Results of Proficiency Test
AZO Dyes in Tattoo Ink
March 2021

Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

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1 INTRODUCTION

Some AZO Dyes are known to metabolise in aromatic amines. These primary aromatic amines have been considered mutagenic and/or carcinogenic for many years. These AZO Dyes can be used to colour Tattoo Ink. In the past years, tattoos have become very popular worldwide, and millions of people have tattoos. Tattoo Ink is especially important as a matrix to avoid AZO colorants that break down to aromatic amines, because it is used directly under the skin, where 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 1 the maximum allowed concentration for Aromatic Amines 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 limit for the individual AZO Dyes is less than 0.5 mg/kg. No reference materials (RMs) for AZO Dyes in Tattoo Ink are available to optimise 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 banned aromatic amines derived from AZO Dyes in Tattoo Ink during the annual testing program 2020/2021.

In this interlaboratory study 10 laboratories in 7 different countries registered for participation. See appendix 4 for the number of participants per country. In this report the results of the AZO Dyes 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 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 real-life sample of Tattoo Ink positive on banned Aromatic Amines derived from AZO dyes and labelled #21544.
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 can be downloaded from 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 Tattoo Ink was obtained from the local market and tested on several banned components like AZO Dyes, Heavy metals and Polycyclic Aromatic Hydrocarbons (PAH). The batch was found to be positive on o-Anisidine and o-Toluidine. It was decided to use this real-life sample without further spiking. After homogenization the batch was divided over 18 subsamples in vials of 8 mL and labelled #21544.
The homogeneity of the subsamples was checked by determination of o-Toluidine using an in-house test method on four stratified randomly selected subsamples.

	o-Toluidine in mg/kg
sample #21544-1	180.40
sample #21544-2	164.73
sample #21544-3	170.30
sample #21544-4	168.13

Table 1: homogeneity test results of subsamples #21544

From the above test results the relative standard deviation was calculated and compared with 0.3 times the relative standard deviation derived from iis PTs on AZO Dyes in Textile in agreement with the procedure of ISO13528, Annex B2, in the next table.

	o-Toluidine
RSD (observed)	3.9%
reference method	iis PTS*
0.3 x RSD (reference method)	5.7%

Table 2: evaluation of the relative standard deviation of subsamples #21544

*) referring to RSD of o-Toluidine from PTs of AZO Dyes in Textile

The calculated relative standard deviation was in agreement with 0.3 times the relative standard deviation derived from iis PTs on AZO Dyes in Textile. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories one sample labelled #21544 was sent on February 24, 2020.

2.5 ANALYZES

The participants were asked to determine on sample #21544 the concentrations of the following aromatic amines:

4-Aminodiphenyl (CAS No. 92-67-1)
Benzidine (CAS No. 92-87-5)
4-Chloro-o-toluidine (CAS No. 95-69-2)
2-Naphtylamine (CAS No. 91-59-8)
o-Aminoazotoluene (CAS No. 97-56-3)
2-Amino-4-nitrotoluene (CAS No. 99-55-8)
4-Chloraniline (CAS No. 106-47-8)
2,4-Diaminoanisol (CAS No. 615-05-4)
4,4'-Diaminodiphenylmethane (CAS No. 101-77-9)
3,3'-Dichlorobenzidine (CAS No. 91-94-1)
3,3'-Dimethoxybenzidine (CAS No. 119-90-4)
3,3'-Dimethylbenzidine (CAS No. 119-93-7)
3,3'-Dimethyl-4,4'-Diaminodiphenylmethane (CAS No. 838-88-0)
p-Cresidine (CAS No. 120-71-8)
4,4'-Diamino-3,3'-dichlorodiphenylmethane (CAS No. 101-14-4)
4,4'-Diaminodiphenylether (CAS No. 101-80-4)
4,4'-Diaminodiphenylsulfide (CAS No. 139-65-1)
2,4-Diaminotoluene (CAS No. 95-80-7)
2,4,5-Trimethylaniline (CAS No. 137-17-7)
o-Anisidine (CAS No. 90-04-0)
2,4-Xylidine (CAS No. 95-68-1)
2,5-Xylidine (CAS No. 95-78-3)
2,6-Xylidine (CAS No. 87-62-7)
Total of Xylidines
o-Aminoazotoluene (CAS No. 97-56-3)
o-Toluidine (CAS No. 95-53-4)
Sum of o-Aminoazotoluene and o-Toluidine
4-Aminoazobenzene (CAS No. 60-09-3)

It was explicitly requested to treat the samples as if they were routine samples 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 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 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 dataset 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 10 laboratories reported 19 numerical test results. Observed were two outlying test results which is 10.5%. In proficiency studies outlier percentages of 3% - 7.5% are quite normal.

All original data sets given in appendix 1 proved to have a normal Gaussion distribution.

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 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 Aromatic Amines derived from AZO Dyes in Tattoo Ink. Therefore, the calculated reproducibility was compared against the estimated reproducibility calculated from the Horwitz equation.

Sample #21544

o-Anisidine (CAS No. 90-04-0):This determination may be problematic at a consensus value of 42 mg/kg. Two statistical outliers were observed. The calculated reproducibility after rejection of the statistical outliers is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

o-Toluidine (CAS No. 95-53-4):This determination may be very problematic at a consensus value of 100 mg/kg. No statistical outliers were observed. The calculated reproducibility is not at all in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

The concentrations reported for all other components 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)
o-Anisidine	mg/kg	7	42.5	50.7	26.3
o-Toluidine	mg/kg	10	100.3	135.7	58.2

Table 3: reproducibilities of components on sample #21542

Without further calculations it can be concluded that for the determined components there is not 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	10
Number of test results	19
Number of statistical outliers	2
Percentage of statistical outliers	10.5%

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 (30 - 100 mg/kg)
o-Anisidine	43%	8 - 14%
o-Toluidine	48%	8 - 14%

Table 5: relative standard deviations (RSD)

4.4 EVALUATION OF THE ANALYTICAL DETAILS

Four participants reported to have used an in house test and four used ISO14362-1 (Textile) or a modified version of the Textile test method.
Seven participants are accredited for the determination of AZO Dyes in Tattoo Ink.
Four participants used 0.1-0.3 grams of sample intake, five other participants used 0.5 – 1 grams.
The influence of these analytical details could not be determined because the group of participants is too small for further subanalyses.

5 DISCUSSION

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

Components	CAS No.	Conc. Limit
o-Anisidine	90-04-0	0.5 mg/kg
o-Toluidine	95-53-4	0.5 mg/kg
3,3'-Dichlorobenzidine	91-94-1	0.5 mg/kg
4-Methyl-m- phenylenediamine	95-80-7	0.5 mg/kg
4-Chloroaniline	106-47-8	0.5 mg/kg
5-Nitro-o-toluidine	99-55-8	0.5 mg/kg
3,3'-Dimethoxybenzidine	119-90-4	0.5 mg/kg
4,4'-Bi-o-toluidine	119-93-7	0.5 mg/kg
4,4'-Thiodianiline	139-65-1	0.5 mg/kg
4-Chloro-o-toluidine	95-69-2	0.5 mg/kg
2-Naphthylamine	91-59-8	0.5 mg/kg
Aniline	62-53-3	0.5 mg/kg
Benzidine	92-87-5	0.5 mg/kg
p-Toluidine	106-49-0	0.5 mg/kg
2-Methyl-p-phenylenediamine	95-70-5	0.5 mg/kg
Biphenyl-4-ylamine	92-67-1	0.5 mg/kg
4-o-Tolylazo-o-toluidine	97-56-3	0.5 mg/kg
4-Methoxy-m- phenylenediamine	615-05-4	0.5 mg/kg
4,4'-Methylenedianiline	101-77-9	0.5 mg/kg
4,4'-Methylenedi-o-toluidine	838-88-0	0.5 mg/kg

Components	CAS No.	Conc. Limit
6-Methoxy-m-toluidine	120-71-8	0.5 mg/kg
4,4'- Methylene-bis-[2-chloro aniline]	101-14-4	0.5 mg/kg
4,4'-Oxydianiline	101-80-4	0.5 mg/kg
2,4,5-Trimethylaniline	137-17-7	0.5 mg/kg
4-Aminoazobenzene	60-09-3	0.5 mg/kg
p-Phenylenediamine	106-50-3	0.5 mg/kg
Sulphanilic acid	121-57-3	0.5 mg/kg
4-Amino-3-fluorophenol	399-95-1	0.5 mg/kg
2,6-Xylidine	87-62-7	0.5 mg/kg
6-Amino-2-ethoxynaphthaline	293733-21-8	0.5 mg/kg
2,4-Xylidine	95-68-1	0.5 mg/kg

Table 6: limits for Aromatic Amines derived from AZO Dyes in Commission Regulation (EU) 2020/2081

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

In this PT the average of the homogeneity test results is not in line with the average (consensus value) from the PT results. There are several reasons for this. First, the goal of the homogeneity testing is very different from the goal of the evaluation of the reported PT results. In order to prove the homogeneity of the PT samples, a test method is selected with a high precision (smallest variation). The accuracy (trueness) of the test method is less relevant.
Secondly, the homogeneity testing is done by one laboratory only. The test results of this (ISO/IEC 17025 accredited) laboratory will have a bias (systematic deviation) depending on the test method used. The desire to detect small variations between the PT samples leads to the use of a sensitive test method with high precision, which may be a test method with significant bias.
Also, each test result reported by the laboratories that participate in the PT will have a bias. However, some will have a positive bias and others a negative bias. These different biases compensate each other in the PT average (consensus value). Therefore, the PT consensus value may deviate from the average of the homogeneity test. At the same time the accuracy of the PT consensus value is more reliable than the accuracy of the average of the results of the homogeneity test.

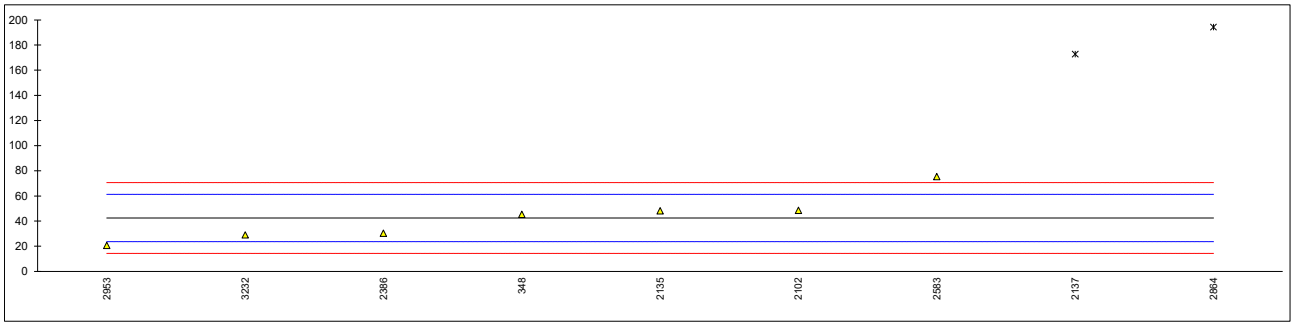
6 CONCLUSION

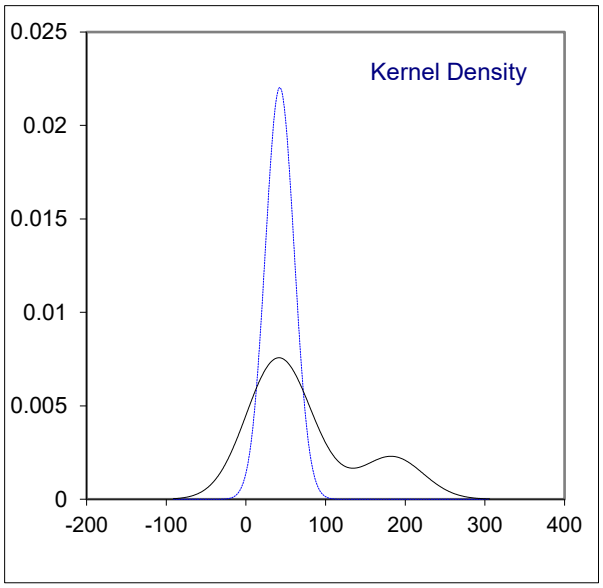
Although it can be concluded that some of the participants have a problem with the determination of o-Ansidine and o-Toluidin in this PT, each participating laboratory will have to evaluate its performance in this study and decide about any corrective actions if necessary.
Therefore, participation on 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 o-Ansidine (CAS no. 90-04-0) in sample #21544; results in mg/kg

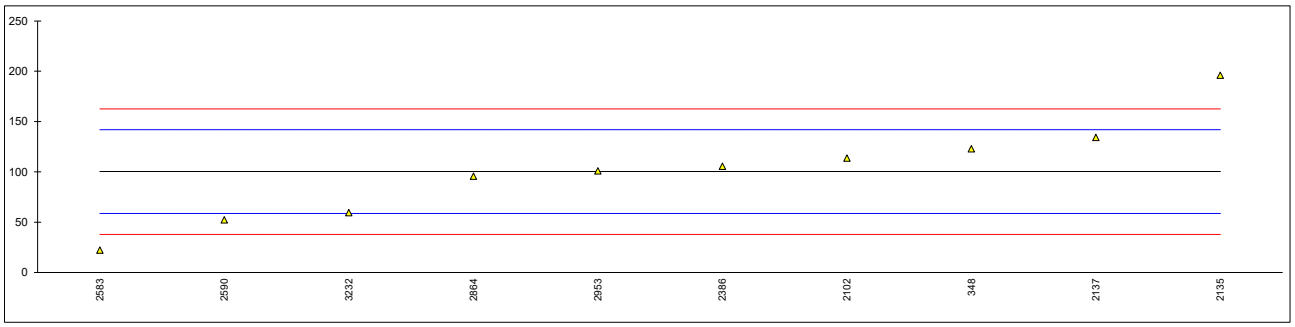
lab	method	value	mark	z'(targ)	remarks
348	ISO14362-1	45.3		0.30	
2102		48.549		0.65	
2135	In house	48.12		0.60	
2137	In house	172.74	DG(0.01)	13.88	
2386	In house	30.30		-1.30	
2583	In house	75.4		3.51	
2590		-----		-----	
2864	EN14362-1	194.29	C,DG(0.01)	16.17	first reported: 292.81
2953	ISO14362-1Mod	20.66		-2.32	
3232	ISO14362-1Mod	28.98		-1.44	
	normality	OK			
	n	7			
	outliers	2			
	mean (n)	42.4727			
	st.dev. (n)	18.10633	RSD = 43%		
	R(calc.)	50.6977			
	st.dev.(Horwitz')	9.38714			
	R(Horwitz')	26.2840			

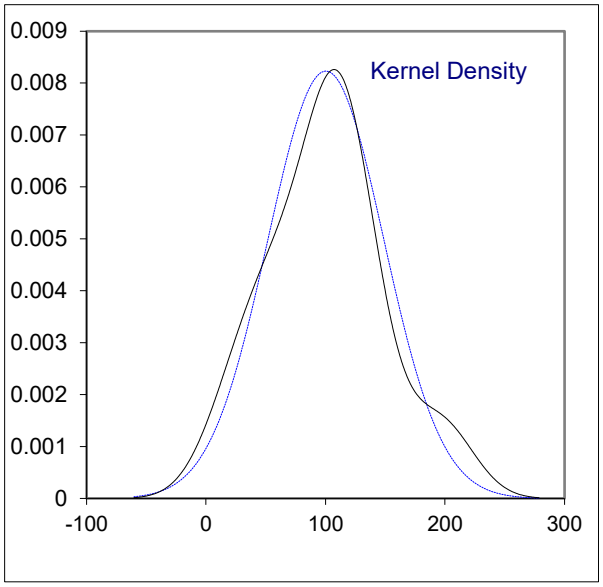




Determination of o-Toluidine (CAS no. 95-53-4) in sample #21544; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
348	ISO14362-1	122.9		1.09	
2102		113.587		0.64	
2135	In house	196.04		4.61	
2137	In house	134.23		1.64	
2386	In house	105.68		0.26	
2583	In house	22.2		-3.76	
2590		52.29		-2.31	
2864	EN14362-1	95.56		-0.23	
2953	ISO14362-1Mod	100.92		0.03	
3232	ISO14362-1Mod	59.31		-1.97	
	normality	OK			
	n	10			
	outliers	0			
	mean (n)	100.2717			
	st.dev. (n)	48.46675	RSD = 48%		
	R(calc.)	135.7069			
	st.dev.(Horwitz')	20.76851			
	R(Horwitz')	58.1518			





APPENDIX 2 Other reported aromatic amines

Abbreviations

4AD	= 4-Aminodiphenyl (CAS No. 92-67-1)
B	= Benzidine (CAS No. 92-87-5)
4CoT	= 4-Chloro-o-toluidine (CAS No. 95-69-2)
2NA	= 2-Naphtylamine (CAS No. 91-59-8)
ANT	= 2-Amino-4-nitrotoluene (CAS No. 99-55-8)
4CA	= 4-Chloraniline (CAS No. 106-47-8)
DAA	= 2,4-Diaminoanisol (CAS No. 615-05-4)
DADM	= 4,4'-Diaminodiphenylmethane (CAS No. 101-77-9)
DCB	= 3,3'-Dichlorobenzidine (CAS No. 91-94-1)
DMoxB	= 3,3'-Dimethoxybenzidine (CAS No. 119-90-4)
DMB	= 3,3'-Dimethylbenzidine (CAS No. 119-93-7)
DDDM	= 3,3'-Dimethyl-4,4'-Diaminodiphenylmethane (CAS No. 838-88-0)
pC	= p-Cresidine (CAS No. 120-71-8)
DDM	= 4,4'-Diamino-3,3'-dichlorodiphenylmethane (CAS No. 101-14-4)
DDE	= 4,4'-Diaminodiphenylether (CAS No. 101-80-4)
DDS	= 4,4'-Diaminodiphenylsulfide (CAS No. 139-65-1)
24DAT	= 2,4-Diaminotoluene (CAS No. 95-80-7)
TMA	= 2,4,5-Trimethylaniline (CAS No. 137-17-7)
24X	= 2,4-Xylidine (CAS No. 95-68-1)
25X	= 2,5-Xylidine (CAS No. 95-78-3)
26X	= 2,6-Xylidine (CAS No. 87-62-7)
TotX	= Total of Xylidines
oAAT	= o-Aminoazotoluene (CAS No. 97-56-3)
SumAAT/Tol	= sum of o-Aminoazotoluene and o-Toluidine
4AAB	= 4-Aminoazobenzene (CAS No. 60-09-3)

Sample #21544; abbreviations explained above

lab	4AD	B	4CoT	2NA	ANT	4CA	DAA	DADM	DCB	DMoxB	DMB	DDDM	pC
348	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
2102	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.
2135	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2137	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2386	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
2583	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2590	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2864	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.
2953	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3232	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.

lab	DDM	DDE	DDS	24DAT	TMA	24X	25X	26X	TotX	oAAT	SumAAT/Tol	4AAB
348	<10	<10	<10	<10	<10	<10	-----	<10	-----	<10	122.9	<10
2102	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	Not det.	113.587	Not det.
2135	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2137	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2386	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
2583	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2590	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2864	not det.	not det.	not det.	not det.	not det.	not det.	-----	not det.	-----	not det.	-----	not det.
2953	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	100.92	-----
3232	not det.	not det.	not det.	not det.	not det.	not det.	not det.	not det.	-----	not det.	-----	not det.

APPENDIX 3 Analytical details

lab	ISO17025 accr.	Sample intake (in g)
348	Yes	0,2
2102	No	0.5 gram
2135	Yes	1 g
2137	No	0.25
2386	Yes	0,5 g
2583	Yes	0,3 g
2590	Yes	1g
2864	Yes	0.5 g
2953	---	---
3232	Yes	0.2 g

APPENDIX 4

Number of participants per country

3 labs in GERMANY
1 lab in INDIA
2 labs in ITALY
1 lab in SOUTH KOREA
1 lab in SPAIN
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 statistical evaluation
fr. = first reported
f-? = possibly a false negative test result?
f+? = possibly a false positive test result?
n.a. = not applicable
n.e. = not evaluated
n.d. = not detected

Literature

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