

PROFICIENCY TEST

Battery of ecotoxicological tests – 2025 Fish Embryo Acute Toxicity (FET) Test

Round 1S-E25

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Introduction

Dear colleagues,

many thanks for your participation and interest in the proficiency test ARPAE 2025 – Battery of Ecotoxicological tests round 1S-E25.

A total of 15 laboratories, both public and private, enrolled in the proficiency testing program, of which 13 submitted their results.

This is the second interlaboratory comparison focused on the FET test. The previous one was conducted in 2023 using a sample prepared by the participants.

In the current round, samples were distributed to participants by the PTP. The solutions were formulated using NaCl, which was provided at a concentration of 20 g/L.

The total number of results received was 14 for the year 2023 and 13 for the year 2025. Eight laboratories participated in both interlaboratory comparisons.

1. Confidentiality

Throughout the proficiency test scheme, the participating laboratories are identified solely and exclusively by a numerical code (Lab.ID), which is provided to each participant via email. This identification code is assigned at the time of registration and it is maintained for subsequent PTs. The communication of a participant's results to third parties occurs only with the prior knowledge and written authorization of the participant.

This authorization is not foreseen if the request is required by law, however the Provider must give written notice to the participant.

2. Samples objects to the PT scheme

The reference toxic substance used for 1S-E25 PT round was sodium chloride (NaCl).

The sample sent to the participants was a solution of NaCl solubilized in dilution water (see ISO 6341:2013, paragraph 6.3) referred to as "ISO water" at a concentration of 20 gr/l.

3. PT Samples preparation

Each participant received a sample containing the reference toxic substance dissolved in dilution water (see ISO 6341:2013, paragraph 6.3) referred to as "ISO water".

Based on the number of samples to be prepared and the selected concentration of the reference toxic substance, the mass of the substance has been weighed in order to achieve the desired concentration in each sample. The toxic substance was added to the matrix and mixed thoroughly until the preparation of the different aliquots, which were then packaged in appropriate polyethylene (PE) plastic containers.

4. Delivery of the PT samples

The samples were despatched on 06/05/2025.

During transportation, the samples were kept at room temperature. Delivery of the items was expected within 24-48 hours. For four laboratories, the initial delivery time exceeded 48 hours, and then replacement samples were dispatched, and delivery was completed within 24-48 hours.

Delivery times and sample integrity were reported by the participating laboratories to the PT coordinator via the Data Return Sheet. Based on this monitoring, all the samples were received within 24-48 hours and not damaged.

5. Results notification

For the Battery of Ecotoxicological Tests, the deadline for submitting results is 30/06/2025. The laboratories must complete the "Data Return Sheet" with the analytical results and return it in a signed PDF format. This sheet also includes other information on the test procedures. Data confidentiality is ensured by the exclusive use of an Excel sheet for each participant. Data traceability is guaranteed by the return of the signed PDF Excel file.

6. Assessment of homogeneity and stability

The study for the control of homogeneity and stability of the samples, was carried out in accordance with the guidelines of the UNI CEI EN ISO/IEC 17043:2023 and ISO 13528:2022.

The homogeneity and stability analyses concerning the samples involved in the PT were carried out at the Ravenna Multisite Laboratory of Arpae, accredited according to UNI CEI EN ISO/IEC 17025:2018, and performed following accredited testing procedures (for surface and discharge waters) under conditions of strict repeatability. The homogeneity assessment was performed on the objects configured in their final form, prior to distribution to the participants. From the total number of prepared objects, 10 samples were randomly selected and analyzed in duplicate, with two aliquots taken from each sample. The PT coordinator assigned a random code to each aliquot to ensure blind duplicate analysis.

The homogeneity check was conducted using a one-way ANOVA test ($\alpha=0.05$) after identifying any abnormal duplicates through the Cochran test and excluding them from the analysis. The results, shown in Figure 1, show that there is no significant difference between the duplicate samples, indicating that the PT objects are sufficiently homogeneous.

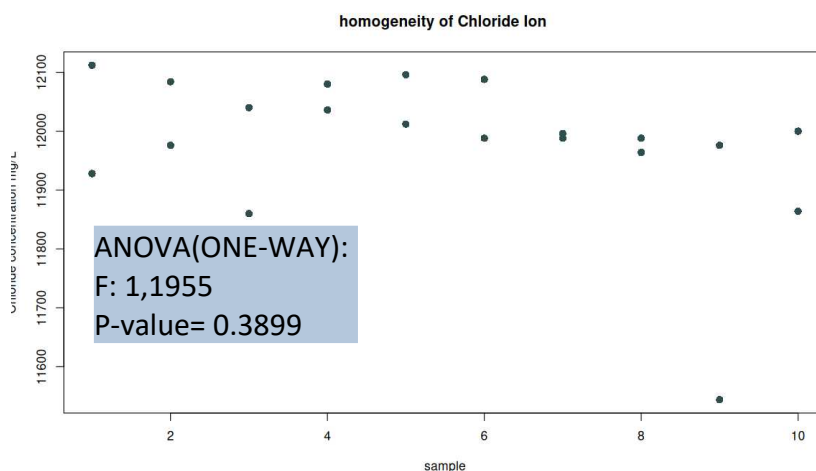


Fig.1: The concentrations, expressed in mg/l, of Chloride ion for the ten replicates analyzed in duplicate. The pair of values found to be anomalous after the Cochran test is highlighted. The result of the one-way ANOVA test ($\alpha=0.05$) is reported in the box.

To assess stability during shipment, three samples are selected from the entire set of objects and analyzed at three different time points:

- Day 1: shipment
- Day 2: 48 hours after shipment, stored at room temperature (shipping temperature)
- Day 3: at the end of the test period (data return), with the aliquot stored in a refrigerator

Samples were analyzed in duplicate by taking two aliquots from each sample and ensuring double-blind analysis under strictly repeatability conditions.

The results of this analysis, presented in Table 1, support the hypothesis that transport at ambient temperature for a maximum duration of 48 hours does not have a significant effect on the stability of NaCl in solution. The stability analysis conducted over the entire duration of the proficiency test (PT), from the day of delivery to the final deadline for data submission (30/06/2025), during which the PT samples were stored under refrigerated conditions, revealed an increase (2.8%), in chloride concentration. Although statistically significant, the observed increase remains within the method's analytical variability (repeatability limit = 3%).

Tab.1: Stability - one-tailed t-test for paired samples results

Stability of Chloride ion	T-test
day 1- day 2 (stability during transport at room temperature)	t value: -1.10938 p-value: 0.1914
day 2- day 3 (stability during PT tests, at refrigerated temperature)	t value:-3,07294 p-value: 0,0458

7. Statistical data analysis

For the present scheme 1S-E25, the minimum number of participants required to apply robust statistics using Algorithm A (Appendix C.3.1 of ISO 13528:2022) for the estimation of the assigned value as a robust mean was set at 13. As established in Section 6.5.2 of ISO 13528:2022, Algorithm A is capable of compensating for the presence of outlying data provided that such outliers do not exceed 20% of the total dataset.

The analysis of the data submitted by the participants in the PT 1S-E25 FET identified 4 outliers out of 13 total results, corresponding to 30.8% of the dataset. Consequently, it was not possible to apply Algorithm A to estimate the robust mean and robust standard deviation.

Given the small number of valid data points (only 9 in total), the relevant standards advise against calculating performance statistics, as the estimation of an assigned value based on consensus among participants would be affected by excessively high uncertainty.

However, considering that in the present scheme the interlaboratory standard deviation σ_{pt} is not derived from participant results but is set as a target value of 30%, the PT coordinator opted to compute performance scores using the z-score formula:

$$z = (x - X_{pt})/\sigma_{pt}$$

where:

x is the result of each lab;

X_{pt} is the assigned value calculated as the mean of the 9 values

σ_{pt} is the standard deviation of the proficiency test calculates as 30% of the assigned value

Due to low number of results, performance scores (z-score), calculated as described above, are shown for information purposes only and does not aim to quantify the participants' performance.

Figure 2 shows the graphical representation of the results.

FET 96%LC50

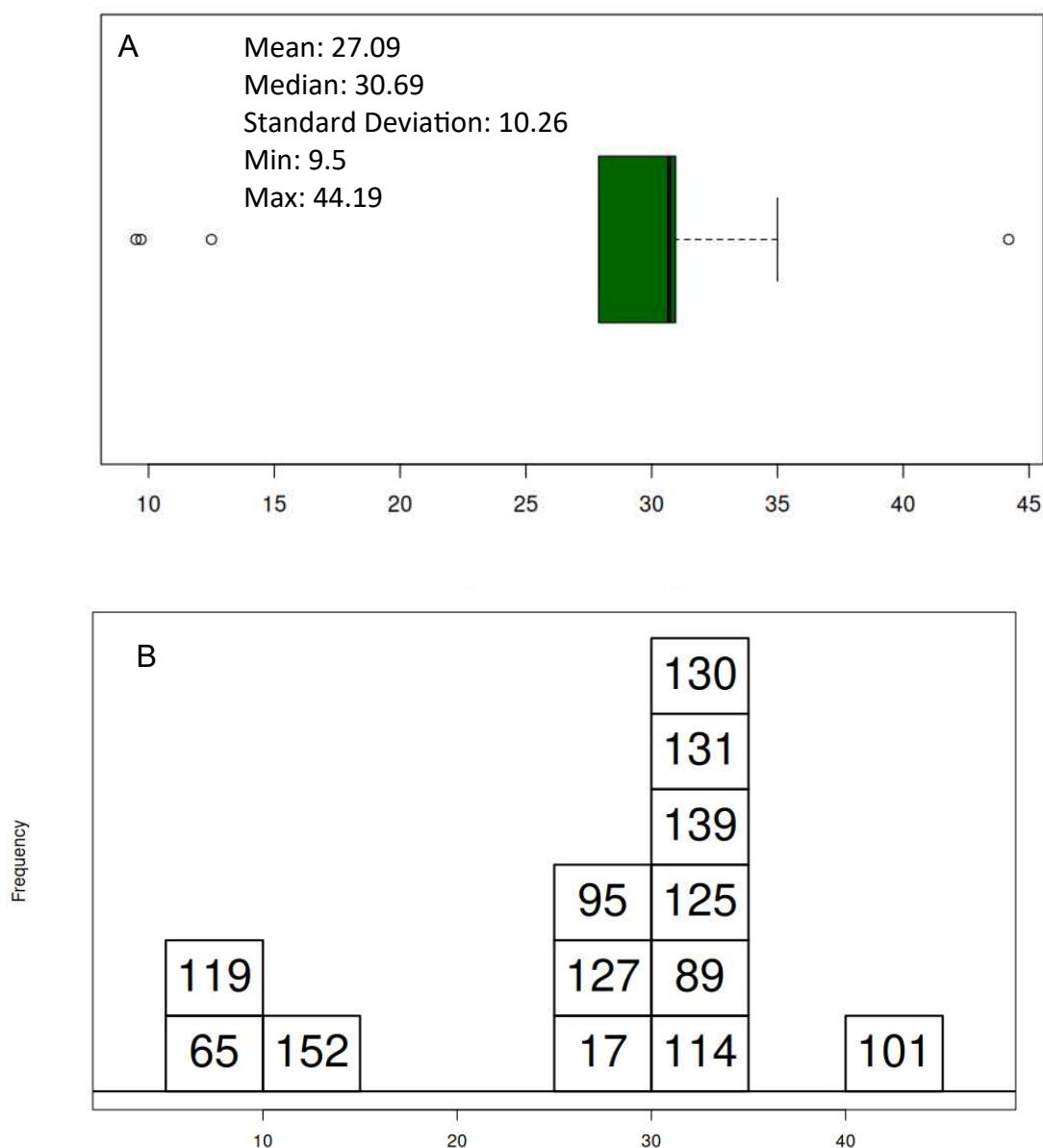


Fig. 2: A) boxplot of the results expressed as percentage. Outliers are shown as dots. B) Blockplot of the results expressed as percentage with the LabID reported in each block.

Descriptive and performance statistics are presented in Table 2, while Table 3 reports the participants' results along with their corresponding z-scores.

Tab 2: Data and performance statistics: Fish Embryo Acute Toxicity Test 96h %LC50 . Mean, median and standard deviation were calculated after the exclusion of the outliers.

Data statistic	value
Number of Result	13
Number of Excluded Results (blunders)	0
Number of outliers	4
Percentage of outliers	30,8 %
Mean (without outliers)	30,69 %
Median (without outliers)	30,78 %
Standard deviation (without outliers)	1,912 %
Intervallo dei risultati	27,89-35 %

Performance Statistics	Valore
Assigned value	30,69 %
Uncertainty of Assigned Value	0,6373 %
Standard Deviation for Proficiency Assessment	9,207%

Tab. 3: Determination of the 96h%LC₅₀ Fish Embryo Acute Toxicity (FET): participants results and z-scores (* the value provided is for information purpose only)

Fish Embryo Acute Toxicity (FET): 96h%LC ₅₀		
LAB ID	96hEC ₅₀ (%)	z-score*
17	27,89	-0,30
65	9,50	-2,30
89	30,78	0,01
95	29,6	-0,12
101	44,1891	1,47
114	30,69	0,00
119	9,7	-2,28
125	30,78	0,01
127	29,5	-0,13
130	35	0,47
131	31,06	0,04
139	30,95	0,03
152	12,50	-1,98

8. Discussion

The results obtained in this round 1S-E25 are comparable to those from the previous 2023 round as illustrated in Fig. 3.

The primary difference between the two rounds concerns the distribution characteristics: in 2023, the dataset exhibited a more symmetrical distribution, whereas in 2025, 9 out of 13 results were clustered within a narrow interval between 27.89% and 35%. This convergence of results in round 1S-E25 resulted in a substantial number of statistical outliers, compromising the applicability of robust statistical methods. Consequently, only an indicative assessment of participant performance could be performed.

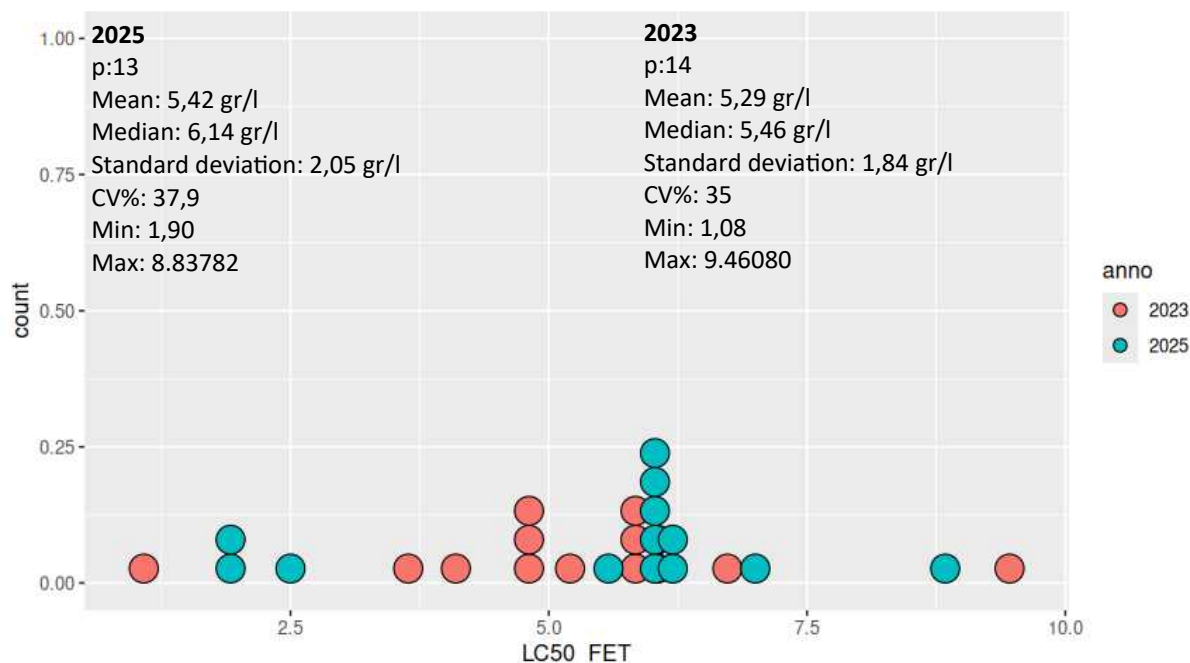
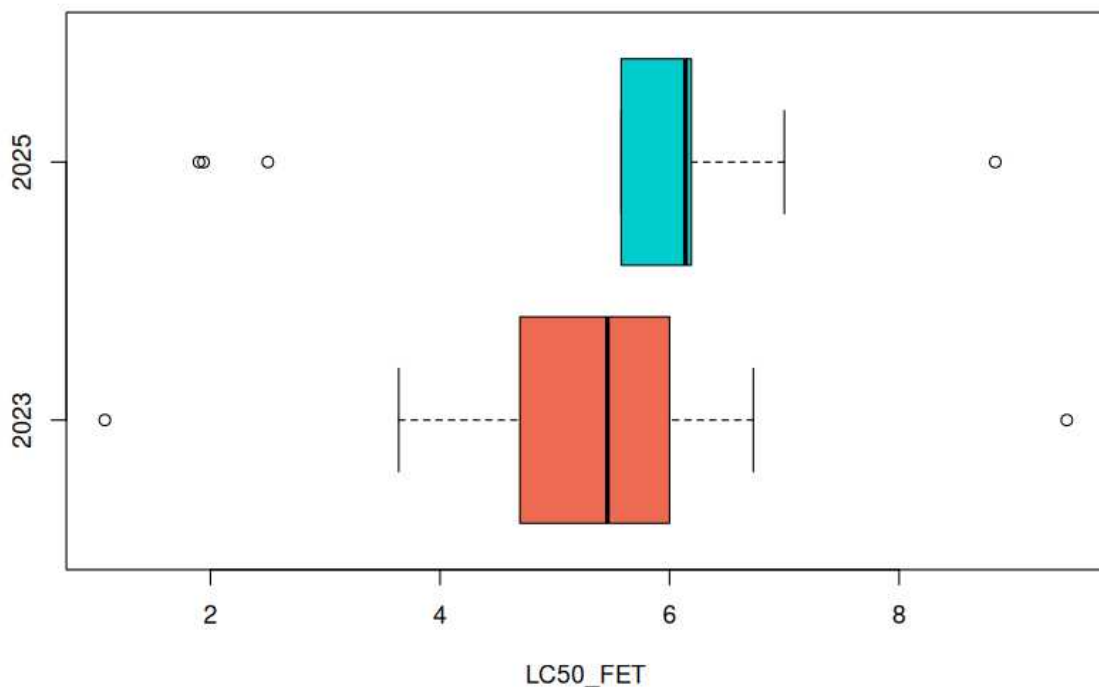


Fig 3: Comparative analysis (boxplot and dotplot) of proficiency test results for the “96h %LC50 Fish Embryo Acute Toxicity (FET)” conducted in 2023 (NaCl 16 g/L) and 2025 (NaCl 20 g/L). Data were transformed from percentage values to concentrations expressed in g/L.

End of the Report: Battery of ecotoxicological tests – 2025 Fish Embryo Acute Toxicity (FET) Test - Round 1S-E25 (Rev 0 28/07/2025)