Institute for
Interlaboratory Studies

Results of Proficiency Test
NDELA in Finger Paint EN71-7/12
May 2022

Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

Author: ing. G.A. Oosterlaken-Buijs
Correctors: ing. R.J. Starink & Mrs. E.R. Montenij-Bos
Approved by: ing. A.S. Noordman-de Neef

Report: iis22V06

August 2022

CONTENTS

1 INTRODUCTION

NDELA stands for N-nitrosodiethanolamine. This chemical may be formed in finger paint when a secondary amine like Diethanolamine or tertiary amines like Triethanolamine is present together with a nitrosating agent such as nitrite (present in preservatives like bronopol). NDELA is considered to be carcinogenic. Finger paint is used by children with direct skin contact and with a possibility of ingestion, therefore exposure to this chemical should be limited or avoided.
The European Union published the test method EN71-12 for the determination of N-nitrosamines and N-nitrosatable substances. The limit for finger paint stated in EN71-12:16 is 0.02 mg/kg N-nitrosamine and 1 mg/kg N-nitrosatable substances.

Since 2020 the Institute for Interlaboratory Studies (iis) organizes a proficiency scheme for the determination of NDELA in Finger Paint. During the annual proficiency testing program 2021/2022 it was decided to continue the proficiency test for the determination of NDELA in Finger Paint EN71-7/12.

In this interlaboratory study 9 laboratories in 6 countries registered for participation, see appendix 4 for the number of participants per country. In this report the results of the NDELA in Finger Paint EN71-7/12 proficiency test are presented and discussed. This report is also electronically available through the iis website www.iisnl.com.

2 SET UP

The Institute for Interlaboratory Studies (iis) in Spijkenisse, the Netherlands, was the organizer of this proficiency test (PT). Sample analyzes for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory.
It was decided to send a yellow finger paint sample positive on NDELA in an 8 mL bottle labelled #22610.
The participants were requested to report rounded and unrounded test results. The unrounded test results were preferably used for statistical evaluation.

2.1 QUALITY SYSTEM

The Institute for Interlaboratory Studies in Spijkenisse, the Netherlands, has implemented a quality system based on ISO/IEC17043:2010. This ensures strict adherence to protocols for sample preparation and statistical evaluation and 100% confidentiality of participant's data. Feedback from the participants on the reported data is encouraged and customer's satisfaction is measured on regular basis by sending out questionnaires.

2.2 PROTOCOL

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation' of June 2018 (iis-protocol, version 3.5). This protocol is electronically available through the iis website www.iisnl.com, from the FAQ page.

2.3 CONFIDENTIALITY STATEMENT

All data presented in this report must be regarded as confidential and for use by the participating companies only. Disclosure of the information in this report is only allowed by means of the entire report. Use of the contents of this report for third parties is only allowed by written permission of the Institute for Interlaboratory Studies. Disclosure of the identity of one or more of the participating companies will be done only after receipt of a written agreement of the companies involved.

2.4 SAMPLES

A batch of yellow finger paint positive on NDELA was obtained from a local supplier. After homogenization 20 bottles of 10 mL were filled and labelled #22610.
The homogeneity of the subsamples was checked by determination of N-nitrosamines in accordance with ISO15819 on 5 stratified randomly selected subsamples.

	N-nitrosamines in mg/kg
sample #22610-1	0.946
sample #22610-2	0.972
sample #22610-3	0.921
sample #22610-4	0.893
sample #22610-5	0.921

Table 1: homogeneity test results of subsamples #22610

From the above test results the repeatability was calculated and compared with 0.3 times the reproducibility of the reference test method in agreement with the procedure of ISO13528, Annex B2 in the next table.

	N-nitrosamines in mg/kg
r (observed)	0.083
reference test method	EN71-12:16
0.3 x R (reference test method)	0.195

Table 2: evaluation of the repeatability of subsamples #22610

The calculated repeatability is in agreement with 0.3 times the reproducibility of the reference test method. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories one finger paint sample labelled #22610 was sent on April 27, 2022.

2.5 ANALYZES

The participants were requested to determine: N-nitrosamines and N-nitrosatable substances.
It was requested to report if the laboratory was accredited for the determined components and to report some analytical details.

It was explicitly requested to treat the sample as if it was a routine sample and to report the test results using the indicated units on the report form and not to round the test results, but report as much significant figures as possible. It was also requested not to report 'less than' test results, which are above the detection limit, because such test results cannot be used for meaningful statistical evaluations.

To get comparable test results a detailed report form and a letter of instructions are prepared. On the report form the reporting units are given as well as the reference test methods (when applicable) that will be used during the evaluation. The detailed report form and the letter of instructions are both made available on the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The participating laboratories are also requested to confirm the sample receipt on this data entry portal. The letter of instructions can also be downloaded from the iis website www.iisnl.com.

3 RESULTS

During five weeks after sample dispatch, the test results of the individual laboratories were gathered via the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The reported test results are tabulated per determination in appendices 1 and 2 of this report. The laboratories are presented by their code numbers.

Directly after the deadline, a reminder was sent to those laboratories that had not reported test results at that moment. Shortly after the deadline, the available test results were screened for suspect data. A test result was called suspect in case the Huber Elimination Rule (a robust outlier test) found it to be an outlier. The laboratories that produced these suspect data were asked to check the reported test results (no reanalyzes). Additional or corrected test results are used for data analysis and the original test results are placed under 'Remarks' in the result tables in appendices 1 and 2. Test results that came in after the deadline were not taken into account in this screening for suspect data and thus these participants were not requested for checks.

3.1 STATISTICS

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation' of June 2018 (iis-protocol, version 3.5).
For the statistical evaluation the *unrounded* (when available) figures were used instead of the rounded test results. Test results reported as '<…' or '>…' were not used in the statistical evaluation.

First, the normality of the distribution of the various data sets per determination was checked by means of the Lilliefors-test, a variant of the Kolmogorov-Smirnov test and by the calculation of skewness and kurtosis. Evaluation of the three normality indicators in combination with the visual evaluation of the graphic Kernel density plot, lead to judgement of the normality being either ‘unknown’, ‘OK’, ‘suspect’ or ‘not OK’. After removal of outliers, this check was repeated. If a data set does not have a normal distribution, the (results of the) statistical evaluation should be used with due care.

The assigned value is determined by consensus based on the test results of the group of participants after rejection of the statistical outliers and/or suspect data.

According to ISO13528 all (original received or corrected) results per determination were submitted to outlier tests. In the iis procedure for proficiency tests, outliers are detected prior to calculation of the mean, standard deviation and reproducibility. For small data sets, Dixon (up to 20 test results) or Grubbs (up to 40 test results) outlier tests can be used. For larger data sets (above 20 test results) Rosner’s outlier test can be used. Outliers are marked by D(0.01) for the Dixon’s test, by G(0.01) or DG(0.01) for the Grubbs’ test and by R(0.01) for the Rosner’s test. Stragglers are marked by D(0.05) for the Dixon’s test, by G(0.05) or DG(0.05) for the Grubbs’ test and by R(0.05) for the Rosner’s test. Both outliers and stragglers were not included in the calculations of averages and standard deviations.

For each assigned value the uncertainty was determined in accordance with ISO13528. Subsequently the calculated uncertainty was evaluated against the respective requirement based on the target reproducibility in accordance with ISO13528. In this PT, the criterion of ISO13528, paragraph 9.2.1. was met for all evaluated tests, therefore, the uncertainty of all assigned values may be negligible and need not be included in the PT report.

Finally, the reproducibilities were calculated from the standard deviations by multiplying them with a factor of 2.8.

3.2 GRAPHICS

In order to visualize the data against the reproducibilities from literature, Gauss plots were made, using the sorted data for one determination (see appendix 1). On the Y-axis the reported test results are plotted. The corresponding laboratory numbers are on the X-axis. The straight horizontal line presents the consensus value (a trimmed mean). The four striped lines, parallel to the consensus value line, are the +3s, +2s, -2s and -3s target reproducibility limits of the selected reference test method. Outliers and other data, which were excluded from the calculations, are represented as a cross. Accepted data are represented as a triangle.

Furthermore, Kernel Density Graphs were made. This is a method for producing a smooth density approximation to a set of data that avoids some problems associated with histograms. Also, a normal Gauss curve (dotted line) was projected over the Kernel Density Graph (smooth line) for reference. The Gauss curve is calculated from the consensus value and the corresponding standard deviation.

3.3 Z-SCORES

To evaluate the performance of the participating laboratories the z-scores were calculated. As it was decided to evaluate the performance of the participants in this proficiency test (PT) against the literature requirements (derived from e.g. ISO or ASTM test methods), the z-scores were calculated using a target standard deviation. This results in an evaluation independent of the variation in this interlaboratory study.

The target standard deviation was calculated from the literature reproducibility by division with 2.8. In case no literature reproducibility was available, other target values were used, like Horwitz or an estimated reproducibility based on former iis proficiency tests.

When a laboratory did use a test method with a reproducibility that is significantly different from the reproducibility of the reference test method used in this report, it is strongly advised to recalculate the z-score, while using the reproducibility of the actual test method used, this in order to evaluate whether the reported test result is fit-for-use.

The z-scores were calculated according to:

$z_{(target)}$ = (test result - average of PT) / target standard deviation

The $z_{(target)}$ scores are listed in the test result tables in appendix 1.

Absolute values for z<2 are very common and absolute values for z>3 are very rare. Therefore, the usual interpretation of z-scores is as follows:

	|z|	< 1	good
1 <	|z|	< 2	satisfactory
2 <	|z|	< 3	questionable
3 <	|z|		unsatisfactory

4 EVALUATION

In this proficiency test no problems were encountered with the dispatch of the samples. One participant did not report any test results and none of the participants reported the test results after the final reporting date.
In total 8 participants reported 8 numerical test results. No outlying test results were observed, which is 0%. In proficiency studies, outlier percentages of 3% - 7.5% are quite normal.

The data set didn’t prove to have a normal Gaussian distribution and is referred to as “unknown”. The statistical evaluation of this data set should be used with due care, see also paragraph 3.1.

4.1 EVALUATION PER COMPONENT

In this section the reported test results are discussed per component. The test methods which were used by the various laboratories were taken into account for explaining the observed differences when possible and applicable. These test methods are also in the tables together with the original data in appendices 1 and 2. The abbreviations, used in these tables, are explained in appendix 5.
Method EN71-12 was used by all of the reporting participants. Regretfully, only a relative interlaboratory standard deviation RSD_R is given in EN71-12:16. Multiplication of RSD_R by 2.8 gives the relative reproducibility.

N-nitrosamines: This determination was not problematic. No statistical outliers were observed. The calculated reproducibility is in agreement with the requirements of EN71-12:16.

The majority of participants agreed on a concentration near or below the limit of detection for the determination of N-nitrosatable substances. Therefore, no z-scores were calculated. The test results are mentioned in appendix 2.

4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

A comparison has been made between the reproducibility as declared by the reference test method and the reproducibility as found for the group of participating laboratories. The number of significant test results, the average, the calculated reproducibility (2.8 * standard deviation) and the target reproducibility derived from reference methods are presented in the next table.

Component	unit	n	average	2.8 * sd	R(lit)
N-nitrosamines	mg/kg	8	1.470	0.630	1.029

Table 3: reproducibility of N-nitrosamines in sample #22610

Without further statistical calculations, it can be concluded that there is a good compliance of the group of participating laboratories with the reference test method. See also the discussion in paragraphs 4.1 and 5.

4.3 COMPARISON OF THE PROFICIENCY TEST OF MAY 2022 WITH PREVIOUS PTS

	May 2022	June 2021	June 2020
Number of reporting laboratories	8	8	12
Number of test results	8	8	12
Number of statistical outliers	0	0	0
Percentage of statistical outliers	0%	0%	0%

Table 4: comparison with the previous proficiency test

In proficiency tests, outlier percentages of 3% - 7.5% are quite normal.

The performance of the determinations of the proficiency test was compared, expressed as relative standard deviation (RSD) of the PTs, see next table.

Component	May 2022	June 2021	June 2020	R(lit)
N-nitrosamines	15%	24%	12%	25%

Table 5: development of the uncertainties over the years

The relative standard deviation observed in this PT is in line with the relative standard deviations observed in previous PTs

4.4 EVALUATION OF THE ANALYTICAL DETAILS

The reported analytical details from the participants are listed in appendix 3.

- Seven participants mentioned to be accredited for the determination of the reported components.
- The participants reported a sample intake between 0.4 and 1 grams.
- The reported time period between the preparation of the extract and the start of the analysis varied from immediately to 4320 minutes.
- In case not analyzed directly, four participants reported that the extract was stored in a dark place at 5±3 °C and two particpants reported that they didn't do this.
- Five participants reported that after mixing of the test solution with hydrochloric acid solution the solution was allowed to stand for 30 minutes at 40°C.

As the group of participants is too small for further sub analyzes and as the performance of the determination of N-nitrosamines is in line with the target reproducibility no separate statistical analysis has been performed.

5 DISCUSSION

The limit stated in EN71-12:16 is 0.02 mg/kg N-nitrosamine and 1 mg/kg N-nitrosatable substances. All reporting laboratories would have rejected the sample for N-nitrosamines. However all reporting participants, except one, would have accepted the sample for N-nitrosatable substances.

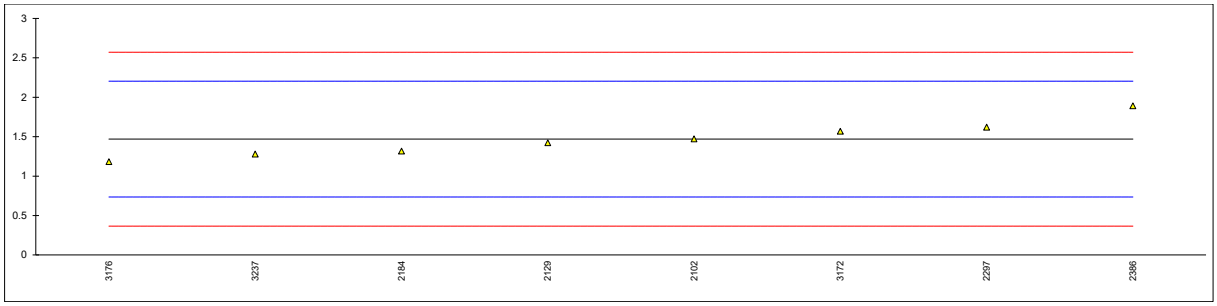
6 CONCLUSION

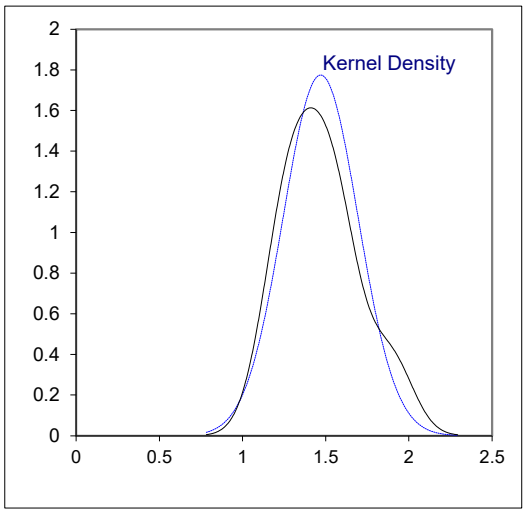
Although it can be concluded that most of the participants have no problem with the determination of NDELA in this PT, each laboratory will have to evaluate its performance in this study and make decisions about necessary corrective actions.
Therefore, participation on a regular basis in this scheme could be helpful to improve the performance and the quality of the analytical results.

APPENDIX 1

Determination of N-nitrosamines in Finger Paint on sample #22610; results in mg/kg

lab	method	value	mark	z(targ)	remarks
2102		1.472		0.01	
2129	EN71-12	1.423		-0.13	
2184	EN71-12	1.317		-0.42	
2297	EN71-12	1.62		0.41	
2363		-----		-----	
2386	EN71-12	1.8921		1.15	
3172	EN71-12	1.5694		0.27	
3176	EN71-12	1.184		-0.78	
3237	EN71-12	1.28		-0.52	
	normality	unknown			
	n	8			
	outliers	0			
	mean (n)	1.4697	RSD = 15%		
	st.dev. (n)	0.22490			
	R(calc.)	0.6297			
	st.dev.(EN71-12:16)	0.36742			
	R(EN71-12:16)	1.0288			





APPENDIX 2
Other reported test results

N-nitrosatable substances in Finger Paint on sample #22610; results in mg/kg

lab	method	value	mark	remarks
2102		Not analyzed		
2129	EN71-12	0.303		
2184	EN71-12	0.146		
2297	EN71-12	2.55	C	first reported 1.72
2363		-----		
2386	EN71-12	not detected		
3172	EN71-12	< 0.01		
3176	EN71-12	0.062		
3237	EN71-12	0.11		

APPENDIX 3

Analytical details

lab	ISO/IEC 17025 accr.	sample intake (g)	time between prep. of extract and start of analysis (minutes)	if not analyzed directly, extract stored in dark place at 5°C before analysis	time to stand solution after mixing with HCL (minutes)	temperature when standing after mixing with HCL (°C)
2102	Yes	1	4320 min	Yes		
2129	Yes	0,5	immediate analysis after preparation	No	30	40
2184	Yes			Yes		
2297	Yes	1.0171	15 min	Yes	30	40
2363	---			---		
2386	Yes	0,5	directly	---	30	40
3172	Yes			---		
3176	Yes	1 + 0,4	5	Yes	30	40
3237	No	0,5	na	No	30	40

APPENDIX 4

Number of participants per country

2 labs in GERMANY

2 labs in HONG KONG

1 lab in ITALY

3 labs in P.R. of CHINA

1 lab in THE NETHERLANDS

1 lab in TURKEY

APPENDIX 5

Abbreviations

C	= final test result after checking of first reported suspect test result
D(0.01)	= outlier in Dixon's outlier test
D(0.05)	= straggler in Dixon's outlier test
G(0.01)	= outlier in Grubbs' outlier test
G(0.05)	= straggler in Grubbs' outlier test
DG(0.01)	= outlier in Double Grubbs' outlier test
DG(0.05)	= straggler in Double Grubbs' outlier test
R(0.01)	= outlier in Rosner's outlier test
R(0.05)	= straggler in Rosner's outlier test
E	= calculation difference between reported test result and result calculated by iis
W	= test result withdrawn on request of participant
ex	= test result excluded from statistical evaluation
n.a.	= not applicable
n.e.	= not evaluated
n.d.	= not detected
fr.	= first reported
f+?	= possibly a false positive test result?
f-?	= possibly a false negative test result?

Literature

1 iis Interlaboratory Studies, Protocol for the Organisation, Statistics & Evaluation, June 2018
2 ISO5725:86
3 ISO5725 parts 1-6:94
4 ISO13528:05
5 M. Thompson and R. Wood, J. AOAC Int, 76, 926, (1993)
6 W.J. Youden and E.H. Steiner, Statistical Manual of the AOAC, (1975)
7 P.L. Davies, Fr. Z. Anal. Chem, 331, 513, (1988)
8 J.N. Miller, Analyst, 118, 455, (1993)
9 Analytical Methods Committee, Technical Brief, No 4, January 2001
10 P.J. Lowthian and M. Thompson, The Royal Society of Chemistry, Analyst, 127, 1359-1364, (2002)
11 W. Horwitz and R. Albert, J. AOAC Int, 79.3, 589-621, (1996)
12 Bernard Rosner, Percentage Points for a Generalized ESD Many-Outlier Procedure, Technometrics, 25(2), 165-172, (1983)