 2018, p. 18).

Table 39: Criteria for HP 14 classification of waste using the calculation method according to Regulation (EU) 2017/997

Criterion for HP 14 classification		Concentration limit	Generic cut-off value[b]
1	H420 (hazardous to the ozone layer)	C(H420) ≥0.1%	–
2	H400 (acutely hazardous to the aquatic environment)	Σ c (H400) ≥25%	0.1%
3	H410, H411, H412 (long-term hazardous to the aquatic environment)[a]	100 × Σ c (H410) + 10 × Σ c (H411) + Σ c (H412) ≥25%	H410: 0.1%
4	H410, H411, H412, H413 (long-term hazardous to the aquatic environment)[a]	Σ c (H410) + Σ c (H411) + Σ c (H412) + Σ c (H413) ≥25%	H410: 0.1% H411, H412, H413: 1%

Σ = Sum, c = concentration of waste constituents (relative to waste fresh weight). [a] H410: very toxic to aquatic life with long lasting effects (chronic 1), H411: toxic to aquatic life with long lasting effects (chronic 2), H412: harmful to aquatic life with long lasting effects (chronic 3), H413: may cause long lasting harmful effects to aquatic life (chronic 4); [b] generic cut-off value: if this value is reached, the concentration of the respective waste constituent must be considered when determining if the waste has to be classified as HP 14 (EU 2018).

The cut-off values used for the calculation method correspond to the generic cut-off values of the CLP Regulation ((EC) 1272/2008) and are relatively high:

- The cut-off value of 0.1% for waste constituents classified as H400 and/or H410 corresponds to 1 g/kg waste fresh weight.

- The cut-off value of 1% for waste constituents classified as H411, H412 or H413 corresponds to 10 g/kg waste wet weight.

When classifying chemical mixtures according to the CLP Regulation, lower cut-off values can be used for very toxic substances, if there is reason to assume that the respective substance is relevant for the classification of the mixture at lower concentrations (see (EC) 1272/2008, EC 2021, p. 194-201). However, this option does not exist for the calculation method for HP 14 classification of waste according to (EC) 2017/997 (EU 2017).

When the calculation method is performed for HP 14 classification, only those substances are included that have a relevant classification under the hazardous substance legislation (H410, H411-413 or H420; UBA 2013, Römbke et al. 2018)[64] or for which self-classifications are available (see above).

Chemical-analytical analyses are the basis for the calculation method. Generally, only substances are analysed, which have been identified as possibly relevant beforehand. Hence, sufficient information on the respective waste sample must be available to analyse all substances or parameters relevant for classification of the waste sample. Sufficiently comprehensive information on the waste composition is often missing. A further shortcoming of the calculation method is that contaminants, which are present in concentrations below the detection limit of the used analytical method, have no impact on the HP 14 classification. Moreover, the classification of hazardous substances is so far based exclusively on aquatic toxicity data. As discussed in section 5.6.1, soil organisms are not sufficiently protected based on aquatic toxicity data alone (Scholz-Starke et al. 2022).

If, for metals and metalloids, chemical analytical data are available on elements (e.g. arsenic, barium, cadmium, lead), for which classification depends on the substances in which they are present, the "reasonable worst-case" species must be determined using expert judgement and considered for HP 14 classification (EU 2018, Annex 4, section 4.2.1). This can lead to an over- or underestimation of toxicity (Römbke et al. 2018). A comparison of the classification based on the calculation method and bioassays would be interesting.

Based on ecotoxicological tests, a statement can be made about the combined effects of all ecotoxic substances (and species) in the waste. This includes substances with concentrations below the chemical-analytical detection limit and substances that are not detected by the selected analytical method, such as degradation products (UBA 2013, Römbke et al. 2018). As mentioned in section 1.1, the bioavailability of the waste constituents shall be considered in the HP 14 classification (EU 2017)[65, 66]. Ecotoxicity tests provide information on the effects of all bioavailable substances in the waste, i.e. matrix effects are taken into account. The results of such biotests also reflect possible interactions between various waste constituents (UBA 2013, Planchon et al. 2015, Römbke et al. 2018, Hennebert 2019).

[64] The contractors currently have no information on the percentage of waste from mirror entries that is assigned to the relevant mirror entry solely on the basis of the criterion HP 14. According to information provided by UBA/BMUV, such cases are assumed to be rare.

[65] Regulation (EU) 2017/997 refers to Article 12(b) of the CLP Regulation (EC 1272/2008). Article 12 deals with special cases requiring further assessment. This is, e.g. the case, if conclusive data show that the substance or mixture to be evaluated is not bioavailable.

[66] There is no guidance on how bioavailability should be taken into account in the evaluation of waste.

Table 40: Possibilities and limitations of the calculation method according to Regulation (EC) 2017/997 and the ecotoxicological test battery according to UBA (2013)

Calculation method	Ecotoxicological test battery[a]
Substances with concentrations below the cut-off values are not considered[b]	Statement on the combined effects of all ecotoxic substances, including substances (a) that are present in concentrations below the cut-off values, (b) that are present in concentrations below the detection limit(s) of the chemical-analytical methods, (c) that cannot be classified under the hazardous substance legislation, because there are no qualitative and/or quantitatively sufficient ecotoxicity data
Substances with concentrations below the detection limits of the chemical-analytical method are not considered	
Substances that cannot be classified under the hazardous substance legislation due to lack of qualitative and/or quantitatively sufficient ecotoxicity data are not considered	
Chemical analysis only covers substances that are being searched for (i.e. not necessarily all substances with a relevant contribution to ecotoxicity)	Statement on combined effects of all ecotoxic substances
For metals and metalloids, the necessary consideration of specification may lead to an underestimation or overestimation of toxicity	Statement on the effects of the metal/metalloid species present in the waste sample or waste eluate
Consideration of the criterion H420 (hazardous to the ozone layer)	H420 is not covered
Consideration of the criterion H400 (acutely hazardous to the aquatic environment)	Toxicity to daphnids and algae is covered, toxicity to fish is not covered[c]
Consideration of the criteria H410 – H413 (long-term hazardous to the aquatic environment)	H410 – H413 are currently not covered. For algae: Derivation of an EC_{10} is possible, for daphnids and fish: chronic tests would be necessary[c]
Terrestrial ecotoxicity not covered	Terrestrial ecotoxicity is covered
Bioavailability of waste ingredients is not considered	Bioavailability is considered

[a] Test battery according to UBA (2013; Table 3); see section 3.3.2; [b] see Table 39; [c] see section 5.6.2.

The comparison in Table 40 clearly shows that the calculation method and the use of bioassays for the HP 14 classification of waste from mirror entries are complementary approaches.

In this context, the procedure for classifying chemical mixtures in accordance with the CLP Regulation is interesting. This classification can be performed (a) using aquatic toxicity tests with the mixture as a whole, (b) using bridging principles (e.g. if mixture has been prepared by dilution of a tested substance with completely non-toxic material) and (c) using the summation method (calculation method). If more than one method has been used to classify a mixture of chemicals, the more conservative result should be used (see (EC) 1272/2008).

In view of this approach and the above-mentioned possibilities and limitations of the calculation method and ecotoxicity tests, it would be desirable to reconsider and further develop the procedure for HP 14 classification of waste from mirror entries. This issue should be addressed at the EU level.

7 List of references

AFNOR (1982): Qualité des sols. Essai d'inhibition de germination de semences par une substance. X31-201. Association Française de Normalisation, La Plaine Saint-Denis [Norm zurückgezogen]

AFNOR (1998a): Déchets. Essai de lixiviation. XP X31-210. Association Française de Normalisation, La Plaine Saint-Denis [Norm zurückgezogen]

AFNOR (1998b): Qualité de l'eau. Détermination de la toxicité chronique des eaux par inhibition de la croissance de l'algue d'eau douce *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). NF T90-375. Association Française de Normalisation, La Plaine Saint-Denis [Norm zurückgezogen]

AFNOR (2000): Water quality. Determination of chronic toxicity to *Ceriodaphnia dubia* in 7 days. Population growth inhibition test. NF T90-376. Association Française de Normalisation, La Plaine Saint-Denis. [Norm zurückgezogen]

AFNOR (2013): Caractérisation des déchets. Détermination de la teneur en éléments et substances des déchets. XP X30-489. Association Française de Normalisation, La Plaine Saint-Denis

AltholzV (2020): Verordnung über Anforderungen an die Verwertung und Beseitigung von Altholz (Altholzverordnung - AltholzV) vom 15. August 2002 (BGBl. I S. 3302), zuletzt geändert durch Artikel 120 der elften Zuständigkeitsanpassungsverordnung vom 19. Juni 2020 (BGBl. I S. 1328)

APA (2020): Guia de classificação de resíduos. Agência Portuguesa de Ambiente, Lisbon

ASTM (2014): Standard guide for conducting terrestrial plant toxicity tests. ASTM E1963-09. ASTM International, West Conshohocken, PA

Austrian Standards International (2018): Herstellung eines Eluates aus ungemahlenen Abfallproben mit einer Korngröße kleiner 10 mm für die Untersuchung der aquatischen Ökotoxizität und der organischen Parameter. ÖNORM S 2117:2018-02. Austrian Standards International, Wien

AVV (2020): Abfallverzeichnis-Verordnung vom 10. Dezember 2001 (BGBl. I S. 3379), die zuletzt durch Artikel 1 der Verordnung vom 30. Juni 2020 (BGBl. I S. 1533) geändert worden ist

Bandarra, B.; Gomes, L.; Pereira, J.L.; Gonçalves; F.J.M.; Martins, R.C.; Quina, M.J. (2019): Characterization of ecotoxicological effects of green liquor dregs from the pulp and paper industry. ACS Sustainable Chemistry & Engineering, 7(17), S. 14707 – 14715

Bandarra, B.S.; Gomes, L.A.; Pereira, J.L.; Gonçalves; F.J.M.; Martins, R.C.; Quina, M.J. (2020): Assessment of hazardous property HP 14 using ecotoxicological tests: a case study of weathered coal fly ash. Environmental Science and Pollution Research, 27, S. 20972 20983

Bandarra, B.S.; Pereira, J.L.; Martins, R.C.; Maldonado-Alameda, A.; Chimenos, J.M.; Quina, M.J. (2021): Opportunities and barriers for valorizing waste incineration bottom ash: Iberian countries as a case study. Applied Sciences, 11:9690

Barbale, M.; Chinaglia, S.; Gazzilli, A.; Pischedda, A.; Pognani, M.; Tosin, M.; Degli-Innocenti, F. (2021): Hazard profiling of compostable shopping bags. Towards an ecological risk assessment of littering. Polymer Degradation and Stability, 188:109592

Beggio, G.; Bonato, T.; Giardina, S.; Grenni, P.; Mariani, L.; Maggi, L.; Hennebert, P.; Loro, F.; Pivato, A. (2021): Challenges and perspectives of direct test methods for assessing waste hazardous properties (HP). Detritus, 15, S. I –IX

Belanger, S.E.; Lillicrap, A.D.; Moe, S.J.; Wolf, R.; Connors, K.; Embry, M.R. (2023): Weight of evidence tools in the prediction of acute fish toxicity. Integrated Environmental Assessment and Management, 19, S. 1220 – 1234

Berger, W.; Kalbe, U.; Krüger, O.; Hennecke, D. (2013): Elutionsverfahren für die Untersuchung von Böden und Abfällen. Aktueller Stand. Vortrag, Altlastenseminar, 13./14.06.2013 Limburg/Lahn

Bernardo, M.; Lapa, N.; Gonçalves, M.; Barbosa, R.; Mendes, B.; Pinto, F.; Gulyurtlu, I. (2010): Toxicity of char residues produced in the co-pyrolysis of different wastes, Waste Management, 30, S. 628 – 635

Bishop, I.; Hennebert, P. (2021): Hazardous waste classification: review of worst case to less worst case metal species with a worked example for a contaminated soil. Detritus, 14, S. 4 – 24

Blaise, C.; Forghani, R.; Legault, R.; Guzzo, J.; Dubow, M.S. (1994): A bacterial toxicity assay performed with microplates, microluminometry and Microtox® reagent. BioTechniques, 16, S. 932 – 937

Blaxter, J.H.S. (1988): Pattern and variety in development. In Hoar, W.S. & Randall, D.J. [Hrsg.]: Fish physiology. Volume XI. The physiology of developing fish. Part A. Eggs and larvae. Academic Press, San Diego, S. 1 – 58

BMNT (2018): Leitfaden des Bundesministeriums für Nachhaltigkeit und Tourismus (BMNT). Bewertung der gefahrenrelevanten Eigenschaft HP 14 "ökotoxisch" gemäß Verordnung (EU) 2017/997 des Rates vom 8. Juni 2018. Bundesministerium für Nachhaltigkeit und Tourismus, Wien

Breitholtz, M.; Bengtsson, B.-E. (2001): Oestrogens have no hormonal effect on the development and reproduction of the harpactacoid copepod *Nitocra spinipes*. Marine Pollution Bulletin, 42, S. 879 – 886

Breitholtz, M.; Ricklund, N.; Bengtsson, B.-E.; Persson, J.N. (2007): Silica gel as a particulate carrier of poorly water-soluble substances in aquatic toxicity testing. Aquatic Toxicology, 84, S. 251 – 264

Breitholtz, M.; Wollenberger, L. (2003): Effects of three PBDEs on development, reproduction and population growth rate of the harpacticoid copepod *Nitocra spinipes*. Aquatic Toxicology, 64, S. 85 – 96

Bundesgesetzblatt (2005): Bekanntmachung der Neufassung des Abwasserabgabengesetzes vom 18. Januar 2005. Bundesgesetzblatt Jahrgang 2005, Teil I Nr. 5, ausgegeben zu Bonn am 25.01.2005

Carlsson, G.; Örn, S.; Larsson, D.G.J. (2009): Effluent from bulk drug production is toxic to aquatic vertebrates. Environ. Toxicol. Chem., 28, S. 2656 – 2662

CEMD (2003): Newsletter 6/2003, Metodický pokyn odboru odpadů ke stanovení ekotoxicity odpadů (auf Tschechisch). Czech Environmental Ministry Directive, Ministry of Environment of the Czech Republic

CEN (2002a): 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). EN 12457-2. European Committee for Standardization, Brussels

CEN (2002b): Characterization of waste. Leaching. Compliance test for leaching of granular waste materials and sludges. Part 4: One stage batch test at a liquid to solid ratio of 10 L/kg for materials with particle size below 10 mm (without or with limited size reduction). EN 12457-4. European Committee for Standardization, Brussels

CEN (2005): Characterization of waste. Sampling of waste materials. Framework for the preparation and application of a sampling Plan. EN 14899:2005. European Committee for Standardization, Brussels

CEN (2006a): Characterization of waste. Sampling of waste materials. Part 1: Guidance on selection an application of criteria for sampling under various conditions. CEN/TR 15310-1:2006. European Committee for Standardization, Brussels

CEN (2006b): Characterization of waste. Sampling of waste materials. Part 2: Guidance on sampling techniques. CEN/TR 15310-2:2006. European Committee for Standardization, Brussels

CEN (2006c): Characterization of waste. Sampling of waste materials. Part 3: Guidance on procedures for sub-sampling in the field. CEN/TR 15310-3:2006. European Committee for Standardization, Brussels

CEN (2006d): Characterization of waste. Sampling of waste materials. Part 4: Guidance on procedures for sample packaging, storage, preservation, transport and delivery. CEN/TR 15310-4:2006. European Committee for Standardization, Brussels

CEN (2006e): Characterization of waste. Sampling of waste materials. Part 5. Guidance on the process of defining the sampling plan. CEN/TR 15310-5:2006. European Committee for Standardization, Brussels

CEN (2010): Characterization of waste. Guidance on the use of ecotoxicity tests applied to waste. Technical report. CEN/TR 16110. European Committee for Standardization, Brussels

CEN (2020): The national practices to assess the hazardous property HP 14 'Ecotoxic' - assessment of the questionnaire. CEN/TC 444/WG 4 ‚Biological characterisation'. CEN/TC 444/WG 4. Document number N 0042. European Committee for Standardization, Brussels

CEN (2021a): The national practices to assess the hazardous property HP 14 'Ecotoxic' – assessment of the questionnaire (updated – October 2021). CEN/TC 444/WG 4 ‚Biological characterisation'. CEN/TC 444/WG 4 N69. European Committee for Standardization, Brussels

CEN (2021b): Characterisation of waste. preparation of waste samples for ecotoxicity tests. EN 14735. European Committee for Standardization, Brussels

Centeno, M.D.F.; Persoone, G.; Goyvaerts, M.P. (1995): Cyst-based toxicity tests IX: the potential of *Thamnocephalus platyurus* as test species in comparison with *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca). Environmental Toxicology and Water Quality, 10, S. 275 – 282

CEMD (2003): Metodický pokyn odboru odpadů ke stanovení ekotoxicity odpadů. Newsletter 6/2003, Czech Environmental Ministry Directive, Ministry of Environment of the Czech Republic

Deprez, K.; Robens, J.; Nobels, I.; Vanparys, C.; Vanermen, G.; Tirez, K.; Michiels, L.; Weltens, R. (2012): DISCRISET: a battery of tests for waste classification - application of tests on waste extracts. Waste Management, 32, S. 2218 – 2228

Destatis (2022): Erhebung der Abfallentsorgung Deutschland. Jahre, Anlagenart, Abfallarten

Devare, M.; Bahadir, M. (1994): Biological monitoring of landfill leachates using plants and luminescent bacteria. Chemosphere, 28, S. 261 – 267

Deventer, K.; Zipperle, J. (2004): Ökotoxikologische Charakterisierung von Abfall - Verfahrensentwicklung für die Festlegung des Gefährlichkeitskriteriums ‚ökotoxisch (H14)'. Landesanstalt für Umweltschutz Baden-Württemberg, Ökologische Umweltbeobachtung

Dias, D.; Lapa, N.; Bernardo, M.; Godinho, D.; Fonseca, I.; Miranda, M.; Pinto, F.; Lemos, F. (2017): Properties of chars from the gasification and pyrolysis of rice waste streams towards their valorisation as adsorbent materials. Waste Management, 65, S. 186 – 194

DIN (1984): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Schlamm und Sedimente (Gruppe S). Bestimmung der Eluierbarkeit mit Wasser (S 4). DIN 38414-4:1984-10. Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (1989a): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Bestimmung der nicht akut giftigen Wirkung von Abwasser gegenüber Fischen über Verdünnungsstufen (L 31). DIN 38412-31:1989-03. Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (1989b): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Bestimmung der nicht akut giftigen Wirkung von Abwasser gegenüber Daphnien über Verdünnungsstufen (L 30). DIN 38412-30:1989-03. Deutsches Institut für Normung e.V., Berlin

DIN (1991a): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Bakterien; *Pseudomonas*-Zellvermehrungs-Hemmtest. DIN 38412-8:1991-03. Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (1991b): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Bestimmung der nicht giftigen Wirkung von Abwasser gegenüber Grünalgen (*Scenedesmus*-Chlorophyll-Fluoreszenztest) über Verdünnungsstufen (L 33). DIN 38412-33:1991-03. Deutsches Institut für Normung e.V., Berlin

DIN (1997): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Bestimmung der Hemmwirkung von Abwasser auf die Lichtemission von *Photobacterium phosphoreum*. Leuchtbakterien-Abwassertest mit konservierten Bakterien (L 34). DIN 38412-34:1997-07. Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (2002): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung - Testverfahren mit Wasserorganismen (Gruppe L). Teil 48: *Arthrobacter globiformis*-Kontakttest für kontaminierte Feststoffe (L 48). DIN 38412-48:2002-09. Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (2003): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Suborganismische Testverfahren (Gruppe T). Teil 6: Giftigkeit gegenüber Fischen; Bestimmung der nicht akut giftigen Wirkung von Abwasser auf die Entwicklung von Fischeiern über Verdünnungsstufen (T 6). DIN 38415-6:2003-08 Deutsches Institut für Normung e.V., Berlin. [Norm zurückgezogen]

DIN (2009a): Untersuchung von Feststoffen. Probenvorbehandlung, -vorbereitung und -aufarbeitung für chemische, biologische und physikalische Untersuchungen. DIN 19747:2009-07. Deutsches Institut für Normung e.V., Berlin

DIN (2009b): Wasserbeschaffenheit. Bestimmung der akuten Toxizität von Abwasser auf Zebrafisch-Eier (*Danio rerio*). Deutsche Fassung EN ISO 15088:2008. DIN EN ISO 15088:2009-06. Deutsches Institut für Normung e.V., Berlin

DIN (2010): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Toxizitätstest zur Bestimmung der Dehydrogenasenaktivitätshemmung in Belebtschlamm (TTC-Test). DIN 38412-3:2010-10. Deutsches Institut für Normung e.V., Berlin

DIN (2014): Untersuchung von Feststoffen. Probenahme von festen und stichfesten Materialien. Teil 1: Anleitung für die segmentorientierte Entnahme von Proben aus Haufwerken. DIN 19698-1:2014-05. Deutsches Institut für Normung e.V., Berlin

DIN (2021): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Algenwachstumshemmtest auf Mikrotiterplatte mit einzelligen Süßwasser-Grünalgen (L 59). DIN 38412-59. Normentwurf. Deutsches Institut für Normung e.V., Berlin

DIN (2022): Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Testverfahren mit Wasserorganismen (Gruppe L). Algenwachstumshemmtest auf Mikrotiterplatte mit einzelligen Süßwasser-Grünalgen (L 59). DIN 38412-59. Deutsches Institut für Normung e.V., Berlin

DIN (2023a): Elution von Feststoffen. Perkolationsverfahren zur gemeinsamen Untersuchung des Elutionsverhaltens von anorganischen und organischen Stoffen. DIN 19528:2023-07. Deutsches Institut für Normung e.V., Berlin

DIN (2023b): Elution von Feststoffen. Schüttelverfahren zur Untersuchung des Elutionsverhaltens von anorganischen und organischen Stoffen bei einem Wasser/Feststoff-Verhältnis von 2 L/kg. DIN 19529: 2023-07. Deutsches Institut für Normung e.V., Berlin

DIN EN (2003a): Charakterisierung von Abfällen. Auslaugung; Übereinstimmungsuntersuchung für die Auslaugung von körnigen Abfällen und Schlämmen. Teil 2: Einstufiges Schüttelverfahren mit einem Flüssigkeits-/Feststoffverhältnis von 10 L/kg und einer Korngröße unter 4 mm (ohne oder mit Korngrößenreduzierung). Deutsche Fassung EN 12457-2:2002. DIN EN 12457-2:2003. Deutsches Institut für Normung e.V., Berlin

DIN EN (2003b): Charakterisierung von Abfällen. Auslaugung; Übereinstimmungsuntersuchung für die Auslaugung von körnigen Abfällen und Schlämmen. Teil 4: Einstufiges Schüttelverfahren mit einem Flüssigkeits-

/Feststoffverhältnis von 10 L/kg für Materialien mit einer Korngröße unter 10 mm (ohne oder mit Korngrößenreduzierung). Deutsche Fassung EN 12457-4:2002. Deutsches Institut für Normung e.V., Berlin

DIN EN (2012): Bodenverbesserungsmittel und Kultursubstrate - Bestimmung der Pflanzenverträglichkeit - Teil 2: Petrischalentest mit Kresse; Deutsche Fassung EN 16086-2:2011. DIN EN 16086-2:2012-01. Deutsches Institut für Normung e.V., Berlin

DIN EN (2022): Charakterisierung von Abfällen. Herstellung von Abfallproben für ökotoxikologische Untersuchungen. Deutsche Fassung EN 14735:2021. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2009): Wasserbeschaffenheit - Bestimmung der Hemmwirkung von Wasserproben auf die Lichtemission von *Vibrio fischeri* (Leuchtbakterientest) - Teil 2: Verfahren mit flüssig getrockneten Bakterien Deutsche Fassung EN ISO 11348-2:2008. DIN EN ISO 11348-2:2009-05. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2012): Wasserbeschaffenheit. Süßwasseralgen-Wachstumshemmtest mit einzelligen Grünalgen (ISO 8692:2012). DIN EN ISO 8692:2012-06. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2013a): Wasserbeschaffenheit. Bestimmung der Hemmung der Beweglichkeit von *Daphnia magna* Straus (Cladocera, Crustacea). Akuter Toxizitäts-Test (ISO 6341:2012). Deutsche Fassung EN ISO 6341:2012. DIN EN ISO 6341:2013-01. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2013b): Bodenbeschaffenheit. Bestimmung der Wirkungen von Schadstoffen auf die Bodenflora. Teil 2: Wirkung von verunreinigten Böden auf Saatauflauf und frühes Wachstum höherer Pflanzen (ISO 11269-2:2012). Deutsche Fassung EN ISO 11269-2:2013. DIN EN ISO 11269-2:2013-05. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2018): Bodenbeschaffenheit. Feststoffkontakttest unter Verwendung der Dehydrogenaseaktivität von *Arthrobacter globiformis* (ISO 18187:2016). Deutsche Fassung EN ISO 18187:2018. DIN EN ISO 18187:2018-07. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2020): Bodenbeschaffenheit. Vermeidungsprüfung zur Bestimmung der Bodenbeschaffenheit und der Auswirkungen von Chemikalien auf das Verhalten. Teil 1: Prüfung von Regenwürmern (*Eisenia fetida* und *Eisenia andrei*) (ISO 17512-1:2008). Deutsche Fassung EN ISO 17512-1:2020. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2021): Bodenbeschaffenheit. Bestimmung der potentiellen Nitrifizierung und Hemmung der Nitrifizierung. Schnellverfahren mittels Ammoniumoxidation. Deutsche Fassung ISO 15685:2012. DIN EN ISO 15685:2021-02. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO (2023): Wasserbeschaffenheit. Bestimmung der Hemmwirkung von Wasserproben auf die Lichtemission von *Vibrio fischeri* (Leuchtbakterientest). Teil 2: Verfahren mit flüssig getrockneten Bakterien (ISO 11348-2:2007 + Amd 1:2018); Deutsche Fassung EN ISO 11348-2:2008 + A1:2018. Deutsches Institut für Normung e.V., Berlin

DIN EN ISO/IEC (2018): Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien (ISO/IEC 17025:2017). Deutsche Fassung EN ISO/IEC 17025:2017. DIN EN ISO/IEC 17025:2018-03. Deutsches Institut für Normung e.V., Berlin

Domene, X. (2007): Methodologies using soil organisms for the ecotoxicological assessment of organic wastes. Dissertation, Universität von Barcelona, Spanien

Domene, X.; Alcaniz, J.M.; Andrés, P. (2007): Ecotoxicological assessment of organic wastes using the soil collembolan *Folsomia candida*. Applied Soil Ecology, 35, S. 461 – 472

EC (2018): Technical guidance for deriving environmental quality standards. Guidance Document No. 27. Updated version 2018. European Commission, Brussels

ECHA (2008): Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment. European Chemicals Agency, Helsinki

ECHA (2017): Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0. European Chemicals Agency, Helsinki

ECHA (2023a): Guidance on information requirements and chemical safety assessment. Chapter R.7b: Endpoint specific guidance. Version 5.0. European Chemicals Agency, Helsinki

ECHA (2023b): Guidance on information requirements and chemical safety assessment. Chapter R.7c: Endpoint specific guidance. Version 4.0. European Chemicals Agency, Helsinki

EG (2014): Beschluss der Kommission vom 18. Dezember 2014 zur Änderung der Entscheidung 2000/532/EG über ein Abfallverzeichnis gemäß der Richtlinie 2008/98/EG des Europäischen Parlaments und des Rates. (2014/955/EU)

EG (2015): Entscheidung der Kommission vom 3. Mai 2000 zur Ersetzung der Entscheidung 94/3/EG über ein Abfallverzeichnis gemäß Artikel 1 Buchstabe a) der Richtlinie 75/442/EWG des Rates über Abfälle und der Entscheidung 94/904/EG des Rates über ein Verzeichnis gefährlicher Abfälle im Sinne von Artikel 1 Absatz 4 der Richtlinie 91/689/EWG über gefährliche Abfälle (2000/532/EG). Konsolidierte Fassung vom 01.06.2015

EG (2018): Richtlinie 2008/98/EG des Europäischen Parlaments und des Rates vom 19. November 2008 über Abfälle und zur Aufhebung bestimmter Richtlinien. Konsolidierte Fassung vom 05.07.2018

EG (2019): Verordnung (EG) 440/2008 der Kommission vom 30. Mai 2008 zur Festlegung von Prüfmethoden gemäß der Verordnung (EG) Nr. 1907/2006 des Europäischen Parlaments und des Rates zur Registrierung, Bewertung, Zulassung und Beschränkung chemischer Stoffe (REACH). Konsolidierte Fassung vom 16.10.2019

EG (2021): Verordnung (EG) Nr. 1272/2008 des Europäischen Parlaments und des Rates vom 16. Dezember 2008 über die Einstufung, Kennzeichnung und Verpackung von Stoffen und Gemischen, zur Änderung und Aufhebung der Richtlinien 67/548/EWG und 1999/45/EG und zur Änderung der Verordnung (EG) Nr. 1907/2006. Konsolidierte Fassung vom 01.10.2021

EG (2022): Verordnung (EG) Nr. 1907/2006 des Europäischen Parlaments und des Rates vom 18. Dezember 2006 zur Registrierung, Bewertung, Zulassung und Beschränkung chemischer Stoffe (REACH), zur Schaffung einer Europäischen Agentur für chemische Stoffe, zur Änderung der Richtlinie 1999/45/EG und zur Aufhebung der Verordnung (EWG) Nr. 793/93 des Rates, der Verordnung (EG) Nr. 1488/94 der Kommission, der Richtlinie 76/769/EWG des Rates sowie der Richtlinien 91/155/EWG, 93/67/EWG, 93/105/EG und 2000/21/EG der Kommission. Konsolidierte Fassung vom 01.03.2022

EU (2017): Verordnung 2017/997 des Rates vom 8. Juni 2017 zur Änderung von Anhang III der Richtlinie 2008/98/EG des Europäischen Parlaments und des Rates in Bezug auf die gefahrenrelevante Eigenschaft HP 14 „ökotoxisch"

EU (2018): Bekanntmachung der Kommission – Technischer Leitfaden zur Abfalleinstufung (2018/C 124/01)

EU (2019): Richtlinie 2010/63/EU des Europäischen Parlaments und des Rates vom 22. September 2010 zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere. Konsolidierte Fassung vom 26.06.2019

Ferrari, B.; Férard, J.F. (1996): Effects of nutritional renewal frequency on survival and reproduction of *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry, 15, S. 765 – 770

Ferrari, B.; Radetski, C.M.; Veber, A.-M.; Ferard, J.-F. (1999): Ecotoxicological assessment of solid wastes: a combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers. Environmental Toxicology and Chemistry, 18, S. 1195 – 1202

Fischer, M.; Belanger, S.E.; Berckmans, P.; Bernhard, M.J.; Bláha, L.; Coman Schmid, D.E.; Dyer, S.D.; Haupt, T.; Hermens, J.L.M.; Hultman, M.T.; Laue, H.; Lillicrap, A.; Mlnaříková, M.; Natsch, A.; Novák, J.; Sinnige, T.L.;

Tollefsen, K.E.; von Niederhäusern, V.; Witters, H.; Županič, A.; Schirmer, K. (2019): Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. Toxicological Sciences 169, S. 353 – 364

Fiskesjö, G. (1985): The *Allium* test as a standard in environmental monitoring. Hereditas, 102, S. 99 – 112

Fiskesjö, G. (1995): *Allium* test. Methods in Molecular Biology, 43, S. 119 – 127

Fiskesjö, G. (1997): The *Allium* test for screening chemicals. In: Wang, W.; Gorsuch, J.W.; Hughes, J.S. [Hrsg.]: Plants for Environmental Studies. Lewis, New York, S. 302 – 331

Flamme, S. (2019): Vortrag am 14. November 2019 auf der BDSV-Jahrestagung in Münster zitiert in: EU-Recycling 01/2020

Garcia Geronasso, J.V. (2010): The assessment of feeding inhibition in *Folsomia candida* (Collembola) as an endpoint for ecotoxicological waste characterization. Dissertation, University of Coimbra, Portugal

Gilbert, F.; Galgani, F.; Cadiou, Y. (1992): Rapid assessment of metabolic activity in marine microalgae: application in ecotoxicological tests and evaluation of water quality. Marine Biology, 112, S. 199 – 205

Götzl, A.; Malissa, H.; Riepe, W. (1999): A rapid toxicological test method with luminous bacteria for laboratory and on-site assessment of wastes and contaminated soils. Field Analytical Chemistry & Technology, 3, S. 329 – 337

Grenni, P.; Ultre, M.; Francese, M.; Baudo, R.; Mariani, L.; Neotti, M.G.; Provenza, F.; Perini, F.; Renzi, M.; Meineri, V.; Tagliati, C.; Zecchini, F.; Perin, F.; Paina, A. (2020): Critical points of the EU Directive on the HP 14 classification: an Italian perspective. Poster presented at SETAC Europe 30rd Annual Meeting (virtual), 03-07 May 2020

Gy, P. (1979): Sampling of particulate materials – Theory and practice, Elsevier, Amsterdam

Gy, P. (1992): Sampling of heterogeneous and dynamic material systems. Theories of heterogeneity, sampling and homogenizing. Elsevier, Amsterdam

Gy, P. (2004a): Sampling of discrete materials – a new introduction to the theory of sampling I. Qualitative approach. Chemometrics and Intelligent Laboratory Systems, 74, S. 7 – 24

Gy, P. (2004b): Sampling of discrete materials – a new introduction to the theory of sampling II. Quantitative approach – sampling of zero dimensional objects. Chemometrics and Intelligent Laboratory Systems, 74, S. 25 – 38

Hennebert, P. (2018): Proposal of concentration limits for determining the hazard property HP 14 for waste using ecotoxicological tests. Waste Management, 74, S. 74 – 85

Hennebert, P. (2019): Hazard classification of waste: review of available practical methods and tools. Detritus, 7, S. 13 – 28

Höss, S.; Römbke, J. (2019): Effects of waste materials on *Caenorhabditis elegans* (Nematoda) using the ISO standard soil toxicity test. Environmental Science and Pollution Research, 26, S. 26304 – 26312

Hund-Rinke, K.; Kördel, W.; Heiden, S.; Erb, R. (2002): Ökotoxikologische Testbatterien – Ergebnisse eines DBU-geförderten Ringtests. Erich Schmidt Verlag, Berlin

INERIS (2016): Classification réglementaire des déchets. Guide d'application pour la caractérisation en dangerosité. Rapport réalisé pour le Ministère de l'Ecologie, du Développement Durable et de l'Energie (MEDDE). INERIS-DRC-15-149793-06416A. (Participation: Pandard, P.)

IPA (2021): Gefährlichkeitseinstufung von Abfällen in den Bundesländern. Informations-Portal-Abfallbewertung.
https://www.abfallbewertung.org/?report=ipa&content=Kurzinfos&subcontent=GefEinst&doc=GefEinst (11.04.2021)

ISO (1987): Water for analytical laboratory use. Specification and test methods. ISO 3696:1987-04. International Organization for Standardization, Geneva

ISO (1995): Water quality. *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test). ISO 10712:1995. International Organization for Standardization, Geneva

ISO (1998): Water quality. Sampling. Part 16: Guidance on biotesting of samples. ISO 5667-16: 1998. International Organization for Standardization, Geneva. [Aktuelle Fassung: ISO 2017]

ISO (2000): Water quality. Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). ISO 10706:2000-04. International Organization for Standardization, Geneva

ISO (2005a): Water quality. Determination of acute toxicity of marine or estuarine sediment to amphipods. ISO 16712:2005. International Organization for Standardization, Geneva

ISO (2005b): Water quality. Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*). Duckweed growth inhibition test. ISO 20079:2005. International Organization for Standardization, Geneva

ISO (2007a): Water quality. Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent bacteria test). Part 2: Method using liquid-dried bacteria. ISO 11348-2:2007. With Amendment 1 from 2018. International Organization for Standardization, Geneva

ISO (2007b): Water quality. Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent bacteria test). Part 3: Method using freeze-dried bacteria. ISO 11348-3:2007. With Amendment 1 from 2018. International Organization for Standardization, Geneva

ISO (2008a): Avoidance test for determining the quality of soils and effects of chemicals on behaviour. Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). ISO 17512-1:2008. International Organization for Standardization, Geneva

ISO (2008b): Water quality. Determination of the chronic toxicity to *Brachionus calyciflorus* in 48 h. ISO 20666:2008-12. International Organization for Standardization, Geneva

ISO (2008c): Water quality. Determination of chronic toxicity to *Ceriodaphnia dubia*. ISO 20665:2008. International Organization for Standardization, Geneva

ISO (2012a): Soil quality. Determination of the effects of pollutants on soil flora. Part 2: Effects of contaminated soil on the emergence and early growth of higher plants. ISO 11269-2:2012. International Organization for Standardization, Geneva

ISO (2012b): Water quality. Fresh water algal growth inhibition test with unicellular green algae. ISO 8692:2012. International Organization for Standardization, Geneva

ISO (2012c): Water quality. Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea). Acute toxicity test. ISO 6341:2012-10. International Organization for Standardization, Geneva

ISO (2012d): Soil quality. Effects of pollutants on earthworms. Part 1: Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei*. ISO 11268-1:2012. International Organization for Standardization, Geneva

ISO (2012e): Soil quality. Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth. ISO 11269-1:2012. International Organization for Standardization, Geneva

ISO (2012f): Soil quality. Determination of abundance and activity of soil microflora using respiration curves. ISO 17155:2012. International Organization for Standardization, Geneva

ISO (2012g): Soil quality. Determination of potential nitrification and inhibition of nitrification. Rapid test by ammonium oxidation. ISO 15685:2012. International Organization for Standardization, Geneva

ISO (2016a): Soil quality. Contact test for solid samples using the dehydrogenase activity of *Arthrobacter globiformis*. ISO 18187:2016. International Organization for Standardization, Geneva

ISO (2016b): Water quality. Marine algal growth inhibition test with *Skeletonem*a sp. and *Phaeodactylum tricornutum*. ISO 10253:2016. International Organization for Standardization, Geneva

ISO (2017): Water quality. Sampling. Part 16: Guidance on biotesting of samples. ISO 5667-16:2017. International Organization for Standardization, Geneva

ISO (2019a): Soil quality. Guidance on the ecotoxicological characterization of soils and soil materials. ISO 15799:2019. International Organization for Standardization, Geneva

ISO (2019b): Soil quality. Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials. ISO 17616:2019. International Organization for Standardization, Geneva

ISO (2020): Water and soil quality. Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda). ISO 10872:2020. International Organization for Standardization, Geneva

ISO (2023a): Soil quality. Effects of pollutants on earthworms. Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei* and other earthworm species. ISO 11268-2:2023. International Organization for Standardization, Geneva

ISO (2023b): Soil quality. Effects of contaminants on Enchytraeidae (*Enchytraeus* sp.). Determination of effects on reproduction. ISO 16387:2023. International Organization for Standardization, Geneva

ISO (2023c): Soil quality. Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants. ISO 11267:2023. International Organization for Standardization, Geneva

ISPRA (2018): Approccio metodologico per la valutazione della caratteristica di pericolo HP14 – Ecotossico. Istituto Superiore per la Protezione e la Ricerca Ambientale/Sistema Nazionale per la Protezione dell'Ambiente

Jensen, J.; Mesman, M. (2006): Ecological risk assessment of contaminated land. Decision support for site specific investigations. RIVM Report No. 711701047, Bilthoven

Kalbe, U. (2020): Wie steht es um die Zukunft der „neuen" Elutionsverfahren? Altlasten Spektrum, 5/2020, 169-170

Kalbe, U. (2021): Wie naturnah simulieren die Elutionsverfahren? Vortrag, online-Altlastenseminar des LLUR ‚Messen, schätzen und bewerten – Theorie und Praxis der Altlastenbewertung', 27.09.2021

Katsiadaki, I.; Ellis, T.; Andersen, L.; Antczak, P.;Blaker, E.; Burden, N.; Fisher, T.; Green, C.; Labram, B.; Pearson, A.; Petersen, K.; Pickford, D.; Ramsden, C.; Rønneseth, A.; Ryder, K.; Sacker, D.; Stevens, C.; Watanabe, H.; Yamamoto, H.; Sewell, F.; Hawkins, P.; Rufli, H.; Handy, R.D.; Maynard, S.K.; Jacobs, M.N. (2021): Dying for change: a roadmap to refine the fish acute toxicity test after 40 years of applying a lethal endpoint. Ecotoxicology and Environmental Safety, 223:112585

Kępys, W.; Sliwka, M.; Pawul, M. (2021): Assessment of ecotoxicity of incinerated sewage sludge ash (ISSA). Minerals, 11:849

Ketelhut, R. (2013): Erarbeitung von grundlegenden Qualitätskriterien für Abfallprobenahmen zum Erhalt von Kenngrößen zur Beurteilung von Untersuchungsergebnissen - Ermittlung fachlicher Grundlagen. Abschluss-bericht LFP-Vorhaben L1.12

Kobeticovà, K.; Hofman, J.; Holoubek, I. (2010): Ecotoxicity of wastes in avoidance tests with *Enchytraeus albidus*, *Enchytraeus crypticus* and *Eisenia fetida* (Oligochaeta). Waste Management, 30, S. 558 – 564

Kočí, V.; Mocová, K.; Kulovaná, M.; Vosáhlová, S. (2010): Phytotoxicity tests of solid wastes and contaminated soils in the Czech Republic. Environmental Science and Pollution Research, 17, S. 611 – 623

KrWG (2023): Gesetz zur Förderung der Kreislaufwirtschaft und Sicherung der umweltverträglichen Bewirtschaftung von Abfällen (Kreislaufwirtschaftsgesetz - KrWG). Zuletzt geändert durch Art. 5 G v. 2.3.2023 I Nr. 5

Kwan, K.K. (1995): Direct sediment toxicity testing procedure using Sediment-Chromotest kit. Environmental Toxicology and Water Quality, 9, S. 193 – 196

LAGA (2004): LAGA PN 98. Richtlinie für das Vorgehen bei physikalischen, chemischen und biologischen Untersuchungen im Zusammenhang mit der Verwertung/Beseitigung von Abfällen. Mitteilung der Länderarbeitsgemeinschaft Abfall (LAGA)

LAGA (2019): LAGA PN 98. Richtlinie für das Vorgehen bei physikalischen, chemischen und biologischen Untersuchungen im Zusammenhang mit der Verwertung/Beseitigung von Abfällen. Mitteilung der Länderarbeitsgemeinschaft Abfall (LAGA). (In Bezug auf rechtliche Verweise überarbeite Version)

Lapa, N.; Barbosa, R.; Morais, J.; Mendes, B.; Méhu, J.; Santos Oliveria, J.F. (2002): Ecotoxicological assessment of leachates from MSWI bottom ashes. Waste Management, 22, S. 583 – 593

LfULG (2014): Richtlinie zur einheitlichen Abfallanalytik in Sachsen. Sächsische Sortierrichtlinie 2014. Landesamt für Umwelt, Landwirtschaft und Geologie, Freistaat Sachsen

Ministerium für Landwirtschaft, Umwelt und Klima (2020): Vollzugshinweise zur Zuordnung von Abfällen zu den Abfallarten eines Spiegeleintrages in der Abfallverzeichnis-Verordnung. Amtsblatt für Brandenburg 31, 699-745

Ministry of the Environment (2019): Classification of waste as hazardous waste- updated guidance. Publications of the Ministry of the Environment 2019:2

MITECO (2021): Guía técnica para la clasificatión de los residuos. Ministerio para la Transición Ecológica y el Reto Demográfico Gobierno de Espaňa

Mocová, K.A.; Sackey, L.N.A.; Renkerová, P. (2019): Environmental Impact of concrete and concrete-based construction waste leachates. IOP Conference Series: Earth and Environmental Science, 290:012023

Moser, H.; Römbke, J. (2009): Ecotoxicological characterization of waste - Results and experiences of an international ring test. Springer Science+Business Media, Heidelberg

MVO (2021): Verordnung zur Einführung einer Ersatzbaustoffverordnung, zur Neufassung der Bundes-Bodenschutz- und Altlastenverordnung und zur Änderung der Deponieverordnung und der Gewerbeabfallverordnung vom 9. Juli 2021. Bundesgesetzblatt Jahrgang 2021 Teil I Nr. 43

Natural Resources Wales, SEPA, Environment Agency (2021): Waste classification. Guidance on the classification and assessment of waste (1st Edition v1.1.GB). Technical Guidance WM3. Natural Resources Wales/Cyfoeth Naturiol Cymru, Cardiff; Scottish Environment Protection Agency (SEPA), Stirling; Environment Agency, Bristol

Neururer, H. (1975): Biotests in der Herbologie. Zeitschrift für Pflanzenkrankheiten, 5(75), S. 316 – 328

OECD (1984): Earthworm, acute toxicity tests. OECD guidelines for the testing of chemicals, No. 207. Organisation for Economic Cooperation and Development, Paris

OECD (2000a): Soil microorganisms: nitrogen transformation test. OECD guidelines for the testing of chemicals, No. 216. Organisation for Economic Cooperation and Development, Paris

OECD (2000b): Soil microorganisms: carbon transformation test. OECD guidelines for the testing of chemicals, No. 217. Organisation for Economic Cooperation and Development, Paris

OECD (2004): *Daphnia* sp., acute immobilisation test. OECD guidelines for the testing of chemicals, No. 202. Organisation for Economic Cooperation and Development, Paris

OECD (2006a): Terrestrial plant test: seedling emergence and seedling growth test. OECD guidelines for the testing of chemicals, No. 208. Organisation for Economic Cooperation and Development, Paris

OECD (2006b): *Lemna* sp. growth inhibition test. OECD guidelines for the testing of chemicals, No. 221. Organisation for Economic Cooperation and Development, Paris

OECD (2010): Activated sludge, respiration inhibition test (carbon and ammonium oxidation). OECD guidelines for the testing of chemicals, No. 209. Organisation for Economic Cooperation and Development, Paris

OECD (2011): Freshwater alga and cyanobacteria, growth Inhibition test. OECD guidelines for the testing of chemicals, No. 201. Organisation for Economic Cooperation and Development, Paris

OECD (2012): *Daphnia magna* reproduction test. OECD guidelines for the testing of chemicals, No. 211. Organisation for Economic Cooperation and Development, Paris

OECD (2013): Fish embryo acute toxicity (FET) test. OECD guidelines for the testing of chemicals. Test No. 236. Organisation for Economic Co-operation and Development, Paris

OECD (2016a): Earthworm reproduction test (*Eisenia fetida*/*Eisenia andrei*). OECD guidelines for the testing of chemicals. Test No. 222. Organisation for Economic Co-operation and Development, Paris

OECD (2016b): Collembolan reproduction test in soil. OECD guidelines for the testing of chemicals. Test No. 232. Organisation for Economic Co-operation and Development, Paris

OECD (2019): Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals. Series on testing and assessment. No 23 (2nd edition). ENV/JM/MONO(2000)6/REV1. Organisation for Economic Cooperation and Development, Paris

OECD (2021): Fish cell line acute toxicity: the RTgill-W1 cell line assay. Test guideline No. 249. Organisation for Economic Co-Operation and Development, Paris

Offhaus, K. (1965): Die Bewertung von Abwasser unter besonderer Berücksichtigung des biologisch abbaubaren Anteiles und der Toxizität. Die Wasserwirtschaft 55, S. 7 – 9

OVAM (2018): HP14: Gepaarde gegevens voor afvalstoffen. OVAM report. Openbare Vlaamse Afvalstoffenmaatschappij (Public Waste Agency of Flanders), Mechelen.

Pandard, P.; Römbke, J. (2013): Proposal for a ‚harmonized' strategy for the assessment of the HP 14 property. Integrated Environmental Assessment & Management 9, S. 665-672

Pandard, P.; Devillers, J.; Charissou, A.-M.; Poulsen, V.; Jourdain, M.-J.; Férard, J.-F.; Grand, C.; Bispo, A. (2006): Selecting a battery of bioassays for ecotoxicological characterization of wastes. Science of the Total Environment, 363, S. 114 – 125

Pinho, I.A.; Lopes, D.V.; Martins, R.C.; Quina, M.J. (2017): Phytotoxicity assessment of olive mill solid wastes and the influence of phenolic compounds. Chemosphere, 185, S. 258 – 267

Pivato, A.; Beggio, G.; Hennebert, P.; Bonato, T.; Favarin, M.; Raga, R. (2020): Proposal of a testing proram for the HP 14 (ecotoxic) classification of automotive shredder residues (ASR) by a battery of ecotoxicological bioassays. Detritus, 13, S. 12 – 22

Planchon, M.; Saïdi, N.; Pandard, P.; Troise, A. (2015): Study to assess the impacts of different classification approaches for hazard property 'HP 14' on selected waste streams. Final report prepared for the European Commission (DG ENV). Bio by Deloitte, INERIS

Radetski, C.M.; Férard, J.F.; Blaise, C. (1995): A semistatic microplate-based phytotoxicity test. Environmental Toxicology and Chemistry, 14, S. 199 – 302

Rebischung, F.; Chabot, L.; Biaudet, H.; Pandard, P. (2018): Cigarette butts: a small but hazardous waste, according to European regulation. Waste Management, 82, S. 9 – 14

Römbke, J. (2018): Testing of 24 potentially hazardous wastes using 6 ecotoxicological tests. Detritus, 4, S. 4 – 21

Römbke, J.; Moser, H. (2007): Ökotoxikologische Charakterisierung von Aschen aus Müllverbrennungsanlagen. VGB PowerTech, 12/2007, S. 62 – 68

Römbke, J.; Ketelhut, R. (2014): Weiterentwicklung der UBA-Handlungsempfehlung zur ökotoxikologischen Charakterisierung von Abfällen. UBA-Texte 19/2014

Römbke, J.; Eisenträger, A.; Hund-Rinke, K.; Jänsch, S.; Neumann-Hensel, H.; Weber, G. (2006): Handlungsempfehlung für die ökotoxikologische Beurteilung von Böden. Sidus-Verlag, Limburg

Römbke, J.; Moser, T.; Moser, H. (2009): Ecotoxicological characterization of 12 incineration ashes (MWI) using 6 laboratory tests. Waste Management, 29, S. 2475 – 2482

Römbke, J.; Moser, T.; Ketelhut, R. (2010): Identifikation umweltgefährlicher Abfälle in Spiegeleinträgen der AVV. FKZ 3708 31 300. Abschlussbericht für das Umweltbundesamt, Dessau

Römbke, J.; Pandard, P.; Wahlström, M.; Weltens, R. (2018): Compilation and evaluation of ecotoxicological data gained in waste testing – results of a literature review. Report for the Austrian Ministry of Sustainability and Tourism

Sander, K.; Schilling, S.; Lüskow, H.; Gonser, J., Schwedtje, A.; Kuchen, V. (2008): Review of the European List of Waste. Final Report. Ökopol GmbH, Argus GmbH

Scheffczyk, A.; Frankenbach, S.; Jänsch, S.; Römbke, J. (2014): Comparison of the effects of zinc nitrate-tetrahydrate and tributyltin-oxide on the reproduction and avoidance behavior of the earthworm *Eisenia andrei* in laboratory tests using nine soils. Applied Soil Ecology, 83, S. 253 – 257

Scholz-Starke, B.; Stibany, F.; Hammers-Wirtz, M. (2022): PROSOIL – Protection of soil organisms: Development of toxicity criteria for soil organisms in the framework of classification of substances and PBT assessment. UBA-Texte 105/2022

Senatsverwaltung für Mobilität, Verkehr, Klimaschutz und Umwelt (2020): Vollzugshinweise zur Zuordnung von Abfällen zu den Abfallarten eines Spiegeleintrages in der Abfallverzeichnis-Verordnung. Berlin

Shaw, L.J.; Burns, R.G. (2006): Enzyme activity profiles and soil quality. In: Bloem, J.; Hopkins, D.; Benedetti, A. [Hrsg.]: Microbiological methods for assessing soil quality. Centre for Agriculture and Biosciences International, Wallingford, S. 158 – 172

SIS (1991): Determination of acute lethal toxicity of chemical substances and effluents to *Nitocra spinipes* (Boeck). Static procedure. SIS SS 02 81 06-1991. Standardiseringskommissionen i Sverige (in Swedish)

SNPA (2020): Linee guida sulla classificazione dei rifiuti. Delibera del Consiglio SNPA. Seduta del 27.11.19. Doc. n. 61/19

Statistisches Landesamt, Freistaat Sachsen (2013): Statistischer Bericht. Verwertung von Abfällen im Freistaat Sachsen 2011 (Q II 4 – j/11)

Stephenson, G.L.; Koper, N.; Atkonson, G.F.; Solomon, K.R.; Scroggins, R.P. (2000): Use of nonlinear regression techniques for describing concentration-response relationships of plant species exposed to contaminated site soils. Environmental Toxicology and Chemistry, 19, S. 2968 – 2981

STN (1999): Testing of dangerous properties of wastes. Ecotoxicity. Acute toxicity tests on aquatic organisms and growth inhibition tests of algae and higher cultivated plants. STN 838303. Slovak technical standard

Stiernström, S.; Wik, O.; Bendz, D. (2015): Critical evaluation of methods for hazard classification of waste ecotoxic properties, Sardinia Conference 2015

TierSchG (2023): Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), das zuletzt durch Artikel 2 Absatz 20 des Gesetzes vom 20. Dezember 2022 (BGBl. I S. 2752) geändert worden ist. Konsolidierte Fassung (Änderung durch Art. 2a G v. 17.8.2023 I Nr. 219 berücksichtigt)

Traas, T.P.; van Leeuwen, C.J. (2007): Ecotoxicological effects. In: van Leeuwen, C.J. & Vermeire, T.G. [Hrsg.]: Risk assessment of chemicals – an introduction. Springer, Dordrecht, S. 281-356

UBA (2013): Recommendations for the ecotoxicological characterization of wastes. 26 February 2013. German Environment Agency, Dessau-Roßlau, Germany

UNI (1998): Compost. Classificazione, requisiti e modalità di impiego. UNI 10780:1998. Ente Italiano di Normazione, Milano

US EPA (1984): Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 3rd ed. EPA/600/4-91/002. U.S. Environmental Protection Agency, Washington, D.C.

US EPA (1988): Protocols for short term toxicity screening of hazardous waste sites. EPA/600/3-88/029. U.S. Environmental Protection Agency, Washington, D.C.

UVM (2002): Zuordnung von Abfällen zu Abfallarten aus Spiegeleinträgen. Vorläufige Vollzugshinweise des Ministeriums für Umwelt und Verkehr Baden-Württemberg auf der Grundlage des Entwurfs einer Handlungshilfe des Abfalltechnikausschusses der LAGA. Reihe Abfall, 69

Vaajasaari, K. (2005): Leaching and ecotoxicity tests as methods for classification and assessment of environmental hazard of solid wastes. Dissertation. Tampere University of Technology, Tampere

Vaajasaari, K.; Joutti, A.; Schultz, E.; Selonen, S.; Westerholm (2002): Comparisons of terrestrial and aquatic bioassays for oil-contaminated soil toxicity. Journal of Soils and Sediments, 2, S. 194 – 202

Wahlström, M.; Laine-Ylijoki, J.; Wik, O.; Oberender, A.; Hjelmar, O. (2016): Hazardous waste classification. Amendments to the European Waste Classification Regulation – what do they mean and what are the consequences? TemaNord 2016:519. Nordic Council of Ministers, Copenhagen

Weltens, R.; Deprez, K.; Michiels, L. (2014): Validation of microtox as a first screening. Waste Management, 34, S. 2427 – 2433

Werle, S.; Dudziak, M. (2015): The assessment of sewage sludge gasification by-products toxicity by ecotoxicologial test. Waste Management & Research, 33, S. 696 – 703

A Appendix

A.1 Key information on sampling that reports must contain to enable the competent authority to evaluate the results

Type of information	Explanation	Details, examples
Considered references	Definition of the reference framework for the study, usually CEN/TR 15310-1 (2006a), DIN EN 14735 (2022), PN 98 (LAGA 2019), DIN 19747 (2009a) and UBA (2013)	This sample protocol is based on the substance data provided by the waste owner and the sampler's expert judgement and assessment in view of the requirements of the relevant guidance.
Waste code	Categorisation of the waste with regard to the List of Wastes and the AVV	—
Identification	All data necessary for an unambiguous identification of the investigated sample	—
Client/responsible person	Identification of the sponsor and the person who defines the objective. Definition of the chain of responsibility	—
Objective	In consultation with the responsible representative from the client	Generally, basic characterisation of ecotoxicological properties
Determination of the amount of material with a particle size <4 mm required for the planned investigations	Irrespective of the recommendations on the size of field and laboratory samples, it must be ensured that the laboratory has sufficient material to perform the analyses	In the present project, the target size for the laboratory sample mass was approx. 5 kg with a particle size <4 mm
Waste owner	The owner of the waste does not necessarily have to be the client. Definition of the chain of responsibility	—
Designation of the expert	Sufficiently competent person who performed the planning of sampling. Definition of the chain of responsibility	—

Type of information	Explanation	Details, examples
Sampler	Designation of the competent person who is able to ensure sampling in order to fulfil the objective. Definition of the chain of responsibility	—
Sampling date, time	Time frame for sampling	—
Sampling point	Local framework conditions for sampling	A photo is always helpful, but the interests and concerns of the waste owner should be considered. No photo of the process without permission
Weather conditions	To assess possible effects of precipitation or temperature	Ambient temperature, degree of coverage and humidity
Relevant background for the sampled waste and description of the sampled material	The objective is to understand and describe the generation of the waste. Is the waste a mixture of different sources or is it a process waste? Is the process always identical or are there batches? How do the batches differ? Is the waste fresh material? What quantities are generated (per hour and per year)? Are there sieve curves? Is the d_{95} defined? Are analytical results available? Are the values stable or fluctuating? Which parameters cause problems? What is the composition of the material (e.g. mineral content, biogenic content, synthetic organic content)?	Fluff-light fraction and dust can occur in batches as fluff-light fraction from steel scrap, aluminium scrap and possibly also electronic scrap. The composition will vary considerably. Flue-gas dust from iron casting can result from various processes (e.g. grey cast iron and lamellar cast iron). All data that the waste producer can provide may contain valuable information on contamination and possible effects.
Information on the presumed variability of the characteristics in the material. Heterogeneity in relation to the characteristics of interest: temporal-spatial particulate	If there are suspicions of possible contamination with hazardous substances, these must be recorded as well as possible. Are there any adhesions? Are there different batches? Are there any additives that may introduce high levels of contamination? Could the effect be concentrated on a few particles?	The type of possible contamination plays an important role in estimating the fraction of particles with a certain characteristic (p)

Type of information	Explanation	Details, examples
Estimation of expected fraction of particles with a certain characteristic (p)	Information on, e.g. a high proportion, superficial contamination, single particles with high load, contamination with metals/metal compounds or plastic additives	Examples: oil on a mineral waste, particles with a high load (e.g. lead carbonate buttons in textiles, phthalates or organotin compounds in PVC)
Determination of the desired coefficient of variation	In view of the objective, the desired reliability should be defined together with the client to determine the required effort for sampling, sample pre-treatment and sample preparation. A CV of 10% is realistic. This means that with a confidence level of 95% the true value is in the range of ±20% around the measured value.	A CV of 10%, as is usually used in the formula for determining the minimum sample mass according to CEN TC 292, requires a sample of at least 100 particles for a probability of a characteristic (p) of 50%. For lower p values, larger sample sizes are required.
Definition of the population	In view of the objective, the population has to be defined in consultation with the responsible representative from the client. A photographic documentation while safeguarding the interests of the waste owner is helpful.	The population can be a batch, a daily or weekly production, or a special batch. It is important to make a selection and justify it conclusively.
Estimation of the bulk density of the material	The estimated bulk density of the material is a criterion for determining the size of the individual sample.	An estimate from a net weight determined with a calibrated balance is usually sufficient. For example: ρ_B = net weight [Mg]/(container volume [m³]·x filling level [%]) Alternatively, orientation values are available, e.g. in the PN 98 (LAGA 2019)
Particle density of particles potentially carrying a certain characteristic	Estimated particle density (ρ_P) of the solids for the particles potentially carrying a certain characteristic. Heavy particles with the characteristic to be determined can introduce high loads into the sample. This parameter is required to determine the minimum sample mass.	Empirical values: Biogenic material: <1 kg/dm³ Wood: 0.6 kg/dm³ Foamed plastics: 0.2-0.3 kg/dm³ Rigid PVC and PET: approx. 1.3 kg/dm³ Mineral waste: approx. 1.8 to 2.0 kg/dm³ Metals depending on elemental composition

Type of information	Explanation	Details, examples
Particle dimension d_{95}	The screen hole diameter allowing 95% of sample weight to pass is an important parameter for dimensioning the samples. If empirical values are available and these can be plausibly transferred to the respective waste, they should be used. The source of the information should be indicated.	The particle dimensions are often known due to the used technology (e.g. screening machines).
Estimation of the correction factor (g)	This correction factor addresses the width of the particle size distribution. The wider the distribution of particle dimensions, the smaller g: $d_{95/d05}$ =1 → g = 1.00 $d_{95/d05}$ >1 and <2 → g = 0.75 $d_{95/d05}$ ≥2 and <4 → g = 0.50 $d_{95/d05}$ ≥4 → g = 0.25	For waste, a uniform distribution of particles is very rare. For heterogeneous waste, d_{95}/d_{05} is routinely expected to be >4.
Determination of the minimum sample mass according to CEN/TR 15310-1	See Figure 13	The minimum sample volume can be derived taking the bulk density of the material into account. It can be compared with the recommendations of the PN 98. Note that the d_{95} must be indicated in cm and the particle density in g/cm³ (= kg/dm³) to determine the correct minimum sample mass.
Determination of the size of the individual samples (random samples, RS) and comparison with the minimum sample mass. Determination of the number of individual samples	$V_{RS} = (3 * d_{95})^3$ To achieve a stable mean value, at least 16 random samples should be taken.	The volume of the individual sample can be compared with the recommendations of the PN 98 for the volume of the individual sample. Multiplying the volume with the bulk density gives the value for the mass.
Determination of the sampling strategy in view of the objectives and the local conditions	To obtain a probabilistic sample, the objective and the specific framework conditions for sampling must be taken into account. It is not always possible to completely represent the population	The population is usually three-dimensional. A mass flow falling from a conveyor belt offers ideal conditions for probabilistic sampling. Depending on the specific case, however, sampling may be temporally and/or spatially restricted. This must be documented.

Type of information	Explanation	Details, examples
Type of sampling performed	Description of: number of individual samples, sampling equipment used, mass and volume of the field sample	As person performing the sampling, it is difficult to avoid being influenced by visual impressions. Hence, it is very useful to use random numbers for determining sampling times or locations. This can e.g. be done, for a waste heap spread out as a flat surface using a wheel loader.
Photographic documentation of the population and the field sample taken (detail)	Photo of details of the material including a scale.	—
Storage	Storage after sampling and mixing of individual samples to obtain the field sample	—
Transport including conditions and start	Definition of the local and temporal framework conditions for transport	—
Type of transport and responsible person	Definition of the chain of responsibility	—
Sample pre-treatment site	Definition of the local framework conditions for sample pre-treatment	—
Arrival at the sample pre-treatment site	Definition of the temporal framework conditions for sample pre-treatment	—

Based on EN 14899 (2005), CEN/TR 15310-1 (2006a), PN 98 (LAGA 2019) and DIN 19747 (2009a)

A.2 Key information on sample pre-treatment that reports must contain to enable the competent authority to evaluate the results

Type of information	Explanation	Details, examples, notes
Waste code	Categorisation of the waste with regard to the List of Wastes and the AVV	If the protocol for sample pre-treatment is a stand-alone document
Identification	All data necessary for an unambiguous identification of the investigated sample	
Client/responsible person	Identification of the sponsor and the person who defines the objective. Definition of the chain of responsibility	
Waste owner	The owner of the waste does not necessarily have to be the client. Definition of the chain of responsibility	
Designation of the expert	Sufficiently competent person who performed the planning of sampling. Definition of the chain of responsibility	
Person performing sample pre-treatment	Sufficiently competent person who is able to ensure an appropriate sample pre-treatment in view of the objectives. Definition of the chain of responsibility.	The requirements for sample pre-treatment should be defined in the sampling plan. How is interfering material defined, which needs to be separated prior to the analysis?
Confirmation that a sampling protocol is in place and that the information contained therein is known	The sampling protocol contains objectives, substance data and information on framework conditions that are also important for sample pre-treatment.	The following information is important: expected particles with a certain characteristic and their fraction (proportion) (p), estimated d_{95}, bulk density ρ_B and particle density ρ_P, desired coefficient of variation.
Date and time	Temporal framework conditions for sample pre-treatment	—
Sample pre-treatment site	Local framework conditions for sample pre-treatment	—
Weather conditions	Only in case that the pre-treatment takes place outdoors	Ambient temperature, degree of coverage and humidity

Type of information	Explanation	Details, examples, notes
Generation of a sieve line	Hand sieving of the field sample is sufficient for generating a sieve line. The type of screen hole has to be specified. Round-hole sieves are typically used. Square-hole sieves have a passage area that is 1.27 times larger at an identical mesh size.	—
Proportion of oversized particles >4 mm	Laboratory samples <4 mm are generally required for biological analyses. If the field sample contains particles >4 mm, a decision must be made on how to handle these particles.	—
Minimum sample mass for the subsample of oversized particles	The sample containing the oversized particles >4 mm is a subsample of the field sample. A determination of the minimum sample mass for this subsample can help to assess the quality of the subsample.	—
Estimation of the correction factor (g)	The correction factor addresses the width of the particle size distribution. The wider the distribution of particle dimensions, the smaller g: $d_{95/d05}$ =1 → g = 1.00 $d_{95/d05}$ >1 and <2 → g = 0.75 $d_{95/d05}$ ≥2 and <4 → g = 0.50 $d_{95/d05}$ ≥4 → g = 0.25	For waste, a uniform distribution of particles is very rare. For heterogeneous waste, d_{95}/d_{05} is routinely expected to be >4.
Determination of the minimum sample mass for the oversized particles according to CEN/TR 15310-1	See Figure 13	Note that the d_{95} must be indicated in cm and the particle density in g/cm³ (= kg/dm³) to determine the correct minimum sample mass.
Decision how to deal with the oversized particles	In view of the desired reliability and the available equipment for crushing/shredding, the person performing sample pre-treatment must decide how to deal with the oversized particles: crushing/shredding and addition to the sample with homogenisation or discarding the oversized particles	—

Type of information	Explanation	Details, examples, notes
Mass balance for sample pre-treatment	In order to document the mass flow of the field sample through the pre-treatment, it is necessary to create and document a mass balance.	Documentation of input, interfering materials, oversized particles, sample material <4 mm and losses during pre-treatment.
Photographic and verbal documentation of the field sample (detail), separated interfering materials and oversized particles, if applicable	Detailed photos of the material including a scale. Material description (type and form), considering mineral, biogenic and, if applicable, synthetic organic components.	—
Minimum sample mass for the subsample of the laboratory sample	The subsample of the particles <4 mm and the laboratory sample to which crushed oversized material has been added are subsamples of the field sample. A determination of the minimum sample mass for these subsamples can help to evaluate the quality of the laboratory sample.	—
Sample division (where applicable)	If a sample division is necessary to obtain samples for several laboratories, this must be shown in the mass balance. Any reserves of sample material must also be documented.	—
Storage	Whereabouts of the laboratory sample after extraction	—
Completion of pre-treatment	Day and time	—
Transport including conditions and start	Local and temporal framework conditions of transport	—
Type of transport and responsible person	Definition of the chain of responsibility	—
Arrival at the laboratory, location of the laboratory, and handover to a responsible person to be named	—	—

Based on EN 14899 (2005), CEN/TR 15310-1 (2006a), PN 98 (LAGA 2019) and DIN 19747 (2009a).

A.3 Key information on elution of waste samples for aquatic biotests that reports must contain to enable the competent authority to evaluate the results

Type of information[a]	Explanation	Details, examples
Identification of the eluted waste sample	All data necessary for an unambiguous identification of the investigated sample	
Dry matter and moisture content of the waste sample	—	—
Sample pre-treatment and storage	The samples should not be stored for more than two months at 4±2°C	—
Sample division in the laboratory (to obtain samples for each elution)	Information on the used method (see also A.2)	—
Test guideline	Guideline number and date	—
Deviations from the test guideline, if any	Brief description of the deviation(s)	—
Date of elution (leaching)	Allows conclusions about the age of the waste sample at elution	—
Amount of waste used	—	—
Type and quantity of eluent (leachant)	—	—
L/S ratio	—	—
Vessel(s) used for elution	Type, size, material	—
Agitation (shaking) device used, setting	—	For instance, end-over-end tumbler or rollertable
Temperature during elution	—	—
Time between the end of the shaking process and the beginning of the liquid/solid separation procedure	—	—

Type of information[a]	Explanation	Details, examples
Liquid/solid separation procedure	Sufficiently detailed description: settling, centrifugation, filtration	Settling: Duration, observations regarding phase separation In the case of centrifugation: g-Value, duration, temperature Filtration: Filtration device, filter material and pore size for pre-filters (if used) and main filters, flow rate
Volume, conductivity and pH of the eluate	—	—
Adjustment of the pH value	Has the pH been adjusted?[a] yes/no (see also next table)	—
Aeration	Was the eluate aerated? yes/no	—

[a] Based on DIN EN 12457-2 (DIN EN 2003), DIN EN 14735 (DIN EN 2022). [b] In the first test run relevant for HP 14 classification, the pH value shall not be adjusted.

A.4 Key information on biotests that reports must contain to enable the competent authority to evaluate the results

Type of information[a]	Explanation	Details, examples
Identification of the examined waste sample or waste eluate	All data necessary for an unambiguous identification of the investigated sample	Place and date of sampling, pH, conductivity, water content (for solid waste)
Sample storage	Description of storage time and temperature	—
Sample division in the laboratory (to obtain the sample for the respective biotest)	Method used to obtain the sample, date of sample division (see also A.2)	The sample mass should, where possible, be greater than the minimum sample mass of the laboratory sample determined during the sample pre-treatment.
Sample pre-treatment in the laboratory	Description of the sample pre-treatment steps performed	For instance, sieving to <2 mm for microbiological tests with soil organisms
Methodology		
Test guideline	Guideline number and date	—
Deviations from the test guideline, if any	Brief description of the deviation(s)	—
Test organisms	Test species, origin/source, batch number and expiry date for luminescent bacteria, strain (algae, *A. globiformis*), clone and age (daphnids), range of the body mass (earthworms)	—
Pre-treatment of the test organisms	Cultivation and preparation for the test	Storage temperature of the bacterial suspension (luminescent bacteria), Start date and duration of the pre-culture (algae), Age of the culture (daphnids)
Date of the test	Allows conclusions about the age of the eluate (aquatic test) or the waste sample (terrestrial tests) at test start	—

Type of information[a]	Explanation	Details, examples
Adjustment of pH[a]	If so, detailed information: Has the pH been adjusted in the eluate or in some/all dilution levels? Which acid or base (type, concentration) was used to adjust pH? Information on pH after adjustment	—
Tested dilution levels of the eluate or waste	—	—
Test medium (aquatic tests) or substrate (terrestrial tests)	Type and quantity (g or ml per replicate) of the test medium or test substrate	—
Number of replicates in the control, the positive control (if applicable; see below) and in the dilution levels	—	—
Number of test organisms per replicate or cell density (algae)	—	—
Exposure vessels	—	—
Exposure duration	—	—
Exposure conditions	Temperature, adjustment of salinity (luminescent bacteria test), oxygen content and pH at test start (luminescent bacteria test) or at test start and test end (algal and *Daphnia* test), light intensity and light quality (algal and plant test), Air humidity and watering (plant test)	—
Observations during exposure	—	For instance, precipitation of material
Test endpoint(s)	—	—

Type of information[a]	Explanation	Details, examples
Method(s) used to determine the test endpoint(s)	Sufficiently detailed description	For instance, used methods for determining biomass or cell density, fluorescence measurement from the top or from the bottom for the algal test (DIN 38412-59)
Consideration of a colour or fluorescence correction	If yes: sufficiently detailed description. Relevant for the luminescent bacteria test (DIN EN ISO 11348-2) and the algal test (DIN 38412-59)	—
Statistical method used to determine the effect concentration (EC_{50})	—	—
All methodological details that are not specified in the respective test guideline	—	—
All circumstances that may have affected the result	—	—
Reference test used (with date) or positive controls used	Substance (chemical name, CAS number, source), concentration of this substance	—
Results		
Compliance with the validity criteria	Statement on compliance with the validity criteria, indication of the result for each validity criterion	Luminescent bacteria test (DIN EN ISO 11348-2): see section 11 of the test guideline Algal test (DIN 38412-59): see section 12 of the test guideline *Daphnia* test (DIN EN ISO 6341): see section 10.2 of the test guideline Solid contact test with *A. globiformis* (ISO 18187): see section 9 of the test guideline Growth inhibition test with *B. rapa* (ISO 11269-2): see section 11 of the test guideline Avoidance test with earthworms (ISO 17512-1): see section 6 of the test guideline
Results of the reference tests or positive controls	Luminescent bacteria test: Results of reference tests for the used batch of bacterial suspension and for the respective test	—

Type of information[a]	Explanation	Details, examples
	Daphnia: Results of the reference test	
Detailed results	Mean values with standard deviation, Data for each replicate	Luminescent bacteria test (DIN EN ISO 11348-2): see section 10 of the test guideline Algal test (DIN 38412-59): fluorescence values for each well and time, growth rates *Daphnia* test (DIN EN ISO 6341): percentage of immobile daphnids (%) Solid contact test with *A. globiformis* (ISO 18187): relative fluorescence, inhibition (%) of dehydrogenase activity Growth inhibition test with *B. rapa* (ISO 11269-2): number of seeds emerged, number of plants and biomass at harvest Avoidance test with earthworms (ISO 17512-1): see sections 8 and 9 of the test guideline
Information on the concentration-response relationship	Graphical and/or tabular presentation	—
Effect concentration (EC_{50}) with 95% confidence interval, as far as possible	Short justification if no confidence interval can be specified	—
Observations on test organisms	—	For instance, bleaching of algal cells, abnormal behavior of daphnids (e.g. reduced swimming activity, floating at the water surface)
Reference	Authors of the report, laboratory	—

[a] Based on ISO 17512-1 (ISO 2008a), DIN EN ISO 11348-2 (DIN EN ISO 2009), ISO 11269-2 (ISO 2012a), ISO 18187 (ISO 2016a), DIN EN ISO 6341 (DIN EN ISO 2023), DIN 38412-59 (DIN 2022). [b] In the first test run relevant for HP 14 classification, the pH value shall not be adjusted.