ETHYLENE OXIDE AND 2-CHLOROETHANOL IN FOOD: AN OVERVIEW OF RESULTS OBTAINED FROM PROFICIENCY-TESTS

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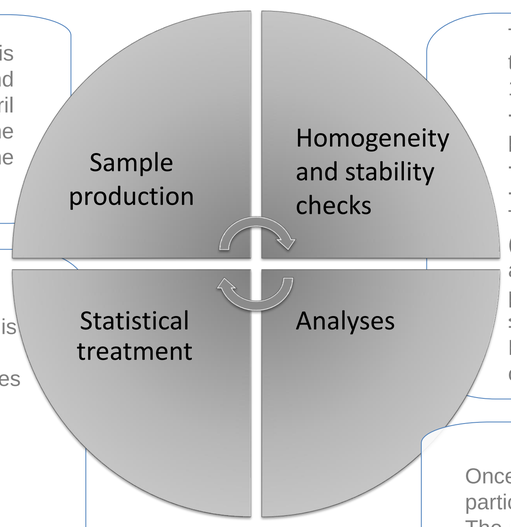
INTRODUCTION

The use of ethylene oxide as fungicide, bactericide and/or insecticide has been banned in Europe since 1991. Nevertheless, lack of uniformity in worldwide regulations (some countries still allow this pesticide) forces European agribusiness operators to continuously monitor foods potentially processed with this gas. In this context, requests of analyses of ethylene oxide and its metabolite 2-chloroethanol have increased in recent years. Laboratories therefore have a key role to play in this process. However, the lack of an official testing method is an obstacle for the comparison of laboratories. To allow laboratories to have an independent monitoring of their performances and to obtain recognition of their analytical procedures by customers and accreditation bodies according to the ISO/IEC 17025 [1], BIPEA organizes proficiency-testing schemes (PTS) for the detection and quantification of these molecules in food. For each test, the statistical treatment of laboratories' results is performed according to ISO 13528 standard [2]. These PTS enable the participating laboratories to have an overview of different methods used, draw up a general inventory of their analytical skills and improve their performances for the detection and quantification of ethylene oxide and 2-chloroethanol in food.

DESIGN & IMPLEMENTATION

Setting up of a Proficiency Test (PT) can be schematized in 4 main steps: sample preparation, homogeneity and stability checks, analyses by the laboratories and statistical treatment of the data (Figure 1).

Raw material is spiked with 2-chloroethanol (spiking range: 50-5000 µg/kg). Spiked product is then handled using a specific equipment to ensure homogeneity during the sampling and correctly conditioned to ensure the stability of the manufactured samples. For the PT of April 2024 on sesame seeds, samples were prepared using a carousel (Figure 2) and a mixer. The principle of turning spreading, which involves a quasi-simultaneous filling, ensures the homogeneity of the product between all the samples.



The homogeneity of the samples is checked by experimental studies on 10 samples in duplicate, taken randomly across the batch of samples, according to the requirements of ANNEX B of the ISO 13528 standard [2]. The results could be analyzed through several statistical tests:
- Study of the ratio of the between-sample standard deviation (s_s) and the standard deviation for proficiency assessment (σ_{pt}): $s_s \leq 0.3\sigma_{pt}$.
- Test of significant non-homogeneity: between-sample variance < critical *c* value;
- Fisher test (variance analysis): observed *F* value < critical *F* value;

The stability of the samples during the test period is checked on the packaged samples stored at (-20±8) °C. According to the ISO 13528 standard [2], the samples can be considered stable if the absolute difference between the means at t_0 and t_1 (t_0+3 weeks, the test period accorded to participants) is inferior or equal to 0.3 x the standard deviation for proficiency assessment ($|y_0 - y_1| \leq 0.3\sigma_{pt}$).
Figures 3 and 4 graphically summarize the results of the homogeneity and stability check on the PT of April 2024 on sesame seeds.

Statistical treatments are conducted according to ISO 13528 [2]. Assigned values (x_{pt}) are estimated using the robust means of the results from application of robust algorithm A. Performances of each laboratory are evaluated using a tolerance value (VT) of 30% of x_{pt}. This value is used to identify an interval around the assigned value. Results in this range are considered as satisfactory. Moreover, laboratory results (x) are also evaluated through z-scores (z). The z-score for a result x_i is calculated as:

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

Where σ_{pt} is the standard deviation for proficiency assessment (VT/2). Laboratories with a "z score ≤ | 2 |" or "z score > | 3|" are considered having reported "Satisfactory" or "Unsatisfactory" results, while the remaining laboratories reported "Questionable" results. Results are published in a specific interlaboratory comparison report broadcasted to all participants who can then classify their results and implement some corrective and/or preventive actions if necessary.

Once the homogeneity and the stability has been demonstrated, the samples are shipped frozen to all participants, who are invited to analyze the samples as soon as possible after reception.
The results of laboratories are collected via a reply form available online over a period of 3 weeks. No instructions are given for extraction analyses, but laboratories are invited to provide information about the method performed in the reply form.

Fig. 1. Description of main steps of the setting up of PTS

RESULTS & DISCUSSION

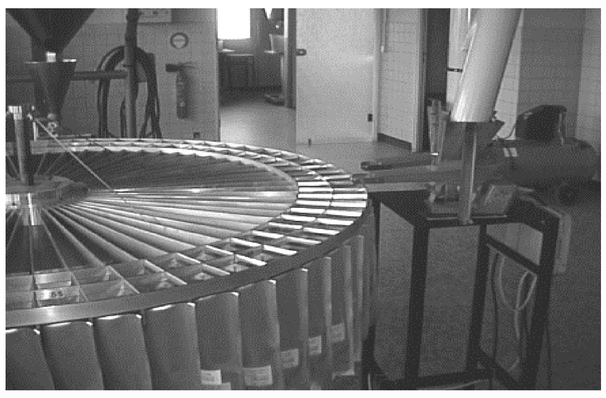


Fig. 2 – Sample production using a carousel equipment

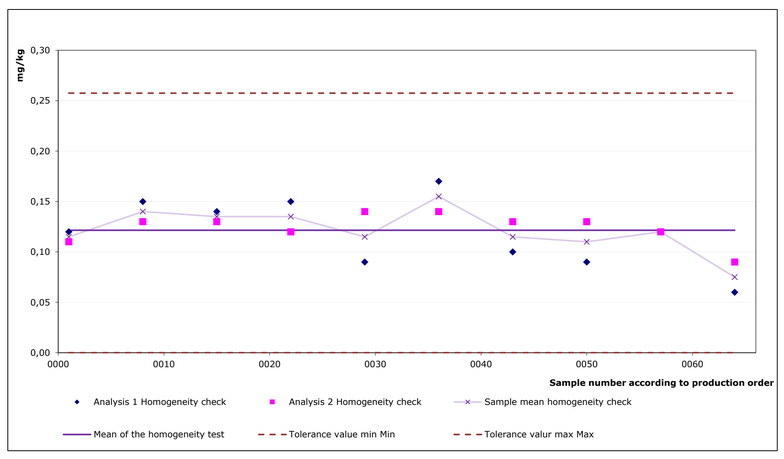


Fig. 3 – PT of April 2024 - Homogeneity check as a function of the sample number (analytical parameter: sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide)

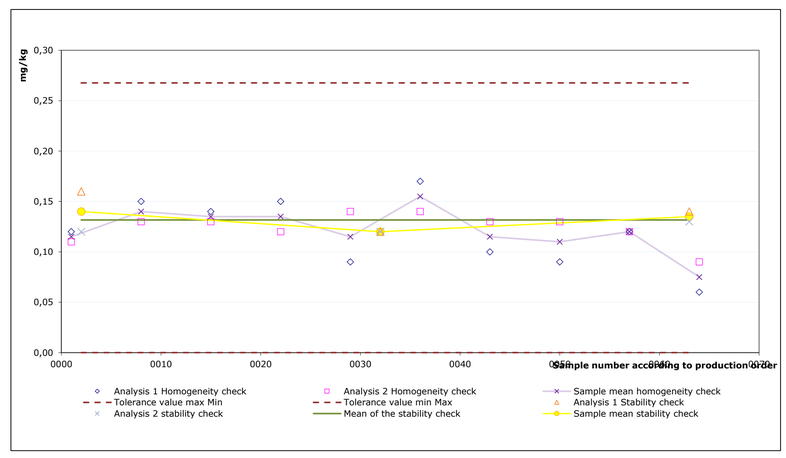


Fig. 4 – PT of April 2024 - Stability check as a function of the date of analysis (analytical parameter: sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide)

2-Chloroethanol (mg/kg)

x_{pt}	$u(x_{pt})$	$s(x_{pt})$	$p(x_{pt})$	$CV(x_{pt})$ %	σ_{pt}	VT
0.453	0.023	0.053	8	12	0.068	0.136

x_{pt} : assigned value
***$u(x_{pt})$:** Standard uncertainty of the assigned value*
***$s(x_{pt})$:** Robust standard deviation of the results*
***$p(x_{pt})$:** Number of results considered for the estimation of the assigned value*
***$CV(x_{pt})$:** Coefficient of variation*
σ_{pt} : Standard deviation for proficiency assessment
***VT :** Tolerance value (30% of x_{pt})*

Table 1 – PT of April 2024 - Statistical summary of the results - 2-Chloroethanol

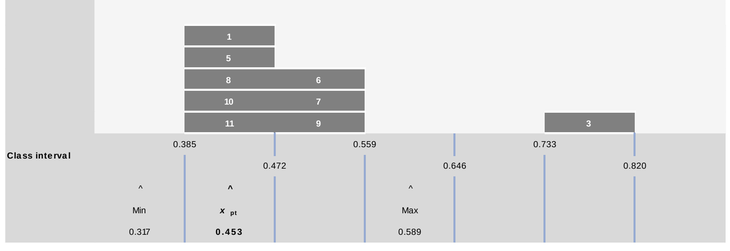


Fig. 5 – PT of April 2024 - Results of 2-chloroethanol represented as a histogram, mg/kg

Laboratory	Result of Ethylene oxide (mg/kg)	Result of 2-Chloroethanol (mg/kg)	z-score 2-Chloroethanol	Analytical principle	Grinding	Test portion (g)	Extraction	Hydrolysis	Derivatisation	Analysis method	Internal standard
1	0.000	0.385	-1.00								
2	0.156			Global iodo-ethanol determination	YES	5	YES	YES	YES	GC/MS & GC-ECD	
3	0.450	0.820	5.40								
4	0.182			Global iodo-ethanol determination	YES	10	YES	YES	YES	GC-MS	NO
5	< 0.010	0.470	0.25	Determination of ETO & 2-CE separately	NO	2	YES	NO	NO	GC	YES
6		0.474	0.31	Determination of ETO & 2-CE separately	YES	5	YES	NO	NO	GC	NO
7	0.281	0.513	0.88	Determination of ETO & 2-CE separately	YES	1	YES	NO	NO	GC	NO
8		0.442	-0.16		YES		YES	YES	NO	GC	NO
9	< 0.010	0.491	0.56		YES		YES	NO	NO	GC	
10		0.386	-0.99	Gloabl 2-CE determination	YES	2	YES	NO	NO	GC	YES
11	0.000	0.462	0.13	Determination of ETO & 2-CE separately		4	YES	NO	NO	GC	YES

Table 2 – PT of April 2024 - Laboratories data

For the PT of April 2024, 11 laboratories out of 14 communicated their results together with useful information for the interpretation of the data. An assigned value (x_{pt}) of 0,453 mg/kg was calculated for 2-chloroethanol from the robust mean of the all returned results except incoherent ones. The main statistical parameters of this parameter are summarized in Table 1. Concerning ethylene oxide, no assigned value could be estimated due to the dispersion of results (raw material was not spiked with ethylene oxide). Table 2 shows the laboratories results and information about the analytical process performed by each participants. These data highlight that different analytical principles are carried out by laboratories: most of participants determine the ethylene oxide and its metabolite 2-chloroethanol separately, one perform a global 2-chloroethylene determination, and two other carry out a derivatization and a global iodo-ethanol determination. These last returned results only for ethylene oxide and not for 2-chloroethanol. All laboratories except one crushed the sesame seeds before analysis. The most used analysis method is gas chromatography (GC). Despite the differences in protocol used by the laboratories, the results obtained are consistent, with only one participant with an unsatisfactory result.

CONCLUSION

PTS for ethylene oxide and 2-chloroethanol have been implemented successfully, both from production of homogeneous and stable samples and statistical point of view. These PT are an important tool for laboratories, answering to a lack of official methods and enabling participants to draw up a general inventory of their analytical skills in term of analyses of 2-Chloroethanol in food. Laboratories can now monitor the reliability of their results and obtain recognition of their analytical procedures. This work and data collected during these interlaboratory tests are also useful for customers and accreditation bodies.

REFERENCES

(1) ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
(2) ISO 13528:2022 - Statistical methods for use in proficiency testing by interlaboratory comparisons.