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LESSONS FROM A LARGE DATASET OF AQUATIC ECOTOXICOLOGICAL TEST RESULTS FOR ROUTINE HP 14 CLASSIFICATION OF WASTES: THE CASE OF BOTTOM AND FLY ASHES IN THE ITALIAN FRAMEWORK

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INTRODUCTION

According to the European Waste Framework Directive (WFD), waste materials classified as hazardous are not suitable for recycling. In this context, an efficient and scientifically sound system for waste classification is crucial to achieve the ambitious goals of the European circular economy (European Parliament and European Council, 2018).

Wastes classified as "mirror entries" (i.e., wastes potentially classified as hazardous if containing hazardous substances above a specific level) on the European List of Waste (LoW, European Commission, 2000) require assessment of their specific Hazard Properties (HP). In this context, "Ecotoxicity" (i.e., HP 14) is acknowledged as the HP most frequently resulting from wastes classified as hazardous.

Current regulation states that HP 14 shall be used to classify wastes that can present or may present immediate or delayed risks for ecosystems (European Council, 2017). In practice, HP 14 can be assigned by an "indirect" approach, based on the total content of hazardous substances (selected according to "expert judgement"), or a "direct" approach, which relies on data from a battery of ecotoxicological laboratory tests. Considering toxic effects due to the speciation of hazardous substances in addition to possible mixture effects, the direct approach can provide more reliable assessments. For this reason, when performed, outcomes from bioassays will prevail over the results of chemical composition analyses.

The EU regulation does not indicate a detailed procedure for the direct test approach. Rather, it suggests that the methods indicated in Regulation 2008/440/EC (European Council, 2008) are compliant when used within the scope of the regulation on Classification Labelling and Packaging of products and substances, (CLP) (European Parliament and European Council, 2008), together with "other internationally recognized guidelines" (European Council, 2017). Among these latter, validated methods developed ad hoc for waste materials may vary from those used for assessing the ecotoxicity of products (i.e., CLP-related methods). These discrepancies, together with the lack of detailed instructions, fostered the development of an unharmonized analytical context within Member States, thus far hindering the use of the direct approach and the creation of a well-defined classification of mirror entries.

In Italy, the institutional/regulatory guideline for the performance of the direct approach is consistent with the Reg. 2008/440/EC and thus the CLP (SNPA, 2021).

The Italian Guideline allows only aquatic bioassays to be used with specific classification limits, as indicated in Table 1. Solid wastes require suitable leaching tests (ECHA, 2017) in which the solid waste sample preparation (i.e., particle size reduction and leaching conditions) is crucial to obtaining reproducible and reliable results. However, no specifications are provided for the treatment of materials containing substances and mixtures that are difficult to extract in aqueous solution, as is frequently the case for solid waste materials. These aspects are a source of variability in the HP14 classification, regardless of the total quantity and speciation of the solid phase chemicals in a waste (Stiernström et al., 2016).

Adopting product-related regulations, the Italian approach relies on the following technical guidelines for waste preparation and leaching test performance:

- OECD N.23 - Guidance document on aqueous-phase aquatic toxicity-testing of difficult test chemicals (OECD, 2019).
- OECD N.29 - Guidance document on transformation/dissolution of metals and metal compounds in aqueous media (OECD, 2001).

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TABLE 1: Battery of biotests and concentration limits used within the testing strategy complying with the CLP Regulation and SNPA (2021) (derived and modified from Beggio et al., 2021).

Organism	Type	Standard	Classification Criteria (waste is hazardous for HP 14 if)
Algae	Acute-Chronic *	OECD 201/2011 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test)	Acute LC50 ≤ 100 mg/L
	Chronic	OECD 221/2006 (Lemna sp. Growth Inhibition Test)	Chronic NOEC ≤ 1 mg/L
Crustacean	Acute	OECD 202/2004 (Daphnia magna, Acute Toxicity Test)	Acute LC50 ≤ 100 mg/L
	Chronic	OECD 211/2012 (Daphnia magna, Chronic Toxicity Test)	Chronic NOEC ≤ 1 mg/L
Fish	Acute	OECD 203/1992 (Fish, Acute Toxicity Test)	Acute LC50 ≤ 100 mg/L
		OECD 236/2013 (Fish, Acute Toxicity Test)	Acute LC50 ≤ 100 mg/L
	Chronic	OECD 210/2013** (Fish, Early-Life Stage Toxicity Test)	Chronic NOEC ≤ 1 mg/L

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

Alternative, internationally recognized guidelines are available, including similar aquatic tests, but with different leaching methods and, consequently, different limits (CEN, 2005; Moser and Römbke, 2009; Pandard and Römbke, 2013). Many authors (Bandarra et al., 2020; Tsiridis et al., 2012) have developed experimental designs that are more consistent with this approach than the CLP design. However, this comparison is beyond the scope of the current report (Beggio et al., 2021).

Two additional critical issues should be considered in the Italian framework. First, several methods are suggested by the guideline regarding the application of CLP criteria to obtain chronic endpoints (ECHA, 2017) but are not explicitly included in the list of methods validated for CLP or the Italian guideline for waste classification (European Parliament and European Council, 2008; SNPA, 2021). This fact still represents a limit because, from the definition of ecotoxicity, the assessment of delayed risks for ecosystems, in other words chronic effects, are needed.

Second, the OECD 236:2013 "Fish Embryo Acute Toxicity test" is suggested as an alternative test to the OECD 203/1992 without any comments on the validity of the method to replace the latter. Sobanska et al. (2018) explicitly affirm that OECD 236:2013 cannot be used as a direct "one-to-one" replacement for the AFT and thus cannot be used alone to meet the information requirement for short-term toxicity testing on fish (REACH Regulation, Annex VIII, 9.1.3). ECHA reached the same conclusions in 2016 (ECHA, 2016).

Among designated mirror wastes, those generated by the incineration of municipal solid waste (MSW), i.e., bottom ash (BA) and fly ash (FA), are of particular interest due to the large quantity of these materials generated yearly. In fact, European MSW production accounts for approximately 492 kg/year/inhabitant (7-10% of the total waste generated in 2018), and 60% of this waste is incinerated (Eurostat, 2020), in line with the recommended MSW management (Dri et al., 2018). Municipal solid waste incineration substantially reduces its volume by 90% (Cheng et al., 2010) by producing bottom ash (BA, approximately 200-300 kg/t of waste) and fly ash (FA, approximately 30-60 kg/t waste) (EU, 2015).

BA and FA can be classified as mirror entries according to the European LoW. Depending on their specific content, they could be classified with the specific entry codes: hazardous BA: 19 01 11* (hazardous) or 19 01 12 (not hazardous); FA: 19 01 13* (hazardous) or 19 01 14 (not hazardous).

Moreover, HP14 classification also has practical consequences regarding incineration plant authorization under the framework of the Seveso Directive (European Parliament, European Council, 2012). In fact, ashes are commonly stored in large quantities and for a long period in incinerator deposits, and if they are classified as dangerous, requirements for the Seveso Directive must be fulfilled. In accordance with the Seveso Directive, waste must be included in the calculation of the quantities of hazardous substances/mixtures for the purposes of verifying the applicability of the directive. In fact, Note 5 of Annex Directive 2012/18/EU states: "In the case of dangerous substances which are not covered by Regulation (EC) No 1272/2008, including waste, but which nevertheless are present, or are likely to be present, in an establishment and which possess or are likely to possess, under the conditions found at the establishment, equivalent properties in terms of major-accident potential, these shall be provisionally assigned to the most analogous category or named dangerous substance falling within the scope of this Directive". Table 2 reports the correspondence between CLP and Seveso classification.

The aim of the current paper was to report the results of routine classification outcomes from a large sample size of BA and FA derived from the results of both indirect and direct testing approaches and performed in compliance with the current Italian regulation. These data can be useful to a) define specific suggestions for laboratory procedures, b) provide a reference dataset for comparison to future data, and c) promote the use of bioassays for a more realistic HP14 classification of wastes.

TABLE 2: Comparison of Environment hazardous codes in CLP and Seveso regulations.

CLP Classification	Seveso Category
Aquatic Acute 1 H400	E1. Hazardous to the Aquatic Environment in Category Acute 1 or Chronic 1
Aquatic Chronic 1 H410	E1. Hazardous to the Aquatic Environment in Category Acute 1 or Chronic 1
Aquatic Chronic 2 H411	E2. Hazardous to the Aquatic Environment in Category Chronic 2
Aquatic Chronic 3 H412	-
Aquatic Chronic 4 H413	-

MATERIAL AND METHODS

179 samples of bottom ash (BA) and fly ash (FA) were collected from 20 different plants and assessed for routine HP14 classification by the "indirect" and "direct" approaches.

The indirect approach relied on the chemical characterization of collected samples. Total concentration data were used as input in the classification criteria laid down in the regulation 2017/997/EU (European Council, 2017). For the speciation of inorganic element concentrations a "worst case with information" approach was used.

For ecotest performance, sample preparation and leaching procedures followed the methods suggested by CLP regulations and Italian guidelines for waste classification (SNPA, 2021). Only acute ecotoxicological tests were performed on the eluates (see Table 1). Wastes showing an EC50 higher than 100 mg/L for each tested organism were evaluated as not hazardous. Conversely, wastes resulting in at least one EC50 lower than 100 mg/L among the performed bioassays were evaluated as ecotoxic and thus classified as hazardous for HP 14.

All analytical procedures were carried out at the "LabAnalysis" and ChemService laboratories (Italy).

RESULTS

According to the "indirect" approach, based on the measured total content of elements and substances already classified as ecotoxic in the CLP, approximately 81% (i.e., 130 of 161) and 89% (i.e., 16 of 18) samples of BA and FA, respectively, were classified as hazardous for HP 14.

Interesting considerations can be drawn from the outcomes of bioassays performed on samples already classified as hazardous by indirect approaches, which are graphically summarized in Figure 1.

Remarkably, all ecotests performed on fish resulted in EC50 values above 100 mg/L.

Among BA samples, the hazard classification by HP 14 was confirmed only for 15.4% (20 of 130) of the samples by the direct test approach. Among the results of single bioassays (Figure 1), the most sensitive test was the Daphnia magna acute toxicity test, with 13.1% (17 of 130) of the samples resulting in an EC50 lower than 100 mg/L, followed by the freshwater algae growth inhibition test, with 3.8% (5 of 130) of the samples showing an EC50 lower than 100 mg/L. There were 2 samples classified as toxic both for algae and daphnia.

In the case of FA, ecotoxicity was confirmed for 56.3% (9 of 16) of the samples by the performed bioassays, resulting in a waste stream with a higher probability of being classified as HP14 when compared to BA. Algae was the most sensitive organism, with 56.3% (9 of 16) of the samples having an EC50 lower than 100 mg/L, followed by the test on Daphnia magna, with 6.3% (1 of 16) of the samples having an EC50 lower than 100 mg/L. There was just 1 sample classified as toxic both for algae and daphnia.

No specific correlations were found among the different ecotoxicological tests.

Finally, a further issue could arise when controversial outcomes result from the performance of ecotests, which can classify a waste sample as hazardous when the same material was already classified as nonhazardous by the

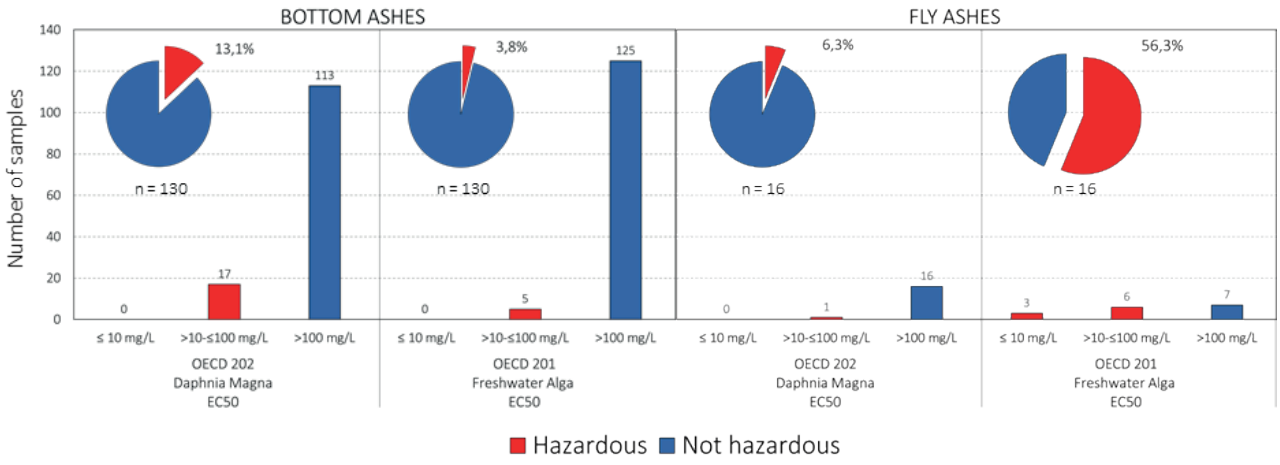

FIGURE 1: Results from the ecotoxicological tests (i.e., the "direct" approach) applied to samples of BA (n = 130 samples) and FA (n = 16 samples), already classified as hazardous for HP 14 by measured substances concentration (i.e., by the "indirect" approach).

indirect approach. In this case, outcomes from bioassays should prevail on the classification. In routine analysis, eco-tests are not usually performed on samples classified as nonhazardous, representing a further possibility to refine the classification designation. Therefore, this issue should be considered only from the perspective of establishing the mandatory performance of ecotoxicological tests.

Considering what has been reported here, a decision tree is proposed (see Figure 2) for HP 14 classification of waste. It is based on a sequence of ecotoxicological tests from the most sensitive to the least sensitive to optimize time and costs in the waste classification process. According to the results obtained, the most sensitive test is represented by the Daphnia magna acute toxicity test and the freshwater algae growth inhibition test for bottom ashes and fly ashes, respectively.

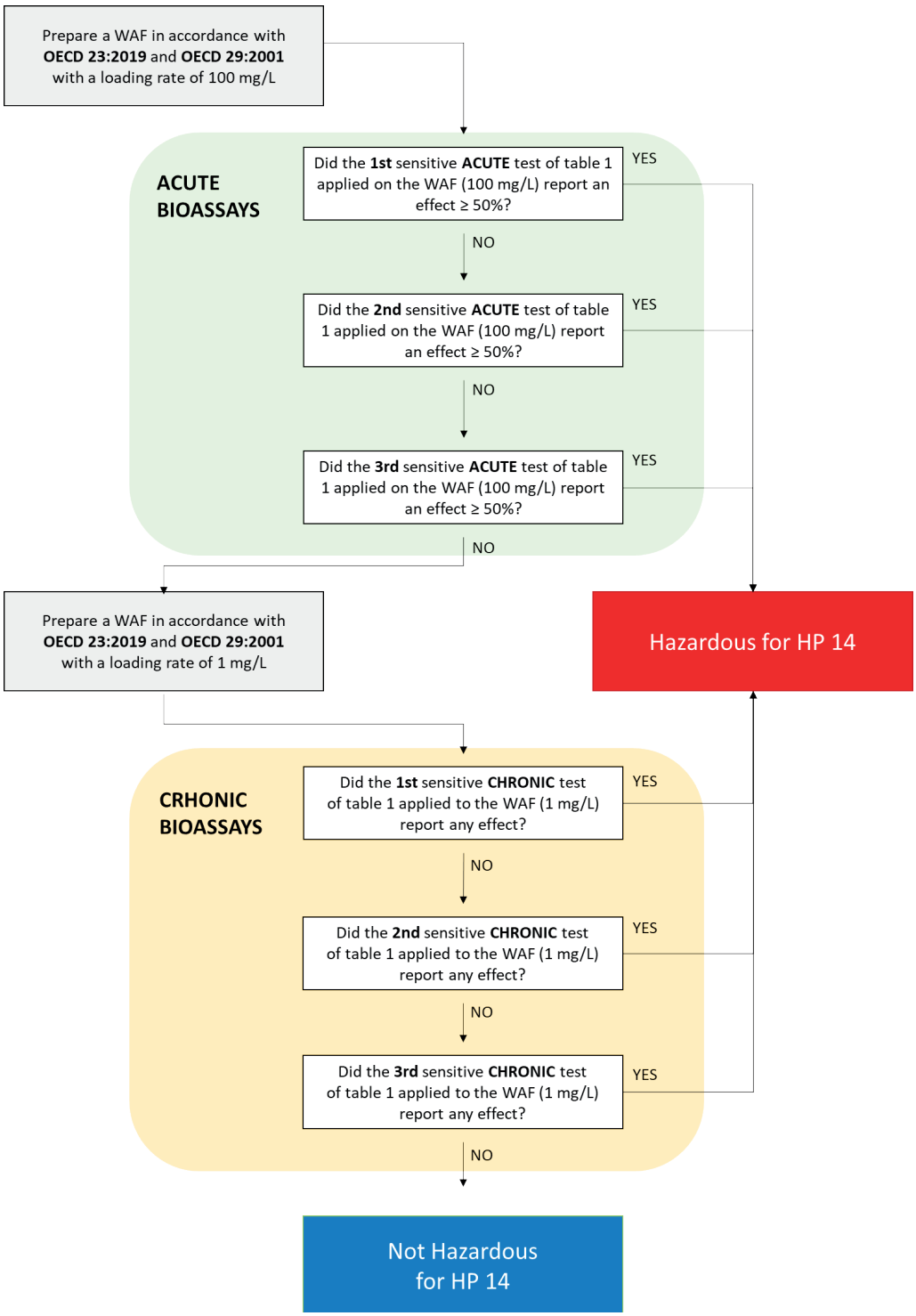

FIGURE 2: Proposed decision tree for HP 14 classification of waste with methods validated for CLP. WAF: Water Accommodated Fractions (derived and modified from Beggio et al., 2021).

CONCLUSIONS

Direct ecotoxicological tests for HP 14 assessment are useful to refine waste ecotoxicity assessments based only on chemical analysis, since variables such as bioavailability of the substances, their interactions, and their ecotoxicological effects on live organisms are immediately evaluated in the results of bioassays.

This work reports findings derived from a large dataset of results from bioassays applied on BA and FA samples that were already classified as hazardous according to their chemical composition. In particular, both water extract preparation and biotest batteries were completely compliant with Italian regulations. Based on the ecotest outcomes, the majority of samples could be reclassified as nonhazardous for HP 14. In addition to being used for future comparison analysis, with the correct awareness, this contribution could help to identify outlier results.

Moreover, the decision tree proposed here for HP 14 classification of wastes can be adopted for the classification of similar materials and optimizing the validity of the classification process. To optimize both time and costs, the decision tree recommends performing ecotoxicological tests from the most sensitive to the less sensitive and including both acute and chronic biotests.

This short communication contributes scientific data to the discussion around the topic of hazardous waste classification and promotes the use of direct ecotoxicological bioassays for the purpose of waste classification within the waste community.

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