



Report on the Interlaboratory Comparison Ring Test

Determination of Acrylamide in Extruded Cereal Products for Infants

June – September 2025

Reported by

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31 October 2025

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OBJECTIVES

As part of project CA 21149 ACRYRED, an interlaboratory comparison (ring test) was organized to evaluate the performance of laboratories in determining acrylamide in cereal products for infants. The aim was to assess reproducibility between laboratories and to investigate the effect of sample homogenization on analytical results. The request for an interlaboratory comparison of samples with expected low values came from a project member from the industrial sector, presented at the General meeting of ACRYRED on 10 June 2025 in Piešťany, Slovakia, within the activities of WG5. The main requirement was to verify the reliability of low acrylamide values in products intended for children, which has a direct impact on their placement on the market.

TEST MATERIAL

Test Samples

Two forms of the same extruded cereal product were tested:

- **SK-2025-1:** extruded products (duck-shape; not pre-ground)
- **SK-2025-2:** ground extruded products

Both samples originated from the same production batch of extruded cereal product for infants:

SK-2025-1 was the original sample;

SK-2025-2 was a sample ground to powder at 5000 rpm for 15 s using a Grindomix GM200 (Retsch, Germany).

Each sample was packed in a sealed plastic bag (15 g) at ambient temperature.

Dispatch

- Samples were distributed on 10 June 2025, at the General Meeting of CA ACRYRED in Piešťany, Slovakia. Participants picked up the samples in person.
- The results were collected until 30 September 2025.

PARTICIPANTS

Seven laboratories (L01–L07) from seven countries participated in the test and submitted results. Each laboratory was assigned a unique code to maintain anonymity. Participation by the laboratories was voluntary.

List of participating countries in alphabetical order:

Czech Republic, Greece, Montenegro, Poland, Serbia, Slovakia, Spain

RESULTS

Analytical Methods

Each participant was requested to provide details of the analytical method:

- technique for determining acrylamide,
- expression of acrylamide in µg/kg in whole numbers – no decimals,
- indication of % yield (if relevant),
- indication of standard deviation (if relevant),
- use of internal standard (if yes, which one),
- use of reference material (if yes, which one),
- indication of LOD and LOQ,
- whether the method is validated (yes/no)
- whether the method is accredited (yes/no)
- date of analysis

Details of analytical methods (techniques, internal standard, reference material, LOD/LOQ, validation/accreditation) are listed in Table 1.

Table 1. Overview of analytical method

Lab	Method	Internal standard	Reference material	LOD (µg/kg)	LOQ (µg/kg)	Validation	Accreditation
L01	GC-MS	D3-acrylamide	no	n.r.	≤25	yes	yes
L02	LC-MS/MS	¹³ C3-acrylamide	FAPAS	4.5	15	yes	no
L03	LC-MS/MS	D3-acrylamide	no	10	25	yes	no
L04	LC-MS/MS	D3-acrylamide	no	3	10	no	no
L05	UHPLC-MS/MS	¹³ C3-acrylamide	no	3	10	yes	yes
L06	LC-MS/MS	D3-acrylamide	Internal, related to FAPAS	5	15	yes	no
L07	LC-MS/MS	D3-acrylamide	FAPAS (biscuits)	10	25	yes	yes

Reported Values

Within the specified period, the participating laboratories submitted their results by email, together with a description of the method and parameters used for the determination. Reported values are listed in Table 2.

Table 2. Reported acrylamide levels ($\mu\text{g}/\text{kg}$) in the samples SK-2025-1 and SK-2025-2

Lab	SK-2025-1 ($\mu\text{g}/\text{kg}$)	SK-2025-2 ($\mu\text{g}/\text{kg}$)	Correction (yes/no)	Recovery %	Date of analysis
L01	85 \pm 13	84 \pm 13	>0.99	89	01/07/2025
L02	75 \pm 6	79 \pm 6	no	98	18/06-01/07/2025
L03	221	230	no	n.r.	06-07/2025
L04	116 \pm 17	n.r.	no	n.r.	25/09/2025
L05	35 \pm 12	33 \pm 12	no	102	27/09/2025
L06	n.r.	100 \pm 6	no	105	29/05/2025
L07	69	71	no	102	July 2025

n.r. = not reported

Robustness

Assigned values were calculated as **robust median** according to ISO 13528. Only reported values were included in the calculations.

Table 3. Robust median of the samples SK-2025-1 and SK-2025-2

Sample	Values included	Robust median ($\mu\text{g}/\text{kg}$)	Robust SD ($\mu\text{g}/\text{kg}$)
SK-2025-1	L01, L02, L03, L04, L05, L07	79.8	64.6
SK-2025-2	L01, L02, L03, L05, L06, L07	81.6	67.8

Calculation of z-score

z-Score = (Lab result – Robust median) / Robust SD

Table 4. z-Scores for SK-2025-1

Lab	Result (µg/kg)	z-Score	Assessment
L01	85	-0.31	Within normal variability
L02	75	-0.07	Within normal variability
L03	221	2.18	Possible high outlier – may need verification
L04	116	0.56	Within normal variability
L05	35	-0.69	Within normal variability
L06	n.r.	–	Cannot be evaluated
L07	69	-0.17	Within normal variability

n.r. = not reported

Table 5. z-Scores for SK-2025-2

Lab	Result (µg/kg)	z-Score	Assessment
L01	84	0.04	Within normal variability
L02	79	-0.04	Within normal variability
L03	230	2.19	Possible high outlier – may need verification
L04	n.r.	–	Cannot be evaluated
L05	33	-0.72	Within normal variability
L06	100	0.27	Within normal variability
L07	71	-0.16	Within normal variability

n.r. = not reported

COMMENTS OF PARTICIPANTS

For the purpose of interlaboratory test, participants were contacted individually to provide comments on this performance.

Participant L01:

The discussion was focused on feedback and visibility preferences for participating laboratories. The Participant L01 emphasized the need for visibility and consistent units in the report, along with the inclusion of z-score interpretations for clarity. The Organizers supported these suggestions and highlighted the necessity for more details on analytical methods and laboratory accreditation.

The Organizers inquired about the department's experience with proficiency tests and the existence of a reference laboratory for validating results, to which the Participant L01 responded that the accredited laboratory has conducted proficiency tests for nearly 50 labs, noting challenges with small sample sizes. The Participant L01 suggested to recalculate median values. This suggestion has been included in the report.

Participant L02:

The discussion focused on the challenges associated with interpreting acrylamide sample analysis results, particularly due to the absence of known true values. The Participant L02 highlighted the significance of understanding detection and quantification limits from participating laboratories, which is essential for accurate comparisons. It was expressed concerns regarding regulatory compliance for infant food products, noting that many results surpassed reference values. The need for clearer methodologies in future Ring tests and the inclusion of more laboratories was also emphasized. The group acknowledged the difficulties in achieving low acrylamide levels in corn products, with the Organizer noting the compound's formation during processing. The Organizer proposed sending samples to independent accredited laboratory and a national authority for comparative analysis. The variability in results from different laboratories was discussed, underscoring the importance of reliable findings for industry use. The participants agreed to continue their collaboration and plan future meetings to enhance their approach and share insights.

Participant L03:

The discussion confirmed including detailed laboratory results and participant names would strengthen the report's credibility and transparency. The data discrepancy arose because some labs used a faster method without solid phase extraction, which Participant L03 flagged as unsuitable for acrylamide determination. The Organizers agreed to add this new, more reliable data to the report, despite it coming from a non-member commercial lab, because it provides useful benchmarking for accredited and research labs. This data was subsequently included in this report. The Participant L03

committed to providing methodological details such as LOD, LOQ, internal standards, and reference materials to support the new data's inclusion and maintain report quality.

Participant L04:

The discussion focused on the management of shifts and the sharing of information related to the Acrylamide Ring test. The Organizer raised concerns about the confidentiality of participant information in their report. The group acknowledged that differences in results could stem from the use of different columns and noted that other accredited laboratories reported similar variability.

The Organizer requested more details on testing methods to ensure accurate evaluation, and the group agreed on the need for a comprehensive report reflecting recommendations from all laboratories involved. They also discussed the extraction process and the potential for acrylamide formation during high-speed grinding, which the Organizer addressed.

Participant L05:

The Participant L05 pointed out the lack of information on how the sample was stored. The sample was analysed after a considerable time lapse. The participant preferred to resend the sample for a control analysis. He agreed to provide all details of the analysis. He suggested a detailed analysis of the chromatograms to rule out interference.

GENERAL COMMENTS

Most laboratories achieved results within ± 1 z-score, indicating good inter-laboratory agreement. One laboratory exceeded the z-score ± 2 which indicates the lack of reliability of this result. However, the absolute differences in reported acrylamide concentrations are considerable. Given the benchmark level for cereal-based foods for infants (40 $\mu\text{g}/\text{kg}$), the uncertainty near this level may have a meaningful impact on the data interpretation and further consequences on product classification.

Despite this overall statistical consistency, the true acrylamide concentration remains uncertain. Some of the participating laboratories are accredited for acrylamide analysis, while others are not, and accreditation itself does not necessarily guarantee the correctness of results. It therefore cannot be excluded that the apparent outliers may in fact reflect more accurate measurements.

Findings from this ring test further highlight that reliable verification of such low benchmark levels for acrylamide in cereal-based foods for infants remains a significant analytical challenge, particularly in view of the quantification limits reported by several participating laboratories.

Finally, the analysis of acrylamide levels in samples SK-2025-1 and SK-2025-2 provided an important practical observation, confirming that grinding under standard conditions did not lead to additional acrylamide formation. This verification was performed following a request arising from collaboration with the food industry during process control.

COMMENTS ARISING FROM THE DISCUSSION FORUM (31 Oct 2025)

- **Ring Test Expansion:** Finalize report with inputs from all 20 participants to improve data quality and representation.
- **Acrylamide Measurement Challenges:** Need validated methods for baby food; current LOQ of 40 micrograms per kilogram is problematic.
- **Sample Stability Issues:** Analyze samples within one month; degradation up to 70% noted in storage over a year.
- **Future Test Improvements:** Set clear deadlines and storage conditions to enhance reliability and data comparability.
- **Increased Participation Goals:** Aim to invite more accredited labs; current seven labs too low for strong statistical confidence.
- **Collaboration for Safety:** Ongoing teamwork essential for enhancing product safety and market confidence in acrylamide analysis.

CONCLUSIONS

- Most laboratories achieved a value **within ± 1 z-score**, indicating **good inter-lab agreement**.
- **L03** showed a **significantly higher value** ($z > 2$), indicating a need to review analytical or procedural practices.
- It was observed that the grinding of samples performed by the participating laboratories did not lead to any additional acrylamide formation.
- Several comments pointed to a problem with sample stability. In further steps, it is necessary to verify the stability of the samples before starting a new round of the ring test.

REFERENCES

ISO/IEC 17043:2023 – *Conformity assessment — General requirements for proficiency testing*

ISO 13528:2022 – *Statistical methods for use in proficiency testing by interlaboratory comparisons*

APPENDICES

Appendix A: Plots of results (Figures 1)

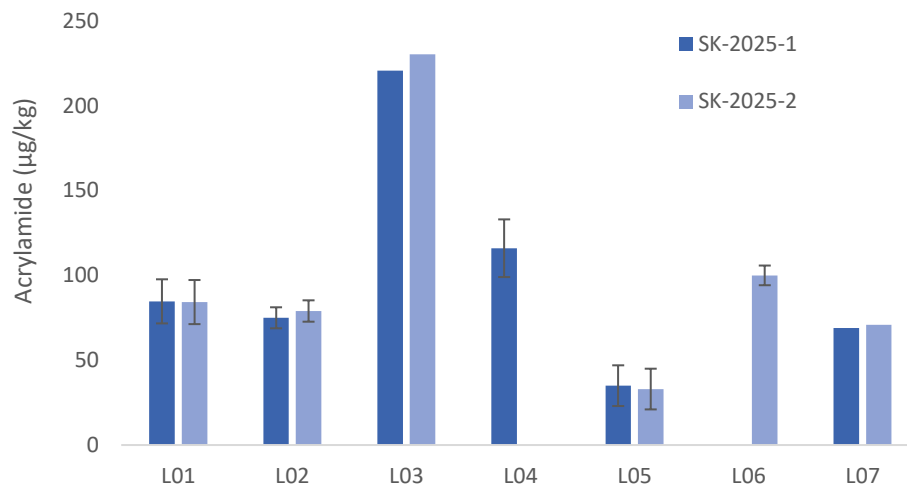


Figure 1. Acrylamide level (µg/kg) in the samples SK-2025-1 and SK-2025-2 provided by seven independent laboratories L01-07

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Appendix B: Plots of z-scores (Figures 2 and 3)

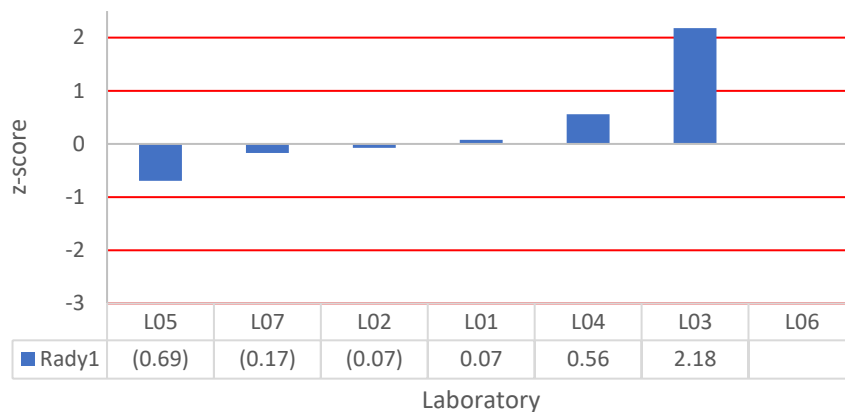


Figure 2. z-Scores of data provided by seven independent laboratories for the sample SK-2025-1

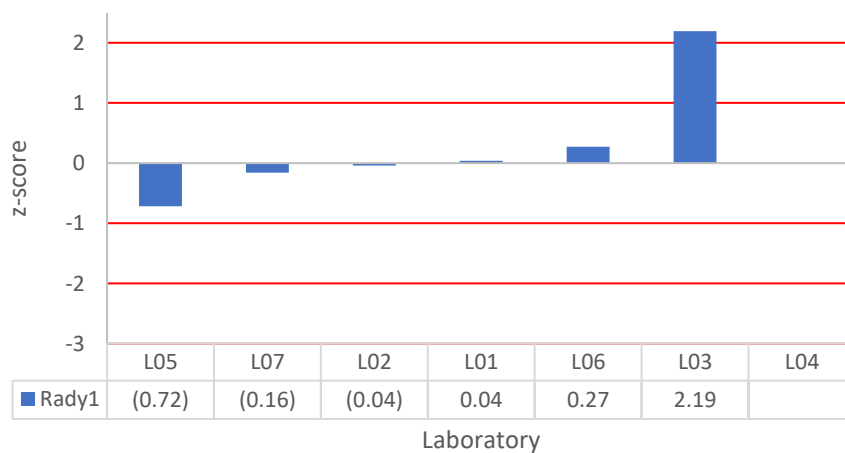


Figure 3. z-Scores of data provided by seven independent laboratories for the sample SK-2025-2