Improving the Ecotoxicity Assessment of Waste from Mirror Entries: Evaluating the Suitability of a Biotest Battery

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Introduction

Waste from mirror entries in the European List of Wastes (2000/532/EC)

Allocation to:
- Hazardous mirror entry* or
- Non-hazardous mirror entry

Hazard property (HP) 14 (ecotoxic)

Bioassays prevail (EU 2017/997), but:
- Very limited guidance at EU level (types of bioassays, test design, limit concentration)
- Different approaches in EU member states (e.g. German recommendations, UBA 2013)

Calculations based on the concentrations of the waste constituents (EU 2017/997)

Objectives

- Compare bioassay-based approaches for HP 14 classification in Europe
- Review the test strategy proposed in the recommendations of the German Environment Agency (UBA 2013) based on sampling, sample preparation and ecotoxicological testing of 10 waste samples from mirror entries
- Elaborate proposals for an update and further development of the UBA recommendations

The present poster focuses on the ecotoxicological testing of the waste samples.

Methods

Waste samples

- 10 Waste samples from 3 mirror entries (Fig. 1, Tab. 2)
- Waste sampling in accordance with CEN/TR 15310-1 and German LAGA PN 98
- Waste samples sieved to < 2 mm for the terrestrial microbial test and < 4 mm for all other tests

10 09 09*/10 09 10: Flue-gas dust

17 05 03*/17 05 04: Soil and stones

19 10 04: Fluff-light fraction and dust

Fig. 1: Examples of the tested waste samples

Test battery

- The used test battery (Tab. 1) corresponds to the recommendations of UBA (2013)
- Exception: algal growth inhibition test in microtiter plates (DIN 38412-59) instead of ISO 8692 (Fig. 2)

Elution

- One-stage batch procedure, 10 L/kg waste dry weight, 24 h (EN 12457-2, EN 14735)

Aquatic bioassays

- With ≥ 5 dilutions of the eluate (50, 25, 12.5, 6.3 and 3.1%)
- No pH-adjustment in tests used for HP 14 classification
- Up to 3 test runs per waste sample to evaluate reproducibility

Terrestrial bioassays

- With 5 dilutions of solid waste (25, 12.5, 6.3, 3.1 and 1.6%)
- One test for each waste sample

Classification as ecotoxic (HP 14)

- If at least one EC_{50} was ≤ 10% eluate content in test medium or waste content in test substrate

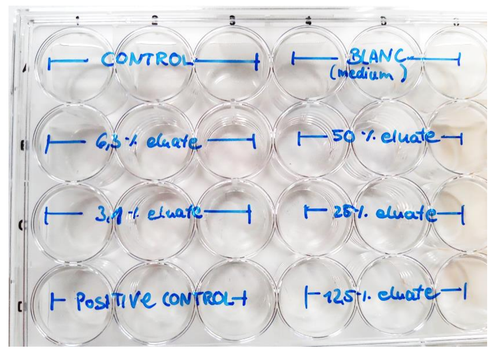

Fig. 2: 24-Well microtiter plate

Tab. 1: Ecotoxicological test battery

Test species	Test endpoint	Test guideline	Test duration
Aquatic bioassays			
Daphnia magna	Immobility	ISO 6341	48 h
Raphidocelis subcapitata	Growth	DIN 38412-59	72 h
Aliivibrio fischeri	Light emission	ISO 11348-2	30 min
Terrestrial bioassays			
Arthrobacter globiformis	Dehydrogenase activity	ISO 18187	6 h
Brassica rapa	Emergence and early growth	ISO 11269-2	14 d
Eisenia fetida	Avoidance behaviour	ISO 17512-1	48 h

Results

Tab. 2: Results of the aquatic and terrestrial tests and resulting HP 14 classification (results of tests without pH adjustment)

Waste code[a]	Waste sample		Classification based on testing	Aquatic tests: EC_{50} (% eluate)								Terrestrial tests: EC_{50} (% waste)		
				D. magna		*R. subcapitata*			*A. fischeri*			*A. globiformis*	*B. rapa*	*E. fetida*
10 09 09*	Flue-gas dust	Batch 1	**Ecotoxic**	5.45	4.26	< 3.1	< 0.4	**0.201**	> 25			1.08	1.66	1.86
		Batch 2	**Ecotoxic**	32.8	19.8	< 3.1	**0.913**		> 25			1.03	3.93	4.49
10 09 10		Plant A	**Ecotoxic**	5.53	**< 3.1**	5.21	**< 3.1**		> 50	> 25		> 25	23.4	21.9
		Plant B	**[Ecotoxic]**	> 50	> 50	43.5	> 50		> 50			**[7.56[b]]**	> 25	10.8
17 05 03*	Soil and stones	Excavated geogenic material	**Ecotoxic**	3.49	**3.15**	7.85	7.77		22.9	> 25		> 25	15.1	7.36
		Material from the side verges of a federal road	**Not ecotoxic**	> 50	> 50	> 50	> 50		> 50	> 50		> 25	> 25	> 25
17 05 04		Material from the side verges of a secondary road	**Not ecotoxic**	> 50		> 50			> 50			> 25	> 25	> 25
19 10 04	Fluff-light fraction and dust	Plant A, batch 1	**Ecotoxic**	< 3.1	**0.678**	< 3.1	1.16		4.08	> 0.8	> 3.1	8.20	8.20	2.94
		Plant A, batch 2	**Ecotoxic**	< 3.1	0.818	< 3.1	**0.287**		23.5	19.7		6.16	7.59	4.53
		Plant B	**Ecotoxic**	> 50	> 50	13.0	17.3		**7.11**	9.52		11.7	13.5	9.61

Highlighted values: most sensitive tests. [a] The classification by the waste owner (red = hazardous, yellow = non-hazardous) was not necessarily based on the HP 14 criterion. [b] Formally not valid due to lack of effect of the reference substance in the Lufa 2.2 soil, but clear effect in the positive control with quartz sand.

Conclusions

- Generally, the test battery recommended by UBA (2013) is well suited for the testing of waste samples from mirror entries.
- However, in the test with *A. globiformis* and heterogeneous waste, variability of the results was often high, most likely due to the small amounts of waste used. Possible alternatives for this test should be further evaluated.
- The aquatic tests are highly reproducible (Tab. 2; reproducibility of the terrestrial tests was not evaluated).
- In most cases, algae and daphnids were more sensitive than luminescent bacteria. The terrestrial tests tended to be slightly less sensitive than the aquatic tests.
- The algal growth inhibition test in microtiter plates showed a high sensitivity. It offers practical advantages (e.g. faster fluorescence measurement compared to ISO 8692).
- The high ecotoxicity of the samples of fluff-light fraction and dust (19 10 04) deserves further attention.
- Suggestions were developed how to update and further develop the German ‘Recommendations for the ecotoxicological characterization of waste’.

References and acknowledgement

UBA (2013). Recommendations for the ecotoxicological characterization of wastes. German Environment Agency.

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