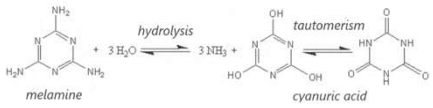


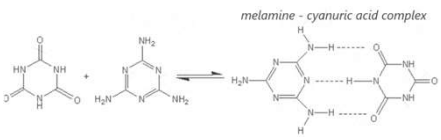


# Analysis of melamine and cyanuric acid in dairy products: interlaboratory tests as a quality tool

*Julien LEPROVOST, Antoine GORSE, Anne TIRARD, François ROUSSEY, Abdelkader BOUBETRA*
*Bureau Interprofessionnel d'Études Analytiques (BIPEA) - 189 rue d'Aubervilliers, 75018 PARIS – France. Tel .+33 1 40 05 26 30*
*Corresponding author: jleprovost@bipea.org*

## OVERVIEW

Melamine and cyanuric acid are low-mass compounds rich in nitrogen.
Their fraudulent use to falsify the protein content of dairy products has been, several times, at the center of health scandals.

Although the lethal toxicity of these compounds is low, their toxicity following chronic exposure is greater.
Cyanuric acid, the hydrolysis product of melamine, forms a crystalline complex with the latter, which can be responsible for kidney failure (stones) and urinary failure.

Quality control is therefore essential, using high-performance analytical measurements. More specifically, as regards powdered milk, these controls are of crucial importance for infant food safety.

In this context, BIPEA (Bureau Interprofessionnel d'Etudes Analytiques) has been organizing interlaboratory comparison tests on this specific topic since 2015.

The many benefits for laboratories of taking part in these tests make these interlaboratory campaigns a powerful quality tool.

## INTRODUCTION

A proficiency testing (PT) involves different laboratories analyzing the same analytical parameters on identical samples. The setting up of a proficiency test, in accordance with the requirements of ISO 17043 standard **[1]**, can be schematized by 4 main steps: test design, preparation of homogenous samples, analyses by the laboratories, statistical treatment of the data (with the estimation of an assigned value and evaluation of the laboratory performance).

For this PT, laboratories were invited to analyze milk powder samples spiked with melamine and cyanuric acid, in well controlled proportions.

The main goal of the test is to provide laboratories with a means of objective assessment, to demonstrate the reliability of the data they produce on samples that are as close as possible to real ones. All this contributes to improve the analytical performance of the participating laboratories, in accordance with ISO 17025 standard **[2]**.

## METHODOLOGY AND RESULTS


Fig. 1 - Organisation of the PT scheme by BIPEA

## SAMPLES PRODUCTION

The manufacturing process involves contaminating powdered products with melamine and cyanuric acid at given concentrations.

A batch of milk contaminated with melamine and cyanuric acid is produced as follows. First, aqueous solutions spiked with melamine and cyanuric acid are prepared (solutions n°1). Then, the powdered milk is dissolved in distilled water (solutions n°2). The desired volumes of contaminated solutions (n°1) are mixed with these solutions (n°2). These batches are then freeze-dried and ground to obtain contaminated milk powder.

These batches of contaminated milk are mixed in well-defined proportions with a matrix of non-contaminated milk using a powder mixer. The target concentration range for melamine and cyanuric acid is 0.1 to 2.5 mg/kg, which corresponds to levels usually encountered by the laboratories.

Samples are divided into series using a carousel combined with a direct distributor. The principle of rotary distribution, which results in progressive filling, ensures the homogeneity of the product between each sample.

## HOMOGENEITY CONTROL

After the samples have been produced and before they are dispatched, a homogeneity check is carried out, in accordance with the requirements of standard ISO 13528 **[3]**. These analyses are carried out by a subcontracted laboratory selected and assessed in accordance with internal procedures.

Ten samples are selected from the production, at regular intervals, and are analyzed in duplicate in random order. The results are analyzed by calculating ratio between the inter-sample standard deviation and half the tolerance value. Also, complementary statistical tests are used: Fisher's test (analysis of variance); Significant inhomogeneity test and Cochran's test (outlier variances).

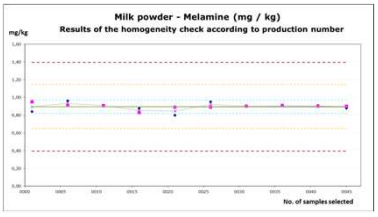


Fig. 2 - Monitoring the results of homogeneity checks for the "melamine" criterion

## ANALYSIS

Samples are sent at room temperature to the laboratories taking part in the test.

A reply form is made available on the BIPEA website, enabling laboratories to return their melamine and cyanuric acid results within 4 weeks.

According to ISO 23970 **[4]**, these analyses are carried out by LC-MS/MS.
However, laboratories can also carry out these analyses using GC-MS/MS or any other method suited to this application.

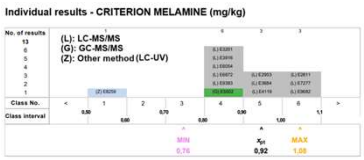


Fig. 3 - Individual melamine results, depending on the analytical technique used

## STATISTICAL TREATMENT

Statistical processing was carried out in accordance with ISO 13528 **[3]**. The assigned value ($x_{pt}$), for each criterion, is estimated using the robust means of the participants' results (x). The performance of each laboratory is evaluated using tolerance values (TV) of twice the standard deviation, delimiting the range in which the participants' results are considered satisfactory.

Each result ($x_i$) can also be evaluated and ranked using z-scores:

- $z \leq |2|$ : satisfactory
- $|2| < z < |3|$ : questionable
- $z \geq |3|$ : unsatisfactory.

where : $Z = (x_i - x_{pt}) / (TV/2)$

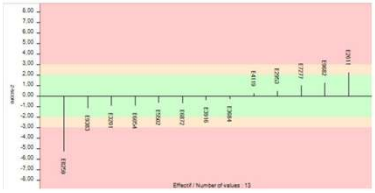

Fig. 4 - Chart showing the z-scores of participating laboratories

## REVIEW 2020-2023 AND CONCLUSIONS

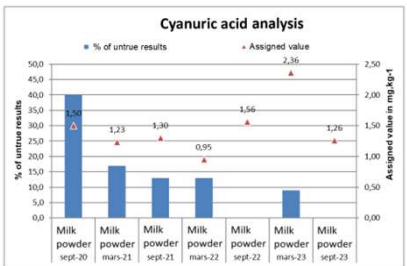


Fig. 5 – Cyanuric acid / Evolution of untrue results (%)

The results obtained over the period (2020-2023) show that the trend for cyanuric acid analysis in milk powder is satisfactory, with the % of "out-of-range" results falling over this period to an average of less than 10% – **Fig.5**.

The average number of laboratories taking part in these tests is around ten.

| | Melamine | | |
|---|---|---|---|
| Test | Spiking value (mg/kg) | Assigned value (mg/kg) | Relative difference |
| September 2020 | 0.90 | 0.66 | -27% |
| March 2021 | 2.03 | 1.51 | -26% |
| September 2021 | 2.25 | 1.81 | -20% |
| March 2022 | 1.31 | 1.34 | 2% |
| September 2022 | 2.16 | 2.17 | 0% |
| March 2023 | 1.08 | 1.11 | 3% |
| September 2023 | 0.90 | 0.92 | 2% |

Fig. 6 – Melamine / Theorical concentrations (spiking) vs assigned values (participants' results)

In regards of melamine analysis, the gap between the spiked values (BIPEA) and the assigned values (participants' results) is satisfactory and narrowing over the period in question (2020-2023) – **Fig.6**. This reflects an improvement in the analytical performance of the laboratories and also a better control of the spiking process.

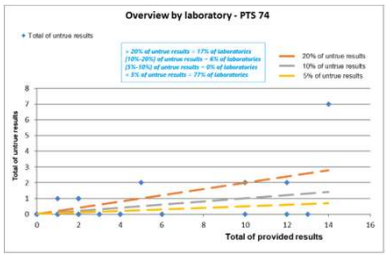


Fig. 7 – Overview by laboratory of the tests from September 2020 to September 2023

Considering the two criteria (cyanuric acid and melamine), the performance of the laboratories is satisfactory, since the large majority (77%) has an untrue results rate of less than 5% – **Fig.7**.

**REFERENCES**

*[1] ISO 17043, Conformity assessment - General requirements for proficiency testing,*
*[2] ISO 17025, General requirements for the competence of testing and calibration laboratories.*
*[3] ISO 13528, Statistical methods for use in proficiency testing by interlaboratory comparisons.*
*[4] ISO 23970, Milk, milk products and infant formula - Determination of melamine and cyanuric acid by liquid chromatography and tandem mass spectrometry (LC-MS/MS).*

**Conclusions:**

- **Infant food safety is a major concern and requires high-performance controls.**
- **Interlaboratory tests are a powerful quality tool for enhancing the performances of laboratories and their measurements.**
- **The results obtained in these interlaboratory tests are good and improve over time.**