Results of Proficiency Test
NDELA in Finger Paint EN71-7/12
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Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

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1 INTRODUCTION

NDELA stands for N-nitrosodiethanolamine. This chemical may be formed in fingerpaint 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. Fingerpaint is used by children with direct skin contact and with a possibility of ingestion, therefore exposure to this chemical should be limited or avoided.
In 2013 the European Union published the test method EN71-12 for the determination of N-nitrosamines and N-nitrosatable substances. The limit stated in this method is 0.02 mg/kg N-nitrosamine and 1 mg/kg N-nitrosatable substances.

During the annual proficiency testing program 2019/2020 the Institute for Interlaboratory Studies (iis) decided to organize a proficiency scheme for the analysis of NDELA in Fingerpaint.
In this interlaboratory study 14 laboratories in 8 different countries registered for participation. See appendix 3 for the number of participants per country. In this report, the results of this 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 one sample with approximately 8 mL of fingerpaint, labelled #20630, positive on NDELA.
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 a regular basis by sending out questionnaires.

2.2 PROTOCOL

The protocol followed in the organisation 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 blue fingerpaint, positive on NDELA for N-nitrosamines, was obtained from a local supplier. After homogenization the batch was divided over 25 small vials of approximately 8 mL each and labelled #20630. The homogeneity of the subsamples was checked by determination of NDELA using test method EN71-12 on five stratified randomly selected subsamples.

	NDELA (N-nitrosamines) in mg/kg
Sample 20630-1	1.666
Sample 20630-2	1.662
Sample 20630-3	1.868
Sample 20630-4	1.737
Sample 20630-5	1.898

Table 1: homogeneity test results of subsamples #20630

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.

	NDELA (N-nitrosamines) in mg/kg
r (observed)	0.311
reference test method	EN71-12:16
0.3 x R (reference test method)	0.371

Table 2: evaluation of the repeatability of subsamples #20630

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 sample of fingerpaint labelled #20630 was sent on May 20, 2020.

2.5 ANALYZES

The participants were requested to determine the NDELA (N-nitrosamines) en NDELA (N-nitrosatable substances) content on sample #20630. It was also requested to report if the laboratory was accredited for the requested 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 to report as much significant figures as possible. It was also requested not to report ‘less than’ results which are above the detection limit, because such results can not 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 appendix 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 reanalysis). Additional or corrected test results are used for data analysis and the original reported test results placed under 'Remarks' in the result tables in appendix 1. 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 organisation 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 results. 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.

According to ISO5725 the original test results per determination were submitted to Dixon’s and/or Grubbs' and/or Rosner’s outlier tests. 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.

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 analysis 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. The Kernel Density Graph 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 was projected over the Kernel Density Graph for reference.

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, 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. In some cases, a reproducibility based on former iis proficiency tests could be used.

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 results is fit-for-use.

The z-scores were calculated in according to:

$z_{(target)}$ = (test result – average of proficiency test) / target standard deviation

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

Absolute values for z<2 are very common and absolute values for z>3 are very rare. 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 severe problems were encountered with the dispatch of the samples. Two participants did not report at all and none of the participants reported the test results after the final reporting date. Not all laboratories were able to report all components requested.
In total 12 numerical test results were reported and no outlying results were observed. In proficiency studies, outlier percentages of 3% - 7.5% are quite normal.

The original data set proved to have a normal Gaussian distribituion.

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 methods are also in the table together with the original data. The abbreviations, used in these tables, are explained in appendix 5.

Method EN71-12 was performed by all 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.

Sample #20630

NDELA (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 NDELA (N-nitrosatable substances). Therefore, no z-scores were calculated for this determination. The test results are given in appendix 2.

4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

A comparison has been made between the reproducibility as declared by the estimated target reproducibility 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 estimated target reproducibility of EN17-12:16 are presented in the next tables.

Component	unit	n	average	2.8 * sd	R(lit)
NDELA (N-nitrosamines)	mg/kg	12	2.04	0.68	1.43

Table 3: overview of results for sample #20630

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 OVERVIEW OF THE PROFICIENCY TEST OF JUNE 2020

The evolution of the uncertainty for NDELA in Fingerpaint as observed in this proficiency scheme is listed in table 4.

Year	Component	Observed RSD%	Target RSD%	Concentration in mg/kg
2020	NDELA (N-nitrosamines)	12%	25%	2.0

Table 4: development of uncertainties in % in NDELA in Fingerpaint

4.4 EVALUATION OF THE ANALYTICAL DETAILS

For this PT also some analytical details were requested and are given in appendix 4.
Based on the answers given by the participants the following can be summarized:
- Nine out of twelve reporting participants mentioned that they are accredited for the determination of NDELA.
- The majority of the participants used less sample intake for the determination of N-nitrosatable substances as for N-nitrosamine. For N-nitrosamines between 0.5 and 1 gram was used and for N-ntirosatable substances 0.15 to 1 gram was used.
- The time between the preparation of the extract and the start of the analysis varied from 15 minutes to 6 hours. Most particpants did store the solutions in a dark place at 5°C.
- All reporting participants used 30 minutes at 40°C to stand the solution with HCl.

Because the amount of analytical details and the number of participating laboratories is small, no conclusions could be drawn from these analytical details.

5 DISCUSSION

The limit stated in EN71-12 is 0.02 mg/kg N-nitrosamine and 1 mg/kg N-nitrosatable substances. All reporting participants were able to detect NDELA (N-nitrosamines) in sample #20630 in quantities higher than the limit, so all would have rejected this sample for NDELA (N-nitrosamines). All reporting participants, but one, did not detect NDELA (N-nitrosatable substances) above the limit. The laboratory that did find a higher test result for N-nitrosatable substances is suspected for not taking into account the N-nitrosamines before reporting the test result.

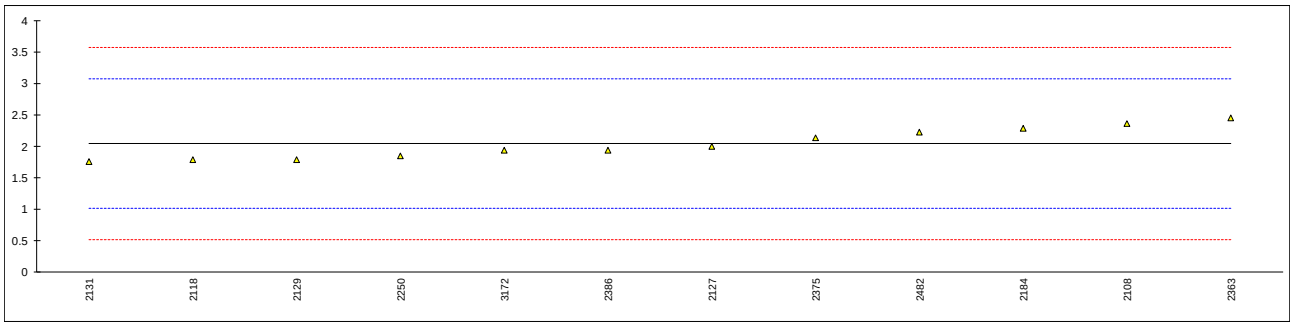
6 CONCLUSION

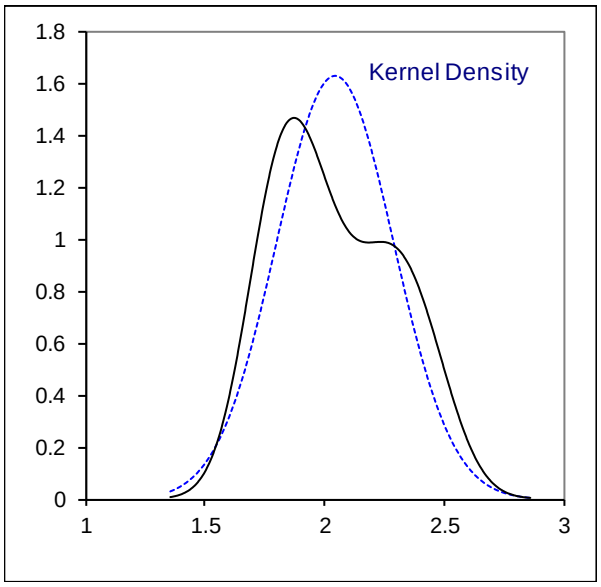
Each laboratory should evaluate its performance in this study and make decisions about necessary corrective actions. Therefore, participation on a regular basis in this scheme could be helpful to improve the performance and the quality of the analytical results.

APPENDIX 1

Determination of NDELA (N-nitrosamines) in Finger Paint sample #20630; results in mg/kg

lab	method	value	mark	z(targ)	remarks
2102		-----		-----	
2108	EN71-12	2.364		0.63	
2118	EN71-12	1.78		-0.52	
2127	EN71-12	2		-0.09	
2129	EN71-12	1.787		-0.50	
2131	In house	1.75833333		-0.56	
2184	EN71-12	2.289		0.48	
2250	EN71-12	1.854		-0.37	
2363	EN71-12	2.457		0.81	
2375	EN71-12	2.14		0.19	
2386	EN71-12	1.9345		-0.21	
2482	EN71-12	2.23		0.36	
3146		-----		-----	
3172	EN71-12	1.933		-0.22	
	normality	OK			
	n	12			
	outliers	0			
	mean (n)	2.0439			
	st.dev. (n)	0.24430	RSD = 12%		
	R(calc.)	0.6840			
	st.dev.(EN71-12:16)	0.51098			
	R(EN71-12:16)	1.4307			





APPENDIX 2

Other reported NDELA in Finger Paint sample #20630; results in mg/kg

lab	method	NDELA (N-nitrosatable substances)	remarks
2102		-----	
2108		-----	
2118	EN71-12	0	
2127	EN71-12	0.15	
2129	EN71-12	<0,01	
2131	In house	1.870	Test result without subtraction of N-nitrosamines content?
2184	EN71-12	0.321	
2250	EN71-12	0.2165	
2363	EN71-12	ND	
2375	EN71-12	ND	
2386	EN71-12	0	
2482		-----	
3146		-----	
3172	EN71-12	n.a.	

APPENDIX 3
Analytical details as reported by the participating laboratories

Lab	ISO 17025 accr.	sample intake (g)	time between prep of extract and start of analysis	stored at in dark place at 5°C before analysis?	time to stand solution after mixing with HCL (min)	temperatyre when standing after mixing with HCL(°C)
2102	---			---		
2108	Yes	1 g 0.5g for n-nitrosamines	approx.30-60 min	Yes	30 min	40°C
2118	No	0.4g for n-nitrosatable subst.	15 minutes	Yes	30 minutes	40°C
2127	Yes	N-nitrosamines ca 0,5g		---	30 Min	40°C
2129	Yes	N-nitrosatable subst. ca 0,15g	*)	No	30 min	40°C
2131	No	0.400	6 hours	No	30	40
2184	Yes	NS: 1g NSBO: 0.4g 0,5g for nitrosamines	n.a.	Yes	n.a.	n.a.
2250	Yes	0,2g for nitrosatable subst.	immediately	Yes	30 min	40°C
2363	Yes	1g Nitrosamine: 1 gram	1H	No	0.5H	40°C
2375	Yes	Nitrosatable: 0.4 gram	4 Hours	Yes	30 Minutes	40 °C
2386	Yes	0,5	2-4h	Yes	30	40
2482	No	0,5	< 10 min	Yes	30	40 °C
3146	---			---		
3172	Yes	1	60	No		

*) Remark lab 2129: The analyzes started immediately, but after measuring the calibration and routine samples (~ 6 hours).

APPENDIX 4

Number of participants per country

1 lab in	BELGIUM
7 labs in	GERMANY
1 lab in	HONG KONG
1 lab in	ITALY
1 lab in	P.R. of CHINA
1 lab in	SWITZERLAND
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	= possibly an error in calculations
W	= test result withdrawn on request of participant
ex	= test result excluded from statistical evaluation
n.a.	= not applicable
n.d.	= not detected
n.e.	= not evaluated
fr.	= first reported

Literature

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