Concawe
Environmental Science
for European Refining

Report

Report no. 16/20

Literature Review: Effects-Based Analysis for Soils, Risk Management, and Waste Disposal

ISBN 978-2-87567-128-8

Literature Review: Effects-Based Analysis for Soils, Risk Management, and Waste Disposal

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Brussels
August 2020

ABSTRACT

Classification of wastes is based on different pieces of legislation including the Waste Framework Directive (Directive 2008/98/EC) (WFD), as well as EU Member State guidance, that can vary between Member States. Waste is assessed for different hazard properties (HP) and ultimately classified as either hazardous or non-hazardous, resulting in different disposal considerations, and associated costs. The current calculation approach to waste classification is based on chemical characterisation, with the possibility of replacement by so-called effect-based tests being discussed here.

Based on an assessment of the currently available literature and the understanding of the base science, this study concludes that it is not appropriate to use effects-based testing as a substitute for the calculation approach. Effects-based tests could have value if used to assess particular site or waste-specific issues on a case by case basis, but cannot be used for some HPs where only animal tests (which are not permitted under the WFD) would be suitable.

KEYWORDS

Classification of wastes, hazard properties, waste framework directive, toxicity, eco toxicity, effects-based tests, soils, non-animal study

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This report is available as an Adobe pdf file on the Concawe website (www.concawe.org).

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SUMMARY

Classification of wastes is a complex process involving many different pieces of legislation and guidance. The revised Waste Framework Directive (Directive 2008/98/EC) (WFD) is the key starting point for waste assessment, but reference to other legislation is also required. Guidance is available at EU and, to various extents, at Member State (MS) level. As guidance varies over time and between (and within) MS it is critical that the most up to date guidance is used when classifying waste. Following the waste classification procedures laid out in the legislation and guidance documents, waste is assessed for different hazard properties (HP) and ultimately classified as either hazardous or non-hazardous, resulting in different disposal considerations, and associated costs. The current approach for waste classification relies on chemical characterisation of the waste, which can be challenging. Effects-based testing (referred to as direct testing in the guidance) can also be utilised and the European guidance on waste classification includes this as an option for most HPs. However, specific guidance on which test methods should be used is limited. Animal testing is not appropriate for use for WFD classification and therefore *in vitro* and non-vertebrate test methods need to be utilised.

This literature review assessed the possibilities for using effects-based testing, instead of the current calculation approach, for classification of waste soils under the WFD, and identified tests that could potentially be included in such an approach. It is clear from this literature review that multiple tests would need to be conducted if an effects-based testing approach was used for classification. Not all HPs are relevant for waste soils contaminated with petroleum hydrocarbons; therefore it could be possible to narrow down the list of HPs assessed if the source of contamination is known. However, for classification purposes all contaminants need to be considered, including those that may come from a different source (e.g. metals).

A number of tests have been identified that could potentially be conducted with waste soil samples. However there are limitations with using a testing approach due to the lack of *in vitro* test methods available for assessing many of the mammalian toxicity HPs. There are also challenges in accounting for changes in availability of hazardous constituents when using methods that require leachates to be tested (rather than direct testing of soils), and the current absence of defined classification thresholds for assessing ecotoxicity test results for wastes.

At the present time, based on an assessment of the currently available literature, and the understanding of the base science, it is not considered appropriate to use effects-based testing as a direct substitute for the calculation approach. For some HPs there are no suitable non-animal studies that could be conducted. For those HPs where a testing approach could be utilised, a number of effects-based tests would be required for classification purposes. Effects-based tests could have value if used to assess particular site or waste-specific issues, and so their use would require careful consideration on a case by case basis.

1. INTRODUCTION

1.1. PROJECT BACKGROUND

The European Union (EU) Waste Framework Directive (WFD) requires that waste materials are assessed for potential environmental or human health hazards based on their composition. Waste materials must therefore be characterised in terms of their component substances (and their concentrations) as a first step in the assessment. Each individual component substance is then assessed to evaluate its potential to infer a hazard on the waste as a whole. The substance-specific evaluation utilises hazard thresholds specified by the EU Classification, Labelling and Packaging Regulations (CLP) and Waste Disposal Regulations. The assessment is designed to highlight the potential hazards of the waste according to a series of hazard categories and ultimately to conclude on the overall hazards of the waste. The final classification of overall waste hazard is then used to ensure appropriate and safe transport and disposal.

The utilisation of so-called 'brownfield' sites for new construction projects requires that potential contamination of the ground by previous uses is assessed and addressed. Sites previously utilised for the exploration, production, storage or retailing of hydrocarbon products have the potential to be contaminated with hydrocarbons, metals and other compounds. Material excavated from such sites during new construction and remediation comprises a matrix composed primarily of soil. Removal of such materials from the originating site can result in them being considered as 'waste' under the WFD, and therefore subject to detailed characterisation and hazard classification. The specific hazards of such waste required to be evaluated (based on their likely chemical composition) are generally related to their toxicological properties (e.g. ecotoxicity; carcinogenic, mutagenic or toxic for reproduction (CMR)).

Concawe wishes to evaluate an alternative approach, using effect-based testing (EBT), for the assessment of potential toxicological hazards from such materials. Such an approach involves the holistic assessment of waste samples for toxicological properties using EBTs, to determine whether this could replace the need for detailed chemical analysis and characterisation of constituent substances.

1.2. AIMS AND OBJECTIVES

The overall project aim is to provide evidence to support the simplification of the testing protocols employed to characterise waste materials by replacement of the current system of multiple tests to whole-sample EBTs. The specific tasks carried out to meet this aim, as outlined in this report, are:

1. Review appropriate regulations and identify the key obligations for compliance for the waste producer with specific reference to testing and characterization;

2. Critically review open and grey literature sources on effects-based testing, including those applied to complex mixtures. This will comprise:

    a. Identification of EBTs that are currently available and reporting of specific toxicological characteristics relevant for the waste assessment of soils and sediments;

    b. Prioritisation of those assays that have been shown to be suitable for use with soil or sediment samples; and

c. A critical evaluation of the prioritised methods for use in waste assessment. The evaluation will consider the available evidence for a response to hydrocarbons, how the response can be interpreted and the technical readiness (validation) of the method.

3. Rank the available effects-based protocols that are deemed appropriate for wastes including reference to likely acceptability and aspects of practical implementation; and

4. Identify any potential technical barriers that may be present to acceptance of effects-based testing and indicate possible options to overcome these.

1.3. REPORT STRUCTURE

Following the brief introductory text included in Section 1, this report comprises the following sections:

- Section 2: This section summarises the relevant legislation for waste assessment, the technical guidance that is available at EU and member state (MS) level, waste producer obligations and the waste classification process.
- Section 3: This section outlines where EBTs could potentially be used in the waste classification process and the approach taken in this project for identifying relevant EBTs.
- Section 4: This section evaluates the identified EBTs, including their applicability to waste classification and their practical implementation, and identifies a shortlist of potentially relevant EBTs.
- Section 5: This section summarises each of the shortlisted EBTs into factsheets outlining the methods and how they can be applied.
- Section 6: This section provides conclusions on the potential for using EBTs for the classification of waste soils, outlines the challenges and proposes potential options for overcoming these.

2. REVIEW OF WASTE REGULATIONS

2.1. LEGISLATION

The legislative framework that controls the classification of waste is complex. Whilst the headline legislation is the revised Waste Framework Directive 2008/98/EC (rWFD), this legislation leads to other legislations, at the EU level and also at MS level, that, used together, dictate how the classifier should classify and dispose of their waste.

The following key pieces of legislation are summarised in Sections 2.1 to 2.5:

- Waste Framework Directive;
- List of Waste;
- CLP regulation;
- REACH regulation and
- Landfill directive.

Such legislation is also dynamic, and is continually updated and expanded by amendments and repeals which lead to:

a) Changes to rules that classifiers have to apply to waste classifications; and

b) Changes to the underlying datasets that classifiers have to use in their waste classifications.

To help explain the legislation and these changes, Figure 2.1 displays a timeline describing the key legislation, standards and guidance that relate to the classification of waste in Europe. The timeline covers the period 2007 to 2019 (x-axis) and indicates how and when the legislation (y-axis) is in force and/or is amended (indicated by arrows) by other (newer) legislation.

There are three main types of legislation (shown with the pale blue background in the left hand column of Figure 2.1) that impact waste classification:

1. **Directives**: e.g. the rWFD – A directive is a legal act of the European Union which requires member states to achieve a particular set of goals without dictating the means of achieving those goals; MS have to devise their own laws to reach the goals set out in a directive – examples of national legislation (UK) that enact the rWFD are shown in pale green in **Figure 2.1**.

2. **Regulations** e.g. the CLP[1] – A regulation applies directly on MS; there is no equivalent national legislation.

3. **Decisions** e.g. the List of Waste (LoW)[2] – a decision is a legal instrument that is binding on those to whom it is addressed (e.g. MS) and is directly applicable. In the case of the LoW, the Commission has defined a Europe-wide list of waste codes that MS must use.

[1] CLP: Classification, Labelling and Packaging of Substances and Mixtures Regulation (EC) No 1272/2008
[2] LoW: List of Waste Decision 2000/532/EC as amended

One key legislative tool is a regulation called an Adaptation to Technical Progress (ATP). ATPs amend or update existing legislation. Figure 2.1 shows how the CLP is updated by ATPs (usually one or two per year). These ATPs can modify the law (i.e. the rules) and/or the underlying data (i.e. the chemical data). For this reason, the classifier cannot utilise the original 2008 CLP document without first checking that it hasn't been modified by one or many ATPs; at the time of writing the CLP has been modified by 14 ATPs.

Shown at the bottom of **Figure 2.1**, in pink, is the GHS (Globally Harmonised System). This is an internationally agreed-upon standard, managed by the United Nations, that was set up to replace the assortment of hazardous material classification and labelling schemes previously used around the world. The CLP is based on this standard. The GHS is the origin of entities like the groups of physical, health and environmental hazards, the hazard statements and safety data sheets (but not the 15 hazard properties, such as HP14 Ecotoxic, that are defined by the rWFD). The GHS is being adopted by most countries of the world, but at different rates and to different extents, and its biannual revisions are a key driver to changes in the European chemical legislation as implemented through the publication of an ATP.

Also shown in **Figure 2.1**, in the darker blue highlight, are two technical guidance documents for the classification of waste:

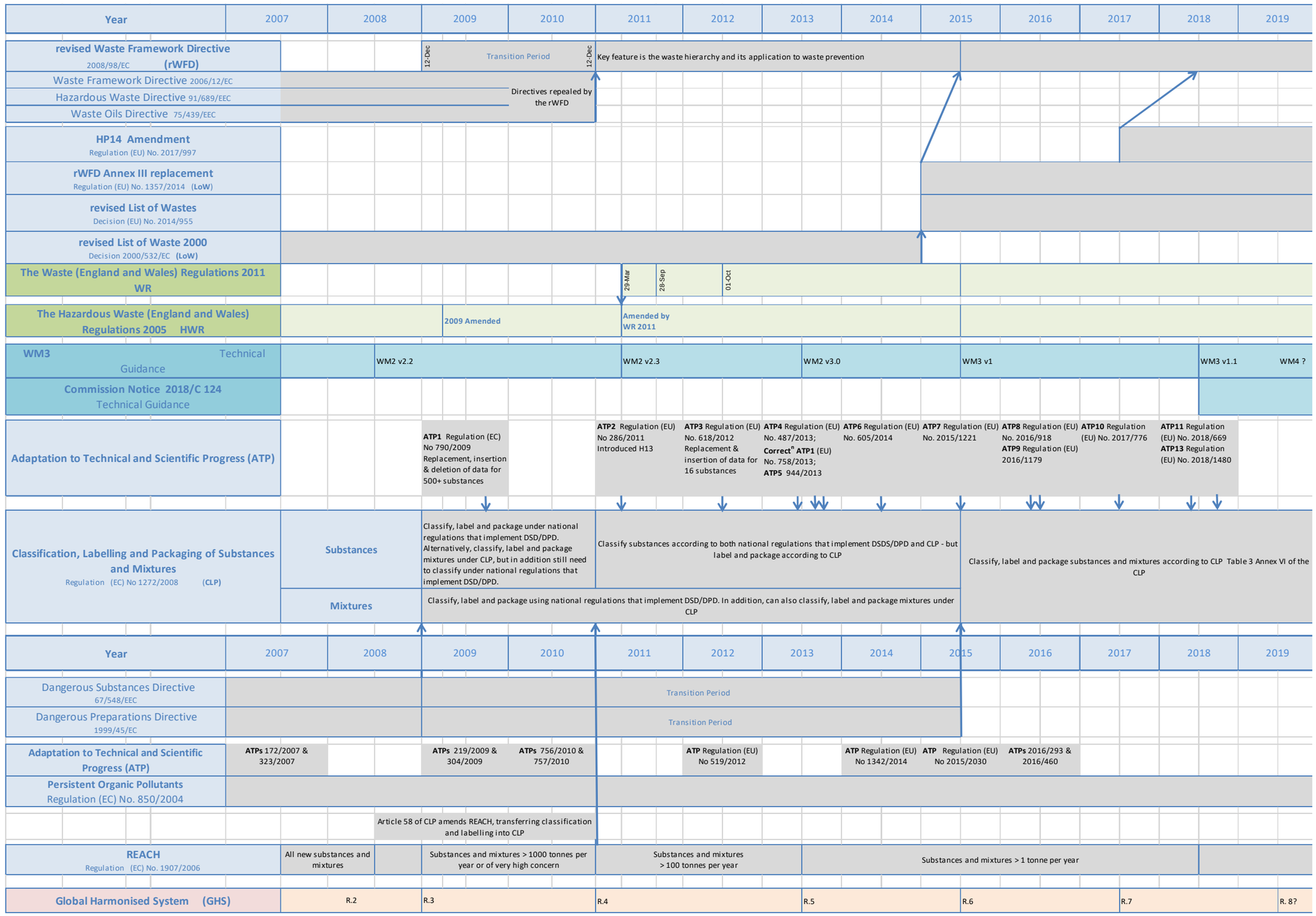
1. The new EU technical guidance published (Commission Notice 2018/C 124); and
2. The UK's established WM3 technical guidance (Environment Agency 2018).

In addition to these pieces of legislation, the Landfill Directive 1999/31/EC and Decision 2003/33/EC (from which the Waste Acceptance Criteria or WAC thresholds are derived) also need to be considered when disposing of waste. This legislation determines whether a particular waste that;

1. Has already been classified as hazardous or non-hazardous, and
2. Is being disposed of to landfill, rather than some other disposal route,

can be accepted at the relevant class of landfill.

Figure 2.1 Timeline for the main EU waste legislation and its relationship to the GHS, technical guidance and selected UK regulations



2.1.1. The revised Waste Framework Directive 2008/98/EC (rWFD)

The rWFD establishes the legislative framework for the handling of waste in the European Community. It defines a waste as

- "any substance or object which the holder discards or intends or is required to discard"

It provides a precise and Europe-wide definition of hazardous waste as

- "waste which displays one or more of the hazardous properties listed in Annex III"

Annex III (as amended) contains a list of the 15 hazard properties, presented below in **Table 2.1.**

Table 2.1 Annex III – Hazardous Properties

Hazard Property	Name
HP1	Explosive
HP2	Oxidising
HP3	Flammable
HP4	Irritant
HP5	Specific Target Organ Toxicity (STOT)
HP6	Acute Toxicity
HP7	Carcinogenic
HP8	Corrosive
HP9	Infectious
HP10	Toxic for Reproduction
HP11	Mutagenic
HP12	Produces toxic gases …
HP13	Sensitizing
HP14	Ecotoxic
HP15	Capable of yielding a hazardous property

Some requirements of the rWFD, relating to waste classification, are outlined below. It:

- Refers to the LoW, established by the Commission Decision 2000/532/EC, as amended;
- States that the waste hierarchy must be considered and applied in priority order when waste is transferred;
- Defines the make-up of the waste transfer note used for the transfer of non-hazardous wastes;
- Makes provision for the controlled management of hazardous waste from the point of production to the final point of disposal or recovery;
- Defines the make-up of the consignment note used for the transfer of hazardous waste;
- States that it is illegal to mix a hazardous waste with a non-hazardous waste, another category of hazardous waste or any other substance or material; and
- States that the mixing of hazardous waste can only be carried out if an appropriate permit is held.

However, the original (un-amended) rWFD is less clear as to how hazard properties are assigned to a particular waste stream, such as a contaminated soil. The relevant clause is found in paragraph 14 of the rWFD which states that:

- "The classification of hazardous waste should be based, inter alia, on the Community legislation on chemicals, in particular concerning the classification of preparations[1] as hazardous."

The main relevant EU chemical legislation is therefore:

- Regulation (EC) No 1272/2008 - Classification, labelling and packaging of substances and mixtures (CLP);
- Regulation (EC) No 1907/2006 - Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and
- Subsidiary legislation for pesticides, biocides, pharmaceuticals and cosmetics, which is not specifically covered in this report.

2.1.1.1. rWFD Annex III replacement - Regulation (EU) No 1357/2014

Regulation (EU) No 1357/2014 was published in December 2014 to align the classification of mixtures (e.g. contaminated soils, sediments, sludges and drill cuttings and muds) with the classification of substances; it marked the end of the CLP's transition period which, between 2009 and June 2015, allowed for the use of the old chemical legislation (Dangerous Substances Directive 67/548/EEC (DSD) and Dangerous Preparations Directive 1999/45/EC (DPD)) for the classification of mixtures.

This regulatory amendment replaced Annex III of the original rWFD; in particular it:

- Renamed the Hazard Properties H1 to H15 to HP1 to HP15;
- Revised some of the hazard property names;

[1] Preparations are called mixtures in the CLP

- Repealed the DSD and DPD, effectively replacing the previous chemical legislation's risk phrases (e.g. Carcinogenic Cat. 1; R45) with the CLP's new hazard statements (e.g. Carc. 1A; H350);
- Listed the new hazard statements, calculations, cut-offs and associated thresholds where applicable; and
- Replaced the term "dangerous" with the term "hazardous".

While paragraph (2) of this regulation has similar reference to the Community's chemical legislation as the rWFD's paragraph 14, it goes one step further, in paragraphs (5), (6), (7), (8) and (9), referring directly to the CLP.

There was one significant exception to these changes; while the Commission undertook a review of the calculation methods for assessing HP14 Ecotoxic, classifiers had to continue to apply the previous R50 - R53 and R59 risk phrases to assess HP14 Ecotoxic. This derogation lasted until July 2018, when the HP14 amendment (Regulation (EU) No 2017/997) was published, which replaced the previous R50 type risk phrases with the new H400 type hazard statements.

2.1.1.2. The HP14 Amendment - Regulation (EU) 2017/997

Following the review of a number of different calculation methods used across the 28 member states for the assessment of HP14 Ecotoxic, the Council of the European Union published Council Regulation (EU) 2017/997, which further amended Appendix III of the rWFD by defining the calculation method required to undertake an assessment of HP14 Ecotoxic. These calculations use the H400 series of hazard statements.

Depending on the chemical composition of a particular waste and the MS's existing approach to the assessment of HP14, the impact of this change varied; some MS found some of their wastes were more hazardous (e.g. Italy) whilst others found some of their wastes to be less hazardous (e.g. UK).

2.1.1.3. Example of equivalent national legislation

As discussed in Section 2 of this report, directives have to be enacted into national law by national legislation. **Table 2.2** shows examples of the legislation that two MS, the UK and Ireland, use to bring the rWFD into law. Each of the EU members states use different national legislation to implement the rWFD, but a detailed review of the approach followed by each member state is outside the scope of this project.

Table 2.2 Examples of national legislation that implement the rWFD

Member State	National Legislation (as amended)	Notes
UK [2]	The Waste (England and Wales) Regulations 2011	Deals with non-hazardous wastes
	The Hazardous Waste (England and Wales) Regulations 2005	Deals with hazardous wastes
Ireland	European Communities (Waste Directive) Regulations 2011 - S.I. no. 126 of 2011	Deals with both hazardous and non-hazardous wastes

[2] Scotland and Northern Ireland both have their own versions of this legislation

2.1.2. The List of Waste - Decision 2000/532/EC as amended

The List of Waste (LoW), also known as the European Waste Catalogue or EWC, provides a complete list of wastes, grouped according to generic industry, process or waste type. The concept is that every waste stream produced in Europe can be given one of the codes listed in the LoW.

The LoW comprises 20 chapters:

- Chapters 1 to 12 and 17 to 20 relate to sources of waste: the industrial process or activity that created it, for example:
  - Chapter 1 Wastes resulting from exploration.
  - Chapter 17 Construction and demolition wastes.
- Chapters 13, 14 and 15 cover waste oils, organic solvents, refrigerants & propellants and waste packaging, for example:
  - Chapter 13 Oil wastes and wastes of liquid fuels.
- Chapter 16 covers "Wastes not otherwise specified in the list" such as:
  - WEEE, tyres, vehicles.

The chapters define 842 six-digit codes that cover all of the hazardous and non-hazardous waste streams. The codes are divided into three types:

- Absolute Hazardous (AH) – marked with an asterisk, are automatically hazardous,
- Absolute Non-hazardous (AN) – not marked with an asterisk, and
- Mirror entries – typically a "pair" of codes, one Mirror Hazardous (MH), one Mirror Non-hazardous (MN); the selection of which depends on whether a waste contains one or more 'hazardous substances' at or above a given threshold.

For example, the following entries are included in the LoW:

- 01 05 05* oil-containing drilling muds and wastes.
- 13 01 01* hydraulic oils, containing PCBs.
- 16 01 17 ferrous metal (end-of-life vehicles).
- 17 05 03* soil and stones containing hazardous substances.
- 17 05 04 soil and stones other than those mentioned in 17 05 03*.

2.1.2.1. Revised List of Waste - Decision 2014/955/EU

Alongside Regulation (EU) No 1357/2014, Decision 2014/955/EU was also published in December 2014 to align the original LoW Decision 2000/532/EC with the terms and approaches defined in the CLP. Apart from listing the 842 waste codes and aligning some definitions, the revised LoW also:

- Refers classifiers to the Testing Regulation (EC) No 440/2008 (and/or other internationally recognised test methods such as those from the OECD), which sets out the test methods which can be applied for the purposes of REACH, as also being appropriate for the attribution of hazard properties;

- Clearly states that certain notes, defined for some substances in Annex VI, Table 3 of the CLP, can be taken into account when establishing the hazardous properties of a waste. A number of these notes (K, L, M and P) refer to chemical markers that can be used to assess whether a waste oil is carcinogenic or mutagenic; and
- Specifically links a set of persistent organic pollutants (POPs) and the concentration limits indicated in the POPs Regulation (EC) No 850/2004 into waste classification.

In the Annex of this decision is the following text which states that a particular hazardous property can be tested either by using the concentration of substances or by EBTs:

"A hazardous property can be assessed by using the concentration of substances in the waste as specified in Annex III to Directive 2008/98/EC or, unless otherwise specified in Regulation (EC) No 1272/2008, by performing a test in accordance with Regulation (EC) No 440/2008 or other internationally recognised test methods and guidelines, taking into account Article 7 of Regulation (EC) No 1272/2008 as regards animal and human testing."

2.1.3. The CLP Regulation (EC) No. 1272/2008

The purpose of the CLP regulation is to ensure a high level of protection for human health and the environment as well as the free movement of substances, mixtures and articles. This Regulation aligns previous EU legislation on classification, labelling and packaging of chemicals to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).GHS is a worldwide standard for the Classification and Labelling of Chemicals. The latest revision of the GHS is R.8 published in 2019.

The CLP complements the REACH regulation (Section 2.4) which requires that manufacturers and importers identify and manage the risks linked to the substances they manufacture and market in the EU.

A key element of CLP is the so called harmonised entries (i.e. the chemical substances) contained in Appendix VI, Table 3 of the CLP. This table contains the meta data for more than 7000 hazardous substances including a substance's CAS[3] Registry number, EINECS[4] number, one or more hazard statements and accompanying notes.

Under CLP, a substance must be self-classified when it has no harmonised classification in Annex VI to CLP and it presents hazardous properties. For a substance that already has a harmonised classification (an entry in Annex VI to CLP), the harmonised hazard classification is legally binding for the hazard classes and differentiations covered in the entry. The hazard classes and differentiations not covered in the entry must be evaluated and self-classified, as appropriate.

[3] CAS Chemical Abstracts Service
[4] EINECS - European Inventory of Existing Commercial Substances

2.1.3.1. The Global Harmonised System (GHS)

The GHS, first published in 2002, is an internationally agreed standard managed by the United Nations. It provides criteria on:

- Classifying pure chemicals and mixtures according to GHS criteria and rules; and
- Communicating the hazards and precautionary information using labelling and packaging.

The CLP is Europe's implementation of the GHS.

2.1.3.2. Adaptation to Technical and Scientific Progress (ATPs)

The CLP (and other regulations) is regularly updated by ATPs that allow the existing legislation to be updated by new scientific knowledge and improvements in technical understanding. In general there are two series of ATPs that can modify the CLP:

1. Amendments to Annex VI, Table 3 (the harmonised entries), which can add, amend or delete the harmonised classifications for chemical substances and mixtures and occur every 12 months or so (e.g. ATP1 (Regulation (EC) No 790/2009) added, amended or deleted the entries for more than 500 substances); and
2. Amendments to the classification criteria and technical annexes which occur every 2 years following a revision to the GHS (e.g. ATP 2 (Regulation (EU) No 286/2011) added Sensitising (HP13)).

It is therefore important for the classifier to make sure that they consult the latest version of both the relevant technical guidance and the harmonised data contained in Annex VI, Table 3 of the CLP (and not the first version of the CLP as published in 2008).

2.1.3.3. Incomplete Entries in Annex VI, Table 3 of the CLP

There are more than 500 entries in Annex VI, Table 3 of the CLP that contain the word "oil", where the oil may be petroleum or coal based. The majority of these entries were labelled "Note H" in older versions of Table 3. Note H is a hangover from the DSD and was used to indicate that the particular substance was missing (hazard) statements for one of more hazard properties including HP14 Ecotoxic.

As an example, kerosene (CAS 8008-20-6) only has a single hazard statement (Asp. Tox. 1; H304, threshold = 10%) in Annex VI, Table 3. It was labelled Note H prior to ATP2. The hazard statements for this entry, like most oils, have not been updated by any subsequent ATP. Examination of REACH compliant SDS from various oil companies, identifies four additional hazard statements: Flam Liq. 3 H226 (HP3); STOT SE 3; H336; Skin Irrit. 2; H315 (HP4) and Aquatic Chronic 2; H411 (HP14). H411 is most significant as it makes kerosene hazardous at only 2.5% (25,000 mg/kg).

Note H was officially deleted by ATP2 as the CLP, considers all harmonised entries as potentially incomplete.

2.1.4. REACH Regulation (EC) No. 1907/2006

The aim of the REACH regulation is to provide a high level of protection of human health and the environment from the use of chemicals; and

REACH requires that companies placing chemicals on the market (manufacturers and importers) are responsible for understanding and managing the risks associated with their use.

The reason that REACH/CLP regulations are important to waste classification is that:

1. The REACH database, published by the European Chemicals Agency (ECHA) in Helsinki, is an important source of classification data for chemical substances. There are only more than 7000 harmonised substances in Annex VI, Table 3 of the CLP while there are more than 100,000 dossiers registered under REACH.

   For the waste classifier, ECHA data sources such as the:

   a. C&L Inventory database[5], and

   b. Registered Substances database[6],

   can be utilised to help determine hazard statements for substances that are either in Annex VI, Table 3 of the CLP or have self-classifications.

2. The Annex II of REACH regulation defines the format of Safety Data Sheets (SDS); these are an important source of information for substances or mixtures that are:

   a. Defined as hazardous under the CLP;

   b. Persistent, bio-accumulative and toxic; or

   c. On ECHA's Candidate List[7] of substances of very high concern for authorisation.

As all the entries for oils in Annex 3, Table 3 of the CLP are incomplete (i.e. missing hazard properties including HP14 Ecotoxic), SDS from the original producer of the substance before it became waste are an important source of more up to date information.

It should be noted that only REACH compliant SDS should be used for waste classification.

2.1.5. Landfill Directive 1999/31/EC and Decision 2004/33/EC

Once the waste has been classified as hazardous or non-hazardous and the correct LoW code selected, the waste can be sent for disposal by either landfill, recovery, recycling, or re-use as per the requirements of the rWFD's waste hierarchy.

If the chosen disposal route is landfill, then the waste falls under the requirements of the Landfill Directive 1999/31/EC and Decision 2003/33/EC. The implementation of this legislation varies between MS but, as a simple example:

[5] Classification & Labelling Inventory Database: https://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

[6] Registered Substances Database: https://echa.europa.eu/information-on-chemicals/registered-substances

[7] ECHA Candidate List – a chemical substance that has been proposed for the use within the European Union, subject to authorisation under the REACH regulation

- if the waste soil is classified as hazardous 17 05 03*, it can go to a hazardous class of landfill, but only if the hazardous waste also passes the Waste Acceptance Criteria (WAC) defined for the hazardous class of landfill; or
- if the waste soil is classified as non-hazardous 17 05 04, it can go to an inert class of landfill, but only if the non-hazardous waste also passes the Waste Acceptance Criteria (WAC) defined for the inert class of landfill.

If the waste fails to pass the WAC for a particular class of landfill, it does not make the waste hazardous, or more hazardous, it simply means that the waste cannot go into that class of landfill without some form of treatment.

It should be noted that most of the WAC thresholds are detailed in Decision 2003/33/EC and vary for the different classes of landfill (hazardous, stable non-reactive hazardous, non-hazardous and inert). Some criteria, like total PAHs, are defined at MS level.

2.2. WASTE CLASSIFICATION – TECHNICAL GUIDANCE

Across the 28 MS, comprehensive technical guidance for the assessment of mixtures (e.g. soils, sludges and filter cakes) as hazardous or non-hazardous has either been non-existent, incomplete or out of date, often scattered between a multitude of sometimes contradicting sources. Where guidance does exist, it varies both between MS and within MS. One exception has been the UK which has published comprehensive guidance for the classification and assessment of waste for many years.

2.2.1. UK Guidance – WM3

The current version of the UK technical guidance is known as WM3 (Environment Agency 2018) and is jointly written by the environmental agencies of England, Wales, Scotland and Northern Ireland.

This guidance provides a comprehensive step-by-step guide to classifying waste as hazardous or non-hazardous based on the waste's chemical composition. It also provides guidance on HP9 Infectious, which is not based on a waste's chemical composition. The guidance document is structured as follows:

- Following a short introduction, Chapter 2 guides the classifier through the basic steps of waste classification.
- Chapter 3 provides examples for specific waste streams including one for soils contaminated by zinc, lead and unknown oil. This chapter includes extra guidance for the classification of unknown oils such as those encountered in contaminated soils, sludges and filter cakes.
- Appendix A describes how to use the LoW to identify the relevant absolute or mirror entry code(s).
- Appendix B provides guidance as to how to determine whether a substance is a hazardous substance, locating data (hazard statements) for substances and how to research substances that are not harmonised entries (i.e. not in Annex VI, Table 3 of the CLP).
- Appendix C of this guidance contains 16 sections, one for each of the 15 hazard properties, plus an extra one for the POPs, including flowcharts and text to describe the assessment of each hazard property.

- Appendix D describes how waste should be sampled and the application of statistics.

2.2.2. European Guidance – Notice 2018/C 124

The European Commission published its first technical guidance in April 2018, several months after publishing Regulation (EU) 2017/997 which defined the calculations for the assessment of HP14. This guidance is heavily based on the UK's WM3 guidance and publishes similar detail.

One key difference between the European guidance and WM3 is that the European guidance doesn't address the case where a waste such as a soil is contaminated by an unknown oil.

The guidance states:

"Regarding organic compounds, sum parameters like PAH, BTEX and hydrocarbons (the latter sometimes referred also as 'mineral oils' or total petroleum hydrocarbons (TPH) are often applied in practical waste analysis. The CLP Regulation does not recognise these as group entries for which a classification could be assigned."

This lack of recognition is because the CLP is chemical legislation written to manage the production and sale of commercial substances and mixtures, rather than for particular wastes streams where different hydrocarbons may have been mixed together.

2.2.3. Waste classification software

Waste classification software, developed based on the classification principles outlined in the waste classification guidance documents, can be used to assess hazard classifications of wastes based on their chemical composition. For example, the commercial software, HazWasteOnline™[8], first released in 2010, contains rule-based classification engines that meet the requirements of both the current UK WM3 guidance and the European guidance for the chemical analysis of waste. Classification engines can be further refined by country to meet the requirements of any national guidance, such as WM3's unknown oils (although the classifier can easily do this themselves if their regulator doesn't have a position). For a given waste stream, the web-based software allows the classifier to import laboratory data directly from registered laboratories and classify their waste as either hazardous or non-hazardous in a few seconds, creating a clear audit trail between the laboratory and the PDF classification report.

2.3. PRODUCER OBLIGATIONS: TESTING AND CHARACTERISATION

Once a material becomes a waste, the waste producer has a duty of care to classify the waste that their activity has produced. This duty needs to be performed:

- Before the waste is collected, disposed of or recovered;
  - And in the case of contaminated soils, the physical extent of the hazardous soil needs to be identified so that (within reason) mixing of non-hazardous soils with hazardous soils does not happen.

[8] www.hazwasteonline.com

- To identify the controls that apply to the movement of the waste;
- To complete waste documents and records;
- To identify suitable authorised waste management options; and
- To prevent harm to people and the environment.

For most wastes, the waste producer will need to ascertain if the waste has any hazardous properties (and which ones) before it can be correctly classified and described.

2.3.1. Testing and Characterisation

Figures 2.2 and **2.3** summarise the basic steps that the waste producer has to go through to complete a hazardous waste assessment. These steps are described in detail in Sections 2.3.1.1 to 2.3.1.5. It should be noted that specific details such as waste transfer documentation and terminology will vary between MS as the rWFD is enacted by domestic legislation.

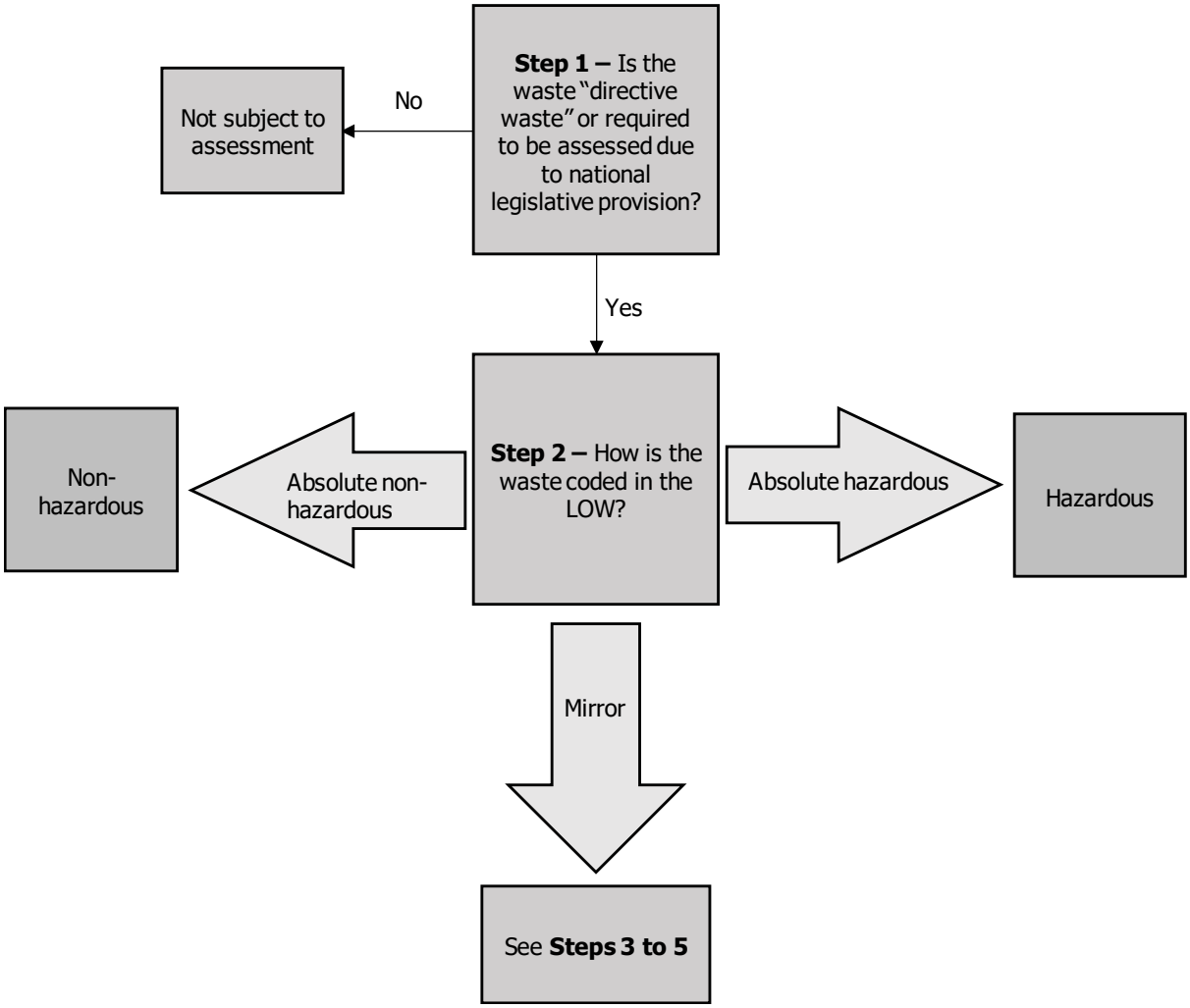
2.3.1.1. Step 1 (Figure 2.2) – Is the waste a directive waste?

A directive waste is waste regulated by the rWFD and not excluded under Article 2 of that directive. Examples of wastes excluded under Article 2 include:

- Most radioactive waste (covered by radioactive legislation);
- Extractive waste - waste resulting from prospecting, extraction, treatment and storage of mineral resources (covered by the mining Directive 2006/21/EC, as amended);
- Land (in-situ) including unexcavated contaminated soil; and
- Sediments relocated inside surface waters for the purposes of managing waters or waterways, if it is proven that the sediments are non-hazardous.

Excavated waste soil is a directive waste.

Figure 2.2 Steps 1 and 2 of a hazardous waste assessment



2.3.1.2. Step 2 (Figure 2.2) - How is the waste coded in the LoW?

The next step concerns the selection of an appropriate (hazardous or non-hazardous) absolute entry code or a pair of mirror entry codes for the particular waste.

The mistake some classifiers make in the selection of a code is to use a key word search approach, for example searching the LoW for any mention of "filter cake" and then selecting a non-hazardous entry (because it's cheaper to dispose of). Instead, the classifier must first identify the process or activity that created the waste (i.e. the relevant chapter in the LoW) and then drill down into that chapter to find the code or codes that best describe the waste.

In the case where an absolute non-hazardous entry is identified, such as 01 05 04 freshwater drilling muds and wastes, the waste can be classified as a non-hazardous waste and the waste transfer note paperwork is used to manage its disposal. Where an absolute hazardous entry is identified, such as 13 05 07* oily water from oil/water separators, the waste is hazardous (even if it doesn't possess any hazardous properties) and a consignment note is used to manage its disposal.

In the case of a mirror entry (i.e. pair of codes) and using waste soils as an example, the review of the LoW first identifies;

Chapter 17 - Construction and Demolition Wastes (including excavated soil from contaminated sites)

and then sub section;

17 05 - Soil (including excavated soil from contaminated sites), stones and dredging spoil

followed by a mirror entry pair of codes i.e. one mirror hazardous code (shown in blue) and one mirror non-hazardous code (shown in green);

17-05-03* soil and stones containing hazardous substances

17-05-04 soil and stones other than those mentioned in 17-05-03

the selection of the code depends on whether the waste has any hazardous properties due to the presence of hazardous substances with concentrations above hazardous levels in the soil.

2.3.1.3. Step 3 (Figure 2.3) – Determine the chemical composition of the waste

To assess whether a waste contains one of more hazardous substances or POPs, the classifier needs to understand the composition of that waste.

Information on composition can come from a number of sources:

1. If it's a waste product and its composition is unchanged, the information should come from the manufacturer's REACH compliant SDS. If the product has changed composition due to, for example, exposure to air, then the composition will have changed and the SDS alone cannot be relied upon (and analysis of the waste may be needed).

2. Where the waste is from a well understood industrial process, then the composition should also be well understood (but further analysis and SDS may still be needed as lines of evidence).

3. Where the composition is not understood, such as a contaminated soil, then the waste will need to be sampled and analysed to better determine its composition.

For example, the assessment of oil-based drill cutting waste arising from a waste treatment plant would include a desktop study to look at the process that created the waste, covering the following:

- What contributed to the composition of the original oil-based drill cuttings (geology, drilling mud, crude oil)?;
- What substances were added to the process (kerosene / diesel / other distillates, conditioners, fatty acid derivatives, calcium chloride etc.)?
- Obtaining the SDS for all additives;
- What was the treatment process (shaker bed, condenser etc.), including information about any further additives (e.g. steam)?; and

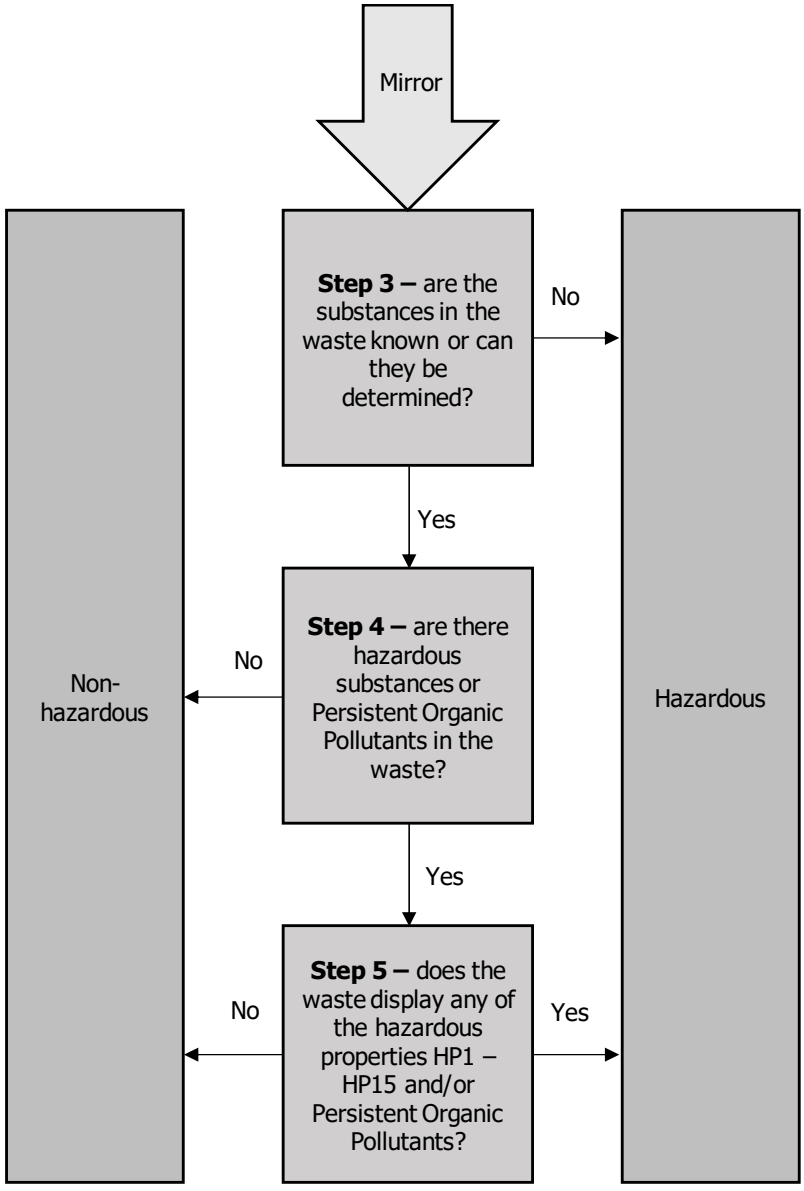
- What was removed from the process as a product (i.e. the recovered oil) or as a separate waste stream (e.g. waste water)?

The results of this review would be used to define a suitable chemical test suite for the treated drill cuttings which might include various metals, plus moisture, suitable TPH tests, and SVOCs[9].

The next step would be to define a suitable sampling plan to collect samples of the waste that is being disposed of (a single sample is not fit for purpose).

Information about designing sampling plans for different waste streams can be found in both the WM3 (Appendix D) and the European (Annex 4) technical guidance.

Figure 2.3 Steps 3 through 5 of a hazardous waste assessment



[9] Semi volatile organic compounds – includes the PAHs

2.3.1.4. Step 4 (Figure 2.3) - Identify if the substances in the waste are hazardous substances or POPs

Once the laboratory test results have been returned, using Appendix B of WM3, Annex 3 of the European technical guidance, or the relevant classification engine in HazWasteOnline™, the classifier can work out whether one or more of the substances in the waste will make the waste hazardous.

The challenge with the results of chemical analysis, especially for mixtures such as soils, is that the laboratories cannot always identify the exact metal compounds present in the soil, and often instead only report individual total cations (e.g. copper) and anions (e.g. sulphates).

This is a different approach to the simpler WAC assessment which just assesses the concentrations of a dozen soluble metals and a few soluble anions against specific threshold concentrations.

The guidance says that if it is not known which form of a metal you have in your waste, a worst case is assumed.

A good classifier can rule out some species of metal compounds by understanding the process that generated the waste, the physical properties of substances and by making sure the laboratory test suite includes tests like speciated chromium (Cr III and Cr VI), and tests for selected total and soluble cations/anions. For example, if the laboratory reports that the concentration of Cr VI is below the limit of detection, then the classifier can rule out the worst case metal chromates.

The total metal concentration (e.g. total nickel) cannot be used to complete a classification. For example, if it is assumed that the nickel is in the form of nickel sulphate, a conversion to the amount of nickel sulphate is required using molecular weights.

2.3.1.5. Step 5 (Figure 2.3) - Assess the hazardous properties of the waste

The waste classification assessment can be done by hand, in a spreadsheet or using commercially available software. The classifier should utilise either the new European guidance and/or the guidance published by the MS regulator if it has been updated since the publication of the HP14 amendment (Regulation (EU) 2017/997).

If the classification determines that one or more substance make the waste hazardous for one or more of the hazard properties, or it contains POPs, at or above the concentration limits, then the mirror hazardous LoW code is selected. If none of the substances exceed hazardous thresholds, then the mirror non-hazardous LoW entry is chosen.

Some of the hazards, in particular the physical hazards (flammable, explosive, oxidising) may have to be tested by physical testing if the waste contains significant concentrations of substances with the relevant hazard statements.

2.3.2. Example: soils contaminated by oils

In order to illustrate the process for classifying waste, this section works through an example for soils contaminated by oils.

Soils contaminated by oils and/or other chemicals are a difficult waste to characterise and classify as either hazardous (17 05 03*) or non-hazardous (17 05 04) because of the many variables that have to be assessed including:

- History – the industries that may have contaminated the site in the past may be unknown.
- Spatial variability – soil types vary from site to site and within a site both laterally and with depth.
  - Soil types relate to texture and grain size - variables include grain size (cobbles, gravel, sand, silt, clay etc), water and air content. Note that engineers, geologists and pedologists use different classification systems to characterise the same soils.

In the case of made-ground or fill, the history of the original soil and what may have contaminated it, is lost.

- Article 2 of the rWFD states that the following shall be excluded from the scope of the directive (i.e. is not a waste):
- Land (in situ) including unexcavated contaminated soil and buildings permanently connected with the land;
- Uncontaminated soil and other naturally occurring material excavated in the course of construction activities where it is certain that the material will be used for the purposes of construction in its natural state on the site from which it was excavated.

Article 3 defines a waste as any substance or object which the holder discards or is required to discard. Article 18 places a ban on the mixing of hazardous waste with non-hazardous wastes and that mixing shall also include the dilution of hazardous substances.

Taking these together means that where soils have to be excavated for disposal, the waste producer has to determine:

1. Which areas of the waste soils are non-hazardous and which are hazardous, and
2. Keep the hazardous soils separate from non-hazardous soils (i.e. not mix them in stockpiles and then characterise the stockpile).

2.3.2.1. Soil testing

The process to identify hazardous substances that might be present in a contaminated soil includes:

- Conducting a desktop type study to look at the history of the site and which industries may have contaminated that site and with which chemicals, for example:
  - Contamination might be from spillages, leaks, burning, disposal or
  - From “ingredients” used in the industrial process, spills of the final product, fuels used to drive machinery, ashes from burning fuels or other materials or asbestos fragments generated from poor demolition practices.
- Observations from a site walk over; or
- Results from intrusive site investigations.

The results of this review should then be used to define a suitable chemical test suite. Indicatively, a typical test suite would comprise a suite of a dozen or more metals, including copper, lead, nickel and zinc, the 16 US EPA PAHs, total petroleum hydrocarbon (TPH), pH, moisture, plus any other substance (e.g. asbestos) that might be contaminating the soil. Note should also be taken of the background metal content of the soils.

Another mistake that some classifiers make when testing soils is to include in the test suite additional metals such as sodium, calcium, magnesium, potassium, aluminium, iron and silicon. While it is not wrong to measure these elements, and there may be cases where one or more of them have to be measured, it is critical that the classifier appreciate that these elements are also the major constituents of clay and other soil minerals (e.g. biotite $K(Mg,Fe)_3(AlSi_3)O_{10}(OH)_2$, limestone $CaCO_3$, haematite Fe_2O_3 etc.). If they are measured, then the classifier will need to understand the mineral composition of the soil and use the concentrations of these minerals in the classification.

This is not to say that if there was a spill of for example, aluminium chloride, that the classifier wouldn't measure the concentrations of aluminium to see if they were above the natural background levels of aluminium in that particular soil.

It should also be noted that it is not (commercially) possible to identify and measure every component in a soil and achieve a mass balance – most practitioners do not measure these additional metals (unless related to contamination) and it is accepted that chemical testing rarely resolves more than one percent of the total composition of the soil.

This work should be followed by the design of a suitable sampling plan that identifies areas of hazardous soil and delineates them from the surrounding non-hazardous soils.

2.3.2.2. Unknown Oils

An unknown oil can be defined as:

- a mixture of two or more (known) oils, or
- an unknown oil found to be contaminating a mixture, such as a soil.

Where the classifier has undertaken chemical testing of a soil using a suitable test for the estimation of the total petroleum hydrocarbon (TPH) contamination in the soil rather than analyses for specific oils, they encounter a technical problem since such a measure will by default meet the definition for an unknown oil.

An unknown oil is not a commercial product and therefore does not have a harmonised entry in Annex VI, Table 3 of the CLP or for that matter, a registration under REACH that is awaiting harmonisation. So the classifier has to define their own substance and research a suitable set of hazard statements to use in the classification.

The UK regulator, for the case where the identity of the oil is unknown and cannot be determined, has addressed this problem in WM3 (Environment Agency 2018) by defining the hazard statements for an unknown oil (**Table 2.3**).

Table 2.3 Unknown Oil - Hazard Statements

Substance	Alias	Hazard Statements
TPH (C6-C40) Petroleum Group	Unknown oil	(HP3 flammable) H304 & H373 (HP5 STOT Aspiration Toxicity) H340 (HP11 Mutagenic) H350 (HP7 Carcinogenic) H361 (HP10 Toxic for Reproduction) H411 (HP14 Ecotoxic)

The UK regulator also allows the classifier to assess the unknown oil as non-carcinogenic / non-mutagenic if;

1. Forensic analysis of the chromatogram (not the carbon bands) by the testing laboratory, indicates that there is no diesel or petrol present in the unknown oil;

2. The waste has been suitably sampled; and

3. The concentration of the marker compound benzo[a]pyrene (BaP) is less than 0.01% w/w of the TPH concentration e.g. for 1000 mg/kg TPH, BaP would need to be less than 0.1 mg/kg).

It should be noted that the use of the marker only mitigates the HP7 and HP11 hazard properties; it does not affect any of the other hazard properties.

2.3.2.3. Petroleum Groups

For incomplete entries and for substances and mixtures that are not present in Annex VI, Table 3 of the CLP, the classifier is required to identify the relevant hazard properties for use in a classification.

For the case where there has been a recent spill of an oil, the REACH compliant SDS can be sourced from the supplier and the hazard statements on the SDS can be used to determine whether the oil is at hazardous levels in the soil.

For the case where the type of oil can be identified, but the exact brand or brands of the particular oil is not known (such as adjacent to a farmer's diesel tank where different brands or types of diesel have been spilt over many years), entries for families of oils can be used called Petroleum Groups; "unknown oil" being one of these petroleum groups and the "diesel petroleum group" being another. The current set of petroleum groups used in the commercially available software HazWasteOnline™ are listed in **Table 2.4**.

Table 2.4 also shows a breakdown of the various hazard properties defined by the hazard statements assigned to these petroleum groups. For this set of oils, and assuming there are no other hazardous substances present in the waste, the table effectively identifies the hazard properties that effects-based testing would need to address.

Table 2.4 Hazard Properties for Petroleum Groups defined in HazWasteOnline™

Petroleum Group	Hazard Properties							
	HP3	HP4	HP5	HP6	HP7	HP10	HP11	HP14
Petrol (Gasoline)[wm3]	H224	H315	H304		H350	H361	H340	H411
Diesel [wm3]	H226	H315	H304 H373	H332	H351			H411
Kerosene [hwol]	H226	H315	H304					H411
Heavy/Residual Fuel Oils [wm3]			H373	H332	H350	H361	H340	H411
Crude Oils [wm3]	H225	H319	H304 H373		H350			H411
TPH (C6-C40) [wm3] (Unknown Oil)*	H226		H304 H373		H350*	H361	H340*	H411

wm3 – hazard statements are defined by WM3 (Environment Agency 2018)
hwol - hazard statements are defined by HazWasteOnline™ based on review of SDS from: Total, Petrobras, Petrochem, Shell
* WM3 allows use of the marker BaP to mitigate the carcinogenic and mutagenic hazard properties in an unknown oil

2.3.2.4. Non-oil contaminants, additivity and physical hazards

While the focus of this work is to identify whether EBTs can be used to determine whether oil-contaminated soils are hazardous, any assessment, whether EBT, chemical testing or a combination of the two has to consider:

- All the contaminants in the soil that could be at hazardous levels e.g. metals such as zinc, lead, arsenic, copper;
- The fact that many of the hazard properties are additive (HP4, HP5, HP6, HP8 and HP14), which means that while the individual concentration of a substance, such as an oil, may not be at a hazardous level, the concentration of two or more substances with the same additive hazards, have to be added together and then compared to the relevant threshold – which can easily lead to a threshold being exceeded for a particular hazard property; and
- That some hazard properties can only be assessed by physical testing – such as flash point testing for HP3 flammable, as defined in the testing Regulation No (EC) 440/2008.

2.4. SUMMARY OF WASTE CLASSIFICATION OBLIGATIONS

Classification of wastes is a complex process and while the rWFD is the key piece of legislation to consider, reference to many other pieces of legislation is also required, including:

- The List of Waste;
- The CLP regulation;
- The REACH regulation and
- The Landfill directive.

Guidance is available at EU and to various extents at MS level, but varies over time and between (and within) member states.

Following the waste classification procedures laid out in the legislation and guidance documents, waste is assessed for different hazard properties and ultimately classified as either hazardous or non-hazardous, resulting in different disposal considerations, and associated costs. The current approach for waste classification relies on chemical characterisation of the waste, which can be challenging.

3. IDENTIFICATION OF EFFECTS-BASED TESTS (EBT)

3.1. POTENTIAL FOR USING EBTS UNDER THE WFD

There are two main approaches for assessing the classification of wastes under the WFD. The first is to classify based on the composition of the waste, taking into account bioavailability of constituents, and this has been discussed in detail in Section 2 of this report. The second approach is effects-based testing (referred to as direct testing in the EU guidance), where a sample of the waste is subjected to one or more tests directly assessing the hazard potential of the whole waste. The benefits of direct testing are that the full characterisation of the waste is not required (therefore it can be used with samples where the all constituents cannot be identified / quantified) and that it takes into account any interactions between the constituents within the waste. The use of EBTs can also mean that bioavailability of hazardous constituents is taken into account, but this is only the case if a whole waste sample is tested or sample preparation mimics realistic scenarios. If sample preparation is required prior to testing (e.g. grinding of the solid sample, preparation of a solution for testing) this can impact on bioavailability of the hazardous constituents, and therefore affect the test results and the ultimate classification.

As defined by the rWFD, the assessment of hazardous properties can be undertaken by chemical testing and also the application of EBTs, or a combination of the two. For some hazard properties, the EC guidance references specific test methods that can be used to assess the hazard, including for physico-chemical hazards (explosive properties, oxidising properties and flammability), irritancy and mutagenicity. For other hazard properties the guidance indicates that the use of EBTs may be possible, but does not specify particular tests methods, and instead refers to the Test Methods Regulation and the European Union Reference Laboratory for alternatives to animal testing. Animal testing should not be used for testing of wastes, and therefore this approach relies on suitable *in vitro* methods, or *in vivo* methods with non-vertebrate species, being available and suitable for classification according to ECHA CLP guidance. For hazard properties where suitable EBTs are available, direct test data prevails over an assessment of waste based on the concentrations of hazardous constituents. **Table 3.1** summarises whether EBTs could potentially be used for each hazard property, according to the EC guidance.

This report covers all HPs included in the WFD, whether relevant to petroleum hydrocarbons or not. When assessing classification of a waste soil, the whole composition must be taken into account and this could include contaminants (e.g. metals) coming from other sources, for which different HPs are applicable. If the source of contamination is known (e.g. a spill), it may be possible to narrow down the list of relevant HPs. For example, those HPs that are relevant for an "unknown oil" according to the UK's WM3 guidance (Environment Agency 2018) are HP3, HP5, HP7, HP10, HP11 and HP14 (see **Table 2.3**).

Some of the WFD HPs cover endpoints also assessed by the ADR[10], relating to the carriage of dangerous goods, including ADR Class 6.1 (Toxic substances) and Class 9 (Miscellaneous dangerous substances and articles), which covers environmental hazards. Under the ADR, wastes are classified in the same way as other substances, therefore EBTs that are applicable for classification under the WFD may also be helpful for assessing ADR classifications.

[10] http://www.unece.org/fileadmin/DAM/trans/danger/publi/adr/adr2017/ADR2017E_web.pdf

Table 3.1 Opportunities for effects-based testing for each hazard property

Hazard Property		Direct testing option listed in EC guidance?	Test methods recommended by EC guidance
HP1	Explosive	Yes	A14 Explosive properties
HP2	Oxidising	Yes	A17 Oxidising properties (solids) A21 Oxidising properties (liquids)
HP3	Flammable	Yes	A10 Flammability (solids) A11 Flammability (gases) A12 Flammability (contact with water)
HP4	Irritant	Yes	pH used as initial screen. Combination of acid / alkali reserve and *in vitro* testing recommended. B.46 Reconstructed human epidermis method specified, and further *in vitro* tests may be available from other sources e.g. based on European Union Reference laboratory for alternatives to animal testing
HP5	Specific Target Organ Toxicity (STOT)	Yes	No test methods specified. *In vitro* tests may be available from other sources e.g. based on European Union Reference laboratory for alternatives to animal testing.
HP6	Acute Toxicity	Yes	No test methods specified. *In vitro* tests may be available from other sources e.g. based on European Union Reference laboratory for alternatives to animal testing.
HP7	Carcinogenic	No direct testing for carcinogenicity envisaged for wastes or mixtures.	Mutagenicity tests considered in many cases to be suitable indicators of carcinogenicity.
HP8	Corrosive	Yes	B.40 *In vitro* skin corrosion, transcutaneous electrical resistance test (TER) B.40 *In vitro* skin corrosion Human skin model test
HP9	Infectious	No	No test methods specified.
HP10	Toxic for Reproduction	Potentially, but there are limited *in vitro* testing options	Limited options for assessing reproductive toxicity *in vitro*. *In vitro* tests may be available from other sources e.g. based on European Union Reference laboratory for alternatives to animal testing

Hazard Property		Direct testing option listed in EC guidance?	Test methods recommended by EC guidance
HP11	Mutagenic	Yes	B.10 Mutagenicity - *In vitro* mammalian chromosome aberration test B.13/14 Mutagenicity- - Reverse mutation test using bacteria B.15 Mutagenicity testing and screening for carcinogenicity gene mutation - *Saccharomyces Cerevisiae* B.17 *In vitro* mammalian cell gene mutation test
HP12	Release of acute toxic gas	Yes	No direct test methods available. Tests for emission of flammable gas under CLP can be used.
HP13	Sensitizing	Yes	No test methods specified. *In vitro* tests may be available from other sources e.g. based on European Union Reference laboratory for alternatives to animal testing.
HP14	Ecotoxic	Yes	No test methods specified. The EC guidance does not currently provide specific recommendations regarding the approach for ecotoxicity characterisation of waste using biotests. Member states can decide on a case by case basis whether waste characterisation based on biotests is acceptable and if the interpretation is appropriate, including assessment of bioavailability considerations.
HP15	Capable of yielding a hazardous property	Yes	No test methods specified. Testing should be in accordance with ECHA CLP guidance.

For most hazard properties the guidance allows the potential for using EBTs, but for some hazard properties (e.g. reproductive toxicity, carcinogenicity) the requirement to use non-animal test methods means that limited direct testing options may be available. There is also the potential for a single test to cover more than one hazard property (e.g. mutagenicity and carcinogenicity), although it is not possible that a single test will be able to screen for all hazard properties. For some HPs, such as the assessment of ecotoxic properties, a single EBT is unlikely to be sufficient for classification purposes without consideration of other species / endpoints or the composition of the waste. A combination of EBTs and calculation based on waste characterisation may, however, be used.

Although the EC waste classification guidance states that EBTs may be used for classification, specific guidance on testing approaches is currently limited and therefore waste producers face uncertainties if choosing to follow a direct testing approach, particularly as there may be inconsistencies in the acceptance of approaches between different MS. A review of published literature has therefore been conducted in order to identify test methods that have been used (or could potentially be applied) for the assessment of soils, and which may be relevant for

one of the HPs assessed under the WFD. The literature search process and approach used for assessing potentially relevant EBTs is outlined in Sections 3.2 to 3.4.

3.2. LITERATURE SEARCHES

In order to identify relevant EBTs that could potentially be applied to waste soils, literature searches were conducted using the following databases and covered the last 10 years to identify test methods that have been recently used for the assessment of soils or similar matrices:

- Derwent Innovation (Clarivate Analytics)[11]
  - Derwent Innovation is a bibliographic database covering scientific literature from Web of Science, Current Contents Connect, Conference Proceedings and Inspec. The Web of Science provides access to current and retrospective multidisciplinary information from more than 10,400 of the most prestigious, high impact research journals in the world in the sciences, social sciences and arts and humanities – with coverage back to 1900 (sciences), 1956 (social sciences) and 1975 (arts & humanities).
- Toxline (United States National Library of Medicine)[12]
  - The TOXLINE database is the National Library of Medicine's (NLM) bibliographic database for toxicology, a varied science encompassing many disciplines. TOXLINE records provide bibliographic information covering the biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals. It contains over 4 million bibliographic citations, most with abstracts and/or indexing terms and CAS Registry Numbers. TOXLINE covers much of the standard journal literature in toxicology, complemented with references from an assortment of specialized journals and other sources.

A number of search strings were developed in order to identify relevant literature. These were built around the HPs which had been identified as potentially being assessed by EBTs, as well as including one more general search string to identify any other assays that could potentially be relevant. The following search strings were used:

- Toxic for reproduction: (Repro OR Reproductive OR Reproduction OR Pregnant OR Pregnancy OR Fertility OR Developmental) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)
- Mutagenic / carcinogenic: (Hyperplas* OR Metaplas* OR Mutagen OR Mutagenic OR Cytotoxic OR Cytotoxicity OR Genotoxic OR Genotoxicity) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)
- Ecotoxic: (Environment OR Ecotox* OR NOEC OR NOEL OR Algae OR Fish OR Invertebrate OR Crustacean OR Daphnia OR Rotifer OR Microorganism) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)
- Irritant / corrosive / sensitising: (Irritat* OR Sensitis* OR Corrosion OR Corrosive) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)
- General toxicity: (Toxicant OR Toxicology OR Toxicity OR Acute OR Chronic OR Subchronic OR Sublethal OR Mortality) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)
- EBTs: (Effects based tools OR EBT OR Effects based OR Biological effect OR Bioassay) AND (Test OR Method OR Assay OR cell OR in vitro OR biomarker)

[11] https://clarivate.com/products/derwent-innovation/
[12] https://toxnet.nlm.nih.gov/

As these search strings are very general, and therefore returned a large number of results from the literature searches (both relevant and irrelevant), they were refined further using one or more of the additional strings outlined below. These additional search strings narrowed down the search results to those methods that are relevant to a suitable matrix (soil or sediment), used for assessment of waste or for regulatory purposes, or have been used with substances that would potentially be relevant for Concawe. These additional search strings were included as required to result in a manageable number of results for further screening and assessment (< 1000 results per string).

- Matrix: Soil OR Sediment
- Waste / regulatory: Effluent OR Regulatory OR Waste
- Substances: Oil OR Gas OR PAH OR Polyaromatic hydrocarbon OR Polycyclic aromatic hydrocarbon OR Heavy Hydrocarbons OR Petroleum hydrocarbons OR BTEX OR Heavy fractions

The literature searches resulted in 3890 combined results, which were then screened for further assessment.

3.3. SCREENING OF LITERATURE

The results obtained from the literature searches were screened initially based on title and abstract, in order to identify papers assessing potentially relevant EBTs. Each of the papers considered potentially relevant were then subjected to a more detailed screen and the following information extracted based on the abstract:

- Name or description of EBTs covered in the paper;
- WFD-relevant HP that could potentially be assessed using the EBT;
- Matrix tested in the study (e.g. soil, sediment, freshwater, sewage sludge);
- Type of test (e.g. whole organism, cell-based / *in vitro*); and
- Duration of test, if stated.

At this stage, we used a broad inclusion approach in order to prepare a longlist of EBTs. Whole organism studies with vertebrates were excluded as animal testing should not be conducted for the purposes of waste classification, according to the WFD guidance. However, all other potentially relevant test methods were screened in, including novel non-standard methods, methods that have not (yet) been used with soil samples or for regulatory / classification purposes and whole organism (non-vertebrate) studies. The methods were not critically assessed at this point. Due to the limited information sometimes available in the literature abstracts, and as some EBTs potentially could be used to assess more than one HP, EBTs were assigned to the longlist based on what was considered to be the most relevant HP, for further assessment during the evaluation and shortlisting process.

As a result of screening the titles and abstracts of the studies identified in the literature searches, around 700 papers were identified as mentioning a potentially relevant EBT. However, the majority of these papers were not relevant for further consideration of the test methods for the purposes of this study (e.g. test method mentioned but limited detail available regarding methodology, not relevant for assessment of soils or wastes). As we only wished to obtain the most relevant literature papers for detailed review and evaluation of EBTs, each of the potentially relevant papers were further screened according to the following criteria:

- The paper appears to include a detailed assessment of the test methodology or its application; and
- The study applied the test method for assessment of soil or sediment samples, wastes or complex mixtures.

This resulted in 76 papers which were obtained for further detailed assessment of the EBTs in order to develop a shortlist of potential EBTs that could be used for the classification of waste soils. Some additional references identified from within the key references were also reviewed, where relevant.

3.4. EVALUATION CRITERIA FOR ASSESSING EBTS

A wide range of EBTs are highlighted in the published literature, but many of these are novel approaches with niche applications, non-validated methods or would not be appropriate for use for regulatory purposes. The identified test methods therefore needed to be critically assessed against a range of evaluation criteria in order to determine which methods may have potential for further use under the WFD. The selected evaluation criteria focus on four main aspects: method development maturity, validation maturity, pedigree and, applicability and availability. These criteria are described in more detail in Sections 3.4.1 to 3.4.4, and summarised in Section 3.4.5.

3.4.1. Method development maturity

Numerous novel test methods are trialled in the literature and claims are often made with regard to the applications of these methods. However, as this project is looking to assess classification of hazards, the requirement is for standardised or potentially standardised test methods are required that can be used routinely, and are reproducible. The endpoints assessed by a method need to be relevant for hazard classification, or have a high chance of being acceptable for classification purposes in the near future. For example, many methods reported in literature studies assess biomarker endpoints which may be useful for assessing a specific effect of a chemical or mixture, but are not yet generally acceptable for classification purposes. For classification, there needs to be a clear link demonstrated between the effect observed in the study and a classifiable endpoint, and any methods selected need to result in standardised endpoints that can be compared to classification criteria.

Another criterion that needs to be considered when assessing the suitability of test methods is the form of the substance that can be tested. Sample preparation may affect the bioavailability of any hazardous constituents, therefore if a method does not allow for direct testing of a whole soil sample without manipulation or processing, the sample preparation needs to be carefully considered. Some methods require testing of a solution and in such cases the preparation of any solution needs to be well thought out to ensure that the bioavailability of hazardous constituents is realistic compared to what would be expected under normal conditions. Some test methods may therefore need to be adapted to account for this.

3.4.2. Validation maturity

In order for waste producers and regulators to have confidence in the use of EBTs, any selected test method should be well validated to ensure that it is suitable for use, and that results are reproducible. The NORMAN network validation guidance (2008) is used to assign a level of validation maturity to a method, either 1 (research laboratory), 2 (expert laboratory) or 3 (routine laboratory).

It also needs to be considered if there are precedents for the method being used for hazard assessment, for example under other regulations, and whether the method has previously been used successfully for similar types of samples. The current guidance on EBTs under the WFD is limited, but approaches are more likely to be accepted by regulators if they have been used previously for regulatory assessment of similar substances or mixtures.

3.4.3. Pedigree and applicability

Any method used needs to be appropriate for the particular samples that will be assessed for waste classification; soil samples containing multiple contaminants. Some methods have been validated for single substances but may not be appropriate for complex mixtures, or may not be suitable for use with soil samples without modification. To assess this criterion the available literature was reviewed to assess if the method has been applied previously to environmental samples including soils or sediments, or waste samples.

For the classification of wastes, multiple samples may need to be tested and any direct testing approach needs to be efficient and not significantly extend the time required beyond that taken with a waste characterisation approach. Methods that enable screening of multiple samples should therefore be prioritised, as long as they are also able to meet the requirements of WFD classification. Information on the classification criteria that should be used for wastes is limited, with suitable thresholds not defined in the EC guidance. However, an assessment as to whether a method is likely to meet the WFD classification requirements will be made based on requirements for classification under other associated regulations (e.g. CLP).

3.4.4. Availability

Any testing approaches that are considered need to be practical to implement, and not place an unnecessary burden on waste producers compared to the waste characterisation process. The evaluation of test methods therefore needs to consider not only the scientific and regulatory robustness of any methods, but also their potential for widespread application by waste producers. When assessing wastes, any testing approaches need to be possible to complete in a reasonable timeframe and not incur excessive costs. This is particularly important for wastes that are not homogenous where multiple samples may need to be tested for classification purposes. The test methods must therefore be commercially available at contract research organisations (CROs), or there must be a prospect of them becoming available in the near future. Ideally, the test methods should be offered by multiple laboratories to ensure sufficient capacity when testing is required and a choice of options for conducting the testing. Alternatively, test kits should be available that will allow straightforward screening of multiple samples in a time and cost-effective manner. Although a detailed assessment of the costs of test methods (e.g. obtaining quotes for tests) is not within the scope of this project, excessive costs would be prohibitive when implementing a testing programme and therefore an assessment of the ballpark costs has been used to evaluate the methods.

3.4.5. Summary of evaluation criteria

Based on the considerations discussed in Sections 3.4.1 to 3.4.4, the following evaluation criteria were selected for assessment of EBTs:

1. Method development maturity
   - Is a standardised method available, or is the method adequately described and amenable to standardisation?
   - Are the endpoints / results reported relevant for hazard classification of wastes?
   - Is sample preparation required, and could this impact on the relevance of the results for waste classification?
2. Validation maturity
   - Is the method validated to the level of routine use?
3. Pedigree and applicability
   - Has the method previously been applied to environmental samples and complex mixtures?
   - Has the method been applied to soil / sediment samples? Can soil / sediment samples be tested directly, or is leachate tested?
   - Is the method suitable for quick screening of multiple samples?
   - Does the method meet the requirements for classification under the WFD?
4. Availability
   - Is the method commercially available at CROs, or are test kits available?
   - Is there an indication of the costs of the methods, and are these likely to be prohibitive for use in waste classification?

The potential EBTs for each HP are assessed against these criteria and discussed in relation to their potential for use for waste classification in Section 4.

4. ASSESSMENT OF EBTS

4.1. SAMPLE PREPARATION

Where EBTs are used to assess any HP, careful consideration of any sample preparation is required. For *in vitro* mammalian toxicology testing, the majority of studies require a solution or suspension to be tested rather than directly testing a solid sample. There is an exception for some irritancy testing, where direct testing of solids can be conducted, but in these cases physical effects can influence the results in addition to the chemical composition of the sample. The preparation of any test solutions must be carefully considered so that the solution is representative of the waste as handled and does not over or underestimate toxicity.

For the HP 14 (ecotoxic), both EBTs using terrestrial organisms and indirect testing of aquatic organisms can be considered (discussed in more detail in Section 4.2.7). Preparation methods for waste samples for ecotoxicity testing are discussed in detail in EN 14735:2005 (Characterisation of waste – Preparation of waste samples for ecotoxicity tests). This standard means that waste samples should be prepared for testing – either directly or indirectly – in a consistent manner. Prior to testing (either terrestrial testing or testing of water extracts), a sample should have a ≥95% (mass) particle size of <4mm. The sample should therefore be sieved or, if necessary, crushed to achieve this but should not be finely ground and no additional sample treatment should be conducted. Samples should only be dried if moisture content does not allow sieving or crushing, and drying temperatures should not exceed 40°C. For tests with aquatic organisms, water extracts should be prepared with a liquid/solid ratio of 10, according to EN12457-2:2202 (Characterisation of waste. Leaching. Compliance test for leaching of granular waste materials and sludges. 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)). The standard recommends no pH adjustment of the water extracts, however if toxicity is observed at concentrations where pH would lead to mortality, the test can be repeated with pH adjustment. Following preparation of the water extracts, these should be thoroughly mixed with dilution medium and expressed as percentages (volume of water extract per total volume).

During assessment of the EBTs, where methods were considered potentially useful for classification purposes, an indication of whether waste soils could be tested directly or whether indirect testing of soil extracts or leachates should be conducted is provided.

4.2. EBTS IDENTIFIED FOR WFD CLASSIFICATION

For each of the WFD HPs, the EBTs that could potentially be used in a direct testing approach were assessed based on information from the published literature and test guidelines according the criteria outlined in Section 3.5.4. Any methods meeting the evaluation criteria were shortlisted. The assessment of each of the EBTs against the evaluation criteria is outlined in Sections 4.2.1 to 4.2.7. Where it was logical to do so, HPs have been discussed together, for example for mutagenic and carcinogenic HPs where some tests give an indication of both. No potential EBTs were identified for the assessment of HP 9 infectious, HP 10 toxic for reproduction, HP 12 Release of acute toxic gas or HP 15 capable of yielding a hazardous property; therefore these HPs are not discussed further in this section.

4.2.1. HP 1 Explosive, HP 2 Oxidising and HP 3 Flammable

The composition of a waste can indicate if it is likely to be classified for physico-chemical endpoints such as explosive, oxidising or flammable. However, direct testing of the waste should be conducted if the composition indicates that one of these classifications might be relevant. Direct testing is a standard approach for classifying substances and mixtures for physico-chemical endpoints and guideline methods are recommended in the WFD guidance, for solids, liquids and gases. The recommended methods for solids are A.14 for explosive properties, A.17 for oxidising properties and A.10 for flammability. As the methods in the WFD guidance are standard methods used for classification purposes, these are recommended if testing is required for assessment of these HPs and physico-chemical HPs are not assessed further.

4.2.2. HP 4 Irritant, HP 8 Corrosive

Under the WFD, irritant and corrosive properties are assessed by HP 4 (irritant) and HP 8 (corrosive). However, the two HPs are linked with HP 4 assigned based on the concentration limits of substances within the waste that are assigned skin corrosion 1A (H314), eye damage 1 (H318), skin irritation 2 (H315) and eye irritation 2 (H319), whilst HP 8 is assigned based on the concentrations of substances with skin corrosion 1A, 1B or 1C classifications (H314). Under the WFD, corrosion can be assessed based on the pH of the waste, in combination with the acid / alkali reserve test and this should be carried out before *in vitro* testing is conducted. If *in vitro* testing is then conducted, consideration of which test method to use should be based on existing knowledge of the composition of the waste, and any indication this may provide as to the likely classification of the waste. Some of the test methods are not able to differentiate between all of the hazard categories and consequently there is the potential that more than one test may need to be conducted.

In recent years a significant number of *in vitro* test methods have been validated for the assessment of corrosive and irritant properties, both for skin and eye. These provide an ethical alternative to animal testing (which cannot be conducted under the WFD) and are generally quick and straightforward to conduct. *In vitro* testing for these endpoints is now included in the standard testing requirements under regulations such as REACH and the Biocidal Products Regulation; *in vivo* testing for skin and eye irritation can no longer be conducted for REACH purposes. Due to the routine application of these *in vitro* tests under other regulations, tests following standard guidelines (OECD or EC methods) are available at many contract research organisations (CROs). In addition to the standard guideline methods, other test methods have been trialled with a range of different substances and mixtures, and methods and results reported in the published literature. Some of these tests may be useful for providing an indication of irritancy potential, however as test results need to be applied for classification purposes under the WFD, well validated methods for which standard test guidelines are available have been prioritised.

Some of the test methods require solutions / suspensions to be tested, whereas other methods recommend testing of solid samples directly, ground to a fine powder. In the case of waste soils, we would recommend testing soil extracts rather than testing the soil samples directly to avoid the possibility of any physical effects contributing to the study results. However, consideration of the sample material would be required before determining the sample preparation method as the presence of material such as limestone fragments in the matrix could change the pH during sample preparation and therefore influence the results of any testing. **Table 4.1** evaluates some of the *in vitro* methods that are available for the assessment of irritant and corrosive properties, and recommends shortlisting the validated methods with standard test guidelines that are regularly used for classification purposes under other regulations such as REACH.

Table 4.1 Evaluation of EBTs for the assessment of HP 4 Irritant and HP 8 Corrosive

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Short-time exposure *in vitro* test method	Eye irritation	Standard method available (OECD 491). The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes.
Bovine corneal opacity and permeability method (BCOP)	Eye irritation	Standard method available (OECD 437) The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Hen's egg test chorioallantoic membrane (HET-CAM)	Eye irritation. Can also provide a useful indication of irritancy to tissues other than the eye.	No guideline method available, but well-described test protocols. Results from the method provide an indication of irritancy potential, but other methods are more routinely used for classification purposes. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 2: Expert laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method could be used for classification purposes under the WFD, but other standard guideline methods would generally be used for classification purposes.	Some availability at CROs.	No. Could be suitable for use for classification purposes, but other standard guideline methods are available for the assessment of irritation.
EpiOcular assay (EO)	Eye irritation	Standard method available (OECD 492) The results from this method are suitable for use for classification purposes. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for direct testing of soils or for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for direct testing of soils and relevant for classification purposes.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
SkinEthic™ Human Corneal Epithelium Eye Irritation Test method (SkinEthic HCE EIT)	Eye irritation	Standard method available (OECD 492) The results from this method are suitable for use for classification purposes. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for direct testing of soils or for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for direct testing of soils and relevant for classification purposes.
Isolated chicken eye (ICE)	Eye irritation	Standard method available (OECD 438; B.48) Results from this method identify substances that should be classified for serious eye damage or not classified, but further testing would be required for substances not falling within these categories. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 3: Routine laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD, for identifying eye damage classification only.	Some availability at CROs.	No. Standard test method, but less suitable for classification purposes compared to other *in vitro* test methods.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Porcine Corneal Ocular Reversibility Assay (PorCORA)	Eye irritation	No guideline method available, but well-described methods. Results from the method provide an indication of irritancy potential, but other methods are more routinely used for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD, but other standard guideline methods would generally be used for classification purposes.	Limited availability at CROs.	No. Could be suitable for use for classification purposes, but other standard guideline methods are available for the assessment of irritation.
Ex Vivo Eye Irritation Test (EVEIT)	Eye irritation	No guideline method available, but well-described methods. Results from the method provide an indication of irritancy potential, but other methods are more routinely used for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD, but other standard guideline methods would generally be used for classification purposes.	Limited availability at CROs.	No. Could be suitable for use for classification purposes, but other standard guideline methods are available for the assessment of irritation.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Chorioallantoic Membrane-Trypan Blue Staining (CAM-TBS) assay	Eye irritation. Can also provide a useful indication of irritancy to tissues other than eye.	No guideline method available, but well-described test methods. Results from the method provide an indication of irritancy potential, but other methods are more routinely used for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD, but other standard guideline methods would generally be used for classification purposes.	Limited availability at CROs.	No. Could be suitable for use for classification purposes, but other standard guideline methods are available for the assessment of irritation.
Fluorescein Leakage Test (FLT)	Eye irritation	Standard method available (OECD 460) The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Reconstructed human epidermis - skin corrosion (RhE method)	Skin corrosion	Standard method available (OECD 431; B.40) The results from this method are suitable for use for classification purposes. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 3: Routine laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes. Mentioned in the WFD guidance for assessment of HP 8.
Reconstructed human epidermis – skin irritation (RhE method)	Skin irritation	Standard method available (OECD 439; B.46) The results from this method are suitable for use for classification purposes. Testing can be conducted directly on solid material (ground to powder); potential for physical effects due to test item form.	Level 3: Routine laboratory	Suitable for testing mixtures. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: < €5000	Yes. Routinely available, suitable for direct testing of soils and relevant for classification purposes. Mentioned in the WFD guidance for assessment of HP 4.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

4.2.3. HP 5 Specific target organ toxicity (STOT)

The assessment of specific target organ toxicity (STOT) currently relies on *in vivo* test methods and therefore is not suitable for use under the WFD. *In vitro* cell-based assays, mainly assessing cytotoxicity with various cell types, can provide an indication of the toxicity of a sample (**Table 4.2**). However, these cell-based assays are not validated or routinely used for classification purposes and any such approach would be likely to require assessment of multiple cell types. Therefore, the assessment of this HP using *in vitro* test methods, while potentially possible in the future, is not currently considered to be pragmatic. In order to pursue an *in vitro* testing approach for this HP considerable time and effort would need to be invested in identifying relevant cell types to test and developing methods for conducting the assays. Use of such tests for classification purposes would also be a major shift from the approach used for other regulations where classification is based on *in vivo* test results or, for mixtures, a calculation approach based on the classification and concentrations of constituents (as is used under the WFD). It is therefore recommended that the standard calculation approach is used for the assessment of this HP, as the use of EBTs is not currently possible.

Table 4.2 Evaluation of EBTs for the assessment of HP 5 STOT

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
In vitro assays with HepG2 cells	Cytotoxicity	Standardised method not available. Results can be used to give an indication of toxicity based on one cell line, but could not be used on their own for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 1: Research laboratory	Method has previously been applied to environmental and waste samples e.g. soil extractable organic matters (EOMs) from contaminated soils (Baderna et al. 2013, Baderna et al. 2014) and bottom ash extracts (Rong et al. 2015). *In vitro* method more suitable for quick screening of samples compared to *in vivo* methods. Method provides an indication of potential toxicity based on one cell line but results cannot be used alone for classification purposes. Baderna et al. (2013, 2014) applied the method alongside chemical characterisation.	Method not routinely available at CROs. Costs likely to be extensive, as testing with one cell line would not be sufficient for classification purposes. Significant research would be required to identify and validate cell lines that could be used and to get these accepted for regulatory assessment.	No. *In vitro* assay could be useful as an indication of toxicity, but could not currently be used for classification purposes.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
In vitro assays with MRC-5 lung fibroblast cells	Cytotoxicity	Standardised method not available. Results can be used to give an indication of toxicity based on one cell line, but could not be used on their own for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 1: Research laboratory	Method has previously been applied to waste samples e.g. bottom ash extracts (Rong et al. 2015). *In vitro* method more suitable for quick screening of samples compared to *in vivo* methods. Method provides an indication of potential toxicity based on one cell line but results cannot be used alone for classification purposes.	Method not routinely available at CROs. Costs likely to be extensive, as testing with one cell line would not be sufficient for classification purposes. Significant research would be required to identify and validate cell lines that could be used and to get these accepted for regulatory assessment.	No. *In vitro* assay could be useful as an indication of toxicity, but could not currently be used for classification purposes on its own.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

4.2.4. HP 6 Acute toxicity

The standard test methods for assessing acute toxicity are *in vivo* studies with either oral, dermal or inhalation routes of exposure (e.g. OECD 420, OECD 423, OECD 402, OECD 403). However, animal testing cannot be conducted for WFD classification purposes and there are limited *in vitro* methods available for assessing this endpoint. Cytotoxicity of different cell lines can provide an indication of potential toxicity. For example, Halwachs et al. (2013) assessed cytotoxicity to HPCT-1E3 cells using 57 substances, and compared the results to LD50 values from oral *in vivo* studies as well as to results from HepG2 cell assays in order to determine if the *in vitro* assay could be used as an indicator of *in vivo* acute toxicity. Although such cell-based assays could be used as indicators of potential toxicity, they cannot currently be used as replacements for *in vivo* toxicity studies for assessing this HP as there is insufficient evidence to demonstrate that these assays provide a reliable indication of *in vivo* toxicity. Therefore, there are currently no *in vitro* options available for using EBTs to assess acute toxicity, and the calculation method based on waste composition will need to continue to be applied for this HP.

4.2.5. HP 7 Carcinogenic, HP 11 Mutagenic

There are a number of potential *in vitro* methods available for assessing HP 11, mutagenic. Test methods for direct assessment of HP 7 carcinogenic are *in vivo*, and therefore cannot be conducted under the WFD, but assessment of mutagenic potential can give an indication of potential genotoxic carcinogenicity. Potential test methods for assessing HP 11 are listed in **Table 4.3**.

The Ames test is one of the most widely used *in vitro* assays for assessing mutagenicity. It is validated, routinely used for regulatory assessment and has been used with environmental samples including soil extracts. The assay uses strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations and can be used to indicate whether a mutagenicity classification is relevant. However, the Ames test should not be used alone in order to conclude on classification and it is recommended that a second *in vitro* study, using mammalian cell lines (micronucleus assay) is also conducted if EBTs are used to assess this HP. The micronucleus assay detects chromosome damage and both *in vivo* and *in vitro* versions of the test can be conducted. Only the *in vitro* version would be relevant for WFD classification, and this can be conducted in either rodent or human cell lines. Taken together, the results from an Ames test and an *in vitro* micronucleus assay could be used for classification assessment of HP 11, and give an indication of carcinogenicity potential for HP 7. Conducting the standard calculation approach in addition to testing would help to strengthen the conclusions of the direct testing approach.

The Comet assay is also a widely-used assay and an *in vitro* version has been applied with different kinds of environmental samples, including soil extracts. The assay assesses DNA strand breaks and the *in vivo* version is used for regulatory purposes. However, the *in vitro* version of the Comet assay is not as widely available and although it can be conducted at CROs with a limited number of cell lines it is not considered to be as relevant for regulatory use as the *in vivo* version.

For regulatory purposes, such as assessment of industrial chemicals under REACH, *in vitro* test methods including the chromosomal aberration test, mouse lymphoma assay and *in vitro* gene mutation in mammalian cells assay can be conducted. These methods all have standard guidelines and can be used to assess classification, and are mentioned in the WFD guidance for assessment of this HP. However, during the

literature searches no information was identified regarding their use with soil or waste samples, and the methods are expensive compared to other *in vitro* tests. They have not therefore been included in the shortlist as they are unlikely to be suitable for screening of multiple samples.

Other *in vitro* test methods are also available, such as the umu-test and SOS chromotest. These tests offer advantages as they are quick screening methods and have been developed to assess a high volume of samples in a short amount of time. The methods have been widely applied to environmental samples and the umu-test has an ISO standard available for its use with water and wastewater samples (ISO 13829). However, in terms of regulatory application for human health classification purposes, the Ames test and *in vitro* micronucleus assay are more widely used and accepted.

A number of plant-based genotoxicity assays are also available, and although these assays can be used for monitoring environmental contaminants they are considered to be less relevant for assessment of HP 11 than the standard bacterial and cell-based assays that are used regularly for human health risk assessment. These assays have therefore not been shortlisted for further assessment.

Although some of these assays are less relevant for the assessment of HP 11, Pandard and Römbke (2013) discuss the assessment of genotoxicity as part of the assessment of HP 14 ecotoxic, and the umu-test is included as one of the tests in a test battery for assessment of HP 14 by Römbke (2018). However, following the assessment made by Römbke (2018), where 24 different waste types were tested, it was recommended that the umu-test not be included in the test battery for HP 14 as none of the samples tested indicated genotoxicity on the basis of this test.

Table 4.3 Evaluation of EBTs for the assessment of HP 11 mutagenic

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Ames test	DNA damage	Standard methods available (OECD 471, EPA OPPTS 870.5265; B.10) Routinely used for classification purposes, but may not be sufficient on its own for concluding on classification. Soil extracts / leachates would be required for testing. Ansari and Malik (2009), Alam et al. (2009) and Anjum and Malik (2012) assessed the impact of different extraction solvents for preparing soil extracts from contaminated soils prior to testing.	Level 3: Routine laboratory	Method used extensively with environmental samples including soil extracts and leachates, sewage sludge extracts and petroleum contaminated wastewaters (e.g. Lah et al. 2008, Man et al. 2013, Park et al. 2008, Courty et al. 2008, Gajski et al. 2011, Steliga 2011, Steliga et al. 2015). *In vitro* assay with relatively quick testing times. Micro-method adaptation also available. Method routinely used for classification purposes; test results can be used for WFD classification but further tests could be required in addition.	Method routinely used at CROs. Cost range: €5 – 10,000	Yes. Routinely used for classification purposes, widely available and previously used with soil extracts. Mentioned in the WFD guidance for assessing this HP.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
In vitro micronucleus assay	Presence of micronuclei	Standard methods available (OECD 487; B.17) Routinely used for classification purposes, but may not be sufficient on its own for concluding on classification. Soil extracts / leachates would be required for testing.	Level 3: Routine laboratory	Method used previously with environmental samples including sediment extracts and landfill leachates (Pinto et al. 2014, Baderna et al. 2019). *In vitro* test, but more time consuming and expensive than other methods. Method routinely used for classification purposes; test results can be used for WFD classification but further tests could be required in addition.	Method routinely used at CROs. Cost range: €15 – 20,000	Yes. Routinely used for classification purposes, widely available and previously used with environmental samples.
Chromosomal aberration test	Chromosome damage	Standard methods available ((OECD 473, B.10). Used for classification purposes, in combination with other tests. Soil extracts / leachates would be required for testing.	Level 3: Routine laboratory	Limited information identified on use with environmental or waste samples or wastes, though mentioned as one of the potential methods to assess this HP in the WFD guidance. *In vitro* test, but more time consuming and expensive than other methods. Method routinely used for classification purposes; test results can be used for WFD classification but results could not be used on their own.	Method routinely used at CROs. Cost range: €15 – 25,000	No. Standard method available and suitable for use for classification purposes in combination with other tests. However, limited information identified on its use with environmental samples and costs are high compared to other *in vitro* methods.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Mouse lymphoma assay	Gene mutations	Standard methods available (OECD 490) Used for classification purposes, in combination with other tests. Soil extracts / leachates would be required for testing.	Level 3: Routine laboratory	No information identified on use with environmental or waste samples or wastes. *In vitro* test, but more time consuming and expensive than other methods. Method routinely used for classification purposes; test results can be used for WFD classification but results could not be used on their own.	Method routinely used at CROs. Cost range: €15 – 25,000	No. Standard method available and suitable for use for classification purposes in combination with other tests. However, limited information identified on its use with environmental samples and costs are high compared to other *in vitro* methods.
In vitro mammalian cell gene mutation test	Gene mutations	Standard methods available (OECD 476; B.17). Used for classification purposes, in combination with other tests. Soil extracts / leachates would be required for testing.	Level 3: Routine laboratory	Limited information identified on use with environmental or waste samples or wastes, though mentioned as one of the potential methods to assess this HP in the WFD guidance. *In vitro* test, but more time consuming and expensive than other methods. Method routinely used for classification purposes; test results can be used for WFD classification but results could not be used on their own.	Method routinely used at CROs. Cost range: €15 – 25,000	No. Standard method available and suitable for use for classification purposes in combination with other tests. However, limited information identified on its use with environmental samples and costs are high compared to other *in vitro* methods.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Comet assay	DNA strand breaks	Standard guideline method is for *in vivo* assay. *In vitro* test can also be conducted, but limited cell lines available for use with this method at commercial laboratories. Results could be applied for classification purposes. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory (for *in vitro* version)	Method used previously with environmental samples including soil extracts and leachates, landfill leachates, sediment extracts and sewage sludge extracts (e.g. Lah et al. 2008, Swati et al. 2017, Gajski et al. 2011, Pinto et al. 2014). In vitro assay has relatively short test time. Results could be used for classification purposes under WFD but other tests would also be likely to be required.	*In vitro* method available at CROs but with limited cell lines available for use in this assay.	No. *In vitro* assay available and previously used with soil extracts, but only limited version of the assay available at CROs and therefore other *in vitro* assays considered more suitable.
Umu-test	DNA damage	Standard method available (ISO 13829) for testing waters and wastewaters. Results could be applied for classification purposes, but other methods would also be required to conclude on classification. Soil extracts / leachates would be required for testing.	Level 3: Routine laboratory	Method previously used with soil extracts (Brinkmann and Eisentraegar 2008). Quick screening method suitable for high throughput of samples. The method could be used for assessment of genotoxicity and was included in a ring test of biotests for waste classification purposes (Moser et al. 2011), which related to the assessment of HP 14. However, for assessment of HP 11 mutagenic the Ames test.	Test kits available.	No Suitable for high throughput screening of multiple samples and test kits available, but not as widely used for regulatory purposes as the Ames test.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
SOS chromotest	DNA damage	No standard guideline available but methods well described. Results could be applied for classification purposes, but other methods would also be required to conclude on classification. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory	Method previously used with sediment extracts (Hilscherova et al. 2010). Quick screening method suitable for high throughput of samples. The method can be used to assess genotoxicity and could be used for classification, but is unlikely to be sufficient on its own.	Test kits available.	No. Suitable for high throughput screening of multiple samples and test kits available but not as widely used for regulatory purposes as the Ames test.
yH2AX assay	DNA damage	No standard guideline available but methods described. Results could be applied for classification purposes, but other methods are also likely to be required. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory	Limited information available on use with environmental samples. Quick screening method suitable for high throughput of samples. The method can be used to assess genotoxicity and could be used for classification, but is unlikely to be sufficient on its own.	Not routinely available at CROs.	No. Suitable for high throughput screening of multiple samples but not as widely used for regulatory purposes as other assays.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Tradescantia micronucleus assay	Presence of micronuclei	Standard guideline not available, but assay used in a number of studies to assess the genotoxic potential of environmental pollutants, and methods well described. Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory	Method used to assess soil leachates and spent pot liner and soil leachates (Lah et al. 2008, Andrade-Vieira et al. 2011). Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays.	Not routinely available at CROs.	No. Not as relevant to assessment of HP 11 as other assays.
Tradescantia stamen hair mutation assay	Mutations	Standard guideline not available, but assay used in a number of studies to assess the genotoxic potential of environmental pollutants, and methods well described. Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory	Method used to assess contaminated spent pot liner and soil leachates (Andrade-Vieira et al. 2011). Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays.	Not routinely available at CROs.	No. Not as relevant to assessment of HP 11 as other assays.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Alium cepa micronucleus test	Presence of micronuclei	Standard guideline not available, but assay used in a number of studies to assess the genotoxic potential of environmental pollutants, and methods well described. Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays. Soil extracts / leachates would be required for testing.	Level 2: Expert laboratory	Method used to assess spent pot liner and soil leachates (Andrade-Vieira et al. 2012, Palmieri et al. 2016). Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays.	Not routinely available at CROs.	No. Not as relevant to assessment of HP 11 as other assays.
Vicia faba micronucleus test	Presence of micronuclei	Standard method available (ISO 29200). Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays. Soils can be tested directly, or leachates can be tested.	Level 3: Routine laboratory	Method used with reference and contaminated soils, and soil leachates (Cotelle et al. 2014, Marcato-Romain et al. 2009). Assay gives an indication of genotoxic potential for environmental pollutants, but is not as directly relevant to HP11 compared to other assays. Test times days – weeks therefore not suitable for quick screening of samples.	Not routinely available at CROs.	No. Not as relevant to assessment of HP 11 as other assays.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Somatic mutation and recombination test (SMART) with Drosophila melanogaster	DNA damage	No standardised method available. Results not directly relevant for classification purposes. Soil extracts / leachates would be required for testing.	Level 1: Research laboratory	Method used with sediment extracts (Jacociunas et al. 2010). Assay gives an indication of genotoxic potential for environmental pollutants, but is not directly relevant to HP 11.	Not routinely available at CROs.	No. Not as relevant to assessment of HP 11 as other assays.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

4.2.6. HP 13 Sensitizing

A number of *in vitro* methods are available for assessing skin sensitisation. No suitable *in vitro* methods are available for assessing respiratory sensitisation. The use of *in vitro* methods for assessing skin sensitisation is relatively recent, but is now accepted under other chemicals regulations such as REACH. Some of the currently available *in vitro* methods are summarised in **Table 4.4**. As new methods continue to be developed and validated it is likely that additional opportunities for using EBTs to assess this HP will arise. However, at this point in time the most commonly used *in vitro* methods are the direct peptide reactivity assay (DPRA) (OECD 422C), assays assessing keratinocyte activation (KeratinoSensTM, LuSens^{TM}) (OECD 422D) and assays assessing activation of dendritic cells (human cell line activation test (hClat), U937 cell line activation test (U-Sens^{TM}) and interleukin-8 reporter gene assay (IL-8 Luc assay)). It is therefore recommended that if *in vitro* testing is conducted for WFD classification that these methods are used, as they have been previously accepted for use for classification purposes. As each of the three OECD methods assess a different part of the adverse outcome pathway (AOP) for sensitisation, all three test methods should be conducted in order to conclude no classification for sensitisation. Conducting *in vitro* testing for this HP can therefore be a costly approach in comparison to the standard calculation method used under the WFD. The *in vitro* sensitisation methods require samples to be soluble; samples that are poorly soluble can lead to inconclusive results in the tests and therefore could not be used to rule out classification for this HP. There are also issues with conducting these tests with samples without defined compositions (particularly for the DPRA assay) and therefore it may be that not all of the assays will be applicable to waste samples, and some compositional information on the sample will be required for testing to be conducted. If *in vitro* testing were to be conducted, it is recommended that soil extracts or leachates are tested to assess sensitisation potential and that sample preparation is discussed with the laboratory beforehand to ensure that it is suitable.

Table 4.4 Evaluation of EBTs for the assessment of HP 13 Sensitising

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Direct Peptide Reactivity Assay (DPRA)	Sensitisation	Standard method available (OECD 442C). The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures, but composition needs to be known, potentially applicable for use with soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: €5 - 10,000 Needs to be conducted alongside OECD 442D and E.	Yes. Routinely available, potentially suitable for testing of soil extracts and relevant for classification purposes. However, generally all 3 OECD 422 *in vitro* assays should be conducted for classification purposes and specific discussions with CROs on the applicability of the test methods for waste samples recommended.
Keratinocyte activation (KeratinoSens™; LuSens™)	Sensitisation	Standard method available (OECD 442D). The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: €5 - 10,000 Needs to be conducted alongside OECD 442C and E.	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes. However, generally all 3 OECD 422 *in vitro* assays should be conducted for classification purposes and specific discussions with CROs on the applicability of the test methods for waste samples recommended.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Activation of dendritic cells (human cell line activation test (hClat), U937 cell line activation test (U-Sens™), interleukin-8 reporter gene assay (IL-8 Luc assay))	Sensitisation	Standard method available (OECD 442E); The results from this method are suitable for use for classification purposes. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 3: Routine laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. The method can be used for classification purposes under the WFD.	Routinely available at CROs. Cost range: €5 – 10,000 Needs to be conducted alongside OECD 442C and D.	Yes. Routinely available, suitable for testing of soil extracts and relevant for classification purposes. However, generally all 3 OECD 422 *in vitro* assays should be conducted for classification purposes and specific discussions with CROs on the applicability of the test methods for waste samples recommended.
HaCaT epidermal model	Sensitisation	Standard test guideline not available. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. Limited acceptability for classification purposes compared to other validated methods.	Limited availability at CROs.	No. Recommended to use validated, standard guideline *in vitro* methods for classification purposes (OECD 422C,D,E) as these have been accepted for use for other regulatory purposes.

Test method	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
SENS-IS assay	Sensitisation	Standard test guideline not available. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. Limited acceptability for classification purposes compared to other validated methods.	Limited availability at CROs.	No. Recommended to use validated, standard guideline *in vitro* methods for classification purposes (OECD 422C,D,E) as these have been accepted for use for other regulatory purposes.
Modified myeloid U937 skin sensitization test (mMUSST)	Sensitisation	Standard test guideline not available. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. Limited acceptability for classification purposes compared to other validated methods.	Limited availability at CROs.	No. Recommended to use validated, standard guideline *in vitro* methods for classification purposes (OECD 422C,D,E) as these have been accepted for use for other regulatory purposes.
Genomic Allergen Rapid Detection (GARD) assay	Sensitisation	Standard test guideline not available. Testing on soil extracts / leachate would be required rather than direct testing of soils.	Level 2: Expert laboratory	Suitable for testing mixtures, would be applicable for soil extracts / leachates. *In vitro* method allows relatively quick screening of test samples. Limited use currently for classification purposes compared to other methods.	Some availability at CROs.	No. Although methods such as this may be used for classification purposes in future, standard guideline *in vitro* methods (OECD 422C,D,E) are recommended at this stage as they are accepted for classification purposes under other regulations.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

4.2.7. HP 14 Ecotoxic

There are a range of considerations that need to be taken into account when assessing ecotoxic potential of soils using EBTs, including the type of organism (terrestrial, aquatic), the endpoint assessed and its relevance to classification, and the sensitivity of the test species or endpoint. Classification as ecotoxic aims to protect the environment from disposal of potentially hazardous wastes; adequate classification for this endpoint is not possible by conducting a single test with one organism as the sensitivity of different organisms to contaminants varies. Conducting a test with a single species when evaluating a complex mixture such as a waste may not therefore be protective of other relevant species. For this reason, instead of a shortlist of potentially relevant test methods to be conducted individually, a battery of ecotoxicity tests is proposed for the assessment of HP14, aiming to balance the need to robustly classify the waste soils with the requirement for a pragmatic testing approach. Alternatively, a combination of the current constituent-based approach where classification is determined based on the classification of constituents identified from the chemical analysis with a more limited confirmatory direct testing programme could be considered.

The use of EBTs for assessing the HP 14 was discussed prior to the HP14 amendment in 2017, which aimed to standardise the approach for assessing this HP across the EU, to reduce variation in waste classification between MS. When the amendment was being developed, an assessment of the different approaches for assessing HP14 was carried out by Deloitte and INERIS in March 2015 and discussed at stakeholder meeting in 2016. The briefing document prepared for the meeting reviewed the approaches followed by nine MS (Austria, France, Belgium, Germany, Italy, Finland, Czech Republic, United Kingdom and Spain), including whether direct testing approaches, characterisation approaches or combined approaches were used. Where direct testing approaches were used, the tests considered in the approaches were compared.

Of the MS included in the sample, four recommended calculation approaches (Austria, Belgium, Finland and the UK), three testing approaches (France, Czech republic and Spain) and two used combined approaches (Germany and Italy). Of the countries using testing or combined approaches, the types of tests varied, with Italy, the Czech Republic and Spain recommending only aquatic tests and Germany and France using both aquatic and terrestrial tests as part of a tiered approach (**Table 4.5**). All MS advocating testing approaches recommended acute *Daphnia* tests as part of the test programme, with algal tests (with *Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus*) and tests with the bacteria *Vibrio fischeri* recommended by all but one MS. Where testing was recommended, standardised ISO test methods were recommended in almost all cases. For MS using EBTs, the threshold values used for classification varied even when the same test was conducted (e.g. the threshold used for a *Daphnia magna* acute study was 10% (v/v) in France and 750 mg L^{-1} in Spain). This lack of standardised classification thresholds for use in waste assessment makes the application of EBTs difficult. For those MS where calculation methods were predominantly used, the UK considered that animal testing of solid wastes leads to ethical concerns and can be of little or no scientific value, and Finland did not apply direct testing due to the lack of standardised thresholds.

Table 4.5 Summary of tests recommended by MS for the assessment of HP14, as stated in the 2015 review

Species	Test method	Member State				
		France[1, 2]	Germany[2]	Italy	Czech Republic	Spain
Daphnia magna (acute)	ISO 6341	X	X	X	X	X
Daphnia magna (chronic)	ISO 10706	-	X	-	-	-
Sinapsis alba	Czech guidelines ISO 8692	-	-	-	X	-
Poecillia reticulata		-	-	-	X	-
Vibrio fischeri	ISO 11348-1/2/3	X	X	X	-	X
Pseudokirchneriella subcapitata/ *Desmodesmus subspicatus*	NF EN ISO 8692	X	X	X	X	-
Ceriodaphnia dubia	NF ISO 20665	-	-	-	-	-
Brachionus calyciflorus	NF ISO 20666	-	-	-	-	-
Lemna minor	ISO 20079	-	X	-	-	-
Eisenia fetida (acute)	ISO 11 268-1	-	-	-	-	-
Eisenia fetida (avoidance)	ISO 17512-1	X	X	-	-	-
Eisenia fetida (chronic)	ISO 12 268-1	-	X	-	-	-
Lactuca sativa	ISO 11269	-	-	-	-	-
Avena sativa / *Brassica rapa*	ISO 11269-2	X	X	-	-	-
Arthrobacter globiformis	ISO/DIS 18187	X	X	-	-	-
Folsomia candida (chronic)	ISO 11267	-	X	-	-	-

[1] This strategy is a combination of the initial French strategy and the German strategy. The initial French strategy also included *Ceriodaphnia dubia* (NF ISO 20665) and *Brachionus calyciflorus* (NF ISO 20666) but did not include *Arthrobacter globiformis* (ISO/DIS 18187) and used *Lactuca sativa* instead of *Avena sativa* / *Brassica rapa*

[2] France and Germany follow a tiered approach, aquatic tests are prioritised and terrestrial tests are conducted only when aquatic tests are inconclusive

Although there was discussion regarding the inclusion of direct testing at the 2016 stakeholder meeting, as a large amount of work would have been required to obtain agreement on the tests to be included and the thresholds that should be used for classification, specific recommendations for the use of EBTs were not included in the HP14 amendment in 2017, or in the European technical guidance in 2018.

The type of tests selected for assessment of HP 14 under the WFD depend on a number of factors, including their practical implementation (commercial availability, applicability to soil samples and cost-effectiveness) and their suitability for use for regulatory purposes, specifically for classification under the WFD. There are, as yet, no specific criteria in the WFD for classifying wastes as ecotoxic based on EBTs which provides an added complication when deciding on a direct testing approach, but references to CLP are made in the WFD guidance. A diverse range of EBTs are available for assessing ecotoxic potential; these can be broadly split into whole organism studies (usually assessing endpoints such as mortality, growth or reproduction but often also assessing biomarker endpoints) and *in vitro* cell-based tests, which usually assess a particular response such as endocrine disrupting potential.

In vitro cell-based assays have a number of advantages for assessment of environmental samples, as they are often quicker to perform allowing multiple samples to be screened quickly and cost-effectively. However, although such tests can be useful for indicating the potential for a hazardous effect, such as endocrine disruption, or the presence of a hazardous constituent, such tests are not routinely used for classification purposes under regulatory schemes. Biomarker endpoints (e.g. genetic markers, indicators of oxidative stress etc), whether measured as part of a whole organism study or an *in vitro* test, cannot usually be directly compared to classification criteria. Environmental classification under CLP is based on determining that a substance or mixture would lead to a population-relevant effect and therefore focuses on population-relevant endpoints (e.g. mortality, reproduction, growth). Studies focussing on these endpoints have therefore been prioritised when preparing the shortlist of EBTs and this generally means relying on whole organism assays.

In order to determine which test species are most relevant, consideration of whether aquatic or terrestrial species, or a combination of both, should be tested is required. To classify a substance or mixture under CLP requires comparison of a relevant endpoint (e.g. EC / LC50 or NOEC) for an aquatic species (usually fish, aquatic invertebrate or algae) with classification thresholds. Although different thresholds may be agreed for use under the WFD, the general classification approach would remain the same. As the classification criteria under CLP refer to aquatic organisms, test results for terrestrial organisms cannot be directly compared with the CLP classification criteria. Therefore, for direct comparison of test results with the CLP classification criteria, testing of aquatic organisms exposed to soil leachates would be required. Tests conducted with terrestrial organisms may be more relevant to the soil matrix but would require specific thresholds to be determined for waste classification purposes.

As a result of these conflicting requirements, and to ensure that the ecotoxic potential of a waste is not underestimated, a battery of ecotoxicity tests are likely to be required if EBTs are used to assess waste classification. As multiple tests are likely to be required, a direct testing approach could lead to far higher testing costs compared to the standard approach of classifying based on composition, particularly if chronic studies are conducted. As tests would be conducted specifically for classification purposes, there is a possibility of conducting any tests at a single threshold concentration, as a limit test. This would simplify the testing procedure

and reduce test costs. However, as there are currently no standardised classification thresholds for WFD classification, this approach could risk not being accepted until such thresholds are agreed at EU level. There is also the potential to conduct testing in a tiered manner – if a positive result is received from the first test, the waste would be classified as ecotoxic and subsequent testing would not be required. Some of the test types can be modified to reduce the time required to run the assay (and therefore the cost) and some assays are available as 'toxkits' which can also increase the efficiency when screening multiple samples. These points are considered when evaluating the test methods.

A number of published studies have assessed the use of EBTs for assessment of HP14 classification (e.g. Pandard et al. 2006, Wilke et al. 2008, Stiernström et al. 2011, Moser et al. 2011, Pandard and Römbke 2013, Römbke 2018). In addition, an assessment of whether EBTs can be used for the assessment of dredged sediments disposed at sea has been conducted in the Netherlands (Schipper et al. 2010). Although the legislation for disposal of dredged sediments differs to the WFD, there are similarities in that contaminated sediments disposed at sea are assessed based on chemical analysis, with analysis of a number of known contaminants, against 'action levels', considered to be acceptable concentrations. A number of EBTs were applied with dredged sediments, including two assessing general toxicity – the solid phase Microtox assay and an assessment of mortality for *Corophium volutator*. However, issues with false positives were identified for both assays (0 – 29% false positives for *Corophium volutator* and 6 – 84% false positives for the solid phase Microtox), due to matrix effects which are not likely to be relevant once conditions change following disposal. Confounding factors were found to include ammonium, pH, salinity, oxygen levels and sulphur for the *Corophium volutator* assay and ammonium, pH, oxygen levels, sulphide and grain size for the Microtox assay. If assays were conducted with extracts rather than whole sediment, the matrix effects could potentially be avoided. Although there are many differences between assessment of sediments and soils, such as the nature of the matrix for testing, selection of relevant test organisms and the criteria applied for disposal or re-use, this detailed assessment of the use of EBTs with dredged sediments highlights some of the challenges that need to be addressed when conducting EBTs with environmental samples.

Pandard et al. (2006) conducted a battery consisting of four aquatic tests, conducted with leachates, and two terrestrial tests, to assess 40 wastes, including excavated soil from a contaminated site. The tests conducted were a *Pseudokirchneriella subcapitata* growth inhibition study (NF T 90-375), an acute *Daphnia magna* study (NF EN ISO 6341), a chronic reproduction study with *Ceriodaphnia dubia* (NF T 90-376), a luminescence inhibition study with *Vibrio fischeri* (NF EN ISO 11348-3), an emergence and growth study with *Lactuca sativa* (ISO 11269-2) and an acute earthworm study with *Eisenia fetida* (ISO 11268-1). For the aquatic tests, leachate was prepared according to the 24 hour leaching procedure described in EN 14735 (2004). The study concluded that a reduced set of these tests could be used to assess HP14, including only the tests with *Lactuca sativa*, *Vibrio fischeri* and *Ceriodaphnia dubia*.

Wilke et al (2008) used a battery of standard ecotoxicity tests, combining both aquatic and terrestrial tests, to assess the toxicity of different wastes, including boiler slag, thin sludge, waste petrol and dried sewage sludge. The terrestrial studies conducted were an acute earthworm study (ISO 11268-1), a Collembolan reproduction study with *Folsomia candida* (ISO 11267), a growth test with the higher plant *Brasica rapa* (ISO 11269-2) and a soil respiration study (ISO 17155). Aquatic studies were conducted using *Lemna minor* (ISO 20079) and luminescent bacteria (EN ISO 11348). Study durations ranged from 30 minutes for the bacteria study to

28 days for the collembola study. Based on the results from the tests, the authors recommended that chronic or sub-chronic endpoints (e.g. reproduction) are used for the assessment of waste as these were found to be most sensitive, and that a battery of tests should be used that includes a terrestrial and aquatic primary producer and consumer, as a minimum.

Stiernström et al. (2011) focused on the use of aquatic tests due to these being directly relevant to CLP classification criteria and used a battery of ecotoxicity tests with decomposers, primary producers and primary and secondary consumers (*Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Nitocra spinipes* and *Danio rerio* embryos) to assess waste ash materials. As aquatic tests were used, the method for preparing the leachate was important and the study assessed two methods; a modified version of a recirculation column test, the ER-H method and the batch test as described under EN 14735:2005 (Characterisation of waste, preparation of waste samples for ecotoxicity tests). Toxicity results were generally comparable for both leaching methods. The study also assessed whether there were differences between assessing classification based on the toxicity tests compared to a constituent-based approach. Differences were observed when only hazardous constituents were included in the assessment, but when both hazardous and non-hazardous constituents were used in the calculations results were consistent between toxicity and calculation approaches.

Moser et al (2011) report the results from a ring test organised by the German Federal Environment Agency to assess a battery of ecotoxicity tests for use with different wastes (municipal waste incineration ash, PAH-contaminated soil and waste wood). Prior to testing the PAH-contaminated soil was dried, sieved (<4 mm) and homogenised. The basic test battery conducted included three tests with eluate and three with the solid phase, and consisted of an algal study with either *Desmodesmus subspicatus* or *Pseudokirchneriella subcapitata* (ISO 8692), an acute *Daphnia magna* study (ISO 6341) and a test with *Vibrio fischeri* (ISO 11348-1/2) with eluate, and an acute earthworm study with either *Eisenia fetida* or *Eisenia Andrei* (ISO 11268), and two plant studies with *Avena sativa* and *Brassica rapa* (ISO 11268-2). In the aquatic tests, the different wastes showed different sensitivity to the test species. The PAH-contaminated soil only affected the luminescent bacteria, with a weak response, and did not demonstrate toxicity to algae or *Daphnia*. The difference in toxicity between terrestrial species was more limited, but plants were more sensitive than earthworms for all of the tested wastes.

Pandard and Römbke (2013) assessed the classification of wastes based on both a calculation and direct testing approach. For the direct testing approach, they selected tests based on criteria including coverage of different taxonomic groups and trophic levels (microorganisms, plants, animals), tests that have high practicability and low effort (e.g. short duration), tests with standardised methods and tests where there is sufficient experience with wastewater or contaminated soils to propose trigger values. Based on these criteria, they included the following tests in their test battery: *Vibrio fischeri* luminescence (ISO 11348-3), algal growth inhibition ((ISO 8692), acute *Daphnia magna* (ISO 6341), dehydrogenase activity of *Arthrobacter globiformis* (ISO 11269-2), emergence and early growth of higher plants (ISO 11269-2) and avoidance tests with earthworms (ISO 17512-1). The tests included cover a range of testing times, from 30 minutes for the luminescent bacteria test to 14 days for seedling emergence.

Römbke (2018) tested 24 potentially hazardous wastes, including waste soils, in a project funded by the German Federal Environment Agency (UBA). Direct testing was conducted by diluting the waste with control substrate to different concentrations so that the results could then be compared to threshold values. The

tests included in the study were the umu-test for genotoxicity, an algal growth inhibition study and an acute *Daphnia* study and the terrestrial tests assessed dehydrogenase activity of *Arthrobacter globiformis*, emergence and early growth of *Brassica napus* and avoidance tests with *Eisenia fetida*. The tests were performed as extended limit tests, with three dilutions of the solid waste or eluate. The criteria used for classification for each test are given in **Table 4.6.**

Table 4.6 Classification criteria for the assessment of HP 14 in Römbke (2018)

Test	Classified as ecotoxic if
Umu-test	Induction rate ≥1.5 at dilution of 25%
Algal growth inhibition	Effect >25% at dilution of 25%
Daphnia magna acute	Effect >20% at dilution of 25%
Dehydrogenase activity of *Arthrobacter globiformis*	Effect >30% at dilution of 12.5%
Seedling emergence with *Brassica napus*	Effect >30% at dilution of 12.5%
Avoidance test with *Eisenia fetida*	Effect >80% at dilution of 12.5%

Following the assessment of the waste samples, it was proposed that the umu-test be replaced by the *Vibrio fischeri* luminescent bacteria test, as none of the wastes tested showed any evidence of genotoxicity in this study. It could be that a more limited test battery could be used if some of the test types appear to be more sensitive, but there is currently insufficient data available for different types of waste samples to make that conclusion.

The Belgian agency, OVAM, assessed ten complex waste materials using both chemical characterisation and EBTs (following the test suite proposed by Pandard and Römbke 2013) (OVAM 2018). To classify, they used the lowest ineffective dilution approach also proposed by Pandard and Römbke (2013). Samples were classified when significant effects were seen at specified dilutions (8 times diluted or 4 times diluted). With 8 times diluted samples, two of the six samples classified based on chemical analysis were not ecotoxic, but at 4 times dilution all HP14 classified samples based on chemical analysis were ecotoxic. One sample that was not classified based on chemical analysis was ecotoxic in most of the EBTs at both dilutions, as toxic substances were present that were not identified in the chemical analysis. Overall, a tiered approach is recommended by OVAM, with classification based on chemical criteria first, then using EBTs conducted with eluate, and finally with EBTs conducted using the solid phase. If a sample is not classified following the three tiers of classification, it is not classified as HP14.

Test methods identified as potentially relevant for assessment of HP 14 are assessed in **Table 4.7** (aquatic) and **Table 4.8** (terrestrial). On the basis of the finding of Römbke (2018) regarding the umu-test, tests assessing genotoxicity are not included. Due to the limited applicability of biomarker endpoints generally in regulatory risk assessment, methods assessing only biomarker endpoints have not been included.

Table 4.7 Evaluation of EBTs for the assessment of HP 14 Ecotoxic (aquatic methods)

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Luminescent bacteria (*Vibrio fischeri*)	Luminescence	Standard method available (EN ISO 11348); MicroTox / solid phase MicroTox toxkits available. Potentially relevant for classification. Test method not used for classification under CLP but has been included in test suites proposed for assessing HP 14 in the literature. Soil leachates would be tested.	Level 3: Routine laboratory	The method has been used extensively with different types of environmental samples and wastes including sludge elutriates (Domene et al. 2010, Alvarenga et al. 2016, Ozcan et al. 2013, Roig et al. 2012), sediments and sediment elutriates (Gonzales-Lozano et al. 2010, Hilscherova et al. 2010), soil and contaminated soil leachates (Alvarenga et al. 2016, Foucault et al. 2013, Beesley et al. 2014, Pivato et al. 2016, Rodrigues-Ruiz et al. 2015) and wastes (Pandard et al. 2006, Wilke et al. 2008, Stierntröm et al. 2011, Moser et al. 2011) Suitable for quick screening of samples (30 minutes). The method could be used for classification purposes under the WFD, in combination with other methods.	MicroTox test kits are widely available and used.	Yes. Quick screening method suitable for use with environmental samples but it would need to be used in combination with other methods.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Duckweed (*Lemna minor*)	Growth	Standard method available (ISO 20079) Potentially relevant for classification. Test method was included in test battery for assessment of wastes by Wilke et al. (2008). Soil leachates would be tested.	Level 3: Routine laboratory	The method has been used with waste samples (Wilke et al. 2008). Longer testing time compared to some other tests (7 days). The method could be used for classification purposes under the WFD, in combination with other methods.	Test method available at CROs.	No. Suitable for assessment of waste soil leachates and could be used for classification, but only included in the test battery for waste assessment in one study and longer test time compared to some other aquatic tests.
Algae (e.g. *Pseudokirchneriella subcapitata*)	Growth	Standard methods available (ISO 8692, OECD 201). Relevant for classification. Soil leachates would be tested.	Level 3: Routine laboratory	Method used to assess waste samples (Pandard et al. 2006, Stiernström et al. 2011, Moser et al. 2001, Pandard and Römbke 2013, Römbke 2018) and soil suspensions with flocculants (Wang et al. 2015). Relatively short test time (days). The method could be used for classification purposes under the WFD, in combination with other methods.	Routinely available at CROs.	Yes. Routinely available at CROs and suitable for hazard classification.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Algae (e.g. *Pseudokirchneriella subcapitata*) - AlgaTox	Oxygen production	AlgaTox device is used for measuring oxygen production. Although EC50 values can be determined, the standard endpoint from algal studies (growth) is used for classification rather than oxygen production. Soil leachates would be tested.	Level 1: Research laboratory	Method used with soil extracts (Buckova et al. 2017). Testing more rapid than standard algal test (12 h oxygen production measurements). Endpoint (oxygen production) not generally used for regulatory classification purposes.	AlgaTox device used for oxygen production measurements.	No. Standard algal tests assessing growth more relevant for classification.
Fish (e.g. *Danio rerio*) embryo - acute test	Mortality	Standard methods available (ISO 15088, OECD 236). Relevant for classification. Soil leachates would be tested.	Level 3: Routine laboratory	Method used with for the assessment of waste ash (Stiernström et al. 2011), river sediments (Hafeli et al. 2011), sediment extracts (Kosmehl et al. 2012) and liquid effluents and sludge elutriates from a deactivated uranium mine (Lourenco et al. 2017). Relatively short test time (days). The method could be used for classification purposes under the WFD, in combination with other methods.	Available at CROs.	Yes. Standard guideline available and suitable for use for classification. Not as widely used for classification as acute fish study, but vertebrate testing cannot be conducted under WFD.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Daphnia magna - acute	Immobilisation	Standard methods available (ISO 6341, OECD 202); plus test kits (DaphToxkit). Relevant for classification. Soil leachates would be tested.	Level 3: Routine laboratory	Method used with different environmental and waste samples, including waste samples (Pandard et al. 2006, Moser et al. 2001, Pandard and Römbke 2013, Römbke 2018), soil-sludge extracts (Garcia-Gomez et al. 2014, Alvarenga et al. 2016), soil suspensions with flocculants (Wang et al. 2015), leachates from soils polluted by metals and metalloids (Foucault et al. 2013), biogas plant digestate mixed with artificial soil (Pivato et al. 2016) and petroleum contaminated wastewaters (Steliga et al. 2015). Relatively short test time (days). The method could be used for classification purposes under the WFD, in combination with other methods.	Available at CROs.	Yes. Routinely available at CROs and suitable for hazard classification.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Daphnia magna - avoidance test	Avoidance behaviour	Standard method not available for avoidance studies with *Daphnia magna*. Results from behavioural studies with Daphnia not generally used for classification. Rosa et al. (2008) compared avoidance responses with results from 21d reproduction studies and found similar responses, although avoidance responses may occur at lower concentrations than those affecting reproduction. Soil leachates would be tested.	Level 1: Expert laboratory	Method used for exposure to pulp mill effluents (Rosa et al. 2008). Shorter test time compared to standard acute *Daphnia* study. Results from avoidance tests alone unlikely to be suitable for WFD classification.	Standardised methods not yet available.	No. Short duration and possibility to use with soil leachates, but the approach is not standardised and behavioural responses with *Daphnia magna* are not generally used for classification and regulatory purposes.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Microorganisms (Microbial assay for risk assessment (MARA) / LumiMARA)	Microorganism growth Luminescence	Routinely used and toxkits available. Results could be used for classification. Soil leachates would be tested.	Level 3: Routine laboratory	Method used with soils with residues from biogas production (Stefaniuk et al. 2015) and petroleum contaminated wastewaters (Steliga et al. 2015). Suitable for quick screening of samples. Results could be used as an indicator of toxicity along with other methods.	Routinely available, toxkits available.	No. Suitable for rapid screening of samples and could potentially be used for classification purposes in combination with other methods. However, method not selected in any of the test batteries used to assess HP 14 in the literature therefore other methods are considered more likely to be accepted for classification purposes.
Thamnocephalus platyurus - acute	Mortality	Routinely used and toxkits available (ThamnoToxkit). Results could be used for classification. Soil leachates would be tested.	Level 3: Routine laboratory	Method used with samples e.g. sludge eluates (Alvarenga et al. 2016). Suitable for relatively quick screening of samples. Results could be used as an indicator of toxicity along with other methods.	Routinely available, toxkits available.	No. Suitable for rapid screening of samples and could potentially be used for classification purposes in combination with other methods. However, method not selected in any of the test batteries used to assess HP 14 in the literature therefore other methods are considered more likely to be accepted for classification.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Dictyostelium discoideum - developmental cycle assay (DDAC)	Amoeba viability and aggregation	No standard guideline available, but method described. Endpoints not regularly used for regulatory hazard assessment. Direct testing or testing of soil leachates.	Level 1: Research laboratory	Method used with soils spiked with diesel and metals (Rodriguez-Ruiz et al. 2016), artificial soils and non-polluted rural soil (Rodriguez-Ruiz et al. 2013), polluted and reference soils (Rodrigues-Ruiz et al. 2015). Relatively quick screening of samples.	Not routinely available at CROs.	No. Method has been used with soil samples and allows relatively quick screening but other methods more suitable for use for hazard classification.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

Table 4.8 Evaluation of EBTs for the assessment of HP 14 Ecotoxic (terrestrial methods)

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Earthworm – acute	Mortality	Standard methods available (ISO 11268, OECD 207). Endpoint used for regulatory risk assessment. Direct testing of waste soils.	Level 3: Routine laboratory	Method used extensively for environmental assessment of wastes and contaminated soils, including soil-sludge mixtures (Garcia-Gomez et al. 2014), sludge from a wastewater treatment plant (Curieses et al. 2016), biogas plant digestate mixed with artificial soil (Pivato et al. 2016), polluted and reference soils (Rodrigues-Ruiz et al. 2015) and waste samples (Pandard et al. 20016, Wilke et al. 2008, Moser et al. 2011). Curieses et al. (2016) also assessed biomarker endpoints on coelomocytes which are unlikely to be directly relevant for classification. Relatively long study length (14 days). Results could be used as an indicator of toxicity along with other methods.	Routinely available at CROs.	Yes. Standard method used for regulatory purposes and provides an indication of direct toxicity from waste soils. Long testing time limits use for rapid screening of samples.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Earthworm - chronic	Reproduction Bodyweight	Standard methods available (OECD 222). Endpoint used for regulatory risk assessment. Direct testing of waste soils.	Level 3: Routine laboratory	Method used for assessment of environmental samples including soil-sludge mixtures (Garcia-Gomez et al. 2014), biogas plant digestate mixed with artificial soil (Pivato et al. 2016), polluted and reference soils (Rodrigues-Ruiz et al. 2015). Long study duration (weeks). Results could be used as an indicator of toxicity along with other methods.	Routinely available at CROs.	No. Standard method available and results suitable for regulatory assessment, but test time too long to allow screening of multiple samples.
Collembola (*Folsomia candida*) - reproduction	Reproduction	Standard methods available (ISO 11267, OECD 232). Endpoint used for regulatory risk assessment. Direct testing of waste soils.	Level 3: Routine laboratory	Method used with oil-sludge mixtures (Domene et al. 2010), soils with residues from biogas production (Stefaniuk et al. 2015), sewage sludge-biochars amended soils (Stefaniuk and Oleszczuk 2016) and waste samples (Wilke et al. 2008). Long study duration (weeks). Results could be used as an indicator of toxicity along with other methods.	Routinely available at CROs.	No. Standard method available and results suitable for regulatory assessment, but test time too long to allow screening of multiple samples.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Higher plants (e.g. *Brassica rapa, Avena sativa, Lolium perenne*)	Seedling emergence Growth Biomass	Standard methods available (ISO 11269-2, OECD 208 plus test kits (PhytoToxkit)). Endpoint used for regulatory risk assessment. Direct testing of waste soils and testing of leachates can be conducted.	Level 3: Routine laboratory	Higher plants used extensively for assessment of soils, contaminated soils and wastes (e.g. Domene et al. 2010, Alvarenga et al. 2016, Baderna et al. 2014, Beesley et al. 2014 Ozcan et al. 2013, Pivato et al. 2016, Rodrigues-Ruiz et al. 2015, Roig et al. 2012, Stefaniuk et al. 2015, Stefaniuk and Oleszczuk 2016, Steliga 2011, Pandard et al. 2006, Wilke et al. 2008, Moser et al. 2011, Römbke 2018). Testing time depends on method used and endpoint assessed. Seedling emergence would be most appropriate for screening as it has the shortest test duration. Results could be used as an indicator of toxicity along with other methods.	Routinely available at CROs, toxkits available.	Yes. Standard method used for regulatory purposes and provides an indication of direct toxicity from waste soils. Long testing time limits use for rapid screening of samples, seedling emergence would be most suitable endpoint.

Test organism	Endpoint assessed	Method development maturity	Validation maturity[1]	Pedigree and applicability	Availability	Shortlist?
Soil respiration	Respiration	Standard methods available (ISO 17155). Endpoint used for regulatory risk assessment. Direct testing of waste soils.	Level 3: Routine laboratory	Method used in the assessment of wastes for HP 14 assessment by Wilke et al. (2008). Shorter test duration compared to some of the other terrestrial tests (days). Results could be used as an indicator of toxicity along with other methods.	Routinely available at CROs.	No. Standard method available and results could be relevant, but other methods are considered to be more appropriate for classification based on direct testing of waste soils.
Earthworm - avoidance	Avoidance behaviour	Standard method available (ISO 17512). Endpoint could be appropriate for regulatory assessment but less regularly used than mortality and reproduction endpoints. Avoidance behaviour can be affected by other properties of the soil (in addition to toxicity). Direct testing of waste soils.	Level 3: Routine laboratory	Method used with waste samples (Römbke 2018), incineration ash, contaminated wood chips and contaminated soil (Kobeticova et al. 2010). Shorter test duration compared to mortality of reproduction tests (days). Results could be used as an indicator of toxicity along with other methods.	Can be conducted at CROs.	Yes. Relevant organism, behavioural endpoints less widely used for regulatory assessment compared to mortality or reproduction but the method has a standard guideline and a shorter test duration compared to other earthworm studies.

[1] Validation maturity assessed on a scale of 1 to 3 based on the NORMAN network guidance document (Norman Network 2008)

Following an assessment of the potential test methods that could be used for assessing HP 14, it is clear that a single test will not be sufficient for classification purposes. Three approaches could potentially be taken for assessing the ecotoxic potential of waste soils; a testing battery including both aquatic and terrestrial tests, a testing battery with aquatic tests only or a limited battery of tests that could be used in combination with the constituent-based calculation approach.

Although there are many potential options for tests that could be conducted for the assessment of HP 14, we would recommend following precedent set for regulatory assessments under other regulatory regimes and using validated test methods covering a range of taxonomic groups. Although environmental classification is generally based on results from aquatic tests, for waste assessment of soils at least one terrestrial test is likely to be required. Combining tests using leachates and terrestrial tests allows both indirect and direct effects of the waste soils to be assessed. This is in line with the approaches followed in the majority of the studies specifically assessing classification approaches under the WFD. The shortlisted tests therefore cover standard tests with a range of organisms, both aquatic (*Vibrio fischeri*, algae, *Daphnia magna*, fish embryo toxicity test) and terrestrial (earthworm acute, earthworm avoidance, seedling emergence). Terrestrial reproduction assays (e.g. with earthworms or collembola) and plant growth studies have not been shortlisted as these tests are long in duration (weeks) and relatively expensive and therefore do not offer a practical alternative to the calculation approach for classification. The full set of shortlisted assays would not need to be conducted, but it is likely that two or three aquatic and at least one terrestrial test would be required to ensure taxonomic coverage, unless it could be demonstrated based on the composition of the waste that some of the species will be more sensitive (and therefore only these could be tested). If the calculation approach is followed for classification, direct testing could still be used for confirmation, for example one of the quick screening assays such as the *Vibrio fischeri* luminescence test could be conducted to confirm that the waste sample does not show direct toxicity.

4.3. SUMMARY OF SHORTLISTED EBTS

Following a review of the identified EBTs against the evaluation criteria, the following test methods have the potential to be used in a direct testing approach for classification purposes under the WFD. Although not all of the listed methods would need to be conducted, and there is the potential to combine testing of the most relevant HPs with the chemical analysis approach, using EBTs for the assessment of HPs would still be considerably more expensive and would take longer to complete (considering not only test duration but also lead in and reporting times) than chemical analysis alone. The shortlisted EBTs covering human health and environmental HPs are summarised in more detail in Section 5.

HP 1 Explosive

- A14 Explosive properties.

HP 2 Oxidising

- A17 Oxidising properties (solids).

HP 3 Flammable

- A10 Flammability (solids).

HP 4 Irritant; HP 8 Corrosive

- Short-time exposure in vitro test method (OECD 491);
- Bovine corneal opacity and permeability method (BCOP) (OECD 491; B.47);
- EpiOcular assay (OECD 492);
- SkinEthic Human Corneal Epithelium (HCE EIT) assay (OECD 492);
- Fluorescein leakage test (OECD 460; B.61);
- Reconstructed human epidermis – skin corrosion (RhE method) (OECD 431); and
- Reconstructed human epidermis – skin irritation (RhE method) (OECD 439; B.46).

HP5 Specific target organ toxicity (STOT)

- No test methods shortlisted.

HP 6 Acute toxicity

- No test methods shortlisted.

HP 7 Carcinogenic

- No test methods shortlisted (see HP 11 for an indication of carcinogenic potential).

HP 9 Infectious

- No test methods shortlisted.

HP 10 Toxic for reproduction

- No test methods shortlisted.

HP 11 Mutagenic

- Ames test (OECD 471; B.13/14); and
- In vitro micronucleus test (OECD 487; B.49).

HP 12 Release of acute toxic gas

- No test methods shortlisted.

HP 13 Sensitizing

- DPRA assay (OECD 442C);
- KeratinoSensTM assay (OECD 442D);
- LuSensTM assay (OECD 442D);
- H-CLAT assay (OECD 442E);
- U-Sens™ assay (OECD 442E); and
- IL-8 Luc assay (OECD 442E).

HP 14 Ecotoxic

- *Vibrio* fischeri luminescent bacteria (ISO 11348);
- Algal growth inhibition (ISO 8692; OECD 201);
- Acute Daphnia magna (ISO 6341; OECD 202);
- Fish embryo acute test (ISO 15088; OECD 236);
- Acute earthworm study (ISO 11268; OECD 207);
- Seedling emergence test (ISO 11269-2; OECD 208); and
- Earthworm avoidance test (ISO 17512).

HP 15 Capable of yielding a hazardous property

- No test methods shortlisted.

5. FACTSHEETS FOR SHORTLISTED EBTS

The EBTs identified as meeting the evaluation criteria and therefore having the potential to be used in a direct testing approach for waste classification are summarised in more detail in factsheets in Sections 5.1 to 5.4.

5.1. HP 4 IRRITANT; HP 8 CORROSIVE

5.1.1. Short-time exposure *in vitro* test method

Test	Short-time exposure *in vitro* test method for identifying i) Chemicals inducing serious eye damage; and ii) Chemicals not requiring classification for eye irritation or serious eye damage
Description	STE test method is a cytotoxicity-based in vitro assay that is performed on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells, cultured on a 96-well polycarbonate microplate. After five-minute exposure to a test chemical, the cytotoxicity is quantitatively measured as the relative viability of SIRC cells using the MTT assay. Decreased cell viability is used to predict potential adverse effects leading to ocular damage.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 491 (2018)
Application to soils / wastes	Can be applied to substances and mixtures; Should be conducted with soil extracts. According to test guideline, sample must be dissolved or suspended uniformly in selected solvent at 5% (w/w), and further diluted to 0.5% and 0.05%
Application for waste classification	A test chemical is classified as UN GHS Category 1 when both the 5% and 0.05% concentrations result in cell viability ≤ 70%; Test chemical is predicted as UN GHS No Category when both 5% and 0.5% result in cell viability > 70%
Is test used alone or in combination?	Used to identify chemicals inducing serious eye damage (UN GHS Category 1 only); further testing may be required.

5.1.2. Bovine corneal opacity and permeability method (BCOP)

Test	Bovine corneal opacity and permeability test method for identifying i) Chemicals inducing serious eye damage and; ii) Chemicals not requiring classification for eye irritation or serious damage
Description	The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. Damage by the test chemical is assessed by quantitative measurements of changes in corneal opacity and permeability. Both measurements are used to calculate an IVIS, which is used to assign an *in vitro* irritancy hazard classification category for the prediction of the *in vivo* ocular irritation potential of a test chemical.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 491 (2017) EC No.440/2008, Method B.47 (2008)
Application to soils / wastes	Can be applied to substances and mixtures; Should be conducted with soil extracts. According to test guideline, liquids are tested undiluted, semi-solids are tested as liquids, surfactant solids are tested at a concentration of 10% (w/v) in a solvent solution, non-surfactant solids are tested as solutions or suspensions at 20% (w/w) in a solvent solution.
Application for waste classification	A test chemical is classified as UN GHS Category 1 when the IVIS result is > 55; Test chemical is predicted as UN GHS No Category when the IVIS result is ≤ 3; No prediction can be made on the test chemical when the IVIS result is > 3 and ≤ 55
Is test used alone or in combination?	Used to identify chemicals inducing serious eye damage (UN GHS Category 1 only); further testing may be required.

5.1.3. EpiOcular assay (EO)

Test	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage: EpiOcular Eye Irritation Test and LabCyte CORNEA-MODEL24
Description	The test chemical is applied topically to tissue construct consisting of at least 3 viable layers of cells and a non-keratinized surface showing a corneal-like structure analogous to that found *in vivo*. Tissue viability is measured following exposure and a post-treatment incubation period. Tissue viability is measured by enzymatic conversion of tetrazolium dye to formazan dye.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline. OECD Guideline for the Testing of Chemicals, Method 492 (2018)
Application to soils / wastes	Can be applied to substances and mixtures. Soil samples could be tested directly or soil extracts tested. According to test guideline, liquids (chemicals that can be pipetted at 37 °C or lower) are tested neat and spread evenly over the tissue surface. Solids (chemicals that cannot be pipetted at 37 °C or lower) are applied as a fine powder and enough should be applied to cover the entire surface of the tissue.
Application for waste classification	EpiOcular: A test chemical is predicted as UN GHS No Category when the mean tissue viability is > 60%; No prediction can be made on the test chemical when the mean tissue viability is ≤ 60% LabCyte CORNEA-MODEL 24: A test chemical is predicted as UN GHS No Category when the mean tissue viability is > 40%; No prediction can be made on the test chemical when the mean tissue viability is ≤ 40%
Is test used alone or in combination?	Used to identify chemicals inducing eye damage; if no prediction can be made, further testing will be required to identify classification.

5.1.4. SkinEthicTM HCE EIT

Test	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage: SkinEthic Human Corneal Epithelium (HCE)
Description	The test chemical is applied topically to tissue construct consisting of at least 4 viable layers of cells including columnar basal cells, transitional wing cells and superficial squamous cells, similar to that of the human corneal epithelium. Tissue viability is measured following exposure and a post-treatment incubation period. Tissue viability is measured by enzymatic conversion of tetrazolium dye to formazan dye.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline. OECD Guideline for the Testing of Chemicals, Method 492 (2018)
Application to soils / wastes	Can be applied to substances and mixtures; Soil samples could be tested directly or soil extracts tested. According to test guideline, liquids (chemicals that can be pipetted at 37 °C or lower) are tested neat and spread evenly over the tissue surface. Solids (chemicals that cannot be pipetted at 37 °C or lower) are applied as a fine powder and enough should be applied to cover the entire surface of the tissue.
Application for waste classification	Liquids: A test chemical is predicted as UN GHS No Category when the mean tissue viability is > 60%; No prediction can be made on the test chemical when the mean tissue viability is ≤ 60%. Solids: A test chemical is predicted as UN GHS No Category when the mean tissue viability is > 50%; No prediction can be made on the test chemical when the mean tissue viability is ≤ 50%.
Is test used alone or in combination?	Used to identify chemicals inducing eye damage; if no prediction can be made, further testing will be required to identify classification.

5.1.5. Fluorescein Leakage Test (FLT)

Test	Fluorescein leakage (FL) test method for identifying ocular corrosives and severe irritants
Description	The FL test method is a cytotoxicity and cell-function based *in vitro* assay that is performed on a confluent monolayer of MDCK CB997 tubular epithelial cells that model the non-proliferating state of the *in vivo* corneal epithelium. The irritancy of the test substance is measured by its ability to induce damage to the impermeable MDCK layer. Increasing the permeability of the corneal epithelium *in vivo* has been shown to correlate with the level of inflammation and surface damage observed as eye irritation develops.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 460 (2017) EC No.440/2008, Method B.61 (2008)
Application to soils / wastes	Can be applied to substances and mixtures. Should be conducted with soil extracts. According to the test guideline, all chemicals to be tested are prepared in sterile calcium-containing, phenol red-free HBSS from the stock solution at five fixed concentrations diluted on a weight per volume basis: 1, 25, 100, 250 mg/mL and a neat solution. For solids, a high concentration of 750 mg/mL should also be included.
Application for waste classification	A test chemical is classified as UN GHS Category 1 when the FL_{20} is ≤ 100 mg/mL; Test chemical is predicted as UN GHS No Category when the FL_{20} is > 100 mg/mL
Is test used alone or in combination?	Used to identify chemicals inducing serious eye damage (UN GHS Category 1 only); further testing may be required

5.1.6. Reconstructed human epidermis - skin corrosion (RhE method)

Test	*In vitro* skin corrosion: reconstructed human epidermis (RHE) test method
Description	The test chemical is applied topically to a three dimensional RhE model, comprised of non-transformed, human-derived epidermal keratinocytes, which form a model of the human epidermis. The test method is based on the premise that corrosive cells are able to penetrate the *stratum corneum* by diffusion or erosion and are cytotoxic to the cells in the underlying layers. Cell viability is measured by the enzymatic conversion of the vital dye MTT into a blue formazan salt.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 431 (2016)
Application to soils / wastes	Can be applied to substances and mixtures. Soil samples could be tested directly or soil extracts tested. According to the test guideline, all chemicals should be applied neat to uniformly cover the epidermis surface. Solids should be tested as a fine powder.
Application for waste classification	Classification parameters depend on model used: EpiSkin, EpiDerm, SkinEthic and epiCS. This test guideline allows the identification of non-corrosive and corrosive substance in accordance with UN GHS. It also supports the sub-categorization of corrosive substances into Sub-categories 1A, 1B and 1C.
Is test used alone or in combination?	Used to identify chemicals inducing corrosive properties on skin (UN GHS Category 1a, 1b and 1c); no further testing required

5.1.7. Reconstructed human epidermis - skin irritation (RhE method)

Test	*In vitro* skin irritation: reconstructed human epidermis (RHE) test method
Description	The test chemical is applied topically to a three dimensional RhE model, comprised of non-transformed, human-derived epidermal keratinocytes, which form a model of the human epidermis. Chemical induced skin irritation, manifested by erythema and edema, is the result of a cascade of events, beginning with penetration through the *stratum corneum* where they may damage the underlying layers of keratinocytes. Cell viability is measured by the enzymatic conversion of the vital dye MTT into a blue formazan salt.
Relevant hazard property	HP 4 Irritant; HP 8 Corrosive
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 439 (2015) EC No.440/2008, Method B.46 (2008)
Application to soils / wastes	Can be applied to substances and mixtures. Soil samples could be tested directly or soil extracts tested. According to the test guideline, all chemicals should be applied neat to uniformly cover the epidermis surface. Solids should be tested as a fine powder.
Application for waste classification	The test chemical is identified as UN GHS Category 2 or 1 if the mean tissue viability is ≤ 50%; Test chemical is predicted as UN GHS No Category when mean tissue viability > 50%; if a test chemical is classified as Non-corrosive, and shows mean tissue viability ≤ 50%, it is considered to be UN GHS Category 3.
Is test used alone or in combination?	Used to identify chemicals inducing irritancy properties on skin (UN GHS Category 2 and 1); cannot distinguish between Category 1 and 2 unless classed as non-corrosive; further testing may be required

5.2. HP 11 MUTAGENIC

5.2.1. Ames test

Test	Bacterial Reverse Mutation test
Description	The bacterial reverse mutation test detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesis an essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain.
Relevant hazard property	HP 11 Mutagenic
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 471 (1997) EC No.440/2008, Method B.13/14 (2008)
Application to soils / wastes	Can be applied to substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	There is no requirement for verification of a clear positive response. There are several criteria for determining a positive result such as: a concentration-related increase over the range tested at one or more concentrations in the number of revertant colonies per plate in at least one strain.
Is test used alone or in combination?	Used to identify chemicals inducing gene mutation (positive result); further testing may be required to confirm classification.

5.2.2. *In vitro* micronucleus test

Test	*In vitro* mammalian cell micronucleus test
Description	The test is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments, or whole chromosomes that are unable to migrate to the poles during the anaphase stage. Therefore, this method provides a basis for investigating chromosome damaging potential. Micronuclei represent damage that has been transmitted to daughter cells, whereas chromosomal aberrations score in metaphase cells may not be transmitted.
Relevant hazard property	HP 11 Mutagenic
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 487 (2016) EC No.440/2008, Method B.49 (2008)
Application to soils / wastes	Can be applied to substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	A test chemical is considered to be clearly positive if all conditions are met: • At least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control; • The increase is dose-related in at least one experimental conditional when evaluated with a trend test; • Any of the results are outside the distribution of the historical negative control data.
Is test used alone or in combination?	Used to identify chemicals inducing chromosomal damage (positive result); further testing may be required to confirm classification.

5.3. HP 13 SENSITISING

5.3.1. DPRA assay

Test	*In Chemico* skin sensitization: Direct Peptide Reactivity Assay
Description	The DPRA is an *in chemico* method which quantifies the remaining concentration of cysteine- or lysine-containing peptide following 24 hours incubation with the test chemical at 25 °C. The relative peptide concentration is measured by HPLC, and cysteine and lysine peptide percent depletion values are then used in a prediction model.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442C (2015) EC No.440/2008, Method B.59 (2008)
Application to soils / wastes	Test method may not be appropriate for complex mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a non-sensitizer if mean % depletion is between 0 – 6.38 %; test chemical is identified as a sensitizer is mean % depletion is between 6.38 and 100 %.
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.3.2. KeratinoSens™ assay

Test	*In vitro* skin sensitization: The ARE-Nrf2 Luciferase KeratinoSens test method
Description	The KeratinoSens test method makes use of an immortalized adherent cell line derived from human keratinocytes stably harbouring a luciferase reporter gene, which is known to be upregulated by skin sensitizers. The cell line contains the luciferase gene under transcriptional control, this allows quantitative measurement of luciferase gene induction. Test chemicals are considered positive if they induce an induction of luciferase activity above a given threshold.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442D (2018) EC No.440/2008, Method B.60 (2008)
Application to soils / wastes	Limited information is currently available on the applicability of the test method to multi-constituent substances/mixtures. Although not evaluated in the validation studies, the test method may nevertheless be technically applicable to the testing of multi-constituent substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a sensitizer if 4 conditions are met in 2/2 or 2/3 replicates: • The I_{max} is ≥ 1.5 fold and statistically different compared to the solvent control; • The cellular viability is > 70% at the lowest concentration with induction of luciferase activity; • The $EC_{1.5}$ is < 1000 µM; • There is a dose-dependent increase in luciferase induction.
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.3.3. LuSensTM assay

Test	*In vitro* skin sensitization: The ARE-Nrf2 Luciferase LuSens test method
Description	The LuSens test method makes use of an immortalized adherent cell line derived from human keratinocytes stably harbouring a luciferase reporter gene, which is known to be up-regulated by skin sensitizers. The cell line contains the luciferase gene under transcriptional control, this allows quantitative measurement of luciferase gene induction. Test chemicals are considered positive if they induce an induction of luciferase activity above a given threshold.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442D (2018) EC No.440/2008, Method B.60 (2008)
Application to soils / wastes	Limited information is currently available on the applicability of the test method to multi-constituent substances/mixtures. Although not evaluated in the validation studies, the test method may nevertheless be technically applicable to the testing of multi-constituent substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a sensitizer if: • The luciferase induction is ≥ 1.5 fold and is statistically significant compared to the solvent control in at least 2 consecutive non-cytotoxic tested concentrations (i.e. cellular viability is ≥ 70%), whereby at least three tested concentrations should be non-cytotoxic.
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.3.4. h-CLAT assay

Test	*In vitro* skin sensitization: Human cell line activation test
Description	The h-CLAT method is an *in vitro* assay that quantifies the changes of CD86 and CD54 cell surface markers expression on a human monocytic leukemia cell line, THP-1 cells, following 24 hours exposure to the test chemical. These surface molecules are typical markers of monocytic THP-1 activation, and the changes of surface marker expression are measured by flow cytometry. Cytotoxicity measurement is also conducted concurrently to assess whether upregulation of the surface marker expression occurs at sub-cytotoxic concentrations.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442E (2018)
Application to soils / wastes	Limited information is currently available on the applicability of the test method to multi-constituent substances/mixtures, but the test method may nevertheless be technically applicable to the testing of multi-constituent substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a sensitizer if at least one of the conditions is met in 2/2 replicates or 2/3 replicates: • The RFI (Relative Fluorescence Intensity) of CD86 is ≥ 150% in at least one tested concentration; • The RFI of CD54 is ≥ 200% in at least one tested concentration.
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.3.5. U-SensTM assay

Test	*In vitro* skin sensitization: U937 cell line activation test (U-SENS)
Description	The U-SENS method is an *in vitro* assay that quantifies the change of CD86 cell surface marker expression on a human monocytic leukemia cell line, U937 cells, following 45 hours exposure to the test chemical. These surface molecules are typical markers of monocytic activation, and the changes of surface marker expression are measured by flow cytometry. Cytotoxicity measurement is also conducted concurrently to assess whether upregulation of the surface marker expression occurs at sub-cytotoxic concentrations.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442E (2018)
Application to soils / wastes	Limited information is currently available on the applicability of the test method to multi-constituent substances/mixtures, but the test method may nevertheless be technically applicable to the testing of multi-constituent substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a sensitizer if: • The S.I. (Stimulation index) of CD86 is ≥ 150% in at least one tested concentration and no interference is observed;
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.3.6. IL-8 Luc assay

Test	*In vitro* skin sensitization: IL-8 LUC assay
Description	The IL-8 Luc assay makes use of the THP-1 cell line. Using this cell line, a THP-1-derived IL-8 reporter cell line, THP-G8, which harbors the Stable Luciferase Orange (SLO) and Stable Luciferase Red (SLR) luciferase genes under the control of the IL-8 glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promotors. This allows quantitative measurement of luciferase gene induction by detecting luminescence from light producing luciferase substrates as an indicator of the activity of IL-8 and GADPH in cells.
Relevant hazard property	HP 13 Sensitizing
Method validation	Validation maturity: Routine Standard test guideline OECD Guideline for the Testing of Chemicals, Method 442E (2018)
Application to soils / wastes	Limited information is currently available on the applicability of the test method to multi-constituent substances/mixtures, but the test method may nevertheless be technically applicable to the testing of multi-constituent substances and mixtures. Should be conducted with soil extracts.
Application for waste classification	The test chemical is identified as a sensitizer if: • The test chemical has an Ind-IL8LA ≥ 1.4
Is test used alone or in combination?	Used to identify chemicals inducing skin sensitization (identified as sensitizer); combination of sensitisation test methods required for classification to address whole AOP.

5.4. ECOTOXIC

5.4.1. *Vibrio fischeri* luminescent bacteria

Test	Luminescent bacteria test (*Vibrio fischeri*)
Description	This study assesses the inhibition in bioluminescence in the marine bacteria *Vibrio fischeri*.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 11348 (2007), Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) Toxkits also available (MicoTox / solid phase MicroTox)
Application to soils / wastes	The method has been used with different types of environmental samples including petroleum contaminated wastewaters (Steliga et al. 2015), sludge elutriates (Domene et al. 2010; Alvarenga et al. 2016; Ozcan et al. 2013), sewage sludge extracts (Roig et al. 2016), and sediments and sediment elutriates (Gonzales-Lozano et al. 2010; Hilscherova et al. 2010; Tsangaris et al. 2014). Leachates from soils have been tested using soils polluted by metals and metalloids (Foucault et al. 2013) and soils amended with compost, biochars and sewage sludge-biochars (Beesley et al. 2014; Stefaniuk and Oleszczuk 2016). The method has also been used directly on polluted and reference soils (Rodrigues-Ruiz et al. 2015; Steliga 2011) and artificial soils mixed with biogas plant digestate (Pivato et al. 2016; Stefaniuk et al. 2015). Should be conducted with soil extracts. The guideline states that this method is applicable to: wastewater, aqueous extracts and leachates, fresh water, marine and brackish water, eluates of sediment (fresh water, marine and brackish), pore water and single substances diluted in water.
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard et al. 2006; Wilke et al. 2008; Stiernström et al. 2011; Moser et al. 2011; Pandard and Römbke 2013).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.2. Algal growth inhibition

Test	Algal growth inhibition test
Description	This study assesses the effects of spiked media on the inhibition of growth of algae exposed for 72 hours. The exposure conditions otherwise allow unrestricted growth (continuous illumination and sufficient nutrient conditions) and effect concentrations are determined based on changes in average specific growth rate and yield compared to an untreated control.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 8692 (2012), Fresh water algal growth inhibition test with unicellular green algae OECD Guideline for the Testing of Chemicals, Method 201 (2011), Freshwater Alga and Cyanobacteria, Growth Inhibition Test
Application to soils / wastes	The method has been used with different types of environmental samples including soil suspensions with flocculants (Wang et al. 2015) and soil extracts prepared following guidelines for the determination of toxicity of waste prepared by the Ministry of the Environment of the Czech Republic (2007) and the EN 12457-4 (2002) standard (Buckova et al. 2017). Should be conducted with soil extracts. The guideline states that this method is applicable for substances that are easily soluble in water and, with modifications, can be applied to poorly soluble organic and inorganic materials, volatile compounds, metals and wastewater.
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard et al. 2006; Stiernström et al. 2011; Moser et al. 2011; Pandard and Römbke 2013; Römbke 2018).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.3. Acute *Daphnia magna* test

Test	Daphnia magna acute test
Description	In this study, the immobilisation (corresponding to mortality) of *Daphnia magna* (<24 hours old) exposed for 48 hours to spiked and control media is assessed.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 6341 (2012), Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test OECD Guideline for the Testing of Chemicals, Method 202 (2004), Daphnia sp. Acute Immobilisation test
Application to soils / wastes	The method has been used with different types of environmental samples including petroleum contaminated wastewaters (Steliga et al. 2015), soil-sludge extracts obtained using standard leaching method (DIN 38414-S4 1984) (Garcia-Gomez et al. 2014; Alvarenga et al. 2016), soil suspensions with flocculants (Wang et al. 2015), leachates from soils polluted by metals and metalloids (Foucault et al. 2013) and biogas plant digestate mixed with artificial soil (Pivato et al. 2016). Should be conducted with soil extracts. The guideline states that this method is applicable for: chemical substances which are soluble under the conditions of the test or can be maintained as a stable suspension or dispersion, industrial or sewage effluents, treated or untreated wastewater, aqueous extracts and leachates, fresh water, eluates of freshwater sediment, and pore water for freshwater sediment.
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard et al. 2006; Moser et al. 2011; Pandard and Römbke 2013; Römbke 2018).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.4. Fish embryo acute test

Test	Fish embryo acute test
Description	In this study, daily observations of lethality (coagulation of fertilised eggs, lack of somite formation, lack of detachment of tail bud from yolk sac and lack of heartbeat) are made on newly fertilised fish embryos exposed for 96 hours to spiked and control media.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. OECD Guideline for the Testing of Chemicals, Method 236 (2013), Fish embryo acute toxicity test
Application to soils / wastes	The method has been used with different types of environmental samples including river sediments (Hafeli et al. 2011), sediment extracts (Kosmehl et al. 2012) and liquid effluents and sludge elutriates from a deactivated uranium mine (Lourenco et al. 2017). Should be conducted with soil extracts.
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Stiernström et al. 2011).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.5. Acute earthworm study

Test	Acute earthworm study
Description	This study investigates the acute toxicity of soil contaminants to adult earthworms following dermal and alimentary uptake by assessing survival of the earthworms in the spiked soil compared to survival in an uncontaminated (reference) or standard (artificial) soil.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 11268-1 (2012), Determination of acute toxicity to *Eisenia fetida/Eisenia andrei* OECD Guideline for the Testing of Chemicals, Method 207 (1984), Earthworm acute toxicity test
Application to soils / wastes	The method has been used with different types of environmental samples including soil-sludge mixtures (Garcia-Gomez et al. 2014) and sludge from a cosmetic wastewater treatment plant and foundry sands (Curieses et al. 2016), as well as polluted and reference soils (Rodrigues-Ruiz et al. 2015) and biogas plant digestate mixed with artificial soil (Pivato et al. 2016).
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard et al. 2006; Wilke et al. 2008; Moser et al. 2011).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.6. Earthworm avoidance test

Test	Earthworm avoidance test
Description	The study is a rapid screening method used to assess the impact of soil contaminants on the behaviour of earthworms. The location of earthworms allowed to move freely between compartments filled with control or treated soils is assessed in this sub-lethal study.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 17512-1 (2008), Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Test with earthworms (*Eisenia fetida* and *Eisenia andrei*)
Application to soils / wastes	The method has been used with different types of environmental and waste samples including incineration ash, contaminated wood chips, contaminated soil (Kobeticova et al. 2010).
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard and Rombke 2013; Rombke 2018).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

5.4.7. Seedling emergence test

Test	Seedling emergence test
Description	Study for evaluating the effect of soils contaminants on plant growth through assessment of the emergence and inhibitory effects on early growth of higher plant species. Seedling emergence, biomass and visual detrimental effects are determined weekly for 14 to 21 days and compared to those of untreated control plants.
Relevant hazard property	HP 14 Ecotoxic
Method validation	Validation maturity: Routine Standard guidelines available. ISO 11269-2 (2012), Effects of contaminated soil on the emergence and early growth of higher plants OECD Guideline for the Testing of Chemicals, Method 208 (2006), Terrestrial plant test: Seedling emergence and seedling growth
Application to soils / wastes	The method has been used with different types of environmental samples including sewage sludge extracts and eluates (Roig et al. 2012, Ozcan et al. 2013), soils and soil mixtures (Domene et al. 2010, Pivato et al. 2016, Rodrigues-Ruiz et al. 2015, Steliga 2011, Stefaniuk and Oleszczuk 2016, Stefaniuk et al. 2015, Baderna et al. 2014). Seed germination has been assessed with sludge eluates (Alvarenga et al. 2016) and seedling emergence and root germination were assessed with extracts from soils amended with composts and biochars (Beesley et al. 2014).
Application for waste classification	No official guidance for classification of HP 14 by direct testing is yet available. However, classification is likely to be based on comparison of results from a combination of tests to toxicity thresholds. This test has been included in the battery of tests proposed in the literature (Pandard et al. 2006; Pandard and Römbke 2013; Römbke 2018).
Is test used alone or in combination?	This test would be conducted as part of a test battery for classification purposes.

6. CONCLUSIONS

Classification of waste soils can be complex, requiring knowledge of different pieces of legislation and guidance documents, at EU and MS level. In addition to the revised Waste Framework Directive (Directive 2008/98/EC), reference to other key legislation is also required, including:

- The List of Waste;
- The CLP Regulation;
- The REACH Regulation and
- The Landfill Directive.

Waste is assessed for different HPs and ultimately classified as either hazardous or non-hazardous. The current approach for waste classification relies on a calculation approach based on chemical characterisation of the waste. This can be challenging due to the difficulties in fully characterising waste samples. EBTs can also be utilised, but animal testing cannot be conducted under the WFD.

This literature review identified tests that could potentially be used in the classification of waste soils and assessed the practicality of implementing these for classification purposes. Tests were selected based on those currently used for other regulatory purposes that could also be applied to waste soils and from an assessment of literature data, identifying test methods previously applied to soils and environmental samples, or to wastes.

It is clear from this assessment that multiple tests would need to be conducted if EBTs were used for classification, due to the varied HPs that need to be assessed under the WFD. Although it may be possible to address some HPs together (e.g. HP 4 irritant and HP 8 corrosive or HP 7 carcinogenic and HP 11 mutagenic), in other cases (e.g. for HP 14 ecotoxic) a number of tests would be needed to address one HP.

This assessment has found that EBTs are available for assessing some of the relevant HPs. If EBTs are used, test methods with standard test guidelines that are already used for classification under other regulatory regimes are recommended to increase the likelihood of any direct testing approach being accepted by regulators. However, at the current time it is not considered appropriate to use direct testing as a replacement for the calculation approach. There are some HPs for which no suitable EBTs have been identified. For those HPs where testing is possible, a number of EBTs would be required for classification purposes and therefore this approach would be time consuming (both in terms of experimental duration and lead in and reporting times) and expensive (thousands of euros per test for effects-based tests) than a calculation approach based on waste composition.

For some HPs the number of tests could potentially be reduced, for example for HP 14 (ecotoxic) it could be possible to reduce the number of tests to just cover the most sensitive species if it can be demonstrated that some test species are more sensitive to particular types of waste soils. However, such an approach would require adequate justification and has not been endorsed by regulators.

Overall, the use of EBTs cannot currently replace the calculation approach for WFD classification. Whilst there is potential to use EBTs for WFD classification to assess particular site or waste-specific issues, this would need careful consideration on a case by case basis.

7. LIST OF ABBREVIATIONS

ATP	Adaptation to Technical Process
C&L	Classification and Labelling Inventory database
CLP	Classification, labelling and packaging of substances and mixtures: Regulation (EC) No 1272/2008
CAS	Chemical Abstracts Service; www.cas.org
DSD	Dangerous Substances Directive 67/548/EEC
DPD	Dangerous Preparations Directive 1999/45/EC
EBT	Effects-Based Test
ECHA	European Chemicals Agency
EC	European Community
EINECS, EC number or EC#	European Inventory of Existing Commercial Substances
EEC	European Economic Community
EU	European Union
EWC	European Waste Catalogue
GHS	Global Harmonised System
LoW	List of Waste
MSDS	Material Safety Data Sheet
MS	Member States
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbons
POP	Persistent Organic Pollutant
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals: Regulation (EC) No 1907/2006
rWFD	revised Waste Framework Directive 2008/98/EC
SDS	Safety Data Sheet
SVOC	Semi Volatile Organic Compounds

TPH	Total Petroleum Hydrocarbons
UK	United Kingdom
US EPA	United States Environmental Protection Agency
WAC	Waste Acceptance Criteria
WM3	WM3 Technical Guidance

8. REFERENCES

DIRECTIVES

- Directive 2008/98/EC of the European Parliament and Council of 19 November 2008 on waste and repealing certain Directives.
- Directive 1999/31/EC of 26 April 1999 on the landfill of waste

REGULATIONS

- Regulation (EU) No 2017/997 of 8 June 2017, amending Annex III to Directive 2008/98/EC of the European Parliament and of the Council as regards the hazardous property HP 14 'Ecotoxic'.
- Regulation (EU) No 1357/2014 of 18 December 2014, replacing Annex III to Directive 2008/98/EC of the European Parliament and of the Council on waste and repealing certain Directives.
- Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006
- Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
- Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC
- Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
- The Waste (England and Wales) Regulations 2011; www.legislation.gov.uk/ukdsi/2011/9780111506462
- The Hazardous Waste (England and Wales)Regulations 2005; http://www.legislation.gov.uk/uksi/2005/894/contents/made
- European Communities (Waste Directive) Regulations 2011, S.I. No. 126 of 2011; www.dccae.gov.ie/en-ie/environment/legislation/Pages/S-I--No--126_2011.aspx

DECISIONS

- Decision 2014/955/EU of 18 December 2014, amending Decision 2000/532/EC on the list of waste pursuant to Directive 2008/98/EC of the European Parliament and of the Council.
- Decision 2003/33/EC of 19 December 2002 establishing criteria and procedures for the acceptance of waste at landfills pursuant to Article 16 of and Annex II to Directive 1999/31/EC

GUIDANCE & STANDARDS

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ISBN 978-2-87567-128-8