Results of Proficiency Test
Metals in Tattoo Ink
March 2021

Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

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1 INTRODUCTION

In the past years tattoos have become very popular worldwide, and millions of people have tattoos. Since tattoos are applied just under the skin, the skin barrier is breached and soluble components of the ink are distributed within hours or days across the entire body. Therefore in 2008 a committee of ministers in the EU adopted a resolution (ResAP(2008)1) on requirements and criteria for the safety of tattoos. In resolution ResAP(2008)1 on table 3 the maximum allowed concentration for Metals in Tattoo Ink is mentioned. In 2015 the EU started investigating Tattoo Inks in relation to the hazardous substances that should not be present in Tattoo Ink. This resulted in Commission Regulation (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 concerning (...) substances in Tattoo Inks or permanent make-up. In this regulation the limits for the different metals are published. No reference materials (RMs) for Metals in Tattoo Ink are available to optimize this determination. As an alternative, participation in a proficiency test may enable the laboratories to check their performance and thus to increase this comparability.

On request of a number of laboratories, the Institute for Interlaboratory Studies (iis) decided to set up a new proficiency test of the determination of Metals in Tattoo Ink during the annual testing program 2020/2021.
In this interlaboratory study 13 laboratories in 9 different countries registered for participation. See appendix 4 for the number of participants per country. In this report the results of the Metals in Tattoo Ink 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. Sample analyzes for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory. It was decided to send one sample of Tattoo Ink positive on a number of metals and labelled #21542.
The participants were requested to report the 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 black Tattoo Ink was obtained from a local market and made positive with Cadmium, Cobalt and Nickel. After homogenization the batch was divided over 30 subsamples in vials of 8 mL and labelled #21542.
The homogeneity of the subsamples was checked by the determination of Cobalt and Nickel on five stratified randomly selected subsamples using method EPA 3052.

	Cobalt in mg/kg	Nickel in mg/kg
Sample #21542-1	27.833	14.273
Sample #21542-2	29.787	15.603
Sample #21542-3	28.651	14.659
Sample #21542-4	28.171	15.169
Sample #21542-5	28.361	14.889

Table 1: homogeneity test results of subsamples #21542

From the above test results the repeatabilities were calculated and compared with 0.3 times the estimated reproducibility calculated with the Horwitz equation in agreement with the procedure of ISO 13528, Annex B2 in the next table:

	Cobalt in mg/kg	Nickel in mg/kg
r (observed)	2.09	1.41
reference method	Horwitz	Horwitz
0.3 x R (ref. method)	2.32	1.33

Table 2: evaluation of the repeatibilities of subsamples #21542

The calculated repeatabilities are in agreement with 0.3 times the estimated reproducibility calculated with the Horwitz equation. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories one sample of #21542 was sent on February 24, 2021.

2.5 ANALYZES

The participants were requested to determine on sample #21542 the concentration of Antimony, Arsenic, Barium, Cadmium, Chromium(III), Chromium(VI), Cobalt, Copper, Lead, Mercury, Nickel, Selenium, Tin and Zinc. It was also 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 to 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 appropriate 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 did not report 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 original test results are placed under 'Remarks' in the test 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 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 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 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, for one or more of the analytes the criterion of ISO13528, paragraph 9.2.1 was not met, therefore, the uncertainty of the assigned value for these analytes is not negligible and will be used to calculate z’-scores (see paragraph 3.3).

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. 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 (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 the z-scores were calculated using a target standard deviation. This results in an evaluation independent of the variation of 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.

The standard uncertainty (u_x) was calculated from the (target) standard deviation in accordance with ISO13528, paragraph 5.6:

$$u_x = 1.25 * (\text{st.dev (n)}) / \sqrt{n}$$

In ISO13528 is stated that if $u_x \geq 0.3$ * standard deviation for proficiency testing, the uncertainty of the assigned value is not negligible and needs to be included in the interpretation of the results of the proficiency test. Therefore, in this PT report, z'-scores were calculated instead of the usual z-scores. The $z'_{(target)}$ scores were calculated in accordance with ISO13528 paragraph 9.5:

$$z'_{(target)} = (\text{test result} - \text{mean of PT}) / \sqrt{((\text{target standard deviation})2 + (u_x)2)}$$

The $z'_{(target)}$ scores are listed in the result tables in 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 interlaboratory study no problems were encountered with the dispatch of the samples. One participant reported test results after the final reporting date. Not all participants were able to report all components requested.
In total 13 laboratories reported 35 numerical test results. Observed were no outlying test results. In proficiency studies, outlier percentages of 3% - 7.5% are quite normal.

Not all original data sets proved to have a normal Gaussian distribution. These are referred to as “not OK” or “suspect”. The statistical evaluation of these data sets should be used with due care, see also paragraph 3.1.

4.1 EVALUATION PER ELEMENT

In this section the reported test results are discussed per element. 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 in appendix 1 together with the original data. The abbreviations, used in these tables, are explained in appendix 5.

Unfortunately, a suitable reference method, providing the precision data, is not available for the determination of Metals in Tattoo Ink. Therefore, the reproducibility was compared against the estimated reproducibility calculated from the Horwitz equation.

Sample #21542

Cadmium: This determination was not problematic at a consensus value of 4.2 mg/kg. No statistical outliers were observed. The calculated reproducibility is in agreement the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Cobalt: This determination was not problematic at a consensus value of 26.8 mg/kg. No statistical outliers were observed. The calculated reproducibility is in agreement the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Nickel: This determination was not problematic at a consensus value of 13.3 mg/kg. No statistical outliers were observed. The calculated reproducibility is in agreement the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

The concentrations reported for all other Elements were near or below the detection limit. Therefore, no z-scores were calculated. See appendix 2 for the reported test results.

4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

A comparison has been made between the reproducibility as declared by the estimated target reproducibility calculated with the Horwitz equation 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 are presented in the next tables.

Component	unit	n	average	2.8 * sd	R(target)
Cadmium	mg/kg	12	4.22	0.83	1.55
Cobalt	mg/kg	12	26.79	8.68	7.96
Nickel	mg/kg	11	13.33	3.12	4.21

Table 3: reproducibilities of elements on sample #21542

Without further calculations, it can be concluded that for the determined elements there is a good compliance of the group of participating laboratories with the target reference method. The problematic tests have been discussed in paragraph 4.1.

4.3 OVERVIEW OF THE PROFICIENCY TEST OF MARCH 2021

	March 2021
Number of reporting laboratories	13
Number of test results	35
Number of statistical outliers	0
Percentage of statistical outliers	0%

Table 4: overview of this PT

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

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

Component	March 2021	Horwitz (3 - 30 mg/kg)
Cadmium	7%	10 - 14%
Cobalt	12%	10 - 14%
Nickel	8%	10 - 14%

Table 5: relative standard deviations (RSD)

4.4 EVALUATION OF THE ANALYTICAL DETAILS

Many different test methods were mentioned by the participants. Five participants reported to have used an in house test method, two used test method EPA3052 (oil and sludge) and two method § 64 LFGB,K 84.00-31 (cosmetics test). The American CPSC-CH-E1003-09.1 (Paint) was used once, as was EN16711-1 (Textile) and EPA3050B (oil and sludge).
Ten out of thirteen participants are accredited for the determination of Metals in Tattoo Ink.
Eight participants used 0.1-0.2 grams of sample intake, four other participants used 0.4 – 1 grams.
The influence of these analytical details could not be determined because the group of participants is too small for further sub analyzes.

5 DISCUSSION

The participants were able to detect the spiked elements in both samples correctly in this proficiency test.
Limits for the presence of Metals in Tattoo Ink and Permanent Make-up have been set in Commission Regulation (EU) 2020/2081 of 14 December 2020.

Element	Concentration limit
Antimony	0.5 mg/kg
Arsenic	0.5 mg/kg
Barium (soluble)	500 mg/kg
Cadmium	0.5 mg/kg
Chromium VI	0.5 mg/kg
Cobalt	0.5 mg/kg
Copper (soluble)	250 mg/kg
Lead	0.7 mg/kg
Mercury	0.5mg/kg
Nickel	5 mg/kg
Selenium	2 mg/kg
Organometallic Tin	0.5 mg/kg
Zinc (soluble)	2000 mg/kg

Table 6: limits for Metals in Commission Regulation (EU) 2020/2081

All participants would have rejected this sample, based on these limits.

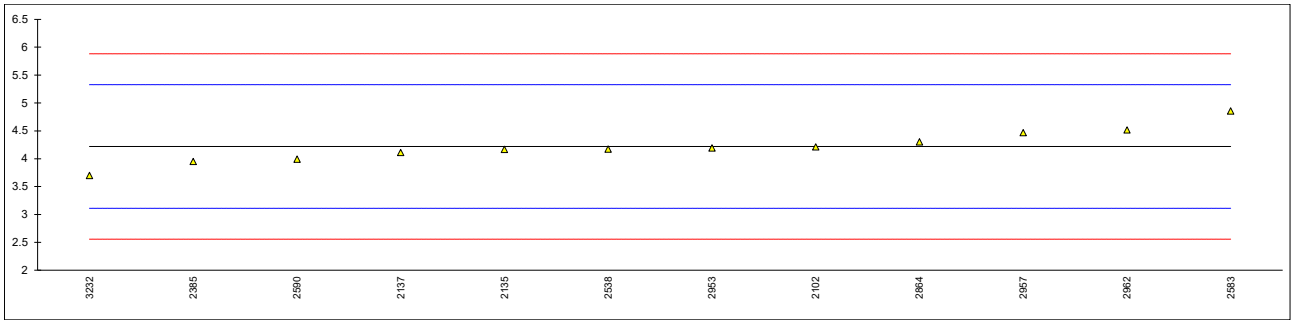
6 CONCLUSION

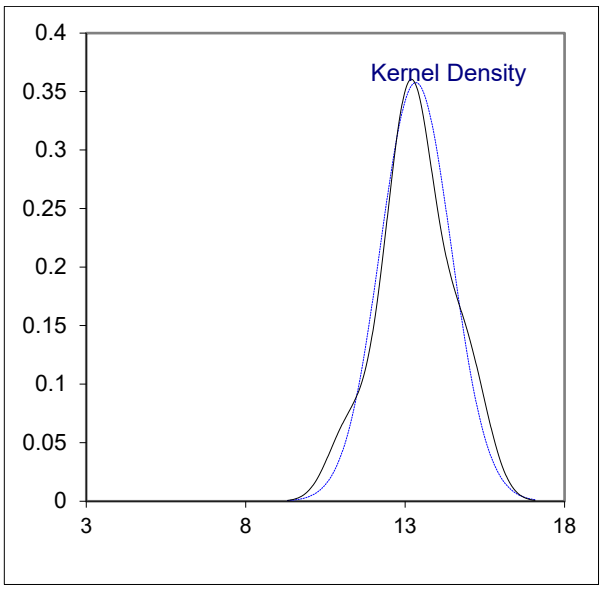
Each participating laboratory will have to evaluate its performance in this study and decide about any corrective actions if necessary. Therefore, participation on a regular basis in this scheme could be helpful to improve the performance and thus increase of the quality of the analytical results.

APPENDIX 1

Determination of Cadmium as Cd on sample #21542; results in mg/kg

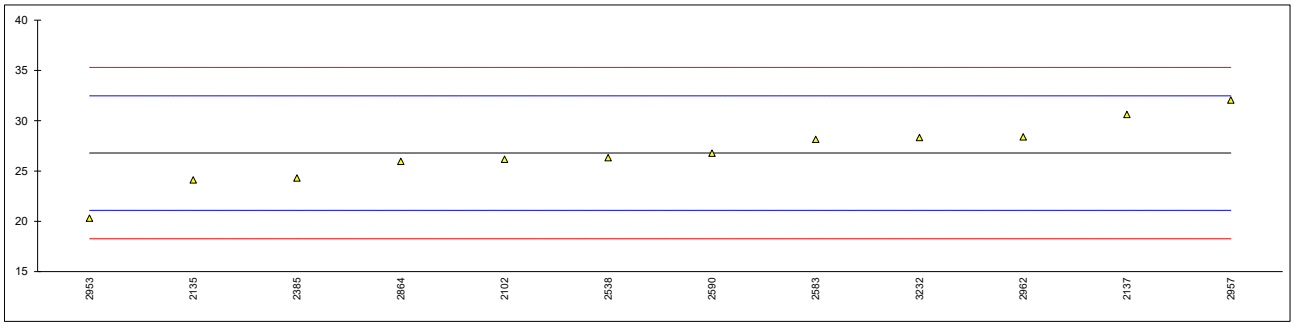
lab	method	value	mark	z'(targ)	remarks
348	CPSC-CH-E1003-09.1	<5		-----	
2102		4.21		-0.02	
2135	In house	4.167		-0.09	
2137	In house	4.11		-0.20	
2385	EPA3052	3.95		-0.49	
2538	§ 64 LFGB,K 84.00-31	4.173		-0.08	
2583	§ 64 LFGB,K 84.00-31	4.855		1.15	
2590	EN16711-1	3.99		-0.41	
2864	EPA3052	4.30		0.15	
2953	In house	4.192		-0.05	
2957	In house	4.466		0.45	
2962	In house	4.5146		0.53	
3232	EPA3050B	3.70		-0.94	
	normality	suspect			
	n	12			
	outliers	0			
	mean (n)	4.2190			
	st.dev. (n)	0.29713	RSD = 7%		
	R(calc.)	0.8320			
	st.dev.(Horwitz')	0.55400			
	R(Horwitz')	1.5512			

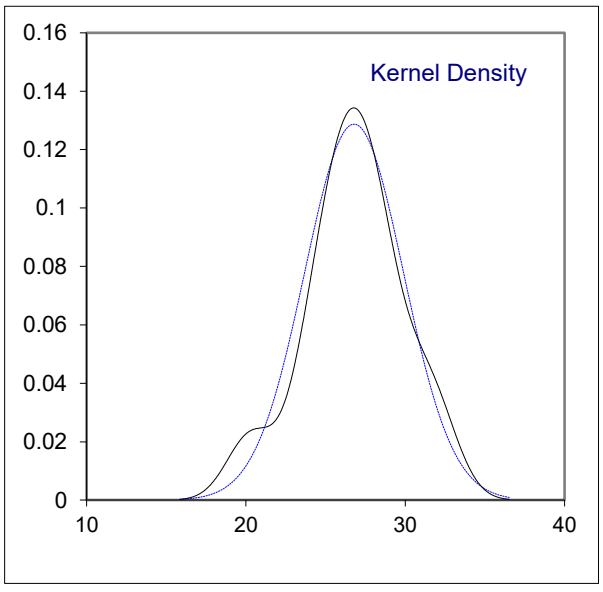




Determination of Cobalt as Co on sample #21542; results in mg/kg

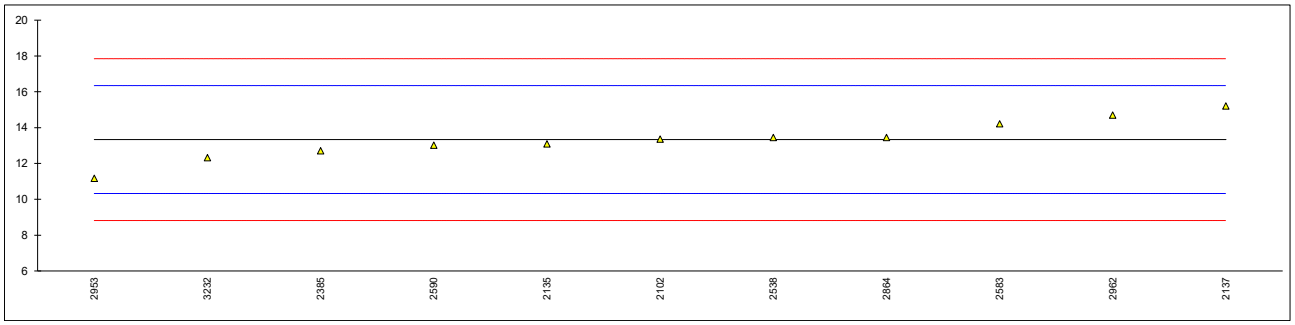
lab	method	value	mark	z'(targ)	remarks
348		-----		-----	
2102		26.16		-0.22	
2135	In house	24.105		-0.94	
2137	In house	30.62		1.35	
2385	EPA3052	24.3		-0.88	
2538	§ 64 LFGB,K 84.00-31	26.32		-0.16	
2583	§ 64 LFGB,K 84.00-31	28.15		0.48	
2590	EN16711-1	26.77		-0.01	
2864	EPA3052	25.96		-0.29	
2953	In house	20.318		-2.28	
2957	In house	32.05		1.85	
2962	In house	28.3810		0.56	
3232	EPA3050B	28.32	C	0.54	first reported: 14.16
	normality	OK			
	n	12			
	outliers	0			
	mean (n)	26.7878			
	st.dev. (n)	3.099288	RSD = 12%		
	R(calc.)	8.67801			
	st.dev.(Horwitz')	2.842226			
	R(Horwitz')	7.95823			

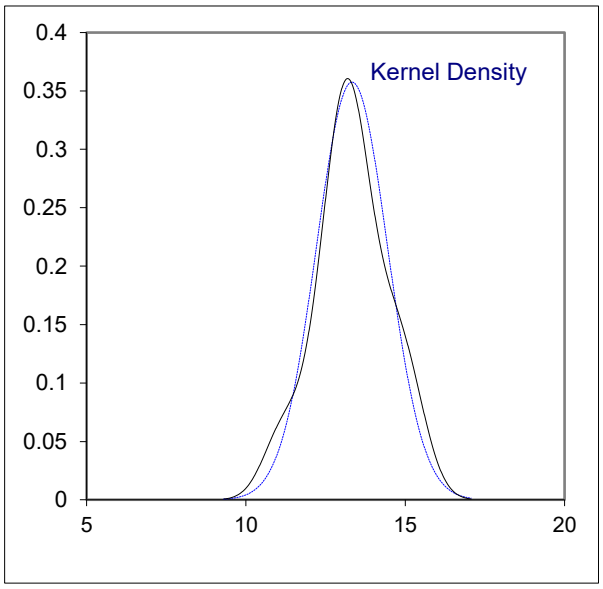




Determination of Nickel as Ni on sample #21542; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
348		-----		-----	
2102		13.35		0.01	
2135	In house	13.093		-0.16	
2137	In house	15.21		1.25	
2385	EPA3052	12.7		-0.42	
2538	§ 64 LFGB,K 84.00-31	13.45		0.08	
2583	§ 64 LFGB,K 84.00-31	14.21		0.58	
2590	EN16711-1	13.01		-0.21	
2864	EPA3052	13.45		0.08	
2953	In house	11.173		-1.44	
2957		-----	W	-----	first reported: 18.739
2962	In house	14.6995		0.91	
3232	EPA3050B	12.32		-0.67	
	normality	OK			
	n	11			
	outliers	0			
	mean (n)	13.3332			
	st.dev. (n)	1.11542	RSD = 8%		
	R(calc.)	3.1232			
	st.dev.(Horwitz')	1.50449			
	R(Horwitz')	4.2126			





APPENDIX 2 Other reported elements

Abbreviations of Metals:

Sb = Antimony
As = Arsenic
Ba = Barium
Cr = Chromium (III)
Cr = Chromium (VI)
Cu = Copper
Pb = Lead
Hg = Mercury
Se = Selenium
Sn = Tin
Zn = Zinc

Determination of Other Metals on sample #21542; results in mg/kg

lab	Sb	As	Ba	Cr(III)	Cr(VI)	Cu
348	-----	-----	-----	<5	<5	-----
2102	Not detected	Not detected	Not detected	Not detected	Not analyzed	0.042
2135	-----	-----	-----	-----	-----	-----
2137	-----	-----	-----	-----	-----	-----
2385	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2538	< 0.50	< 0.50	< 5.0	-----	-----	< 4.0
2583	< our det. limit	< our det. limit	< our det. limit	-----	-----	< our det. limit
2590	<L.O.Q	<L.O.Q	<L.O.Q	<L.O.Q	-----	<L.O.Q
2864	not detected	not detected	not detected	-----	not detected	2.36
2953	-----	-----	2.151	-----	-----	-----
2957	0.204	0.006	0.297	-----	< 0,2	2.159
2962	not detected	not detected	<0.1180	not analyzed	not analyzed	not detected
3232	-----	-----	-----	-----	not detected	0.61

lab	Pb	Hg	Se	Sn	Zn	
348	<10	-----	-----	-----	-----	
2102	Not detected	Not detected	Not detected	Not analyzed	0.041	
2135	-----	-----	-----	-----	-----	
2137	-----	-----	-----	-----	-----	
2385	<0.1	<0.1	<0.1	<0.5	<1	
2538	< 1.0	< 0.5	< 0.50	-----	< 5.0	
2583	-----	< our det. limit	< our det. limit	-----	-----	
2590	<L.O.Q	<L.O.Q	<L.O.Q	<L.O.Q	<L.O.Q	
2864	not detected	not detected	not detected	not detected	not detected	
2953	5.80	-----	-----	-----	-----	
2957	0.218	0.161	0.021	0.110	-----	W
2962	not detected	<0.1250	not detected	<0.1000	not detected	
3232	not detected	-----	-----	-----	-----	

Lab 2957 first reported for Zn: 7.654

APPENDIX 3 Analytical details

lab	ISO17025 accr.	Sample intake (in g)
348	Yes	0.1
2102	Yes	0.1
2135	Yes	0,4
2137	No	0.2
2385	Yes	0.5
2538	Yes	0.1- 0.2
2583	Yes	0,224 to 0,338
2590	Yes	0.2g
2864	Yes	0.2 g
2953	No	0.2g
2957	Yes	0,5 g
2962	Yes	100 mg
3232	No	1

APPENDIX 4

Number of participants per country

4 labs in GERMANY
1 lab in INDIA
2 labs in ITALY
1 lab in SLOVAKIA
1 lab in SOUTH KOREA
1 lab in SPAIN
1 lab in SWITZERLAND
1 lab in TAIWAN
1 lab in THE NETHERLANDS

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
W	= test result withdrawn on request of participant
ex	= test result excluded from the statistical evaluation
n.a.	= not applicable
n.e.	= not evaluated
n.d.	= not detected
fr.	= first reported

Literature

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