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# Ecotoxicological evaluation of construction products: inter-laboratory test with DSLT and percolation test eluates in an aquatic biotest battery

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## Abstract

**Background:** A European inter-laboratory test with 29 participating laboratories investigated whether a battery of four ecotoxicological tests is suitable for assessing the environmental compatibility of construction products. For this purpose, a construction product was investigated with the dynamic surface leaching test (DIN CEN/TS 16637-2) and the percolation test (DIN CEN/TS 16637-3). The eluates were produced centrally by one laboratory and were tested by the participants using the following biotests: algae test (ISO 8692), acute daphnia test (ISO 6341), luminescent bacteria test (DIN EN ISO 11348), and fish egg test (DIN EN ISO 15088). As toxicity measures,  $EC_{50}$  and LID values were calculated.

**Results:** Toxic effects of the eluates were detected by all four biotests. The bacteria test was by far the most sensitive, followed by the algae test and the daphnia test; the fish egg test was the least sensitive for eluates of both leaching tests. The toxicity level of the eluates was very high in the bacteria, daphnia, and algae test, with lowest ineffective dilution values of  $LID = 70$  to  $LID = 13,000$  and corresponding  $EC_{50}$  values around or even below 1 volume per cent. The reproducibility (*approximated by interlaboratory variability*) of the biotests was good (< 53%) to very good (< 20%), regardless of the toxicity level of the eluates. The reproducibility of the algae test was up to 80%, and thus still acceptable.

**Conclusion:** It can be confirmed that the combination of leaching and ecotoxicity tests is suitable to characterize with sufficient reproducibility the environmental impact posed by the release of hazardous substances from construction products.

**Keywords:** Inter-laboratory test, Construction products, Leaching tests, Eluates, Ecotoxicity tests, Grouts

## Background

Construction products that are exposed to rain, seepage water, or groundwater during their service life may release hazardous substances through leaching. To assess the potential impacts of these emissions on the environment, both chemical analysis [1, 2] and ecotoxicity tests

are applied [3–6]. The rationale for applying ecotoxicity tests with leachates is that the identification of hazardous substances by chemical analysis may not cover all contaminants, especially when assessing organic substances for which no reference or limit values for water quality exist. As a tool supplementing chemical analyses, bioassays detect the joint impacts of ingredients by their effects on living organisms. Under a mandate from the European Commission, the Technical Committee CEN/TC 351 “Construction Products—Assessment of release

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of *dangerous substances*” has developed several leaching tests (CEN/TS 16637 part 1–3, conversion to DIN EN expected in 2021 [7–9]) and the “Guidance on the use of ecotoxicity tests applied to construction products” (CEN/TR 17105 [10]). The inter-laboratory test comprised two different leaching methods, the horizontal dynamic surface leaching test (DSLTL) according to CEN/TS 16637-2 [8] and the horizontal up-flow percolation test according to DIN CEN/TS 16637-3 [9]. The DSLTL (CEN/TS 16637-2) [8] is a tank test for assessing the release of dangerous substances from monolithic or plate-like construction products while the up-flow percolation test (CEN/TS 16637-3 [9]) is a leaching test that can be used to determine the leaching behaviour of inorganic and non-volatile organic substances from granular construction products. This toolbox provides a basis for the harmonization of product standards at the European level.

Harmonized product standards and European assessment documents are foreseen under the EU Construction Products Regulation (CPR [11]) to remove technical trade barriers in the common market of the EU. Different test methods in different countries as a prerequisite for entering the market are trade barriers from the viewpoint of manufacturers. Thus, harmonization of test methods is one of the key goals of the CPR. So far, test methods for the environmental performance of construction products has not been a priority, but now the situation is changing. In the currently planned revision of the CPR, environmental aspects, like ecotoxicity, are tackled prominently. To ensure fairness for the manufacturers and reliability for the users the test methods under CPR deserve a thorough validation. This paper describes the challenges and results of the current work carried out for validation of ecotoxicological testing of construction products.

First tests provided by CEN/TC 351 have been put into practice. For several groups of construction products, technical specifications are defined in European Assessment Documents (EAD), which enable manufacturers to declare product performance including data gained from leaching and ecotoxicity tests (EOTA 2021 [12]). Recently, new award criteria for the German Blue Angel ecolabel for concrete products containing recycled aggregates for outdoor flooring (DE-UZ 216, January 2021 [13]) have been published, including the combination of leaching with ecotoxicity testing. For application in a regulatory context, both leaching and ecotoxicity tests must be fit for purpose and provide reproducible results. For this, a previous inter-laboratory test was organized in the year 2015 to assess the reproducibility of the overall process, including preparation of eluates and bioassays for the ecotoxicological characterization of construction products [14]. To this end, two construction products, both made of EPDM polymers (rubber), were eluted by each

participant in the one-stage batch test (DIN EN 12457-1, granular product [15]) or dynamic surface leaching test (DSLTL, CEN/TS 16637-2, sheet-like product [8]). A total of 17 laboratories from five countries participated in the round robin test using a biotest battery of four standardized ecotoxicity tests with algae, daphnia, luminescent bacteria, and zebrafish eggs. For the very toxic eluate from the granular product, a relatively high variability with a coefficient of variation (CV) of 73% to 110% was observed for  $EC_{50}$  in all biotests, while for the less toxic eluate from the sheet-like product, the inter-laboratory variability of  $EC_{50}$  was very good (9.3% to 36.4%). Considering the complex overall process, including the elution step performed by the participating laboratories, the reproducibility of bioassays with eluates from construction products was confirmed to be acceptable.

However, for granular products, CEN/TR 17105 [10] refers to the horizontal up-flow percolation test (CEN/TS 16637-3 [9]) as the preferred leaching test, while CEN/TS 16637-1 [7] mentions the one-stage batch test only as an indirect method that can be applied as a simplified procedure for production control purposes. The reason for choosing the one-stage batch test in the first inter-laboratory test in 2015 was that, at that time, the test conditions of the up-flow percolation tests were still not fully agreed upon. CEN/TS 16637-3 [9] was published in 2016.

Therefore, another inter-laboratory test reported on here was organized by combining the up-flow percolation test and the DSLTL, respectively, with ecotoxicity tests, using a grout made of mineral components and an epoxy resin binder as the sample under investigation. In contrast to the previous inter-laboratory test, this time the elution was centrally performed in one laboratory (BAM, Federal Institute of Materials Research and Testing), and eluates were distributed to the participating laboratories. The objective was to obtain quantitative figures about the reproducibility of data obtained from ecotoxicity testing of eluates and to enlarge the data basis.

## Materials and methods

### Participating laboratories and the organization of the inter-laboratory test

A total of 29 laboratories from nine countries participated in the inter-laboratory test that was performed in 2020: Austria (1), Belgium (2), Czech Republic (1), France (1), Germany (18), Italy (1), Lithuania (1), Portugal (1), and Switzerland (3). The laboratories belong to governmental institutes, contract laboratories, research institutes, and universities (Table 1). The laboratories are anonymized by laboratory codes L01–L32 (in total, 32 laboratories registered for the inter-laboratory test, of which 29 laboratories submitted data). Most laboratories maintain a quality assurance system. The biotests for this

**Table 1** List of participants in alphabetical order (not related to laboratory codes)

	DE	INERIS	FR
AKS—Aqua-Kommunal-Service GmbH—Labor			
Analysen Service GmbH Privates Institut für Umweltanalytik	DE	ISSeP—Laboratory of Ecotoxicology	BE
Analytisches Labor der SGL mbH	DE	Laboratory of Aquatic Ecotoxicology, Institute of Botany, Nature Research Centre (NRC)	LT
aQuaTox-Solutions	CH	Landesamt für Natur, Umwelt und Verbraucherschutz NRW	DE
Arcadis Schweiz AG—Ökotoxikologie Labor	CH	Landesanstalt für Umwelt Baden-Württemberg, Referat 23	DE
BASF SE	DE	Lenzing Aktiengesellschaft—Prüfstelle UAL	AT
Bayerisches Landesamt für Umwelt (LUB), Ref. 73	DE	Microbiotests Inc	BE
BioChem agrar GmbH	DE	Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz	DE
Bioplan GmbH	DE	SGS Institut Fresenius GmbH—Labor Ecotox	DE
Bundesanstalt für Gewässerkunde	DE	SOLUVAL	CH
ECT Oekotoxikologie GmbH	DE	T. G. Masaryk Water Research Institute	CZ
Eurofins Biolab S.r.l	IT	Umweltanalytisches Laboratorium (ISA RWTH Aachen)	DE
Hydrotox GmbH	DE	University of Aveiro	PT
Hygiene-Institut des Ruhrgebiets, Institut für Umwelthygiene und Toxikologie	DE	WESSLING GmbH	DE
IDUS—Biologisch Analytisches Umweltlabor GmbH	DE		

inter-laboratory test, however, did not need to be subjected to these systems. A high quality of the data set was assured by data consolidation before statistical evaluation (see the section “[Evaluation of the inter-laboratory test](#)” for detailed information).

The inter-laboratory test was organized by the Hydrotox GmbH laboratory ([www.hydrotox.de](http://www.hydrotox.de)), supported by the Federal Institute for Materials Research and Testing (BAM), which prepared the eluates and distributed them to the participating laboratories. The work was part of a research project on the ecotoxicological characterization of construction products funded by the German Environment Agency (Project number FKZ 3719 37 302 0). The required conditions for the sample pre-treatment and performance of bioassays were described in a detailed study plan. Participants were provided with templates for reporting data from the ecotoxicity tests and accompanying test protocols to document all relevant test parameters.

### Construction product

As construction product, a pavement joint grout (anonymized with MOE1) was used consisting of two components, a mineral component and a liquid binder (epoxy resin). Under standard use conditions, water can make contact with the surface of the grouted joints, but also penetrate them, as the product is water-permeable. These exposure routes were considered by eluting the grout in both types of leaching test, as a planar specimen in the DSLT and as a granular

product (size reduced to  $\leq 1$  cm particles) in the percolation test. The selection of the construction product MOE1 was based on the results of a broader testing programme with more than 20 construction products from different product types, which is still not completed (publication in preparation). Here, MOE1 showed clear ecotoxicity effects in the laboratory of Hydrotox GmbH. Another objective was to directly compare different elution procedures to address both exposure routes.

Preparation of test specimens was carried out by BAM. Both components of the grout were mixed according to the manufacturer’s instructions in the technical data sheet and dried for at least the given minimum duration. The mixture was poured into prepared moulds (25 cm \* 14 cm \* 0.7 cm internal dimensions). To prevent the grout from sticking to the mould, it was lined with cling film beforehand. In total, four test specimens were prepared to be able to produce enough eluate volume in the DSLT for all participants of the inter-laboratory test. After the test specimens had cured for 4 days under ambient air conditions, they were removed from the moulds and then conditioned for 38 days at  $21.5 \pm 1.0$  °C,  $60 \pm 5\%$  relative moisture. The rest of the mixture dried under ambient conditions for 27 days and was gently size-reduced (particles < 1 cm) in order to enable filling of the glass columns. Four glass columns (ID 10 cm, length 44 cm) containing 2.4 to 2.7 kg of the particles were prepared

for the percolation test to obtain sufficient eluate for the round robin test.

### Leaching tests

For the DSLT, the test specimens were placed in an all-glass aquarium and exposed to deionized water (conductivity  $< 5 \mu\text{S cm}^{-1}$ ) at a liquid to surface area ratio (L/A) of  $25 \text{ L m}^{-2}$  ( $2.5 \text{ mL cm}^{-2}$ ) which is close to the lower limit of the DSLT ( $20 \text{ L m}^{-2}$ ). The test was performed in the dark at  $20\text{--}22 \text{ }^\circ\text{C}$ . Following the standard procedure, this test is carried out for 64 days. The eluent is replaced at distinct time intervals to obtain a total of eight eluate fractions. For the inter-laboratory test, only the first two sampling events after 6 h and 24 h were carried out, and the two eluate fractions were combined to equal parts from the four test specimens to produce one eluate sample for the inter-laboratory test.

Granular construction products are subjected to percolation with deionized water (conductivity  $< 5 \mu\text{S cm}^{-1}$ ) as a function of liquid to solid ratio (L/S) under specified percolation conditions. In the standard procedure, seven eluates are collected with the L/S ratios 0.1, 0.2, 0.5, 1, 2, 5, and 10. The accumulated eluates at a L/S ratio of 2 L/kg was combined from the four columns and taken as the sample for the inter-laboratory test.

The eluates produced centrally at BAM were investigated for conductivity, pH, turbidity (according to DIN EN ISO 7027 [16]), total organic carbon (TOC), and total organic nitrogen (TN) (according to DIN EN 1484 [17]) immediately after sampling. For the participants, the two eluates (DSLIT eluate and PERC eluate) were aliquoted into 50 mL PP (polypropylene) containers (for algae, daphnia, luminescent bacteria, and umu test) and 150 mL PET (polyethylene terephthalate) containers (for fish egg test) and frozen at  $\leq -18 \text{ }^\circ\text{C}$ . The eluates were shipped in frozen condition to the participating laboratories that stored the samples at  $\leq -18 \text{ }^\circ\text{C}$  until the start of the ecotoxicity tests, which had to be performed within 2 months.

For the DSLIT, the blank control was run with deionized water (Milli-Q<sup>®</sup>) in an additional leaching vessel with the same treatments as for the test with the construction product. A sample of the test water (Milli-Q<sup>®</sup>) that was used for the percolation test was taken as a blank control.

### Ecotoxicity tests

The participants conducted a battery of four standardized ecotoxicity tests with algae, daphnia, zebrafish eggs, and luminescent bacteria. The testing strategy was in line with the technical guidance on the use of ecotoxicity tests applied to construction products [10].

The algae growth inhibition test was carried out according to ISO 8692 (2012) [18] with the algae species *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*). The test design included the following parameters: initial cell density was  $\leq 10^4$  algae  $\text{mL}^{-1}$  (nominal or measured start concentration), 3 replicates per dilution and 6 per control, the light intensity should be in the range of  $60\text{--}120 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , the pH of the eluate samples was not adjusted, as only high dilutions of the eluate samples were tested (and the pH values were not very high, see Table 2). The inhibition of growth was determined after 72 h via measurements of algae cell counts or chlorophyll fluorescence.

The acute daphnia toxicity test was applied according to ISO 6341 (2012) [19] with *Daphnia magna* using synthetic dilution water. The pH was only adjusted to the pH of the synthetic dilution water when it was outside pH 6–9. The test design consisted of 2 to 4 beakers per concentration with 5 daphnids each (Annex F of ISO 6341 [19]). The mobility of the daphnids was evaluated after 24 h and 48 h. The participants were asked to report the results of a sensitivity check with potassium dichromate (valid range  $0.6\text{--}2.1 \text{ mg L}^{-1}$ ).

The luminescent bacteria test was realized according to ISO 11348 part 1–3 (2007) [20]. Preferably, freshly grown luminescent bacteria (part 1) or liquid-dried luminescent bacteria (part 2) should be used, but freeze-dried luminescent bacteria (part 3) were also accepted. The evaluation of the decrease of luminescence of the marine bacterium *Aliivibrio fischeri* (formerly *Vibrio fischeri*) was determined after an exposure time of 30 min. Adjustment of the pH was not required, because only very high dilutions of the eluate samples were tested. The test design considered two replicates per concentration. The sensitivity of the bacteria batch used should be checked with reference substances (by the supplier or by one's own measurements).

The fish egg test was carried out according to ISO 15088 (2007) [21]. The pH of the eluates was adjusted to  $7.0 \pm 0.2$ . The fertilized eggs were exposed in 24-well cell culture plates at  $26 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ . The study design comprised 10 replicates (wells) per concentration, four internal negative controls per plate, an additional external negative

**Table 2** Chemical parameters of eluates

	DSLIT eluate	PERC eluate
pH	9.0	9.2
Conductivity ( $\mu\text{S cm}^{-1}$ )	150	957
Turbidity (FNU)	14	6
TOC ( $\text{mg L}^{-1}$ )	267	2114
TN ( $\text{mg L}^{-1}$ )	46	350

control, and a positive control with 10 replicates each. The evaluation after 24 h and 48 h ( $\pm 30$  min) considered the endpoints “coagulated eggs”, “no tail detachment”, and “no heartbeat”.

Further, the umu test, a genotoxicity test with the bacterial strain *Salmonella typhimurium* TA1535/pSK1002, was also performed, following ISO 13829 (2000) [22]. The bacteria were exposed for 2 h to the eluates with and without metabolic activation (with or without S9), followed by a growth phase of 2 h, and the genotoxin-dependent induction of the umuC gene was compared with the spontaneous activation of the control culture. The result given is the smallest dilution step at which an induction rate  $< 1.5$  is measured. However, as only five laboratories performed the umu assay within the inter-laboratory test, a statistical analysis of the results was not deemed meaningful, and results are only qualitatively summarized.

#### Evaluation of the inter-laboratory test

The participants received Excel and Word templates for raw data documentation. The statistical evaluation was performed centrally according to ISO 5725-2 (2019) [23], using Microsoft Excel® and the statistical software ToxRat® Professional 3.3.0 (ToxRat Solutions GmbH & Co. KG, Alsdorf, Germany).

As a first step, the database was checked for compliance with the required experimental conditions (e.g. test design, temperature, pH) and the validity criteria of the respective guidelines. The validity criteria refer to the maximum mortality in the controls (daphnia and fish egg test), the effects observed with a reference substance (all tests), the oxygen content (daphnia test, fish egg test), the growth rates and variability of the controls (algae test), the variability of replicates and correction factors (luminescent bacteria), and the pH shift (algae test).

For the valid data,  $EC_{50}$  values (volume percentage causing 50% effects) and LID values (lowest ineffective dilutions) were determined as measures of toxicity. The  $EC_{50}$  values were calculated with ToxRat Professional via non-linear regression (algae test) or linear regression with Probit analysis (daphnia, bacteria, fish eggs). If no  $EC_{50}$  could be determined with Probit analysis, the Weibull function or moving average was used instead. The LID corresponds to the lowest dilution factor  $D$ , at which effects below the specific threshold were determined. The following effect threshold values were applied for the LID: algae test 5%; daphnia test 10%; fish egg test 10%; and bacteria test 20%.

According to DIN ISO 5725-2 [22], a systematic outlier analysis should be performed including the Cochran test, Mandels- $k$  statistics, Mandels- $h$  statistics, and the Grubbs test [24]. However, the first two methods are

based on intra-laboratory variance and could not be applied, since in the present inter-laboratory test, no repeated measurements were performed. Thus, Mandels- $h$  statistics and the Grubbs test [24] were used. Both tests require normal distribution, so they were performed with log-transformed data. To make use of a third measure, the data were additionally checked for values beyond the 95% and 99% tolerance limits (warning charts concept, Guidance Document of Environment Canada (2005) [25]) (see Figs. 1 and 2). Data identified as outliers on the 5% significance level are called stragglers, data identified as outliers on the 1% level are called statistical outliers. Test results that were classified as statistical outliers with at least one of the three methods were excluded from further evaluations.

After data consolidation, the round robin test statistics were carried out. Because of the logarithmic characteristics of  $EC_{50}$  and LID values, all data were log-transformed ( $Y = \ln(X)$ ) prior to the following calculations:

$$\text{Mean value } \mu = y_{-} = \frac{\sum_{i=1}^n y_i}{n},$$

$$\text{Standard deviation } \sigma = s_y = \sqrt{\frac{\sum_{i=1}^n (y_i - y_{-})^2}{n - 1}}.$$

It should be noted that to calculate standard deviation according to ISO 5725-2 [23], the data measured by each laboratory should be weighted depending on the numbers of repeated measurements. Since in the present inter-laboratory test no repetitions were performed, the simplified formula was applied.

By re-transformation (anti-log) of  $\mu$  and  $\sigma$  using the formulas for log-normal distribution, the following statistical characteristics are obtained for the original scale  $X$ :

$$\text{Geometric mean} = \text{EXP}(\mu),$$

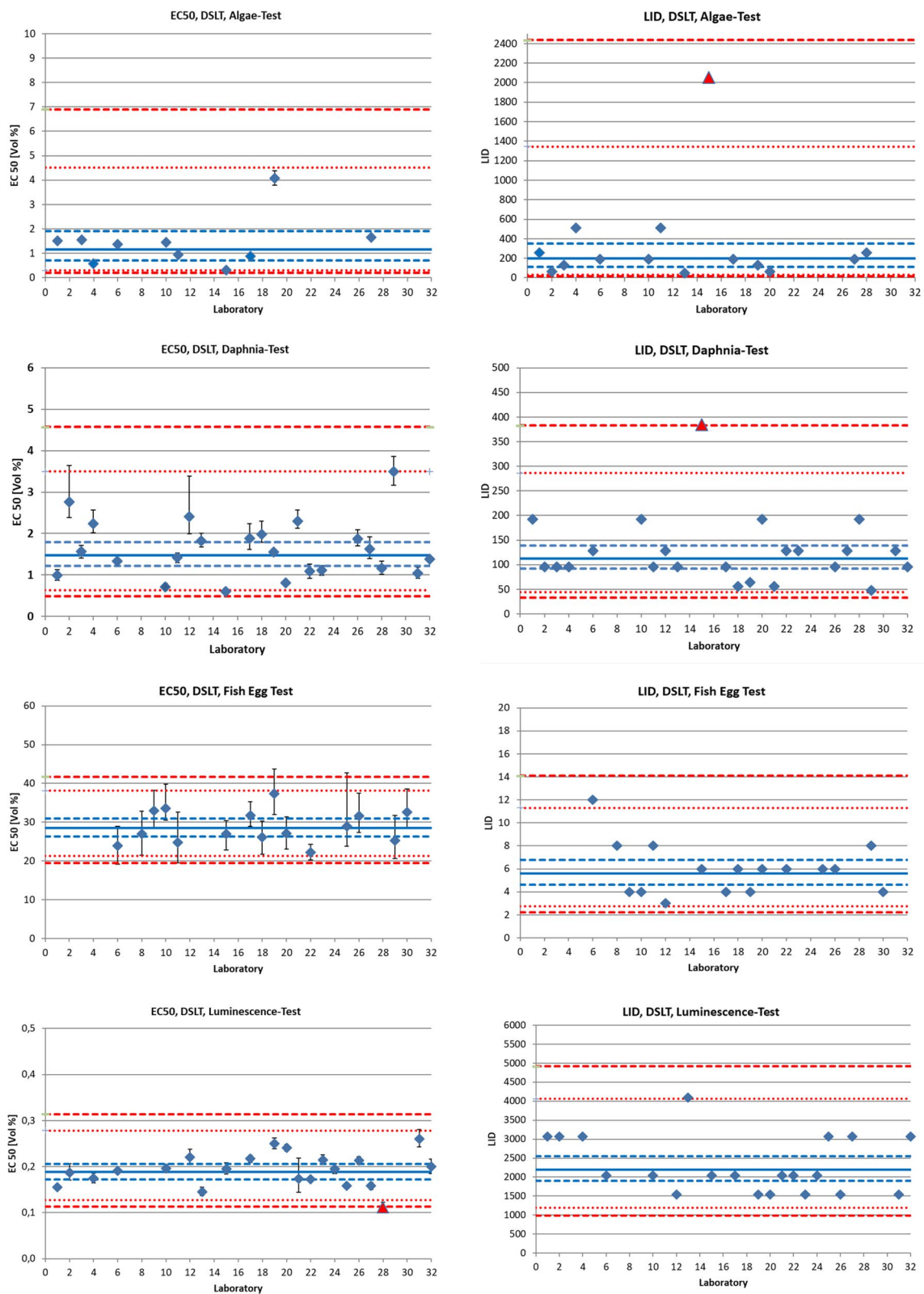
$$95\% \text{ confidence limit} = \text{EXP}\left(\mu \pm \frac{\sigma}{\sqrt{n}} * t(0.05; n - 1)\right),$$

$$95\% \text{ or } 99\% \text{ tolerance limits} = \text{EXP}(\mu \pm \sigma * z) \\ \text{with } z = 1.96 \text{ for } 95\%; z = 2.57 \text{ for } 99\%,$$

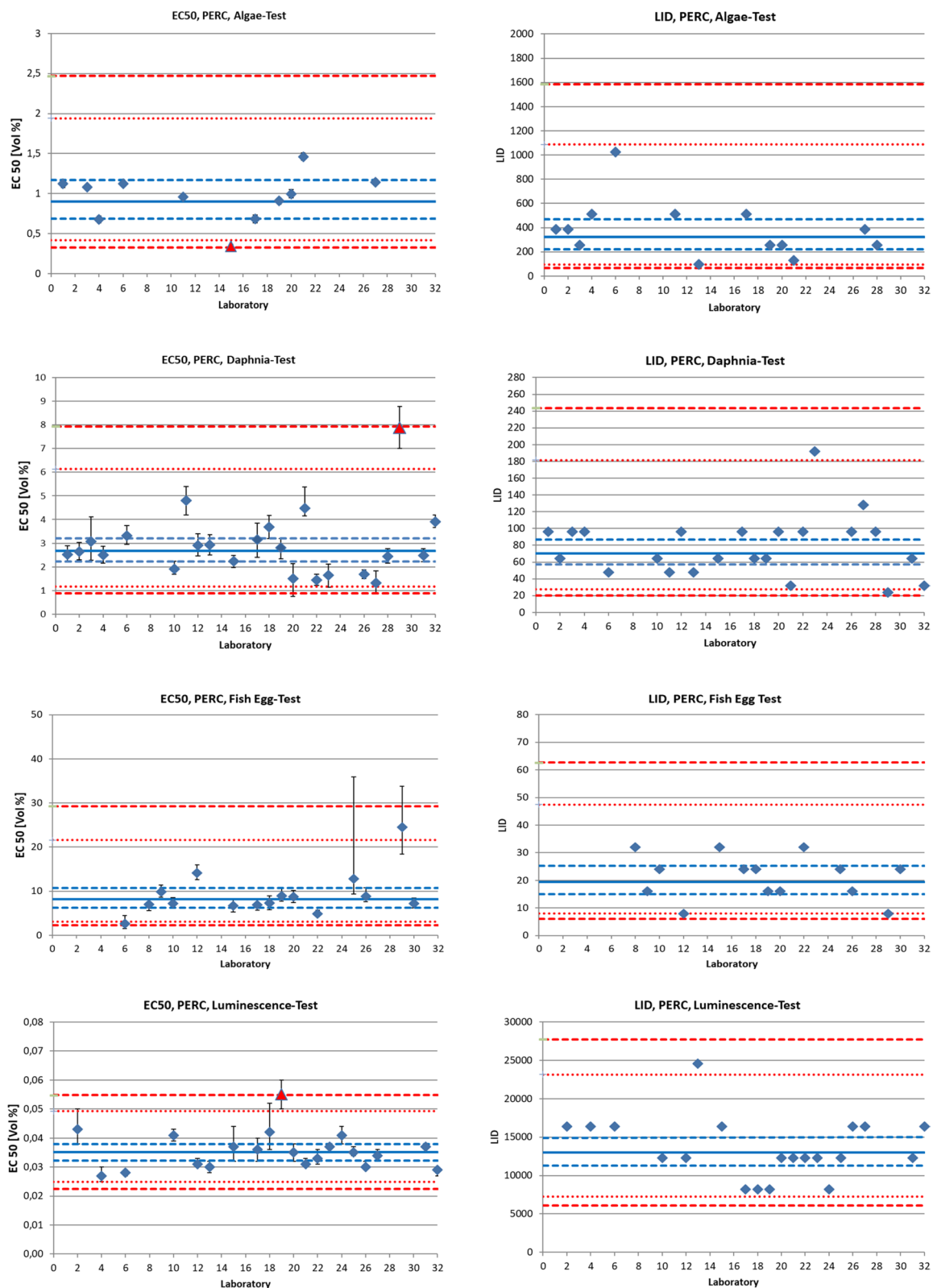
$$\text{Variation coefficient CV [\%]} = \sqrt{e^{\sigma^2} - 1}.$$

All calculations were performed using MS Excel.

According to ISO 5725-2 (2019) [23], intra-laboratory variability (repeatability) and inter-laboratory variability sum up to the total variability (reproducibility). Since the inter-laboratory test participants performed each test only once, repeatability could not be calculated. Thus,



**Fig. 1** Results for valid EC50 (left) and LID (right) from all tests with DSLT eluate. Blue diamonds: laboratory-specific EC50 or LID; Whisker: 95% CI for EC50; Blue lines: geometric mean and its 95% CI. Red dotted and broken line: 95% and 99% prediction interval (=“Tolerance limits”) of single measurements; Red triangle: data that were finally excluded as outliers



**Fig. 2** Results for valid EC50 (left) and LID (right) from all tests with PERC eluate. Blue diamonds: laboratory-specific EC50 or LID; whisker: 95% CI for EC50; blue lines: geometric mean and its 95% CI. Red dotted and broken line: 95% und 99% prediction interval (= "Tolerance limits") of single measurements; red triangle: data which were finally excluded as outliers

the reproducibility of test results is approximated by the inter-laboratory variability as the lower limit of the expected total variability of the examined process. In the following, it will nevertheless be termed “reproducibility”. The relative reproducibility of the toxicity values is calculated as relative reproducibility standard deviation (sR%).

**Results**

Although the same product (2-component grout) was examined with both leaching methods, the centrally produced eluate samples from DSLT and the percolation test differed significantly in their chemical composition with respect to conductivity, total organic carbon, and total nitrogen (Table 2). The PERC eluate, which was obtained by percolating the crushed product (particle size ≤ 1 cm), showed six- to eightfold higher values of these parameters than the DSLT eluate, which was generated by exposing the surface of casted grout plates to water.

The results of the statistical evaluation of the biotests are summarized in Tables 3 and 4. The data set obtained from all submitted results consisted of 81 biotests per eluate sample. After data consolidation and excluding the invalid tests, outliers, and missing LID or EC<sub>50</sub> values, the number of results for the inter-laboratory test statistics was 10 or 13 for the algae test and between 14 and 23 for the other tests. In total, 12 tests were excluded because the required experimental conditions

were not fulfilled, and 3 tests were not valid. In some cases, the tests (although valid) did not produce any usable results, i.e. the LID value could be given only as “greater than” (4 tests) or no EC<sub>50</sub> value could be determined (8 tests, of which 7 tests were algae tests). The outlier tests resulted in the exclusion of six EC<sub>50</sub> or LID results obtained from four laboratories.

The results of EC<sub>50</sub> and LID of all valid biotests are shown in Figs. 1 and 2, which include the outliers marked as red triangles. Both eluates had significant ecotoxicological effects in all biotests. EC<sub>50</sub> and LID values are inversely proportional, i.e. the smaller the EC<sub>50</sub> and the higher the LID, the greater the toxicity. The biotests proved to have differing sensitivity and the eluates to have differing toxicity (Fig. 3). The luminescent bacteria test was by far the most sensitive, followed by the algae test and the daphnia test; the fish egg test was the least sensitive for both eluates. In absolute terms, the toxicity level in the bacterial test, algae test, and daphnia test was very high: in the daphnia test, the observed effects fell below the critical threshold limit from a dilution of 1:70 (PERC eluate) or 1:100 (DSLT eluate), and in the luminescent bacteria test even only from dilutions of more than 1:2000 (DSLT eluate) or almost 1:13,000 (PERC eluate). The corresponding EC<sub>50</sub> values were in the single-digit volume percentage range and for the bacteria test substantially below 1 volume

**Table 3** Inter-laboratory test results for DSLT eluate

Test	DSLT eluate							
	EC50				LID			
	Algae growth inhibition test	Acute daphnia test	Acute fish egg test	Bacteria lumines. test	Algae growth inhibition test	Acute daphnia test	Acute fish egg test	Bacteria lumines. test
Tests performed (number of labs)	17	25	16	23	17	25	16	23
Tests considered	14	23	16	22	14	23	16	22
Invalid tests	0	0	0	1	0	0	0	1
LID or EC50 n.d	4	0	1	0	0	0	0	1
Outlier	0	0	0	1	1	1	0	0
Number of tests for statistics	<b>10</b>	<b>23</b>	<b>15</b>	<b>20</b>	<b>13</b>	<b>22</b>	<b>16</b>	<b>20</b>
Min./max	0.31–4.07	0.60–3.59	22.18–37.35	0.15–0.26	48–512	48–192	3–12	1536–4096
Geometric mean	<b>1.16</b>	<b>1.48</b>	<b>28.54</b>	<b>0.19</b>	<b>166</b>	<b>107</b>	<b>5.6</b>	<b>2196</b>
95% CI	0.71–1.91	1.22–1.79	26.30–30.97	0.18–0.21	107–260	89–127	4.6–6.8	1897–2543
95% PI	0.30–4.52	0.63–3.50	21.37–38.12	0.14–0.27	39–703	49–235	2.8–11.3	1189–4059
Reproducibility sR%	<b>78.5</b>	<b>46.1</b>	<b>14.9</b>	<b>16.4</b>	<b>84.7</b>	<b>41.9</b>	<b>37.1</b>	<b>32.1</b>

n.d. not determined, CI confidence interval, PI prediction interval



**Table 4** Inter-laboratory test results for PERC eluate

Test	PERC eluate							
	EC50				LID			
	Algae growth Inhibition Test	Acute daphnia test	Acute fish egg test	Bacteria lumines. test	Algae growth inhibition test	Acute daphnia test	Acute fish egg test	Bacteria lumines. test
Tests performed (number of labs)	17	25	16	23	17	25	16	23
Tests considered	14	23	16	22	14	23	16	22
Invalid tests	0	0	1	2	0	0	1	2
LID or EC50 n.d.	3	0	0	0	1	0	1	0
Outlier	1	1	0	1	0	0	0	0
Number of tests for statistics	<b>10</b>	<b>22</b>	<b>15</b>	<b>19</b>	<b>13</b>	<b>23</b>	<b>14</b>	<b>20</b>
Min./max	0.68–1.46	1.31–4.80	2.63–24.55	0.027–0.043	96–1024	24–192	8–32	8192–24,576
Geometric mean	<b>0.99</b>	<b>2.54</b>	<b>8.17</b>	<b>0.034</b>	<b>323</b>	<b>71</b>	<b>19.4</b>	<b>12,973</b>
95% CI	0.84–1.17	2.16–2.98	6.21–10.76	0.032–0.037	222–469	57–87	14.9–25.3	11,298–14,897
95% PI	0.62–1.58	1.25–5.16	3.09–21.62	0.026–0.045	96–1086	27–182	8.0–47.4	7270–23,149
Reproducibility sR%	<b>24.0</b>	<b>37.4</b>	<b>52.9</b>	<b>14.2</b>	<b>68.4</b>	<b>51.2</b>	<b>48.0</b>	<b>30.2</b>

n.d. not determined, CI confidence interval, PI prediction interval

percent. The PERC eluate proved to be more toxic than the DSLT eluate by a factor of 6 in the bacterial test, by a factor of 3.5 in the fish egg test, and by a factor of 1.2–2 in the algae test. In the daphnia test, the DSLT eluate was slightly more toxic than the PERC eluate (by a factor of about 1.6).

The reproducibility of the toxicity values can be assessed using the relative reproducibility standard deviation (sR%). The smaller the sR%, the better the reproducibility. With the exception of the algae test, sR% for both EC<sub>50</sub> and LID were below 53% in all bioassays (Tables 3 and 4, Fig. 4). In the bacteria test, reproducibility was 15% (EC<sub>50</sub>) and 30% (LID), in the daphnia test around 40%, and in the fish egg test once 15%, otherwise 40–53%. In the algae test, a reproducibility of 24% was observed once (EC<sub>50</sub> PERC eluate), otherwise the reproducibility was between 70 and 80%.

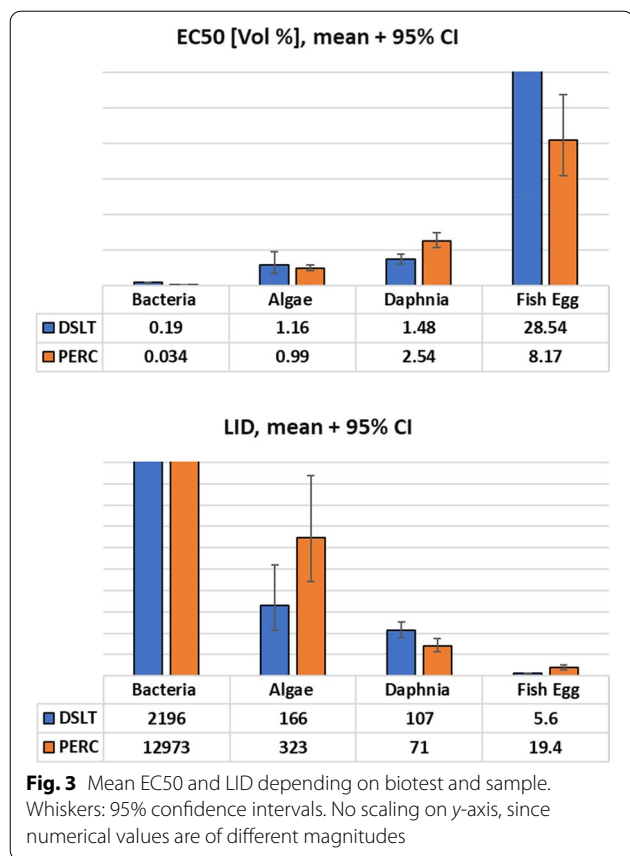
The results of the umu test revealed that both the PERC and the DSLT eluate were slightly genotoxic, with LID = 3 without metabolic activation (S9) and LID = 1.5–3 (DSLT) and LID = 3 (PERC) with S9 (data not shown). This demonstrates that the umu assay also produced consistent results, although the data base did not allow a statistical evaluation.

The blank samples were examined at the Hydrotox laboratory with a dilution of 1:2 ( $D=2$ ). The blanks did

not show any effects in the daphnid, fish egg, and bacteria tests. In the algae test, however, the blanks showed significant toxic effects in dilution level D2. The blanks were then examined with the dilution ranges that were applied to the eluate samples, i.e. in the dilution range D64 to D1024 for PERC eluate and D48 to D768 for DSLT eluate. In these tests, the blanks did not show any effects, i.e. there was no indication of toxic effects in the relevant dilution ranges. Thus, the occurrence of false-positive tested samples due to artificial effects can be excluded. The reason of the conspicuous and repeatable algae toxicity in the blank samples has not been identified so far, although applying extensive analytical means. Normally, no algae toxicity effects are found in the D2 dilution [14]. However, this is an indication that testing of blank samples in the biotest battery is useful to identify false-positive results caused by contamination or other artefacts, and due to the methodical execution of the test.

## Discussion

Due to the comprehensive database of 10–13 valid laboratory results per toxicity measure for the algae test and 14–23 for the other biotests, the determined statistical parameters of the inter-laboratory test evaluation can be regarded as unreservedly meaningful. All four biotests showed significant effects in both eluates.



A toxic potential of the examined eluates was thus detected by all biotests. However, the sensitivity of the test organisms varied considerably in the following order: bacteria test >> algae test > daphnia test >> fish egg test. This applies to both eluates, with the PERC eluate showing higher toxicity in all tests except the daphnia test.

As applied in former studies [14, 26] and recommended by CEN/TR 17105 [10], the DSLT eluate fraction corresponded to  $L/A = 25 \text{ L m}^{-2}$ , while for the percolation test the fraction was set to  $L/S = 2 \text{ L kg}^{-1}$ . This ratio is also recommended by CEN/TR 17105 [10] with an option to test eluates at higher L/S ratios. The L/S ratio of  $2 \text{ L kg}^{-1}$  is considered to be informative about ecotoxicological effects of leachates that may contain readily biodegradable substances that can degrade before the L/S ratio 10 is reached. Product-specific guidance for the collection of eluates can be required, depending on the properties of the leached substances and the leaching processes. Currently, there are no stipulations on the L/S which must be considered. The relevant product-specific L/S may be defined by the responsible product TCs in the future.

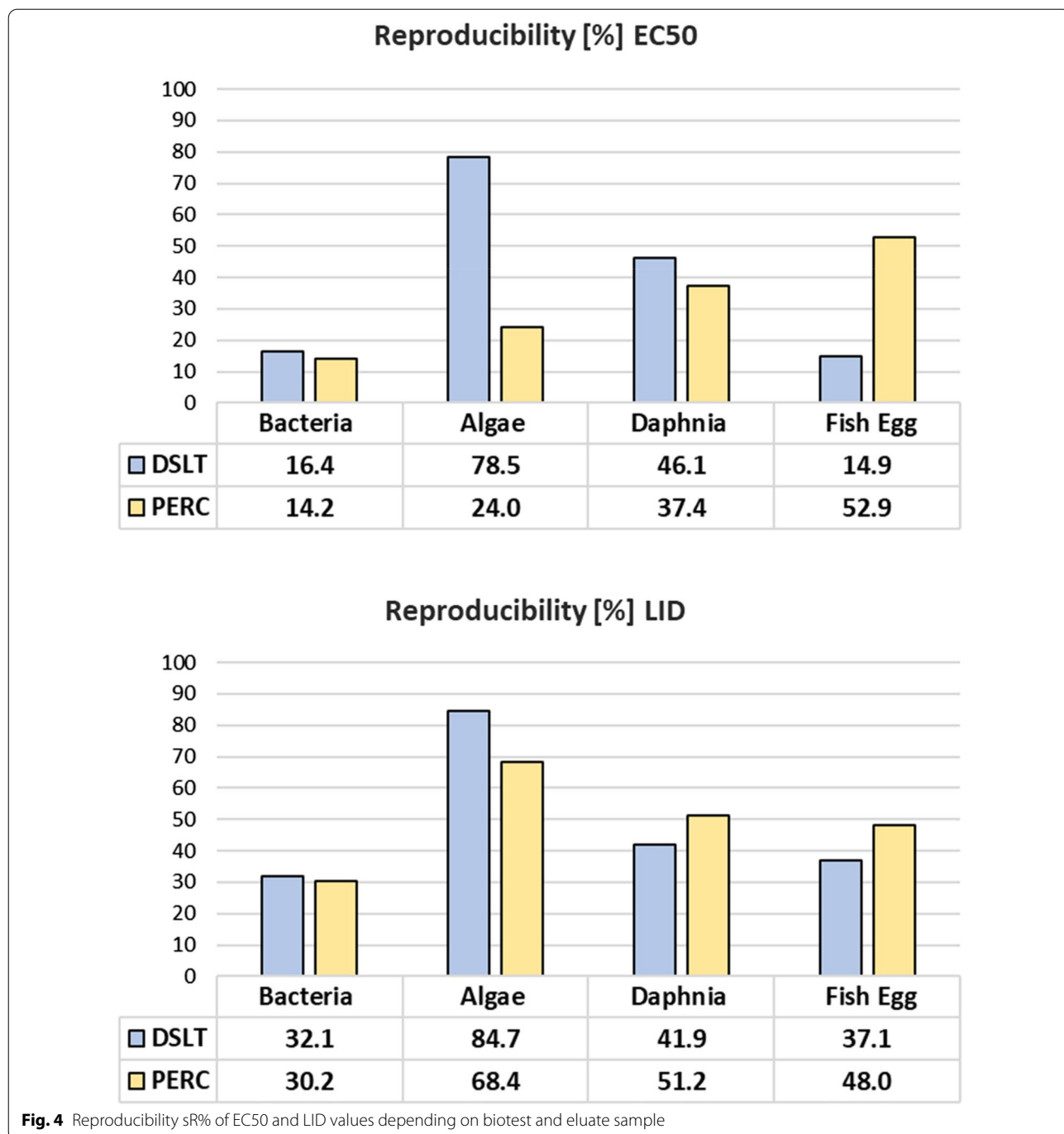
As a rule, the ecotoxicity of the PERC eluate was greater than that of the DSLT eluate (up to a factor of

6). This is in line with the results of the chemical basic analytics (TOC, conductivity, total nitrogen, see Table 2), which also resulted in higher values for the percolation test. This demonstrates that both leaching methods can result in different ecotoxicity of the obtained eluate from the same construction product.

Higher concentrations of released substances in the eluates from the percolation test compared to the DSLT, are certainly caused by the different test scenarios. The contact area for water is higher if a material is percolated compared to leaching of surfaces only. However, not only surface area but also the contact time differ for the two procedures. Therefore, the results from DSLT and percolation test cannot be transferred to each other. Results of ecotoxicity tests must always be interpreted in conjunction with the leaching test applied.

The observed order of sensitivity in the present inter-laboratory test is the same as that obtained for an EPDM roofing membrane in the 2015 round robin test [14]. In contrast, the much more toxic EPDM granules showed no uniform sensitivity pattern for EC<sub>50</sub> and LID, but the fish egg test was by far the least sensitive test. When determining criteria for assessing the ecotoxicological potential of the eluates of construction products, the differing sensitivity of the biotests must therefore be taken into account, which may also differ when testing other construction products. The results of the present inter-laboratory test, like the round robin test in 2015, underline that a biotest battery is useful for testing the ecotoxicological potential of eluates to avoid overlooking or underestimating any effects. The application of a biotest battery is an adequate approach to determine the ecotoxicity of construction products. By the application of biotests with different test species from different trophic levels, effects on the aquatic community can be addressed appropriately.

The observed reproducibilities sR% for both toxicity measures EC<sub>50</sub> and LID were below 53% in all bioassays, except for the algae test (Fig. 4). For the bacteria test, reproducibilities were even 15% to 30%, continuing the decreasing trend reported by Ribo [27] and below the range (36% to 78%) observed by Ross et al. [28]. A universally valid value for a minimum reproducibility is not considered reasonable for the different toxicity tests and therefore recommendations for reasonable variation of ring test results have not been specified [29]. Our results resemble those presented and discussed by other authors with the same type of questions for similar bioassays mostly in the reproducibility range from 15 to 40%, but also including occasionally higher figures [30–36]. Thus, reproducibility in this study can be judged as very good for bacteria, daphnia, and fish egg tests. The LID values spread more widely than the EC<sub>50</sub>



values, which can be explained by the discrete character of the LID levels. The reproducibility of the algae test was between 70 and 80%, except for the EC<sub>50</sub> PERC eluate (24%). The reason for this greater variability in the algae test is unclear. A possible explanation might be the lack of exponential growth in some tests. Since no measurements after 24 h and 48 h were performed, this cannot be checked. In general, we should not lose

sight of the fact that a reproducibility of 80% suggests a relatively high level of variability, however—measured in absolute numbers—the fluctuations in the algae test results are in the range of a few percent by volume. When compared with data in other studies a reproducibility of 80% can be seen as adequate when considering the purpose of testing. For example, Eichbaum et al. [36] calculated a between-laboratory reproducibility

of 83% for the H4IIE micro-EROD assay, a value that, they conclude, qualifies the assay for implementation into testing and management guidelines for dredged materials.

The reproducibilities determined for the toxicity measures were usually similar for both eluates, regardless of toxicity level (Fig. 4). Only in the fish egg and algae tests were noticeable differences observed between the reproducibility of  $EC_{50}$  obtained for DSLT and PERC eluate (fish egg test: 14.9% vs. 52.9%; algae test: 78.5% vs. 24.0%). This can be explained by the presence of nearly outlier values (“stragglers”) in the algae test with DSLT eluate and in the fish egg test with PERC eluate (Figs. 1 and 2).

In general, it must be noted that the obtained reproducibilities represent the lower limit of the actual reproducibility of the applied biotests, since with the given round robin test design without repeated measurements, no repeatability variance, but only inter-laboratory variance could be determined.

In contrast to the present inter-laboratory test with central leaching procedure and eluate production, in the round robin test from 2015, each of the participating laboratories performed the leaching tests on their own [14]. The obtained reproducibilities were 73–110%, and thus significantly greater than in the present inter-laboratory test. This points to the leaching process as an additional source of variability. Especially with construction products of high toxicity, small differences in the leaching process and thus varying amounts of released hazardous substances will result in large differences in the toxicity of the eluate. This does not impede the use of the test procedure in practice, as it is not necessary to determine high toxicities precisely.

Overall, the results clearly show that, even in the case of additional sources of variance such as in the leaching process and in the repeatability of the ecotoxicological tests, acceptable overall reproducibility is to be expected.

## Conclusions

The present inter-laboratory test confirms and supplements the findings of the round robin test of 2015. The applied biotest battery again proves to be suitable for measuring the toxic effects of eluates from construction products. In the present inter-laboratory test with central eluate production, the biotests, with the exception of the algae test, showed good (<53%) to very good (<20%) reproducibility, regardless of the toxicity level of the tested eluate.

There were no significant differences in the reproducibilities of toxic metrics obtained from the two eluates, which confirms the homogeneity of eluate samples and the robustness of ecotoxicity tests at different toxicity levels. Both leaching tests are suitable for producing

eluates from construction products for ecotoxicological testing. In practice, the selection of the leaching test type must be adapted to the exposure scenario of the construction product under investigation (see CEN/TS 16637-1 [7]). The central production of the eluates showed that a significant part of the variability of the results of combined leaching and biotests derives from the production of the eluate itself, which should be taken into account when planning a test setup.

Particularly because of the complex nature of eluates from construction products, the combination of leaching and ecotoxicity tests provides meaningful results that allow the reliable characterization of their environmental hazards.

It is recommended to consider the results in the ongoing harmonization of technical standards developed under CEN/TC 351, next to the implementation of these standards for the ecotoxicological assessment of leachable substances in the EADs for construction products exposed to water.

## Abbreviations

BAM: Bundesanstalt für Materialforschung und -prüfung; CEN/TC: European Committee for Standardization/Technical Committee; CEN/TR: European Committee for Standardization/Technical Report; CEN/TS: European Committee for Standardization/Technical Specification; CPR: Construction Products Regulation; CV: Coefficient of variation; DE-UZ: Umweltzeichen Blauer Engel; DIN: Deutsches Institut für Normung; EN: European Standard; DSLT: Dynamic surface leaching test; EAD: European Assessment Document;  $EC_{50}$ : Volume percentage causing 50% effect; EPDM: Ethylene propylene diene monomer; ID: Inner diameter; ISO: International Organization for Standardization; LID: Lowest ineffective dilution; L/A: Liquid-to-area ratio; L/S: Liquid-to-solid ratio; PET: Polyethylene terephthalate; PERC: Percolation test; PP: Polypropylene; sR%: Percent reproducibility standard deviation; TOC: Total organic carbon; TN: Total nitrogen.

## Acknowledgements

Not applicable.

## Authors' contributions

IH and SG organized the interlaboratory test and drafted the manuscript; MR performed the statistical analysis of the test results; US and UK were responsible for the leaching tests, chemical analysis and distribution of samples to the participants; OI was involved in the study design. All authors contributed to the manuscript. All authors read and approved the final manuscript.

## Funding

Open Access funding enabled and organized by Projekt DEAL. This study was carried out on behalf of the Federal Environment Agency (UBA) within the framework of the “Umweltforschungsplan”—FKZ 3719 37 302 0 and supported by federal funds.

## Availability of data and materials

The original datasets used during the current study are available from Ines Heisterkamp on reasonable request. The aggregated data for the statistical evaluation are available from Monika Ratte.

## Declarations

### Ethics approval and consent to participate

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Received: 28 April 2021 Accepted: 10 June 2021

Published online: 29 June 2021

**References**

- Baderna D, Lomazzi E, Passoni A, Pogliaghi A, Petoumenou MI, Bagnati R, Fanelli R (2015) Chemical characterization and ecotoxicity of three soil foaming agents used in mechanized tunnelling. *J Hazard Mater* 296:210–220. <https://doi.org/10.1016/j.jhazmat.2015.04.040>
- Burkhardt M, Zuleeg S, Vonbank R, Schmid P, Hean S, Lamani X, Boller M (2011) Leaching of additives from construction materials to urban storm water runoff. *Water Sci Technol* 63(9):1974–1982. <https://doi.org/10.2166/wst.2011.128>
- Bell AM, Baier R, Kocher B, Reifferscheid G, Buchinger S, Ternes T (2019) Ecotoxicological characterization of emissions from steel coatings in contact with water. *Water Res* 173:115525. <https://doi.org/10.1016/j.watres.2020.115525>
- Gartiser S, Heisterkamp I, Schoknecht U, Burkhardt M, Ratte M, Ilvonen O (2017) Recommendation for a test battery for the ecotoxicological evaluation of the environmental soundness of construction products. *Chemosphere* 171:580–587. <https://doi.org/10.1016/j.chemosphere.2016.12.115>
- Heisterkamp I, Gartiser S, Kalbe U, Bandow N, Gloßmann A (2019) Assessment of leachates from reactive fire-retardant coatings by chemical analysis and ecotoxicity testing. *Chemosphere* 226:85–93. <https://doi.org/10.1016/j.chemosphere.2019.03.044>
- Vermeirssen ELM, Dietschweiler C, Werner I, Burkhardt M (2017) Corrosion protection products as a source of bisphenol A and toxicity to the aquatic environment. *Water Res* 123:586–593. <https://doi.org/10.1016/j.watres.2017.07.006>
- CEN/TS 16637-1 (2014) Construction products—assessment of release of dangerous substances—part 1: guidance for the determination of leaching tests and additional testing steps. Remark: conversion to DIN EN 16637-1 expected in 2021
- CEN/TS 16637-2 (2014) Construction products—assessment of release of dangerous substances—part 2: Horizontal dynamic surface leaching test. Remark: conversion to DIN EN 16637-2 expected in 2021
- CEN/TS 16637-3 (2016) Construction products—assessment of release of dangerous substances—part 3: horizontal up-flow percolation test. Remark: conversion to DIN EN 16637-3 expected in 2021
- CEN/TR 17105 (2017) Construction products—assessment of release of dangerous substances—guidance on the use of ecotoxicity tests applied to construction products
- Regulation (EU) No 305/2011 of the European Parliament and of the Council of 9 March 2011 laying down harmonised conditions for the marketing of construction products and repealing Council Directive 89/106/EEC. *Official Journal of the European Union*, 4.4.2011, L 88/5–43
- EOTA (2021) European Assessment Document (EAD). European organisation for Technical Assessment. <https://www.eota.eu/en-GB/content/eads/56/>. Accessed 6 Mar 2021
- DE-UZ 216 (2021) Concrete products containing recycled aggregates for outdoor flooring. Blue Angel ecolabel, version 1, January 2021. <https://www.blauer-engel.de/en/companies/basic-award-criteria>. Accessed 6 Mar 2021
- Gartiser S, Heisterkamp I, Schoknecht U, Burkhardt M, Ratte M, Ilvonen I, Brauer F, Brückmann J, Dabrunz A, Egeler P, Eisl A-M, Feiler U, Fritz I, König S, Lebertz H, Pandard P, Pötschke G, Scheerbaum D, Schreiber F, Soldán P, Weiß R, Weltens R (2017) Results from a round robin test for the ecotoxicological evaluation of construction products using two leaching tests and an aquatic test battery. *Chemosphere* 175:138–146. <https://doi.org/10.1016/j.chemosphere.2017.01.146>
- DIN EN 12457-1 (2003) Characterization of granular waste: Compliance test for leaching of granular waste materials and sludges. One stage batch test at a liquid to solid ratio of 2 L/kg for materials with high solid content and with particle size below 4 mm (without or with size reduction)
- DIN EN ISO 7027 (2016–11) Wasserbeschaffenheit – Bestimmung der Trübung, Water quality, determination of turbidity
- DIN EN 1484 (2019-04) Wasseranalytik – Anleitungen zur Bestimmung des gesamten organischen Kohlenstoffs (TOC) und des gelösten organischen Kohlenstoffs (DOC), Water analysis—guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)
- ISO 8692 (2012-02) Water quality—fresh water algal growth inhibition test with unicellular green algae
- ISO 6341 (2012-10) Water quality—Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)—acute toxicity test
- ISO 11348 (2007–12) Water quality—determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)—Part 1: method using freshly prepared bacteria, Part 2: method using liquid-dried bacteria, Part 3: method using freeze-dried bacteria
- ISO 15088 (2007–10) Water quality—determination of the acute toxicity of waste water to zebrafish eggs (*Danio rerio*)
- ISO 13829 (2000–03) Water quality—determination of the genotoxicity of water and waste water using the umu-test
- ISO 5725-2 (2019) 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
- Grubbs FE (1969) Procedures for detecting outlying observations in samples. *Technometrics* 11:1–21
- Environment Canada (2005) Guidance document on statistical methods for environmental toxicity tests. Report EPS 1/RM/46, Ottawa, Ontario. [http://publications.gc.ca/collections/collection\\_2012/ec/En49-7-1-46-eng.pdf](http://publications.gc.ca/collections/collection_2012/ec/En49-7-1-46-eng.pdf)
- Bandow N, Jürgens F, Schoknecht U (2018) Beregnete Bauteile und Bauprodukte: Entwicklung von Vergabekriterien für den Blauen Engel mit Hilfe von Auslaugtests. Final report research project FKZ 3715 37 324 0, UBA-Texte 67/2018
- Ribo JM (1997) Inter laboratory comparison studies of the luminescent bacteria toxicity bioassay. *Environ Toxicol Water Qual* 12:283–294. [https://doi.org/10.1002/\(SICI\)1098-2256\(1997\)12:4%3c283::AID-TOX2%3e3.0.CO;2-4](https://doi.org/10.1002/(SICI)1098-2256(1997)12:4%3c283::AID-TOX2%3e3.0.CO;2-4)
- Ross P, Burton GA Jr, Greene M, Ho K, Meier P, Sweet L, Auwarter A, Bispo A, Doe K, Erstfeld K, Goudey S, Goyvaerts M, Henderson D, Jourdain M, Lenon M, Pandard P, Qureshi A, Rowland C, Schipper C, Schreurs W, Trotter S, Van Aggelen G (1999) Interlaboratory precision study of a whole sediment toxicity test with the bioluminescent bacterium *Vibrio fischeri*. *Environ Toxicol* 14:339–345. [https://doi.org/10.1002/\(SICI\)1522-7278\(1997\)14:3%3c339::AID-TOX7%3e3.0.CO;2-R](https://doi.org/10.1002/(SICI)1522-7278(1997)14:3%3c339::AID-TOX7%3e3.0.CO;2-R)
- Donnevert G, Uhlig S, Moser T (2009) Ring test data evaluation. In: Ecotoxicological characterization of waste. Results and experiences of an international ring test, pp 47–60
- Araújo CVM, Tornero V, Lubián LM, Blasco J, van Bergeijk SA, Cañavate P, Cid Á, Franco D, Prado R, Bartual A, López MG, Ribeiro R, Moreira-Santos M, Torreblanca A, Jurado B, Moreno-Garrido I (2010) Ring test for whole-sediment toxicity assay with -a- benthic marine diatom. *Sci Total Environ* 408:822–828. <https://doi.org/10.1016/j.scitotenv.2009.10.018>
- Eom H, Park M, Jang A, Kim S, Oh SE (2021) A simple and rapid algal assay kit to assess toxicity of heavy metal-contaminated water. *Environ Pollut* 269:116135. <https://doi.org/10.1016/j.envpol.2020.116135>
- Feiler U, Arts G, Bazin C, Brauer F, Casado C, Dören L, Eklund B, Gilberg D, Grote M, Gonsior G, Hafner C, Kopf W, Lemnitzer B, Liedtke A, Matthias U, Okos E, Pandard P, Scheerbaum D, Schmitt-Jansen M, Stewart K, Teodorovic I, Wenzel A, Pluta H-J (2014) Inter-laboratory trial of a standardized sediment contact test with the aquatic plant *Myriophyllum aquaticum* (ISO 16191). *Environ Toxicol Chem* 33:662–670. <https://doi.org/10.1002/etc.2483>

33. Gorbi G, Invidia M, Savorelli F, Faraponova O, Giacco E, Cigar M, Buttino I, Leoni T, Prato E, Lacchetti I, Sei S (2012) Standardized methods for acute and semichronic toxicity tests with the copepod *Acartia tonsa*. *Environ Toxicol Chem* 31:2023–2028. <https://doi.org/10.1002/etc.1909>
34. Hettwer K, Jähne M, Frost K, Giersberg M, Kunze G, Trimborn M, Reif M, Türk J, Gehrmann L, Dardenne F, De Croock F, Abraham M, Schoop A, Waniek JJ, Bucher T, Simon E, Vermeirssen E, Werner A, Hellauer K, Wallentits U, Drewes JE, Dietzmann D, Routledge E, Beresford N, Zietek T, Siebler M, Simon A, Bielak H, Hollert H, Müller Y, Harff M, Schiwiy S, Simon K, Uhlig S (2018) Validation of *Arxula* Yeast Estrogen Screen assay for detection of estrogenic activity in water samples: results of an international interlaboratory study. *Sci Total Environ* 621:612–625. <https://doi.org/10.1016/j.scitotenv.2017.11.211>
35. Mehinto AC, Jia A, Snyder SA, Jayasinghe BS, Denslow ND, Crago J, Schlenk D, Menzie C, Westerheide SD, Leusch FDL, Maruya KA (2015) Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water Res* 83:303–309. <https://doi.org/10.1016/j.watres.2015.06.050>
36. Eichbaum K, Brinkmann M, Nuesser L, Gembé C, Ohlig M, Buchinger S, Reifferscheid G, Hecker M, Hollert GJP (2018) In vitro tools for the toxicological evaluation of sediments and dredged materials: intra- and interlaboratory comparisons of chemical and bioanalytical methods. *Environ Sci Pollut Res* 25:4037–4050. <https://doi.org/10.1007/s11356-017-0094-z>

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