Results of Proficiency Test
PAH 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 with mainly black colors. Black tattoo inks are usually based on soot. These inks are not regulated and may contain hazardous Polycyclic Aromatic Hydrocarbons (PAH). Tattoo Ink is especially important as a matrix 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 3 the maximum allowed concentration for PAH 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 PAHs is less than 0.5 mg/kg and for Benzo[a]pyrene 0.005 mg/kg.
No reference materials (RMs) for PAHs 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 PAHs in Tattoo Ink during the annual testing program 2020/2021.

In this interlaboratory study 8 laboratories in 6 different countries registered for participation. See appendix 4 for the number of participants per country. In this report the results of the PAHs 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 (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 8 mL Tattoo Ink positive on PAHs and labelled #21540.
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 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 the local market and tested on several banned components like Heavy Metals and PAH. The batch was found to be positive for PAH. After homogenization the batch was divided over 20 subsamples in vials of 8 mL each and labelled #21540.
The homogeneity of the subsamples was checked by determination of Naphthalene, Pyrene and Benzo[a]pyrene using an in-house test method on 4 stratified randomly selected subsamples.

	Naphthalene in mg/kg	Pyrene in mg/kg	Benzo[a]pyrene in mg/kg
Sample #21540-1	0.964	14.836	0.249
Sample #21540-2	0.950	14.407	0.244
Sample #21540-3	0.955	14.287	0.276
Sample #21540-4	0.993	14.812	0.272

Table 1: homogeneity test results of subsamples #21540

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 ISO13528, Annex B2 in the next table.

	Naphthalene in mg/kg	Pyrene in mg/kg	Benzo[a]pyrene in mg/kg
r (observed)	0.054	0.784	0.045
reference method	Horwitz	Horwitz	Horwitz
0.3 x R (reference method)	0.130	1.310	0.043

Table 2: evaluation of the repeatabilities of subsamples #21540

The calculated repeatabilities were 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 labelled #21540 was sent on February 24, 2021.

2.5 ANALYZES

The participants were asked to determine on samples #21540 the concentrations of any of the following PAH (CAS No.)

- Total PAH
- Naphthalene (91-20-3)
- Acenaphthylene (208-96-8)
- Acenaphthene (83-32-9)
- Fluorene (86-73-7)
- Phenanthrene (85-01-8)
- Anthracene (120-12-7)
- Fluoranthene (206-44-0)
- Pyrene (129-00-0)
- Sum of Phenanthrene, Anthracene, Fluoranthene and Pyrene
- Benzo[a]anthracene (56-55-3)
- Chrysene (218-01-9)
- Triphenylene (217-59-4)
- Sum of Chrysene and Triphenylene
- Benzo[b]fluoranthene (205-99-2)
- Benzo[j]fluoranthene (205-82-3)
- Benzo[k]fluoranthene (207-08-9)
- Sum of [b],[j] and [k] Benzofluoranthenes
- Benzo[e]pyrene (192-97-2)
- Benzo[a]pyrene (50-32-8)
- Indeno[1,2,3-c,d]pyrene (193-39-5)
- Dibenzo[a,h]anthracene (53-70-3)
- Benzo[g,h,i]perylene (191-24-2)
- Cyclopenta[c,d]pyrene (27208-37-3)

Also, it was requested 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 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 cannot be used for meaningful statistical evaluations.

To get comparable 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 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 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 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'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, 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 these 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 did not report any test results at all and one other participant reported test results after the final reporting date. Not all participants were able to report all components requested.
In total 7 laboratories reported 52 numerical test results. Observed were two outlying test results which is 3.8%. 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 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 PAH in Tattoo Ink. Therefore, the calculated reproducibility was compared against the estimated reproducibility calculated from the Horwitz equation.

Total PAH: Only four participants reported a test result for Total PAH, therefore no z-scores were calculated.

Naphthalene: This determination may be problematic at a consensus value of 1.1 mg/kg. No statistical outliers were observed. The calculated reproducibility is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Acenaphthylene: This determination may be problematic at a consensus value of 0.8 mg/kg. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Phenanthrene: This determination may be problematic at a consensus value of 0.8 mg/kg. No statistical outliers were observed. The calculated reproducibility is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Fluoranthene: This determination may be problematic at a consensus value of 1.6 mg/kg. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Pyrene: This determination may be problematic at a consensus value of 8.9 mg/kg. No statistical outliers were observed. The calculated reproducibility is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Sum of Phenanthrene, Anthracene, Fluoranthene and Pyrene: Only four participants reported the same test result for this sum as was calculated by iis based on the reported test results. Therefore no z-scores were calculated.

Benzo[a]pyrene: This determination may be problematic at a consensus value of 0.2 mg/kg. No statistical outliers were observed. The calculated reproducibility is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

Benzo[g,h,i]perylene: This determination may be problematic at a consensus value of 2.0 mg/kg. No statistical outliers were observed. The calculated reproducibility is not in agreement with the estimated reproducibility calculated with the combined Horwitz equation and the uncertainty as explained in paragraph 3.3.

The participants did agree on a concentration near or below the limit of detection for the other PAH. Therefore, no z-scores were calculated for these components and 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 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 table.

Component	unit	n	average	2.8 * sd	R(target)
Total PAH	mg/kg	4	n.e.	n.e.	n.e.
Naphthalene	mg/kg	6	1.12	2.00	1.14
Acenaphthylene	mg/kg	6	0.77	0.72	0.51

Component	unit	n	average	2.8 * sd	R(target)
Phenanthrene	mg/kg	5	0.81	0.71	0.55
Fluoranthene	mg/kg	6	1.59	2.00	1.22
Pyrene	mg/kg	7	8.86	11.23	6.03
Sum of Ph, An, Fl and Py *)	mg/kg	5	n.e.	n.e.	n.e.
Benzo[a]pyrene	mg/kg	5	0.19	0.31	0.20
Benzo[g,h,i]perylene	mg/kg	6	1.99	5.44	2.89

Table 3: reproducibilities of components on sample #21540

*) Sum of Phenanthrene, Anthracene, Fluoranthene and Pyrene

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	7
Number of test results	52
Number of statistical outliers	2
Percentage of statistical outliers	3.8%

Table 4: comparison with previous proficiency tests

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 proficiency tests. The conclusions are given in the next table.

Component	March 2021	Horwitz 0.1 - 10 mg/kg
Naphthalene	64%	13-23%
Acenaphthylene	33%	13-23%
Acenaphthene	n.e.	13-23%
Fluorene	n.e.	13-23%
Phenanthrene	31%	13-23%
Anthracene	n.e.	13-23%
Fluoranthene	45%	13-23%
Pyrene	45%	13-23%
Benzo[a]anthracene	n.e.	13-23%
Chrysene	n.e.	13-23%
Triphenylene	n.e.	13-23%
Benzo[b]fluoranthene	n.e.	13-23%

Component	March 2021	Horwitz 0.1 - 10 mg/kg
Benzo[j]fluoranthene	n.e.	13-23%
Benzo[k]fluoranthene	n.e.	13-23%
Benzo[e]pyrene	n.e.	13-23%
Benzo[a]pyrene	59%	13-23%
Indeno[1,2,3-c,d]pyrene	n.e.	13-23%
Dibenzo[a,h]anthracene	n.e.	13-23%
Benzo[g,h,i]perylene	98%	13-23%
Cyclopenta(c,d)pyrene	n.e.	13-23%

Table 5: development of uncertainties (RSD)

4.4 EVALUATION OF THE ANALYTICAL DETAILS

For this PT some analytical details were requested and are given in appendix 3. Based on the answers given by the participants the following can be summarized:
- Five of the participants mentioned that they are accredited for determination of PAH.
- Two of the participants mentioned to have used 0.25 grams, three used 0.5 grams and one used 1 gram.

The influence of these analytical details could not be determined because the group of participants is too small for further analysis.

5 DISCUSSION

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

Components	Conc. Limit
Benzo[a]pyrene (CAS no. 50-32-8, 66-71-7)	0.005 mg/kg
Polycyclic-aromatic Hydrocarbons (PAH), classified in Part 3 of Annex VI to Regulation (EC) No 1272/2008 as carcinogen or germ cell mutagen category 1A, 1B or 2	0.5 mg/kg (individual component)

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

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

6 CONCLUSION

Although it can be concluded that some of the participants have a problem with the determination of PAHs 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.

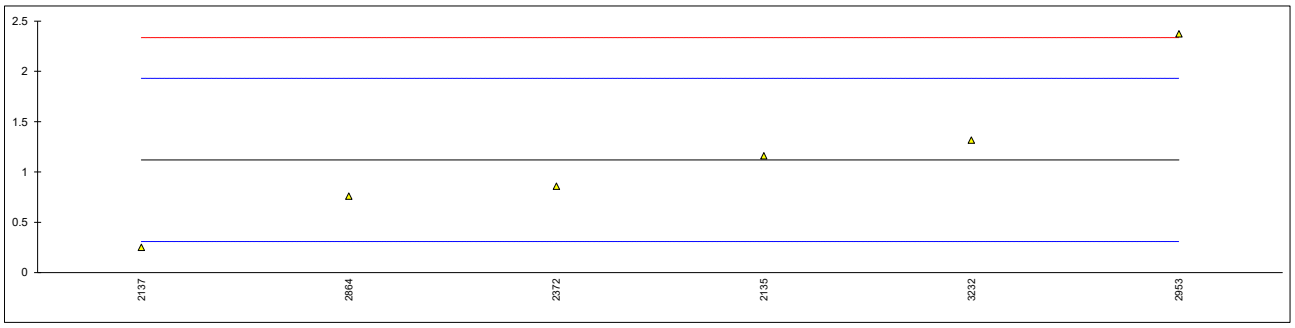
APPENDIX 1

Determination of Total PAH in sample #21540; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		24.816		-----	
2137	In house	6.96		-----	
2372	AfPS GS 2014	20.9036		-----	
2590		-----		-----	
2864		-----		-----	
2953		-----		-----	
3232	AfPS GS 2014	16.626		-----	
	n	4			

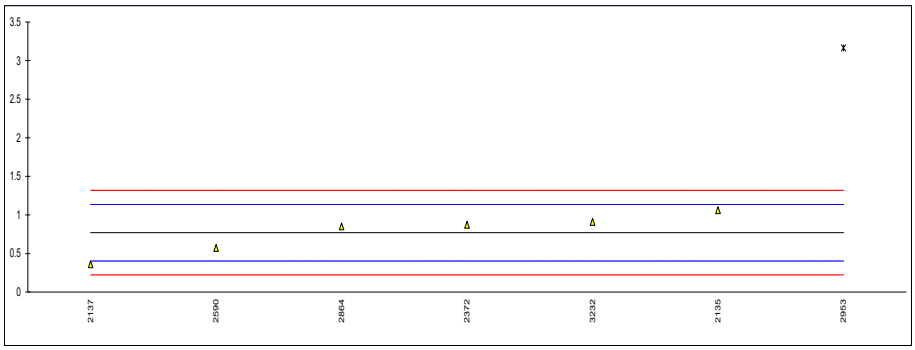
Determination of Naphthalene in sample #21540; results in mg/kg

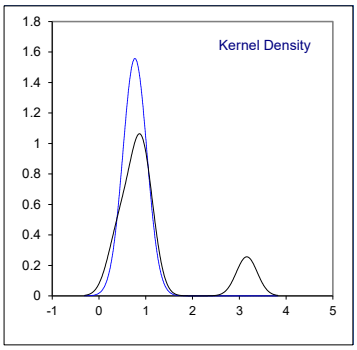
lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		1.16		0.10	
2137	In house	0.25		-2.14	
2372	AfPS GS 2014	0.8586		-0.64	
2590		-----		-----	
2864	AfPS GS 2014	0.76		-0.89	
2953	AfPS GS 2019	2.372		3.09	
3232	AfPS GS 2014	1.316		0.48	
	normality	unknown			
	n	6			
	outliers	0			
	mean (n)	1.1194			
	st.dev. (n)	0.71587	RSD = 64%		
	R(calc.)	2.0044			
	st.dev.(Horwitz')	0.40554			
	R(Horwitz')	1.1355			



Determination of Acenaphthylene in sample #21540; results in mg/kg

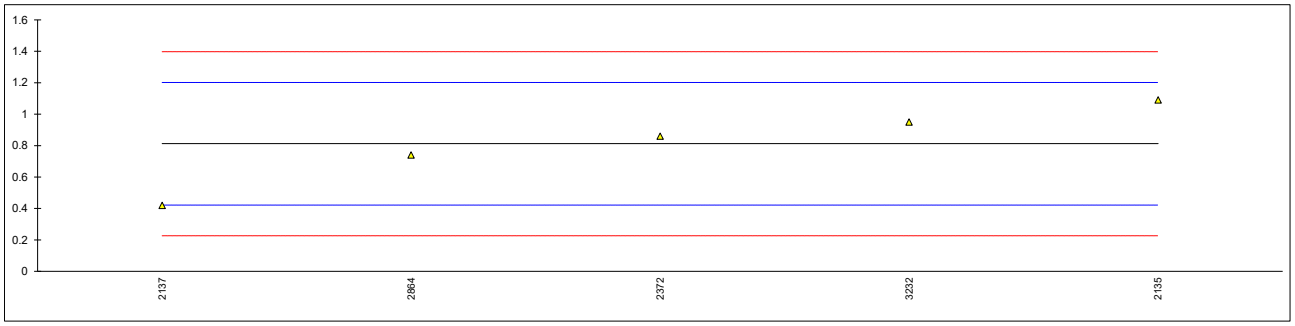
lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		1.06		1.58	
2137	In house	0.36		-2.24	
2372	AfPS GS 2014	0.8710		0.55	
2590	AfPS GS 2014	0.571		-1.09	
2864	AfPS GS 2014	0.85		0.44	
2953	AfPS GS 2019	3.162	D(0.01)	13.06	
3232	AfPS GS 2014	0.910		0.76	
	normality	unknown			
	n	6			
	outliers	1			
	mean (n)	0.7703			
	st.dev. (n)	0.25613	RSD = 33%		
	R(calc.)	0.7172			
	st.dev.(Horwitz')	0.18307			
	R(Horwitz')	0.5126			





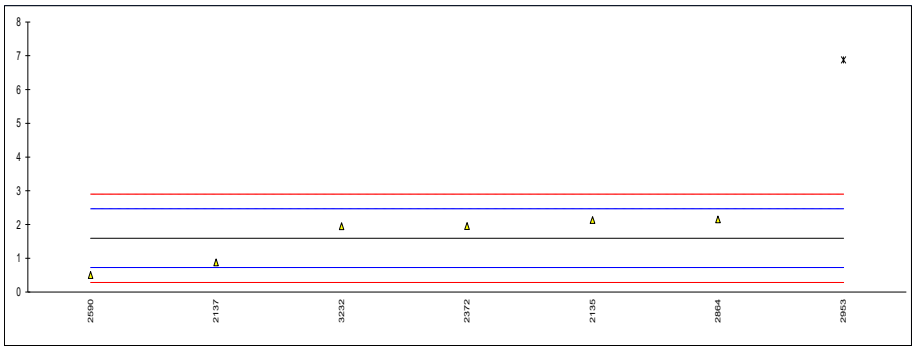
Determination of Phenanthrene in sample #21540; results in mg/kg

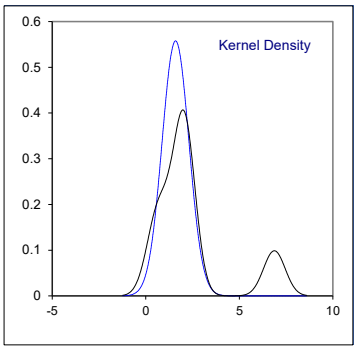
lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		1.09		1.42	
2137	In house	0.42		-2.01	
2372	AfPS GS 2014	0.8599		0.25	
2590		-----		-----	
2864	AfPS GS 2014	0.74		-0.37	
2953		-----		-----	
3232	AfPS GS 2014	0.950		0.71	
	normality	unknown			
	n	5			
	outliers	0			
	mean (n)	0.8120			
	st.dev. (n)	0.25371	RSD = 31%		
	R(calc.)	0.7104			
	st.dev.(Horwitz')	0.19515			
	R(Horwitz')	0.5464			



Determination of Fluoranthene in sample #21540; results in mg/kg

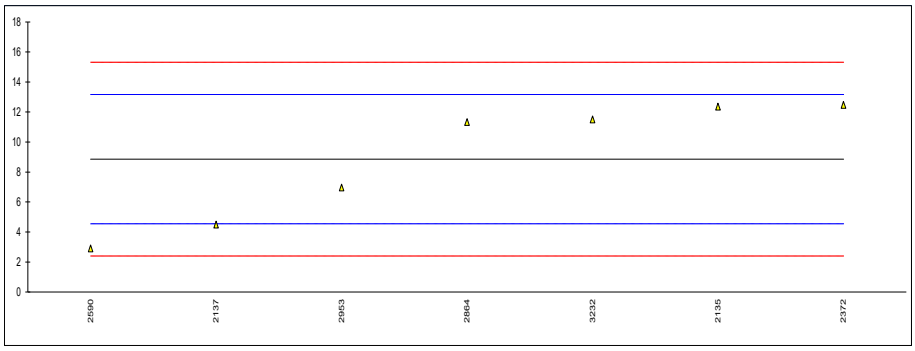
lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		2.13		1.23	
2137	In house	0.88		-1.64	
2372	AfPS GS 2014	1.952		0.82	
2590	AfPS GS 2014	0.5	C	-2.51	first reported: 0.74
2864	AfPS GS 2014	2.15		1.28	
2953	AfPS GS 2019	6.877	G(0.01)	12.13	
3232	AfPS GS 2014	1.95		0.82	
	normality	unknown			
	n	6			
	outliers	1			
	mean (n)	1.5937			
	st.dev. (n)	0.71526	RSD = 45%		
	R(calc.)	2.0027			
	st.dev.(Horwitz')	0.43559			
	R(Horwitz')	1.2196			

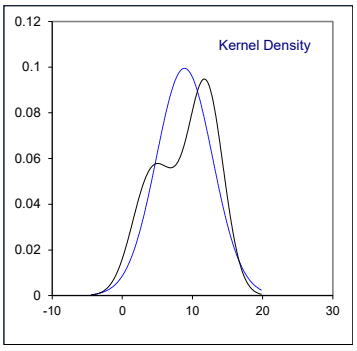




Determination of Pyrene in sample #21540; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		12.36		1.63	
2137	In house	4.49		-2.03	
2372	AfPS GS 2014	12.47		1.68	
2590	AfPS GS 2014	2.9	C	-2.77	first reported: 4.227
2864	AfPS GS 2014	11.32		1.14	
2953	AfPS GS 2019	6.957		-0.88	
3232	AfPS GS 2014	11.50		1.23	
	normality	unknown			
	n	7			
	outliers	0			
	mean (n)	8.8567			
	st.dev. (n)	4.01130	RSD = 45%		
	R(calc.)	11.2316			
	st.dev.(Horwitz')	2.15245			
	R(Horwitz')	6.0269			





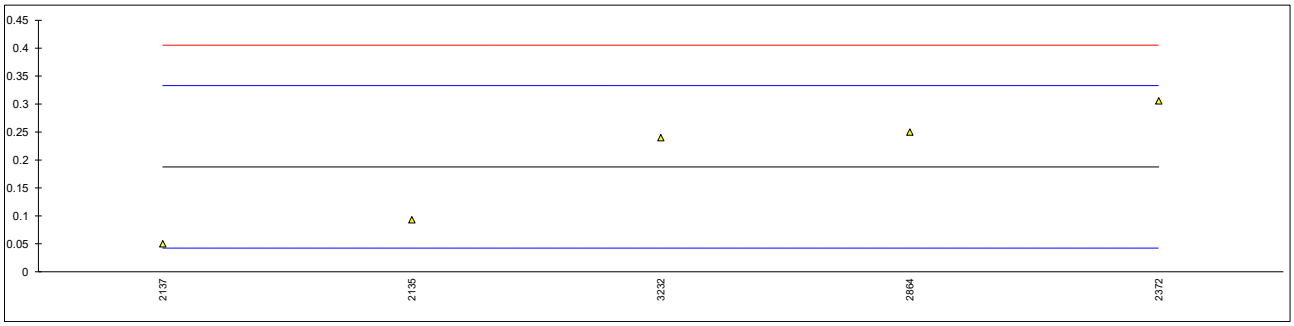
Determination of Sum of Phenanthrene, Anthracene, Fluoranthene and Pyrene in sample #21540; results in mg/kg

lab	method	sum reported		sum calc by iis	remarks
2102		-----		-----	
2135		15.675	C	15.675	
2137	In house	5.87		5.86	
2372	AfPS GS 2014	14.422	E	15.282	
2590	AfPS GS 2014	-----		3.400	
2864	AfPS GS 2014	-----		14.210	
2953	AfPS GS 2019	16.758		16.758	
3232	AfPS GS 2014	14.40		14.40	
	n	5		7	

Lab 2135 first reported: not detected
Lab 2372: calculation difference, it appears that the laboratory did not use the test result of Phenanthrene in calculating the sum

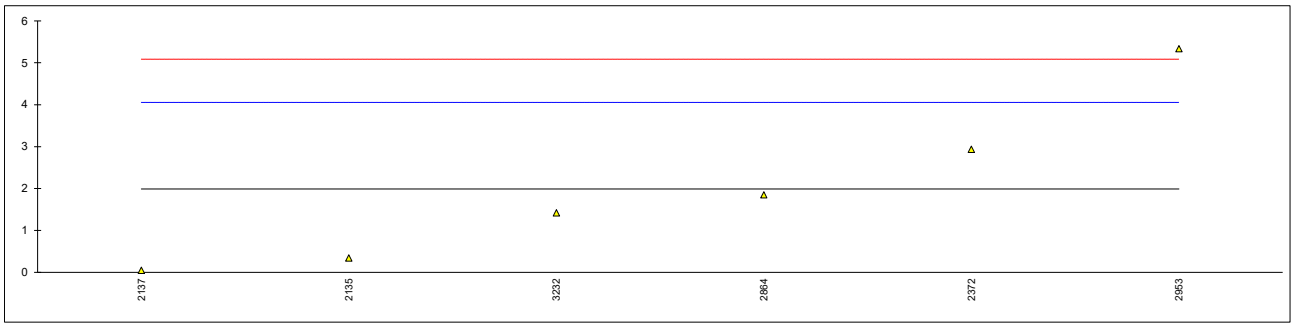
Determination of Benzo[a]pyrene in sample #21540; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		0.093		-1.30	
2137	In house	0.05		-1.90	
2372	AfPS GS 2014	0.3056		1.62	
2590		-----		-----	
2864	AfPS GS 2014	0.25		0.86	
2953		-----		-----	
3232	AfPS GS 2014	0.24	C	0.72	first reported: not detected
	normality	unknown			
	n	5			
	outliers	0			
	mean (n)	0.1877			
	st.dev. (n)	0.11005	RSD = 59%		
	R(calc.)	0.3081			
	st.dev.(Horwitz')	0.07265			
	R(Horwitz')	0.2034			



Determination of Benzo[g,h,i]perylene in sample #21540; results in mg/kg

lab	method	value	mark	z'(targ)	remarks
2102		-----		-----	
2135		0.344	C	-1.59	first reported: not detected
2137	In house	0.05		-1.88	
2372	AfPS GS 2014	2.934		0.91	
2590		-----		-----	
2864	AfPS GS 2014	1.85		-0.13	
2953	AfPS GS 2019	5.336		3.24	
3232	AfPS GS 2014	1.42	C	-0.55	first reported: not detected
	normality	unknown			
	n	6			
	outliers	0			
	mean (n)	1.9890			
	st.dev. (n)	1.94444	RSD = 98%		
	R(calc.)	5.4444			
	st.dev.(Horwitz')	1.03293			
	R(Horwitz')	2.8922			



APPENDIX 2 Other reported PAH in sample #21540; results in mg/kg

Sum C&T = sum of Chrysene and Triphenylene
Sum [b]/[j]/[k] = sum of [b]/[j]/[k]Benzofluoranthenes

lab	Acenaphthene	Fluorene	Anthracene	Benzo[a] anthracene	Chrysene	Triphenylene	Sum C & T	Benzo[b] fluoranthene
2102	-----	-----	-----	-----	-----	-----	-----	-----
2135	not detected	not detected	0.095	not detected	not detected	not detected	not detected	not detected
2137	-----	0.07	0.07	0.07	0.06	-----	-----	0.06
2372	not detected	not detected	not detected	not detected	not detected	not analyzed	not analyzed	not detected
2590	-----	-----	-----	-----	-----	-----	-----	-----
2864	not detected	not detected	not detected	not detected	not detected	-----	-----	not detected
2953	-----	-----	2.924	0.791	-----	-----	-----	1.178
3232	not detected	not detected	not detected	not detected	not detected	-----	-----	not detected

lab	Ben[j] fluoranthene	Ben[k] fluoranthene	Sum [b]/[j]/[k]	Benzo[e] pyrene	Indeno[1,2,3-c,d]pyrene		Dibenzo[a,h] anthracene	Cyclopenta [c,d]pyrene	
2102	-----	-----	-----	-----	-----		-----	-----	
2135	not detected	not detected	not detected	0.158	0.05		not detected	1.36	C
2137	0.02	0.02	0.10	0.05	0.03		-----	-----	
2372	not detected	not detected	not detected	0.4690	0.1835		not detected	not detected	
2590	-----	-----	-----	-----	-----		-----	-----	
2864	not detected	not detected	-----	0.39	0.20	C	not detected	-----	
2953	-----	-----	1.178	1.581	-----		-----	-----	
3232	-----	not detected	-----	-----	0.30	C	not detected	-----	

Lab 2135 first reported: 6.62
Lab 2864 first reported: not detected
Lab 3232 first reported: not detected

APPENDIX 3 Analytical Details

lab	ISO/IEC17025	Intake sample
2102	---	---
2135	Yes	0,5 g
2137	No	1
2372	Yes	0.5g
2590	Yes	0.5 g
2864	Yes	0.25 g
2953	---	---
3232	Yes	0.25 g

APPENDIX 4

Number of participants per country

1 lab in GERMANY
1 lab in INDIA
2 labs in ITALY
1 lab in SOUTH KOREA
2 labs 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
n.a. = not applicable
n.e. = not evaluated
n.d. = not detected
fr. = first reported

Literature

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12 Bernard Rosner, Percentage Points for a Generalized ESD Many-Outlier Procedure, Technometrics, 25(2), 165-172, (1983)
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14 Commission Regulation (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattoo inks or permanent make-up