

Research to Industry and Industry to Research

CHALLENGES AND PERSPECTIVES OF DIRECT TEST METHODS FOR ASSESSING WASTE HAZARDOUS PROPERTIES (HP)

Giovanni Beggio ^{1,*}, Tiziano Bonato ², Silvana Giardina ³, Paola Grenni ⁴, Livia Mariani ⁴, Lorenzo Maggi ⁵, Pierre Hennebert ⁶, Francesco Loro ⁷ and Alberto Pivato ¹

¹ Department of Civil, Environmental and Architectural Engineering, University of Padova, via Marzolo 9, 35131, Padova, Italy

² Department of Environmental Sciences, Informatics and Statistics (DAIS), Ca' Foscari University of Venice, via Torino 155, 30172 Venice, Italy

³ Complife Italia - Lab Analysis Group, via Monsignor Angelini, 21, 27028 S. Martino Siccomario, Pavia, Italy

⁴ Water Research Institute, National Research Council, via Salaria km 29.300, 00015 Monterotondo Scalo (RM), Italy

⁵ Chemservice S.r.l. - Lab Analysis Group, via F.lli Beltrami, 15, 20026 Novate Milanese, Milan, Italy

⁶ Ineris, French National Institute for Industrial Environment and Risks, BP 2, F-60550 Verneuil-en-Halatte, France

⁷ WasteandChemicals Srl - Circ. Gianicolense 216/E 00152 Rome, Italy

* email: giovanni.beggio@unipd.it

Waste generation is a global problem, not only environmentally but also the economic loss it represents. Annual waste generation is projected to increase by 70% by 2050 (OECD, 2019a). Consequently, waste management should be planning and managing a circular economy, to ensure that resources used remain in the EU economy for as long as possible, while ensuring the best degree of environmental safety (European Commission, 2020). In this context, hazard waste classification plays a crucial role.

Hazard waste classification entails safe handling and disposal of discarded materials, with significant impacts on waste producers' budgets, their legal conduct, and public perception. The regulatory criteria should be realistic and scientifically sound ensuring full transparency while providing a level-playing field for all industrial sectors. According to European regulations, waste is defined as hazardous if satisfies at least one of the 15 hazard properties (HP) or contains concentrations of certain persistent organic pollutants over specific legal thresholds (European Commission, 2014; European Parliament and European Council, 2019). Equally, wastes are classified as hazardous according to the 6-digits codes enlisted in the European Waste Catalog, established by the European Commission, (2000). Accordingly, among "absolute non-hazardous" waste, "absolute hazardous" waste, and the so-called "mirror entries" (i.e., waste streams potentially classified as hazardous by their composition), only these latter require an effort to assess specific HPs.

HPs can be assigned by an "indirect" approach, from the total content of hazardous substances (selected according to "expert judgment"), or a "direct" approach, which relies on outcomes of single HP-specific laboratory tests (European Commission, 2014). Based on widespread analytical methods, the "indirect" approach is cheap and

currently the most adopted. Notably, it is characterized by some challenges: the subjectivity of the "expert judgment," the impossibility of detecting all substances and elements that compose the waste material, and the so-called "worst-case" approach, which considers the waste constituents detected as in the most hazardous form (Bishop and Hennebert, 2021; Hennebert, 2019). These drawbacks have been limited by the development of non-targeted organic and mineral analyses, giving an analytical mass balance > 90% (Hennebert et al., 2013), and the speciation of so-called "worst-case with information" pre-calculated approaches (Hennebert, 2019). However, the classification as hazardous can sometimes be judged as incomplete or unrealistically conservative. In these cases, specific testing methods to evaluate "directly" (i.e., without further assumptions) different HP-related effects, closely associated with the real speciation (and environmental fate) of waste constituents. The European legislator suggested the latter approach given the information about the waste composition is not sufficient for a correct evaluation. The European legislation affirms that direct test results will prevail over the results from chemical composition analyses (European Commission, 2018). The EU law-maker also suggests the methods used to be guided by the CLP regulations for performing direct testing, toward the harmonization of products and wastes law frameworks (European Council, 2008).

There are still some challenges to be faced:

- A limited number of laboratories are accredited for the methods available, increasing in costs but not in use;
- The methods designed for classifying products under CLP Regulation (European Council, 2008) can be unsuitable for testing wastes;

TABLE 1: Approaches available for assessing waste Hazard Properties.

Hazard Property		Evaluation approach
HP 1	Explosive	Direct
HP 2	Oxidising	Direct
HP 3	Flammable	Direct
HP 4	Irritant – skin irritation and eye damage	Indirect and Direct
HP 5	Specific Target Organ Toxicity	Indirect
HP 6	Acute Toxicity	Indirect
HP 7	Carcinogenic	Indirect
HP 8	Corrosive	Indirect and Direct
HP 9	Infectious	Not available
HP 10	Toxic for the reproduction	Indirect
HP 11	Mutagenic	Indirect
HP 12	Release of an acute toxic gas	Direct
HP 13	Sensitising	Indirect
HP 14	Ecotoxic	Indirect and Direct
HP 15	Waste capable of exhibiting a hazardous property listed above not directly displayed by the original waste	Indirect

- For some HPs, there is no EU-harmonized list of direct test methods;
- The need to validate in-vitro methods as alternatives to in vivo and ex-vivo animal testing.

Experts from analytical and industrial sectors, with representatives of environmental research institutions, discuss the state-of-the-art direct test procedures while building an efficient and correct waste classification framework. The meeting happened during a specific workshop session held virtually during the SUM Symposium (Fifth Symposium on Urban Mining and Circular Economy Virtual Event/18–20 November 2020). Subsequent meetings involved experts to further widen the main topics discussed during the symposium and are presented here.

This paper describes the most discussed direct decision trees, namely, the methods for assessing HP 4 (Irritant), HP 8 (Corrosive), and HP 14 (Ecotoxic). These HPs can be assessed with the “indirect” and the “direct” approaches, while the remaining properties are evaluated using the calculation method or direct testing only (Table 1). Finally some suggestions prompting the introduction of validated test methods for HP 10 (Toxic for reproduction) are included as an example of the research path taken to provide data for future regulation updates.

HP 4 (irritant) and HP 8 (corrosive)

Both HP 4 and HP 8 have potentially negative effects on human tissues (i.e., skin and eyes), but with an increasing degree of severity, from reversible damages for HP 4 to irreversible injuries in the case of HP 8 (European Commission, 2014). According to experts interviewed, the procedure for evaluating HP 4 and HP 8 using direct testing is harmonized and well-established at the EU level, which is based on validated in-vitro test methods that are in the EU technical guidelines for waste classification (European Commission, 2018).

The decision tree consists of a combined assessment of outcomes from the conventional acid/alkali reserve test

and *in-vitro* assays. Practically, only wastes characterized by inherent $\text{pH} \leq 2$ or ≥ 11.5 can be classified with HP 8 or HP 4. In this case, if the buffer capacity is high (i.e., $(\text{pH} - 1/12 \text{ acid reserve (g NaOH/100g)}) < -0.5$ or $((\text{pH} + 1/12 \text{ alkaline reserve})) > 14.5$; or, equivalently, $(\text{pH} - 1/3 \text{ buffer capacity (mol H+/kg)}) < -0.5$ or $(\text{pH} + 1/3 \text{ buffer capacity (mol H+/kg)}) > 14.5$) the waste is classified with HP 8. Instead, waste characterized by inherent $\text{pH} \leq 2$ or ≥ 11.5 but showing low buffer capacity can undergo further in-vitro testing for HP 4 and/or HP 8 classification. Both pH and buffer capacity are measured following the instructions of OECD 122 (OECD, 2013a). In particular, for solid wastes, the established method requires inherent pH and acid/alkali reserves to be measured in a 1% (w/v) (i.e., 10 g/L) solution with distilled or deionized water (OECD, 2013a). Another approach is to use the classical batch waste leaching test according to EN 12457 series, typically with 100 g/L (CEN, 2004).

A stepwise process was proposed to decrease the costs related to performing in-vitro tests (Figure 1). In-vitro assays related to the “irritation and eye damage” endpoint (i.e., HP 4) should be performed before skin corrosiveness tests (i.e., HP 8). If waste is not classified as HP 4, it cannot be classified also as HP 8, while the contrary is not true. This procedure ensures that mirror entries not requiring any classification for HP 4 and HP 8 undergo just one round of in-vitro tests (i.e., for HP 4 assessment).

The in-vitro test method for assessing skin irritation (i.e., OECD 439) is performed with a time-limited application of the material tested on models of reconstructed human Epidermis (RhE) (OECD, 2013b). Any irritation potential is identified by the loss of cell viability over a specified threshold, by indirectly measuring the enzymatic activity of the RhE model after exposure. Similarly, in-vitro assays for skin corrosion are conducted according to OECD 430, from the exposure of epidermal surfaces of rat skin disks (OECD, 2013c) or, more preferably, by OECD 431, through

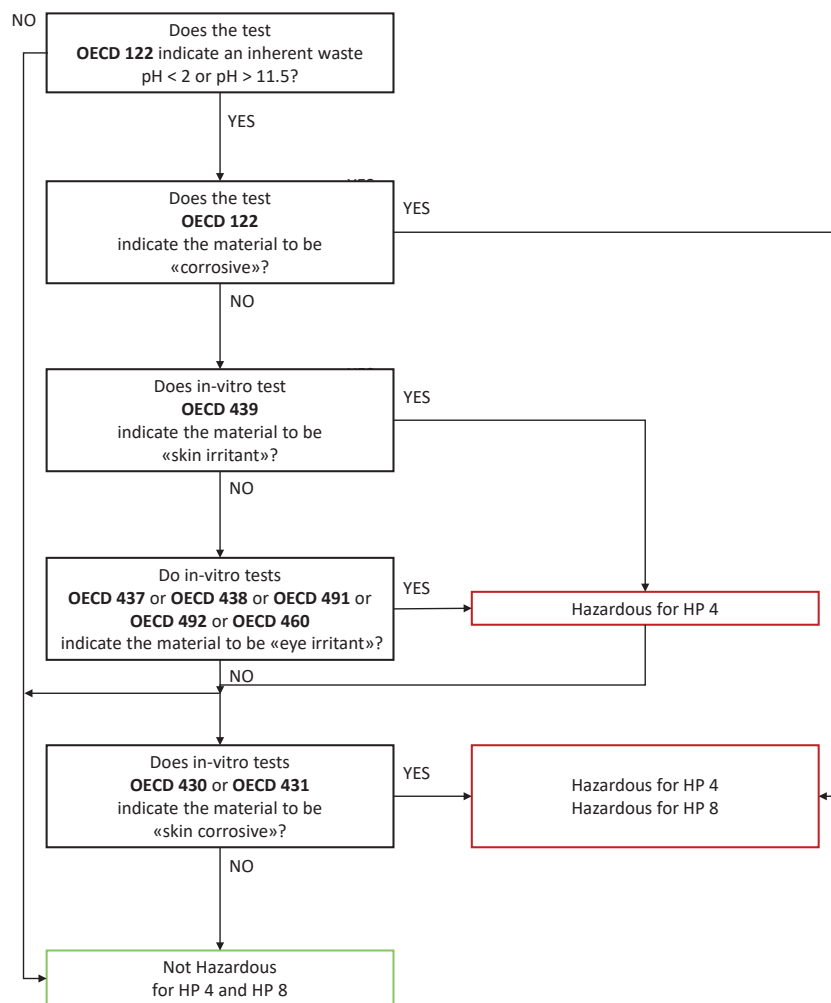


FIGURE 1: Proposed decision tree for waste HP 4 and HP 8 classification by direct tests.

exposing RhE models (OECD, 2015). Both methods measure the consequent response in the loss of cell viability and integrity. Specifically, in-vitro tests for evaluating skin irritation can use the same RhE models used to test in-vitro skin corrosion. However, skin corrosion is assessed by different procedures and classification limits than skin irritation.

Furthermore, the proposed approach includes in-vitro tests for assessing “eye irritation or serious eye damage” since waste having such effects falls within the definition of HP 4 (European Commission, 2014). However, the methods suggested are excluded in either the EU technical guidelines on waste classification or the regulation of analytical methods allowed for CLP (ECHA, 2017; European Council, 2008). However, to strictly adhere to the definition of HPs and in the absence of alternative tests, five valid OECD in-vitro test methods are used to address “eye irritation or serious eye damage,” and are consistently suggested in this study (Figure 1). All in-vitro assays considered are performed by placing the waste test portion on biological layers reconstructed and cultured from bovine cornea cells as in OECD 437 (OECD 2020a), chicken eyes as in OECD 438 (OECD 2018), rabbit cornea cells as in OECD 491

(OECD, 2020), human cornea-like epithelium as in OECD 492 (OECD, 2019a), and canine kidney as in OECD 460 (OECD, 2017). Waste classification can then be assigned by comparing the chemical-induced damage measured at the end of each test.

HP 10 (toxic for reproduction)

Waste is classified as HP 10 if it has negative effects on the reproductive functions of adults or the sexual development of offspring. No strategy for directly assessing this property is within the EU waste law framework. In particular, technical guidelines for waste classification state that “there are limited options for testing reproductive toxicity properties in-vitro.” (European Commission, 2018).

From experts, several in-vitro tests are validated and suited to classify chemical products for the category “reproductive toxicity.” Other in-vitro methods are under investigation with a view to full regulatory acceptance. A full list of in-vitro tests useful for the classification of substances and information on their acceptance status is in the EURL ECVAM dataset on alternative methods to animal experimentation (DB-ALM) (European Commission and Joint Research Center, 2019). However, pending the

transposition to the related waste regulations, only the calculation method should be applied (European Commission, 2014).

A possible solution is a direct decision tree based on already validated in-vitro tests. With the approach developed for chemical products, a multi-step analytical strategy should be performed for a reliable classification of reproductive toxicity. In particular, the biological concept of “reproductive cycle” has been broken down into three elements that are estimated by the combined assessment of three biometabolic effects, namely, loss of male fertility, decrease of implantation capacity (i.e., female fertility), and hindered prenatal development (Hareng et al., 2005). A specific battery of in-vitro assays can also be proposed for waste, whose results cover the sub-endpoints range identified.

Research that focuses on solving the critical issues that occur when applying the validated in-vitro methods for waste characterization is our interest. The latter relates to the adequacy and adaptability of methods to the heterogeneous nature of wastes compared to the more homogeneous features of chemical products. Solubility mechanisms and solid wastes extraction conditions appear the most challenging to be solved before full adoption of available in-vitro assays.

Similar considerations are also valid for direct testing of HP 7 (carcinogenic) and HP 11 (mutagenic) classification of wastes.

HP 14 (ecotoxicity)

Despite the extensive debate on the ecotoxicity classification of wastes, there is no agreement among scientific and industrial communities on the most suitable decision tree to be applied. In this regard, efforts toward a widespread agreement and harmonization at the EU level are essential, since HP 14 “Ecotoxic” is the most frequent HP classifying mirror entries as hazardous (Hennebert et al., 2014). Consequently, the EU regulation does not precisely define the direct test methods but considers methods for CLP appropriate (i.e., European Council, 2008), with “other

internationally recognized test methods and guidelines” (European Council, 2017). This has contributed to establish a non-harmonized framework of regulations among EU Member States.

Each decision tree for the ecotoxicological classification of waste is built on three main elements: the sample preparation procedure such as the leaching tests for preparing liquid samples from solid test portions; the battery of bioassays to be performed; finally, the reference thresholds triggering hazardous classification. If at least one ecotest is non-compliant with the limits, the waste is classified as “ecotoxic”. Two main approaches emerged from the discussion.

First, the “CLP-based approach,” based on a protocol issued by the Italian Institute of Environmental Protection (ISPRA, 2018) and included in the guidelines for waste classification (SNPA, 2020), entails only methods and classification limits developed and validated for chemical products within the CLP regulations, regarding exclusively aquatic toxicity (ECHA, 2017; European Council, 2008). The method for liquid test sample preparation from solid samples (i.e., leaching test) was originally developed for dissolving metal compounds to an aqueous medium, i.e., OECD 29 (OECD, 2001). Solid waste eluates, termed Water Accommodated Fractions (WAF), are produced through a leaching test characterized by loading rates of 100 mg/L, 10 mg/L, and 1 mg/L and tested individually (i.e., without further dilutions) using the battery of biotests. Thus, the concentration limits, indicated in terms of EC50 and NOEC, are expressed in terms of “loading rate” (i.e., mg/L). The bioassays that are composed of the test battery proposed for this approach are listed in Table 2 together with the corresponding concentration limits triggering an HP 14 classification. A potential decision tree adopted following this approach is also depicted in Figure 2.

Second, the “Waste-based approach,” adopts internationally acknowledged methods developed and validated specifically for waste testing, unlike CLP-related methods (CEN, 2005; Moser and Römbke, 2009; Pandard and Römbke, 2013). The test battery proposed includes terres-

TABLE 2: “CLP-based approach.” Battery of biotests and concentration limits used within the testing strategy complying with the CLP Regulation.

Organism	Type	Standard	Classification Criteria (waste is hazardous for HP 14 if)	Source
Algae	Acute-Chronic ***	OECD 201 * (Freshwater Alga and Cyanobacteria Growth Inhibition Test)	Acute LC50 ≤ 100 mg/l	(OECD, 2011)
	Chronic	OECD 221* (Lemna sp. Growth Inhibition Test)	Chronic NOEC ≤ 1 mg/l	(OECD, 2006)
Crustacean	Acute	OECD 202 * (Daphnia magna, Acute Toxicity Test)	Acute LC50 ≤ 100 mg/l	(OECD, 2004)
	Chronic	OECD 211 * (Daphnia magna, Chronic Toxicity Test)	Chronic NOEC ≤ 1 mg/l	(OECD, 2012)
Fish	Acute	OECD 203 * (Fish, Acute Toxicity Test)	Acute LC50 ≤ 100 mg/l	(OECD, 1992)
	Chronic	OECD 210 ** (Fish, Early-Life Stage Toxicity Test)	Chronic NOEC ≤ 1 mg/l	(OECD, 2013)

* Method included in the list of tests validated within the scope of the CLP regulation (European Council, 2008) and SNPA, (2020).

** Test not reported within European Council, (2008) and SNPA, (2020), but present in ECHA, (2017).

*** According to ECHA, (2017) “The algal growth inhibition test is a short-term test that provides both acute and chronic endpoints. However, EC50 is treated as an acute value for classification purposes.”

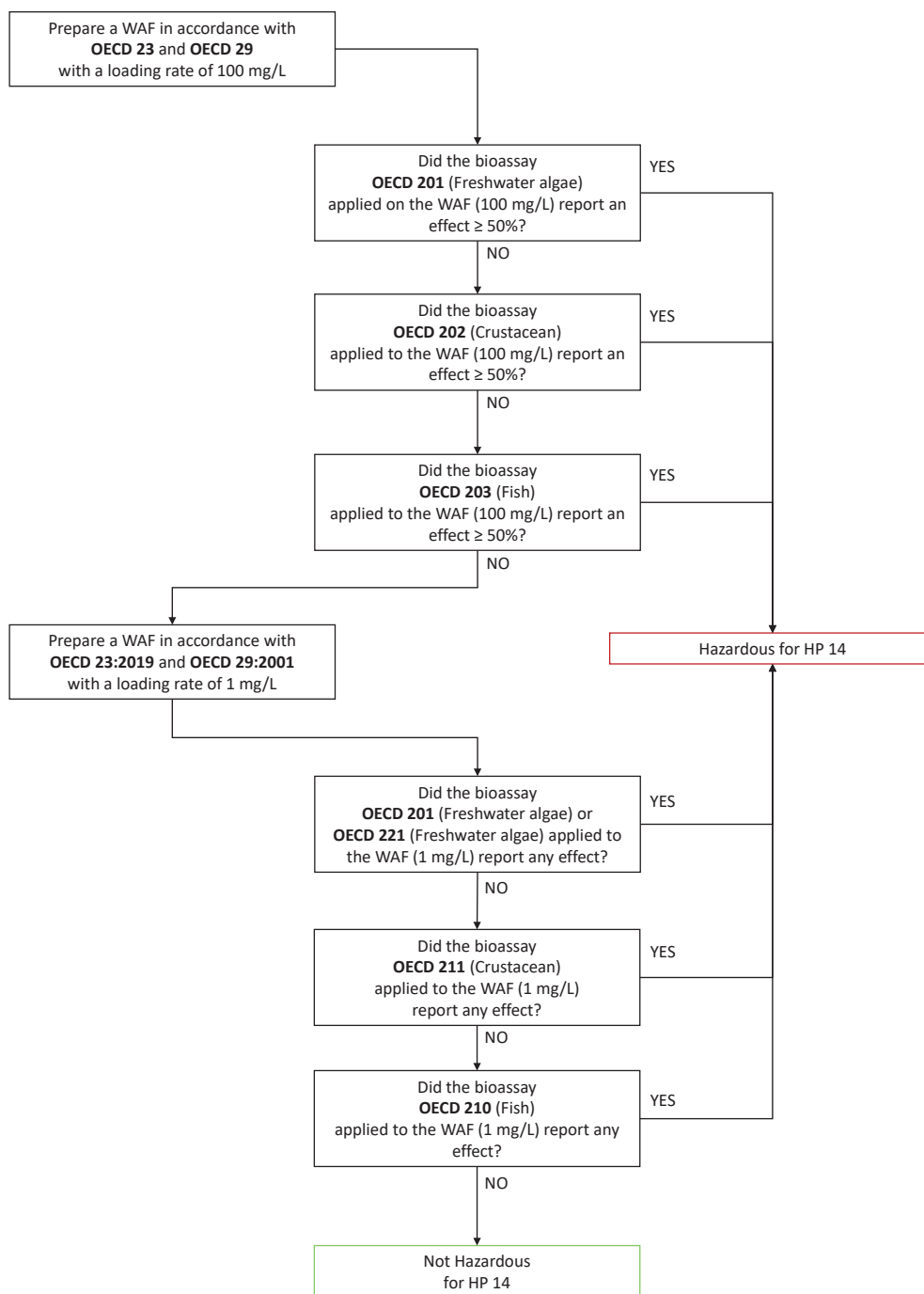


FIGURE 2: “CLP-based approach.” Proposed decision tree for HP 14 classification of waste with methods validated for CLP. WAF: Water Accommodated Fractions. OECD 23: Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures; OECD 29: Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; OECD 201, 202, 203, 210 and 211: see Table 2.

trial and aquatic tests, listed in Table 3. Test portions are prepared following the waste-specific standard EN 14735 (CEN, 2005). In particular, aquatic bioassays are conducted on several volumetric dilutions of solid waste eluates from a leaching test adopting a loading rate of 100 g/L, which corresponds to a Liquid-to-Solid ratio of 10 L/kg, conducted as prescribed in EN 12457-2 (CEN, 2004). Concentration limits, expressed as EC50 (i.e., %vol/vol), were proposed for this approach by Hennebert, (2018). A proposed decision tree is shown in Figure 2. A practical application to

the HP 14 classification of typical mirror entry waste (i.e., Automotive Shredder Residue) is also proposed in Pivato et al., (2020).

A discussion occurred among the participants about the comparison between the two approaches.

In particular, it is worth mentioning that the water extracts are derived according to two distinct sets of applied conditions (L/S ratio, particle size, test duration, leaching media etc.), simulating a wide range of occurred leaching conditions.

The size and the granulometry of the solid test portions indicated by the leaching methods were discussed: despite the significant efforts to reduce particle size up to 1 mm and massive subsampling (from the laboratory sample to 100 mg test portion), test portions can seldom be thought of as representative of the waste batch to be classified. From this, the application of the validated standard EN 14735, recalling the instructions laid down in EN 12457-2, can solve the issues discussed.

Finally, other challenges were specified during the discussion, whose solutions can lie within the outcomes of future research in the field:

- Waste water extracts frequently feature extreme pH values, both lower and higher than the survival range of organisms used in the test batteries suggested (i.e., pH range of 6–9). Therefore, the results of bioassays can be predicted without performing the tests. Further research to identify more roles of pH (and its adjustment in the second set of repeated tests) on aquatic toxicity mechanisms for each matrix tested. Regulations and specific technical standards are open to adjusting the pH of the sole test portion (i.e., not the dilutions) if extreme pH influenced the outcomes of the biotests performed. Nonetheless, pH adjustment should be reported in any test reports produced, together with results of biotests without pH adjustment.
- From Table 2, several methods to obtain chronic endpoints are suggested only by the guideline on the application of CLP criteria (ECHA, 2017), but are neither included in the list of methods validated for CLP (European Council, 2008) nor the Italian guideline for waste classification (SNPA, 2020), which applies a CLP-consistent approach for HP 14 assessment. Similarly, no chronic endpoints can be assessed by the abovementioned testing approach described in Table 3 and Figure 3, consistent with the propositions of Pandard and Römbke,

(2013). This is due to the efforts required by the main laboratories for chronic testing that hindered its use for conventional purposes like waste classification.

- High intra-laboratory and inter-laboratory variability characterize outcomes of the aquatic ecotoxicological bioassays of the test batteries. No data on repeatability and reproducibility are available in the scientific literature or institutional reports for terrestrial tests (Hennebert and Beggio 2021). This issue is crucial for adopting a conventional harmonized decision tree for the HP 14 classification of waste. Besides the intrinsic variability of the method, the low reproducibility and repeatability can be related to the preparation of the test portion analyzed, highlighting the influence of the specific leaching test adopted. This issue was evident in the algae test conducted on waste WAF prepared following the CLP-leaching test. In particular, an Italian institutional report, resulting from inter-laboratory tests conducted according to ISO 5725-2 (ISO, 2019) at 23 laboratories on a prepared 100 mg/L WAF, calculated relative repeatability and reproducibility values for the algae test as 78% and 104%, respectively (ISPRA, 2017). If the results of repetitions of that test are normally distributed, the confidence interval of the mean with a 95% probability level [mean – 1.96*standard deviation; mean + 1.96*standard deviation] includes zero and negative values. Therefore, the results cannot be significantly different from zero nor used for regulatory purposes.

Conclusions and future trends

In the current work, a state-of-the-art method on the most important direct tests for waste classification is presented. Drawbacks are discussed and proposals suggested in a systematic and organic approach.

The recommended test(s) to assess a given hazard property, which covers all aspects of that specific hazard

TABLE 3: “Waste-based approach.” Eco tests and corresponding limits for HP 14 classification of waste according to (Hennebert, 2019; Hennebert, 2018; Pandard and Römbke, 2013; Moser and Römbke, 2009).

Organism	Type	Standard	Classification Criteria (waste is hazardous for HP 14 if) ¹	Source
Aquatic Bacteria	Acute	EN ISO 11348-3 (Determination of the inhibitory effect of water samples on the light emission of <i>Vibrio fischeri</i> (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria)	EC50 < 15%(vol/vol) (original data 15.8%)	(ISO, 2008)
Algae	Acute	EN ISO 8692 (Fresh water algal growth inhibition test with unicellular green algae)	EC50 < 10%(vol/vol) (original data 7.03%)	(ISO, 2012a)
Crustacean	Acute	EN ISO 6341 (Determination of the inhibition of the mobility of <i>Daphnia magna</i> Straus)	EC50 < 10%(vol/vol) (original data 7.95%)	(ISO, 2012b)
Soil Bacteria	Acute	EN ISO 18187 (Contact test for solid samples using the dehydrogenase activity of <i>Arthrobacter globiformis</i>)	EC50 < 5%(w/w) (original data 2.25%)	(ISO, 2018)
Plants	Acute	EN ISO 11269-2 (Determination of the effects of pollutants on soil flora - Part 2: Effects of contaminated soil on the emergence and early growth of higher plants)	EC50 < 15%(w/w) (original data 13.7%)	(ISO, 2013) (ISO, 2013) (ISO, 2013)
Soil Invertebrates	Acute	ISO 17512-1 (Avoidance test for determining the quality of soils and effects of chemicals on behavior. Test with earthworms)	EC50 < 5%(w/w) (original data 3.75%)	(ISO, 2020)

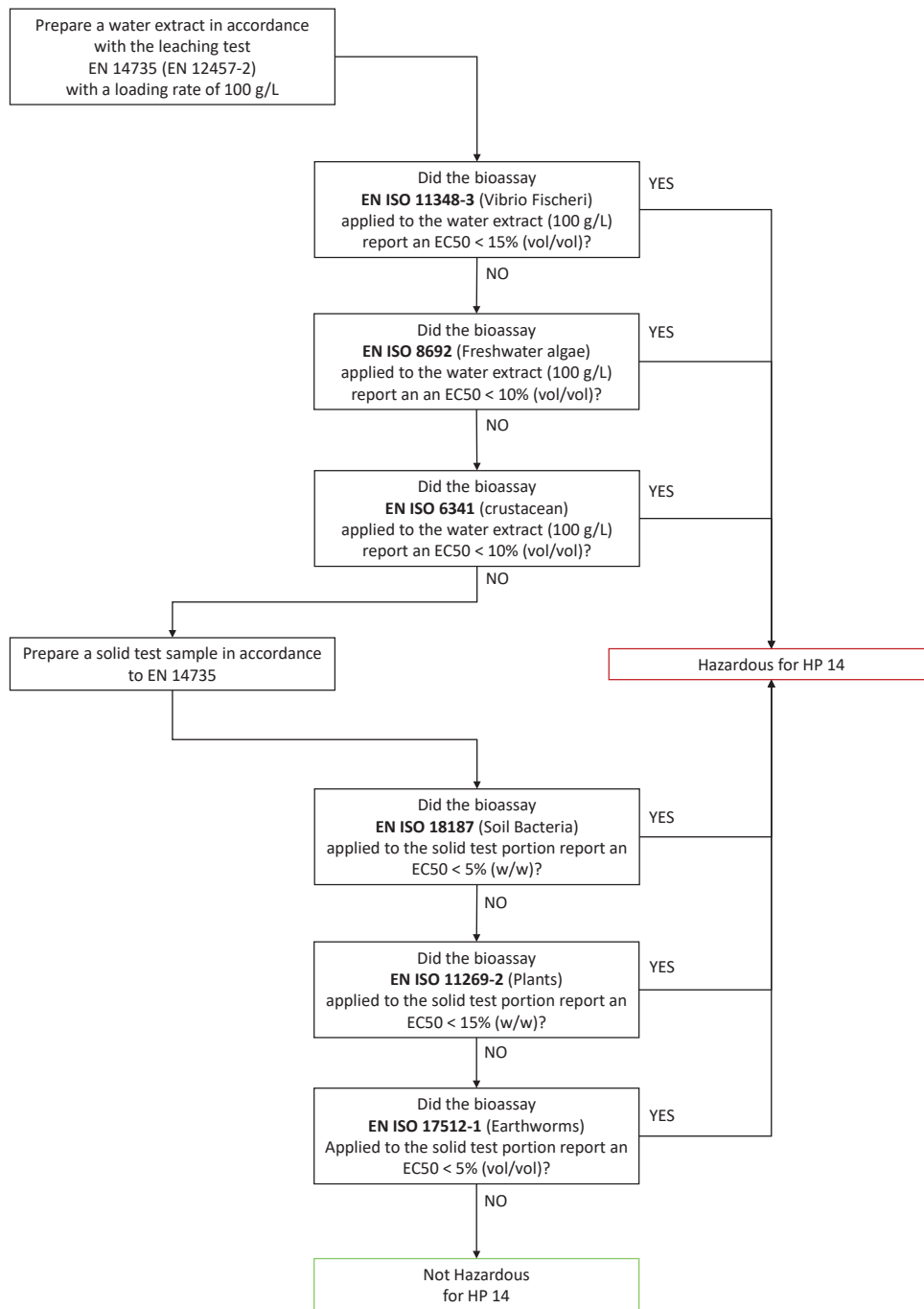


FIGURE 3: “Waste-based approach.” Proposed decision tree for HP 14 classification of waste according to the international standards proposed in Pandard and Römbke, (2013) and the classification limits established in Hennebert (2018, 2019).

is agreed upon by lab specialists. For instance, the six tests proposed for HP 14 are non-correlated, indicating different functional toxic actions in the living organisms. The choice of concentration limits or thresholds that trigger the hazard classification is a distinct subject. Limits must be harmonized and conventional, when dealing with intrinsic hazard and not with risk. They cannot be derived for a given scenario (i.e., emission-transfer-target) of a given use or fate of the waste. Site-specific scenarios are different, and the corresponding thresholds would be multiple too. Thus, the objective is not just to achieve a “zero

effect” but to consider an “acceptable” effect. The best solution is achieved by aligning the concentration limits or thresholds of the tests to match the resulting classification with an existing and well-established reference. This has been done for pH and buffering capacity for irritancy and corrosiveness by the UK industry of soaps and detergents (Young et al. 1988, 1994) and for HP 14 (Hennebert 2018). This approach is also valid when proposing ecotoxicity classification of fertilizers from the circular economy (e.g., digestates, composts). For this latter, an ecotoxic effects at the agronomic rate can be considered accept-

able if its results are not higher than that caused by millenary-used manure, organic matter, and some minerals at their respective agronomic rates. From this standpoint, a consensus can be reached, and the implementation of the regulations improved.

REFERENCES

- Bishop, I., Hennebert, P., 2021. Hazardous waste classification: Review of worst case to less worst case metal species with a worked example for a contaminated soil. *Detritus* 14, 4–24. <https://doi.org/10.31025/2611-4135/2021.14065>
- CEN, 2005. EN ISO 14735 - Preparation of waste samples for ecotoxicity tests.
- CEN, 2004. EN 12457-2 - Leaching - Compliance test for leaching of granular waste materials and sludges. Part 2: One stage batch test at a liquid to solid ratio of 10 l/kg for materials with particles size below 4 mm (with or without size reduction).
- ECHA, 2017. Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0. European Chemicals Agency, July 2017. <https://doi.org/10.2823/124801>
- European Commission, 2020. Circular economy action plan. *Eur. Comm.* 28. <https://doi.org/10.2775/855540>
- European Commission, 2018. Commission notice on technical guidance on the classification of waste. URL https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.C_.2018.124.01.0001.01.ENG
- European Commission, 2014. Commission Regulation 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. URL <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32014R1357>
- European Commission, 2000. Commission Decision of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of C.
- European Commission, Joint Research Centre, 2019. EURL ECVAM dataset on alternative methods to animal experimentation (DB-ALM). European Commission, Joint Research Centre (JRC) [Dataset] [WWW Document]. URL <http://data.europa.eu/89h/b7597ada-148d-4560-9079-ab0a5539cad3>
- European Council, 2017. Council Regulation 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'. URL <https://eur-lex.europa.eu/eli/reg/2017/997>
- European Council, 2008. Council Regulation 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).
- European Parliament and European Council, 2019. Regulation (EU) 2019/1021 of the European Parliament and of the Council of 20 June 2019 on persistent organic pollutants. URL <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32019R1021>
- Hareng, L., Pellizzer, C., Bremer, S., Schwarz, M., Hartung, T., 2005. The Integrated Project ReProTect: A novel approach in reproductive toxicity hazard assessment. *Reprod. Toxicol.* 20, 441–452. <https://doi.org/10.1016/j.reprotox.2005.04.003>
- Hennebert P. 2019. Hazard classification of waste: review of available practical methods and tools. *Detritus.* 07, 1-16. <https://doi.org/10.31025/2611-4135/2019.13846>
- Hennebert, P., 2018. Proposal of concentration limits for determining the hazard property HP 14 for waste using ecotoxicological tests. *Waste Manag.* 74, 74–85. <https://doi.org/10.1016/j.wasman.2017.11.048>
- Hennebert, P., van der Sloot, H.A., Rebuschung, F., Weltens, R., Geerts, L., Hjelmar, O., 2014. Hazard property classification of waste according to the recent propositions of the EC using different methods. *Waste Manag.* 34, 1739–1751. <https://doi.org/10.1016/j.wasman.2014.05.021>
- Hennebert P, Papin A, Padox J-M, Hasebrouck B. 2013. The evaluation of an analytical protocol for the determination of substances in waste for hazard classification. *Waste Management* 33 (2013) 1577–1588. <http://dx.doi.org/10.1016/j.wasman.2013.03.013>
- Hennebert P, Beggio G. 2021. Sampling and sub-sampling of granular waste: Part 1 - Size of a representative sample in terms of number of particles and application to waste containing rare particles in concentration. Submitted to *Detritus*.
- ISO, 2020. EN ISO 17512-1 - Soil quality. Avoidance test for determining the quality of soils and effects of chemicals on behaviour. Test with earthworms (*Eisenia fetida* and *Eisenia andrei*).
- ISO, 2019. ISO 5725-2 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.
- ISO, 2018. EN ISO 18187 - Contact test for solid samples using the dehydrogenase activity of *Arthrobacter globiformis*.
- ISO, 2013. EN ISO 11269-2 - Determination of the effects of pollutants on soil flora Part 2 : Effects of contaminated soil on the emergence and early growth of higher.
- ISO, 2012a. EN ISO 8692 - Water quality - Fresh water algal growth inhibition test with unicellular green algae.
- ISO, 2012b. EN ISO 6341 - Water quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera , Crustacea) - Acute toxicity test.
- ISO, 2008. EN ISO 11348-3 - Water quality - Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria.
- ISPRA, 2017. Rassegna dei Confronti interlaboratorio in ambito ecotossicologico: l'esperienza di ISPRA (2003-2016). <https://www.isprambiente.gov.it/it/publicazioni/rapporti/rassegna-dei-confronti-interlaboratorio-in-ambito-ecotossicologico-lesperienza-di-ispra-2003-2016>
- ISPRA. 2018. Approccio metodologico per la valutazione della caratteristica di pericolo HP14 - Ecotossico. (in Italian). Available at: https://www.isprambiente.gov.it/files2018/notizie/ApprocciometodologicoHP14ISPRA_100718.pdf
- Moser, H., Römbke, J., 2009. Ecotoxicological Characterization of Waste. Results and Experiences of an International Ring Test. Springer. <https://doi.org/10.1007/978-0-387-88959-7>
- OECD, 2020a. Test No. 437: Bovine Corneal Opacity And Permeability Test Method For Identifying i) Chemicals inducing Serous Eye Damage And ii) Chemicals Not Requiring Classification For Eye Irritation Or Serious Eye Damage.
- OECD, 2020b. Test No. 491: 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.
- OECD, 2019a. Global Material Resources Outlook to 2060: Economic Drivers and Environmental Consequences, OECD Publishing. Paris. <https://doi.org/10.1787/9789264307452-en>
- OECD, 2019b. Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage.
- OECD, 2018. Test No. 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants. (2018). <https://doi.org/10.1787/9789264076310-en>
- OECD, 2017. Test No. 460: Fluorescein Leakage (FL) Test Method for Identifying Ocular Corrosives and Severe Irritants.
- OECD, 2015. Test No. 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method. <https://doi.org/10.1787/9789264242753-en>
- OECD, 2013a. Test No. 122: Determination of pH, Acidity and Alkalinity. OECD Guid. Test. Chem. 1–6.
- OECD, 2013b. Test No. 439: In Vitro Skin Irritation - Reconstructed Human Epidermis Test Method. <https://doi.org/10.1787/9789264203884-en>
- OECD, 2013c. Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) 1–15. <https://doi.org/10.1787/9789264203808-en>
- OECD, 2013d. Test No. 210: Fish, Early-Life Stage Toxicity Test. Test No. 210 Fish, Early-Life Stage Toxic. Test 1–24. <https://doi.org/10.1787/9789264070103-en>
- OECD, 2012. Test No. 211: *Daphnia magna* Reproduction Test. <https://doi.org/10.1787/9789264070127-en>
- OECD, 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Guidel. Test. Chem. Sect. 2.
- OECD, 2006. Test No. 221: *Lemna* sp. Growth Inhibition Test.

- OECD, 2004. Test No. 202: Daphnia sp. Acute Immobilisation Test. OECD Guidel. Test. Chem. Sect. 2. <https://doi.org/10.1787/9789264069947-en>
- OECD, 2001. OECD Series on testing and assessment - Document nr. 29 - Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media. <https://doi.org/dx.doi.org/10.1787/9789264078451-en>
- OECD, 1992. Test No. 203: Fish, Acute Toxicity Test. OECD Guidel. Test. Chem. Sect. 2. <https://doi.org/https://doi.org/10.1787/9789264069961-en>
- Pandard, P., Römbke, J., 2013. Proposal for a "Harmonized" strategy for the assessment of the HP 14 property. *Integr. Environ. Assess. Manag.* 9, 665–672. <https://doi.org/10.1002/ieam.1447>
- Pivato, A., Beggio, G., Hennebert, P., Bonato, T., Favarin, M., Raga, R., 2020. Proposal of a testing program for the HP 14 (Ecotoxic) classification of automotive shredder residues (ASR) by a battery of ecotoxicological bioassays. *Detritus*. <https://doi.org/10.31025/2611-4135/2020.14032>
- SNPA, 2020. Linee guida sulla classificazione dei rifiuti. Delibera del Consiglio SNPA. Seduta del 27.11.19. Doc. n. 61/19.
- Young J R, How M J, Walker A P and Worth W M. 1988. Classification as corrosive or irritant to skin of preparations containing acidic or alkaline substances, without testing on animals. *Toxic. In Vitro*, Vol 2, No 1, pp. 19-26
- Young J.R., How M.J. 1994. Product classification as corrosive or irritant by measuring pH and acid/alkali reserve. B. *In Vitro Approaches, I. Physico-chemical Methods, Method BI-1*. In *Alternative Methods in Toxicology vol. 10 - In Vitro Skin Toxicology: Irritation, Phototoxicity, Sensitization*, eds. A.Rougier, A.M. Goldberg and H.I Maibach, Mary Ann Liebert, Inc. pp. 23-27.