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ICI report 2nd round substances

Pesticides/round_02/2020

Pesticide biomarkers in urine

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1 Summary

Within the framework of the HBM4EU project, a 2nd interlaboratory comparison was organised for the determination of nine pesticide biomarkers in urine. Included were biomarkers for glyphosate and AMPA (glyphosate and AMPA), chlorpyrifos (TCPy), and pyrethroids (3-PBA, 4-F-3-PBA, cis-DBCA, cis-DCCA, trans-DCCA, and ClF3CA).

The study was performed in February/March 2020 and was conducted to assess the comparability and reliability of analytical methods across the participating expert laboratories.

The HBM4EU QAU had selected four expert laboratories for pesticide biomarkers in urine. The expert laboratories were from four different countries in Europe. For glyphosate/AMPA, two additional laboratories analysed the samples (the lab preparing the control material, and an external laboratory).

Each participant received two control materials of human urine to be analysed for glyphosate and AMPA (present at 0.4-1.9 ng/ml), and two other control materials, which both contained the chlorpyrifos biomarker (0.6-3.6 ng/ml) and pyrethroid biomarkers (0.1-1.4 ng/ml). The laboratories were requested to perform a single analysis and the submit results to the organiser within 3-4 weeks.

A first assessment of comparability of results was done by calculation of the mean, the RSD, and the relative uncertainty of the mean. Results were compared against the mean through a Z-score when the relative uncertainty of the mean was within 17.5%. In case the relative uncertainty exceeded this value, no objective reliable quantitative comparability assessment could be done.

For glyphosate and AMPA, five out of six laboratories reported results. Results were comparable for both biomarkers in both control materials.

For the chlorpyrifos and pyrethroid biomarkers, three laboratories reported results for all seven biomarkers, and one for five biomarkers (no results for cis-DBCA and ClF3CA). In nine out of 14 cases, comparability of results could be demonstrated. In five cases, all for the same control material, the relative uncertainty of the mean was too high for a quantitative assessment of comparability.

The outcome of this 2nd interlaboratory comparison for pesticide biomarkers in urine is summarised in **Table 1**.

Recommendations were made to further improve comparability of results in the next round.

Table 1. Comparability of results for pesticide biomarkers in urine obtained in interlaboratory comparison/round 2.

Biomarker	Test material	Consensus (ng/ml)	Comparable results for X out of Y labs
Glyphosate	R2A	0.389	5[a]/6
	R2B	2.42	5[a]/6
AMPA	R2A	1.25	5[a]/6
	R2B	0.524	4[a]/6
TCPy	R2A	[0.42] [b]	[c]
	R2B	4.30	4/4
3-PBA	R2A	0.279	4/4
	R2B	1.76	4/4
4-F-3-PBA	R2A	1.56	4/4
	R2B	0.102	3/4
cis-DBCA	R2A	[0.57] [b]	[a, c]
	R2B	0.627	3[a]/4
cis-DCCA	R2A	[0.16] [b]	[c]
	R2B	1.36	4/4
trans-DCCA	R2A	[1.23] [b]	[c]
	R2B	0.126	3/4
ClF3CA	R2A	[1.1] [b]	[a, c]
	R2B	0.418	3[a]/4

[a] one laboratory did not report results

[b] no consensus value due to too high variability. [xx] = concentration as determined during homogeneity study.

[c] results not comparable.

2 Introduction

Pesticides have been included in HBM4EU as substances in the 2nd prioritisation round. The selection of the target pesticides and their most relevant biomarkers was previously done in WP9, and has been described in Deliverable report 9.5 v2.0 [1]. Based on this, and further considerations by the QAU and experts in the field, it was decided to include nine pesticide biomarkers in the anticipated analyses of samples from aligned studies in HBM4EU (see **Table 2**).

For the 2nd round substances, it was decided by WP9 to select a limited number of expert laboratories for analysis of HBM4EU samples. Laboratories were selected by the QAU according to criteria described in HBM4EU-SOP-QA-005 [2]. The selection criteria included:

1. Experience in analysis of all selected parameters in (the selected) human matrices at levels expected in the general population (proven experience, papers, reports, etc.)
2. Capacity for analysis (number of samples/time for analysis)
3. Limit of quantification of the method, i.e. sufficiently low for HBM4EU samples
4. Historical data of the successful participation in interlaboratory comparison exercises for the target substance (selected parameters)

This interlaboratory comparison is intended to assess the comparability and reliability of the analytical methods that laboratories will use for determination of the nine biomarkers from **Table 2** in samples analysed in the frame of HBM4EU. It forms an integral part of quality control, in addition to initial and ongoing in-house method validation.

Table 2 Pesticide biomarkers in urine included in the interlaboratory comparison

Abbreviation	Target biomarker	Biomarker of exposure for
Glyphosate	Glyphosate	glyphosate
AMPA	Aminomethylphosphonic acid	AMPA (glyphosate environmental metabolite)
TCPy	3,5,6-trichloro-2-pyridinol	chlorpyrifos, chlorpyrifos-methyl (triclopyr)
3-PBA	3-phenoxybenzoic acid	all pyrethroids containing this moiety (>10)
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid	cyfluthrin, flumethrin
cis-DBCA	cis-(2,2-dibromovinyl)-2,2-dimethyl cyclopropanecarboxylic acid	deltamethrin
cis-DCCA	cis-3-(2,2-dichlorovinyl)2,2-dimethyl cyclopropane1-carboxylic acid	cyfluthrin, cypermethrin, permethrin, transfluthrin
trans-DCCA	trans-3-(2,2-dichlorovinyl)2,2-dimethyl cyclopropane1-carboxylic acid	cyfluthrin, cypermethrin, permethrin, transfluthrin
ClF3CA	cis-3-(2-chloro-3,3,3trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylic acid	bifenthrin, (lambda-)cyhalothrin, tefluthrin

This study has been organised by Wageningen Food Safety Research (WFSR) in the Netherlands, as part of the Quality Assurance program for biomonitoring analyses within the frame of HBM4EU. Participation in this exercise is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the outcome of the 2nd round of interlaboratory comparisons for pesticide biomarkers in urine.

2.1 Confidentiality

In this report, the identity of the participants is treated as confidential. However, lab codes of the participants will be disclosed to the HBM4EU-QAU for performance assessment.

3 Control material

3.1 Preparation of control material

For this study, two sets of two control materials were prepared. One set (material B3 = R2A, and B4 = R2B) to be analysed for glyphosate and AMPA (prepared and tested by Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, IPA), and one set (material C = R2A, and D = R2B) to be analysed for the biomarkers for chlorpyrifos and pyrethroids (prepared and tested by WFSR). In both cases burdened urine samples were used that were known to contain most of the target analytes. This means that the biomarkers were present as conjugates where applicable. 4-F-3-PBA, cis-DCCA, and ClF3CA had to be spiked to urine sample C and D, because the material used did not contain this biomarker, or only at very low levels. Additions were also done for trans-DCCA and 3-PBA, to material C and D respectively, to enhance the levels and introduce more significant differences between the two materials. As analytical standards of conjugates were not available, the free acids were used in case of spiking.

The control materials were mixed and then aliquoted into coded polypropylene tubes with screwcap (10 ml for glyphosate/AMPA; 5 ml for chlorpyrifos/pyrethroid biomarkers). The tubes were stored in the freezer (<-18°C). Part of the tubes were stored at -80°C as reference for future stability testing.

3.2 Homogeneity of control material

Homogeneity testing was done as described in HBM4EU-SOP-QA-002 [3]. For glyphosate and AMPA, ten tubes of each control material were randomly selected from the freezer and analysed in duplicate. For the chlorpyrifos/pyrethroid biomarkers, 5x2 tubes were randomly selected from the freezer and analysed. The analysis results were processed by the organiser according to the SOP using an Excel macro ("HBM4EU macro homogeneity test v1.xlsm"). The mean concentrations and relative standard deviations (RSD) as obtained during homogeneity testing, are included in Appendix 1. For all biomarkers, it could be concluded that homogeneity was adequate in both control materials.

3.3 Stability of control material

For assessment of storage stability the procedures have been described in HBM4EU-SOP-QA-002 [3]. For glyphosate/AMPA, samples stored at -18°C were analysed at two occasions in separate batches, 48 days apart. For glyphosate in material B3 and AMPA in material B4, results did not significantly differ. A statistically significant lower level was found for glyphosate in material B4 (8%), and for AMPA in material B3 (17%). The differences, although statistically significant in these two cases, were minor with respect to the acceptable interlaboratory variability and therefore were considered not to impact the outcome of the study. For the chlorpyrifos and pyrethroid biomarkers, repeated analysis of the control materials after 17 days did not indicate instability, as expected from previous assessments. It was concluded that the biomarkers were stable in the control materials when stored at -18°C during the period of the conduct of the interlaboratory comparison.

4 Organisational details

4.1 Participants

For the organisation of the interlaboratory comparison exercises, WFSR contacted the four selected expert laboratories (HBM4EU laboratories from four different countries in Europe) and sent them an announcement letter by e-mail on 17th December 2019. The biomarkers to be determined and the required LOQs were listed. It was indicated that the laboratories would receive in total four test samples, one set of two samples to be analysed for glyphosate/AMPA, and one set of two samples to be analysed for chlorpyrifos/pyrethroid biomarkers. Participation was free of charge. The letter also included the schedule for three rounds of interlaboratory comparisons. An update of this schedule was sent to the participants by email on 7th February (see **Appendix 2**). Test results had to be submitted within the stipulated deadline (9th March 2020). For glyphosate/AMPA, one external laboratory from Canada volunteered to also analyse samples for these parameters, and an additional set of results was obtained from the laboratory preparing the control materials.

Results were received from all laboratories in time.

4.2 Dispatch and instructions

The test materials for determination of glyphosate/AMPA (10 ml each) were dispatched to the participants by IPA on 17th February. The test materials for determination of chlorpyrifos and pyrethroid biomarkers (5 ml each) were dispatched by WFSR on 11th February. The samples were packed in an insulation box with ice packs and sent by courier. Instructions were included in the box and also sent by e-mail (see **Appendix 3**). Participants were asked to check the content of the box upon receipt, to store the samples in the freezer, and to carry out a single analysis of the samples according to their routine method. The deadline for submission of results was 9th March 2020.

For reporting of results an excel sheet was provided. In this excel sheet the participants were asked to report the biomarker concentration in ng/ml, with at least three significant figures. In addition, the participants were asked to provide method details for each of the biomarkers (i.e. LOQ, deconjugation, cleanup, analysis technique, internal standards used, precision data).

4.3 Deviations from SOPs

For the interlaboratory comparison, the HBM4EU-QA-SOPs [2,3] were followed. There were no deviations from the SOPs, with the exception of the use of 5 replicate analysis (instead of 10) for homogeneity testing in case of the chlorpyrifos/pyrethroids biomarkers. The reason was the limited amount of control material available. This deviation was considered not to have an effect on the study outcome.

5 Data evaluation

Evaluation of comparability of the data was done according to HBM4EU-SOP-QA-005 [2]. This involves establishing a consensus value and assessing the deviation of the individual results from the consensus value by calculation of Z-scores.

5.1 Consensus value

The mean concentration derived from the expert laboratories is considered an acceptable consensus value in the interlaboratory comparison study when the relative uncertainty of the mean is ≤17.5%.

The relative uncertainty of the mean, is given by:

u = RSD / sqrt(N)

with u = relative uncertainty of the mean concentration from the expert labs

RSD = relative standard deviation of the mean concentration

N = the number of expert labs (after exclusion of outliers if applicable)

In case the uncertainty of the mean exceeds 17.5%, the results are checked for outliers using a Grubbs' test. If an individual value is identified as an outlier, it is rejected from the data set and the relative uncertainty calculated again when N is still ≥3. If u is still >17.5%, then no meaningful consensus expert value can be derived, and no objective reliable quantitative comparability assessment can be done.

It is recognised that with the small number of participants it is not very likely that outliers can be identified through statistical tests.

5.2 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD_R) of 25% of the consensus value was used as target standard deviation.

5.3 Z-scores

The Z-score (Z) was calculated as follows:

$$Z = \frac{x - C}{\sigma_T}$$

with x = result submitted by the laboratory;

C = consensus value;

σ_T = target standard deviation, here 0.25*C

When the Z-score is within -2 and +2 (-2 ≤ Z≤ 2), the results are considered sufficiently comparable.

6 Results and discussion

6.1 Results submitted by participants

In total, four laboratories from four European countries participated in this study. The individual results of the laboratories are included in **Appendix 4**. Quantitative results were reported for almost all biomarkers with the following exceptions:

- one laboratory was not able to report results for glyphosate and AMPA. Their existing method as used in round-1 was not suited for determination of AMPA (see report of the first round). The lab made an attempt to adjust the method (different SPE step) in order to determine both glyphosate and AMPA, but this was not successful. Consequently, no results could be reported for this round.
- One laboratory was not able to report results for two pyrethroid biomarkers: cis-DBCA and ClF3CA. For cis-DBCA it was remarked by this lab that matrix effects in the materials were very high, also in comparison to other samples in their experience, and since the lab did not use the corresponding isotope internal standard, the lab could not adequately correct for this and decided not to report results for this biomarker in both control materials. For ClF3CA, as in the first round, the lab still had problems that could not yet be solved.

For glyphosate/AMPA, results from two other laboratories were received and included in **Appendix 4**.

6.2 Analysis methods

The method details as provided by the laboratories are included in **Appendix 5**.

For glyphosate and AMPA the same methods were used as in the first round, with the exception of one laboratory that changed the method in order to incorporate AMPA in their existing method for glyphosate (see also 6.1). The laboratories used various methods. With one exception, all laboratories determined both compounds by one method. The volume of urine needed varied from 0.05 to 1 ml. Four laboratories used LC-MS/MS based methods. In these cases sample preparation involved an LLE or SPE cleanup and three of the four labs derivatised the compounds before analysis. Two laboratories used GC-NCI-MS based methods. Here sample preparation involved evaporation of a small aliquot of urine, followed by derivatisation. All laboratories used the corresponding isotope internal standards, which were added to the urine before sample processing. Quantification was based on calibration standards prepared in blank urine, solvent/eluent or water, after normalisation of response against the internal standard. For the three expert labs that reported results, the LOQ met the requirement of ≤0.1 ng/ml for glyphosate. For AMPA, the required LOQ of ≤0.2 ng/ml was met by two of the three expert labs. The third laboratory indicated an LOQ of 0.5 ng/ml, but did report a result below that value in control material R2B.

For the biomarkers of chlorpyrifos and pyrethroids various methods were used. Compared to the first round, two laboratories (slightly) adjusted their method. One laboratory changed the enzyme used for deconjugation from *E.coli*-based in round-1 to *Helix Pomatia*-based in this round. Another laboratory changed the procedure for quantification from procedural calibration using a fixed blank urine, to a standard addition procedure. To briefly summarize the methods used: the volume of urine needed varied from 1-5 ml. Three laboratories determined all seven biomarkers by one method. One laboratory used a separate method for TCPy (chlorpyrifos biomarker). In all cases, a deconjugation step was done, in most cases enzymatic, one used acid hydrolysis for the pyrethroids biomarkers. Cleanup was done by either LLE or SPE. Three laboratories analysed the extracts by LC-MS/MS, one lab by GC-MS after derivatisation. Although the isotope internal standards are commercially available for all seven biomarkers except ClF3CA, they were not used in a number of cases.

Especially in LC-MS/MS based methods, this may have affected the quantification. Quantification was based on calibration standards prepared in blank urine processed as the samples (2 labs), in solvent/eluent (1 lab), or using a standard addition procedure (1 lab for all biomarkers, another lab only for ClF3CA). One laboratory having issues with the determination of ClF3CA in the first round, still had issues in this round and did not report results (see also 6.1). The LOQs as reported by the labs met the requirements in most cases, with the exception of one lab (0.25 ng/ml for cis-DBCA and cis-DCCA, requirement was 0.1 ng/ml)

6.3 Consensus values

For all biomarkers the mean, RSD and the relative uncertainty of the mean were determined. The mean was used as consensus value when the relative uncertainty did not exceed 17.5%. The results are included in **Appendix 4**.

For glyphosate/AMPA the calculations were done for the expert labs assigned by WP9 (four, of which three submitted results), and for all five laboratories that provided results. A consensus value could be established in all cases, i.e. for both glyphosate and AMPA, in both control materials, when using the data from the three expert labs, and also when using the data from all five labs.

For the chlorpyrifos and pyrethroids biomarkers, the uncertainty of the mean was too high for five out of seven biomarkers in control material R2A, and no meaningful consensus values could be derived in these cases. In contrast, for material R2B, it was possible to derive consensus values for all seven biomarkers (after exclusion of one result identified as Grubbs' outlier). The difference in comparability of results observed for the two materials was not due to the levels, in both materials both lower and higher concentrations of the biomarkers were present. Altogether, consensus values were obtained for nine out of 14 biomarker/control material combinations.

6.4 Assessment of laboratory performance

The performance of the individual laboratories for each of the biomarkers could only be assessed when a consensus value could be derived. In these cases, a Z-score was determined. It should be noted that with the approach used for determination of the consensus value, Z-scores will be within -2 and +2 in most cases when it is possible to establish a consensus value. Nevertheless, it does provide a way of quantitative assessment. For information, as additional indication for comparability, the percentage deviation of the individual results relative to the consensus value is also included in Appendix 4.

For glyphosate and AMPA, the results submitted by the three expert laboratories were comparable ($-2 \leq Z \leq 2$) in both control materials. This was also true when using the data from all five laboratories, with the exception of AMPA in R2B (one lab Z-score 2.2).

For the chlorpyrifos and pyrethroids biomarkers an assessment was possible for nine out of 14 biomarker/control material combinations. In most of those cases, the results were comparable. Exceptions were:

- 4-F-PBA in material R2B: the result of one lab was identified as Grubbs'outlier, and consequently received a Z-score >2.
- trans-DCCA in material R2B: one lab reported "<0.05 ng/ml" (consensus value based on the other three was 0.126 ng/ml). In this case, no Z-score could be assigned. For information, a proxy-Z-score was calculated using the LOQ value as result.

6.5 Conclusions and recommendations

A 2nd interlaboratory comparison was carried out for nine pesticide biomarkers in urine amongst four selected HBM4EU laboratories. For glyphosate and AMPA results of two additional labs were included in the evaluation.

Glyphosate/AMPA:

- One expert lab was unable to report results.
- Good comparability of results submitted by the three remaining expert labs.
- Good comparability of results for all five labs (one deviating result for one lab).

Chlorpyrifos/pyrethroids biomarkers:

- Comparability of results for nine out of 14 biomarker/control material combinations.
- For five biomarkers in one control material, variability was high, the results were not comparable.
- One lab reported issues with determination of cis-DBCA and ClF3CA.

Overall, the outcome of this 2nd round of interlaboratory comparisons was more favourable compard to the first round. However, comparability of method could not be demonstrated in all cases, and some labs (still) has issues with determination of certain biomarkers.

Recommendations

All or specific laboratories are recommended to:

Glyphosate/AMPA:

- PEL24: to do further efforts to come up with a method for determination of glyphosate and AMPA (or consider a separate method for AMPA in addition to their existing glyphosate method).
- PEL1: to lower the LOQ for AMPA to 0.2 ng/ml.

Chlorpyrifos/pyrethroids biomarkers:

- for (further) improvement of comparability of results, especially for labs using LC-MS/MS, it is strongly recommended to use the corresponding isotope internal standard for each of the biomarkers, rather than generic internal standards or the isotope label of one of the other biomarkers (isotope labels are commercially available for all biomarkers, except ClF3CA).
- PEL24: to lower the LOQ for cis-DBCA and cis-DCCA to the required 0.1 ng/ml.
- PEL2: to do a root cause analysis to identify possible causes of the outlier result for 4-F-3-PBA.
- PEL1: besides the first recommendation, do further attempts to solve the issue with ClF3CA.

7 References

[1] Deliverable Report D 9.5 Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances, v2.0. https://www.hbm4eu.eu/deliverables/

[2] HBM4EU-SOP-QA-005 “Organisation of the Quality Assurance and Quality Control Program for the 2nd prioritized substances”

[3] HBM4EU-SOP-QA-002 “Preparation of test materials for ICI / EQUAS”

Appendix 1 Homogeneity data

	Control material B3		Control material B4		Control material B3		Control material B4	
	Glyphosate		Glyphosate		AMPA		AMPA	
	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2
1	0.357	0.336	1.915	1.919	1.509	1.485	0.810	0.890
2	0.349	0.376	1.887	1.866	1.616	1.503	0.980	0.830
3	0.380	0.378	1.912	1.845	1.621	1.529	0.900	0.800
4	0.341	0.357	1.826	1.938	1.486	1.474	0.920	0.800
5	0.356	0.354	1.872	2.021	1.568	1.456	0.860	0.820
6	0.358	0.362	1.872	1.907	1.475	1.502	0.820	0.810
7	0.358	0.361	1.880	1.835	1.662	1.588	0.830	0.850
8	0.353	0.362	1.835	1.854	1.690	1.559	0.880	0.810
9	0.343	0.375	1.883	1.922	1.696	1.482	0.850	0.810
10	0.378	0.381	1.944	1.856	1.515	1.551	0.810	0.830
grand mean	0.361		1.889		1.548		0.846	
Stdev	0.013		0.047		0.075		0.047	
VC%	4%		2%		0.048		6%	
Cochran's test								
C	0.395		0.423		0.437		0.361	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit →	No outliers detected		No outliers detected		No outliers detected		No outliers detected	
target σ_{FFP}	0.090		0.472		0.387		0.211	
s_x =	0.0110		0.0292		0.0543		0.0251	
s_w =	0.0113		0.0512		0.0724		0.0558	
s_s =	0.0075		0.0000		0.0182		0.0000	
critical=0.3σ_{FFP}	0.0271		0.1417		0.1161		0.0634	
s_s < critical?	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited		Method suited		Method suited		Method suited	

Appendix 1 Homogeneity data (continued)

	Control material C			Control material D	
	TCPy			TCPy	
	replicate-1	replicate-2		replicate-1	replicate-2
1	0.429	0.427		3.484	3.712
2	0.401	0.414		3.711	3.607
3	0.413	0.432		3.575	3.755
4	0.416	0.427		3.547	3.565
5	0.412	0.402		3.479	3.466
6					
7					
8					
9					
10					
grand mean	0.417			3.590	
Stdev	0.011			0.105	
VC%	3%			3%	
Cochran's test					
C	0.457			0.543	
Ccrit	0.841			0.841	
C < Ccrit →	No outliers detected			No outliers detected	
target σ_{FFP}	0.104			0.898	
s_x =	0.0095			0.0795	
s_w =	0.0085			0.0980	
s_s =	0.0074			0.0390	
critical=0.3σ_{FFP}	0.0313			0.2693	
s_s < critical?	Homogeneity adequate			Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited			Method suited	

Appendix 1 Homogeneity data (continued)

	Control material C		Control material D		Control material C		Control material D	
	3-PBA		3-PBA		4-F-3-PBA		4-F-3-PBA	
	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2
1	0.258	0.248	1.431	1.394	1.222	1.270	0.097	0.096
2	0.247	0.260	1.499	1.440	1.180	1.133	0.097	0.097
3	0.247	0.258	1.451	1.429	1.218	1.234	0.100	0.100
4	0.249	0.255	1.393	1.464	1.185	1.200	0.092	0.099
5	0.253	0.254	1.389	1.396	1.234	1.223	0.092	0.097
6								
7								
8								
9								
10								
grand mean	0.253		1.429		1.210		0.097	
Stdev	0.005		0.037		0.038		0.003	
VC%	2%		3%		3%		3%	
Cochran's test								
C	0.405		0.475		0.445		0.648	
Ccrit	0.841		0.841		0.841		0.841	
C < Ccrit →	No outliers detected		No outliers detected		No outliers detected		Outliers detected	
target σ_{FFP}	0.063		0.357		0.302		0.024	
s_x =	0.0008		0.0291		0.0356		0.0020	
s_w =	0.0065		0.0325		0.0229		0.0028	
s_s =	0.0000		0.0178		0.0317		0.0000	
critical=0.3σ_{FFP}	0.0190		0.1071		0.0907		0.0073	
s_s < critical?	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited		Method suited		Method suited		Method suited	

Appendix 1 Homogeneity data (continued)

	Control material C		Control material D		Control material C		Control material D	
	DBCA		DBCA		DCCA-cis		DCCA-cis	
	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2
1	0.464	0.570	0.696	0.452	0.186	0.154	1.131	1.312
2	0.598	0.540	0.538	0.556	0.145	0.171	1.145	1.159
3	0.636	0.552	0.589	0.433	0.166	0.141	1.040	1.126
4	0.605	0.577	0.498	0.589	0.153	0.171	1.338	1.172
5	0.606	0.559	0.485	0.555	0.142	0.151	1.285	1.297
6								
7								
8								
9								
10								
grand mean	0.571		0.539		0.158		1.201	
Stdev	0.047		0.077		0.015		0.100	
VC%	8%		14%		9%		8%	
Cochran's test								
C	0.460		0.611		0.371		0.482	
Ccrit	0.841		0.841		0.841		0.841	
C < Ccrit →	No outliers detected		No outliers detected		No outliers detected		No outliers detected	
target σ_{FFP}	0.143		0.135		0.039		0.300	
s_x =	0.0315		0.0246		0.0088		0.0835	
s_w =	0.0498		0.0986		0.0166		0.0823	
s_s =	0.0000		0.0000		0.0000		0.0598	
critical=0.3σ_{FFP}	0.0428		0.0404		0.0118		0.0900	
s_s < critical?	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited		Method not suited		Method suited		Method suited	

Appendix 1 Homogeneity data (continued)

	Control material C		Control material D		Control material C		Control material D	
	DCCA-trans		DCCA-trans		ClF3CA		ClF3CA	
	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2	replicate-1	replicate-2
1	1.320	1.275	0.115	0.106	0.988	1.140	0.296	0.291
2	1.240	1.266	0.107	0.126	1.114	1.030	0.312	0.276
3	1.169	1.251	0.106	0.115	0.991	1.100	0.281	0.255
4	1.209	1.220	0.110	0.105	1.122	1.173	0.295	0.270
5	1.106	1.211	0.098	0.106	1.088	1.265	0.284	0.275
6								
7								
8								
9								
10								
grand mean	1.227		0.109		1.101		0.283	
Stdev	0.059		0.008		0.084		0.016	
VC%	5%		7%		8%		6%	
Cochran's test								
C	0.535		0.593		0.411		0.477	
Ccrit	0.841		0.841		0.841		0.841	
C < Ccrit →	No outliers detected		No outliers detected		No outliers detected		No outliers detected	
target σ_{FFP}	0.307		0.027		0.275		0.071	
s_x =	0.0519		0.0055		0.0573		0.0109	
s_w =	0.0453		0.0078		0.0871		0.0162	
s_s =	0.0409		0.0000		0.0000		0.0000	
critical=0.3σ_{FFP}	0.0920		0.0082		0.0826		0.0213	
s_s < critical?	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited		Method suited		Method suited		Method suited	

Appendix 2 Announcement letter / adjusted schedule

A general announcement letter and schedule for all three rounds of interlaboratory comparisons for pesticide biomarkers was send on 17th December 2019. A copy of this letter can be found in the report of the 1st round. In this letter, the requirements regarding the LOQs were indicated, and are given below for information again.

TCPy:	0.5 µg/L or lower
Pyrethroid biomarkers:	0.1 µg/L or lower for each of the individual biomarkers
Glyphosate	0.1 µg/L or lower
AMPA	0.2 µg/L or lower

For the next rounds, no specific announcement letter had been sent. An update on the schedule of shipment of samples for the 2nd round was sent by email. A copy of this mail is included below.

Fri 07-Feb-20 17:28

MH

Mol, Hans

HBM4EU ICI pesticides next rounds adjusted schedule

To Mol, Hans

Cc Dam, Ruud van; WFSR, PT; 'ipasum-hbm4eu@fau.de'; 'isciii_hbm4eu@isciii.es'; Koslitz Stephan; 'koch@ipa-dguv.de'

Dear all,

Thank you all for your efforts and submission of your results for the first round.
Due to some delay in receipt of results, the feedback report will follow next week.

Since it is considered important to take notice of the results from round 1 before starting the analysis of the samples for round 2, the ICI schedule has been adjusted.
The new schedule is outlined below.

With kind regards,
Hans Mol

Distribution of test samples round 2:
TCPy/PYR (from WFSR, Netherlands): 11th Feb **[please store in the freezer and do not start before you have received and read the feedback report of round 1]!**
Gly/AMPA (from IPA, Germany): 17th Feb

Deadline for submission of results round 2: 9th March
Feedback report round 2: 17th March

Distribution of test samples round 3:
TCPy/PYR (from WFSR, Netherlands): 17th March
Gly/AMPA (from IPA, Germany): 17th March

Deadline for submission of results round 3: 6th April
Feedback report round 3: 14th April

Letters of approval/certificates send to participants: 21st April

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Dr. J.G.J. (Hans) Mol | Senior Scientist | Wageningen Food Safety Research (previously RIKILT)
Wageningen Campus, Akkermaalsbos 2 (building 123), 6708 WB Wageningen
E hans.mol@wur.nl **T** +31(0)317 480 318 **W** www.wur.eu/food-safety-research

WAGENINGEN
UNIVERSITY & RESEARCH

Our disclaimer: www.disclaimer-uk.wur.nl

Appendix 3 Copy of letter of instructions sent together with test samples

Postbus 230 | 6700 AE WAGENINGEN The Netherlands

WFSR

DATE
February 11th, 2020

SUBJECT
Instruction letter for ICI study pesticide biomarkers in urine

OUR REFERENCE
HBM4EU ICI-PES R2

POSTAL ADDRESS
Akkermaalsbos 2
6708 WB, Wageningen
The Netherlands

INTERNET
www.wur.nl

CoC NUMBER
09098104

HANDLED BY
Hans Mol

TELEPHONE
+31 317 480318

EMAIL
hans.mol@@wur.nl

Dear participant,

Thank you for participation in the interlaboratory comparison study **HBM4EU ICI-Pesticides/round_02** for the determination of pesticide biomarkers in human urine.

As indicated in the announcement sent on 17th December, and as in the first round, you receive two sets of two samples in each round:

1) Two urine samples (approx. 10 ml each) for determination of glyphosate and AMPA. These samples are send to you by IPA/Germany. Shipment 17 February 2020.
2) Two urine samples (approx. 5 ml each) for determination of biomarkers of chlorpyrifos (TCPy) and pyrethroids (3-PBA, 4 F 3 PBA, cis-DBCA, cis-DCCA, trans-DCCA, ClF3CA). Shipment 11 February 2020 by WFSR/Netherlands.

Instructions:

- Upon receipt, store the samples in the freezer until analysis.
- Confirm the receipt by email to hans.mol@wur.nl
  You should receive the samples in frozen condition. If not or in case of damage, please indicate that in your mail.
- Before analysis, thaw and re-homogenize the samples according to your laboratory's procedure.
- Please carry out a **single analysis** for each sample.
- Please report all results to hans.mol@wur.nl
- Results are to be reported in ng/ml urine, using at least 3 significant figures.
- For reporting, please use the excel file provided by us through email: "ICI-study Pesticides-round_02_results and method information v1.xlsx'. Also provide your method details through this excel sheet.
- The deadline for submission of results is strict and is **9th March 2020**.

100years
1918 — 2018

Wageningen Research Foundation/WFSR is part of Wageningen University & Research. WFSR carries out research into the safety and reliability of food and feed. WFSR is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

DATE
February 11th, 2020

PAGE
2 of 2

Please contact us if you have any questions or need any assistance.

With kind regards,

Hans Mol hans.mol@wur.nl
Ruud van Dam ruud.vandam@wur.nl
Ingrid Elbers pt.wfsr@wur.nl

Appendix 4: Result tables.

Results for the four selected expert labs

Biomarker	Glyphosate						AMPA					
Control material	R2A = B3			R2B = B4			R2A = B3			R2B = B4		
Conc. hom. test (ng/ml)	0.361			1.89			1.55			0.846		
Assigned value (ng/ml)	0.408			2.61			1.16			0.442		
Rel. uncertainty	8%			6%			10%			9%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	0.380	-7%	-0.3	2.86	9%	0.4	0.942	-19%	-0.8	0.369	-16%	-0.7
PEL2	0.473	16%	0.6	2.290	-12%	-0.5	1.257	8%	0.3	0.446	1%	0.0
PEL3	0.370	-9%	-0.4	2.69	3%	0.1	1.29	11%	0.4	0.510	15%	0.6
PEL24	n/a			n/a			n/a			n/a		
mean	0.408			2.61			1.16			0.442		
RSD	14%			11%			17%			16%		

Results for all six participants

Biomarker	Glyphosate						AMPA					
Control material	R2A = B3			R2B = B4			R2A = B3			R2B = B4		
Conc. hom. test (ng/ml)	0.361			1.89			1.55			0.846		
Assigned value (ng/ml)	0.389			2.42			1.25			0.524		
Rel. uncertainty	6%			7%			7%			14%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	0.380	-2%	-0.1	2.86	18%	0.7	0.942	-25%	-1.0	0.369	-30%	-1.2
PEL2	0.473	22%	0.9	2.290	-5%	-0.2	1.257	0%	0.0	0.446	-15%	-0.6
PEL3	0.370	-5%	-0.2	2.69	11%	0.4	1.29	3%	0.1	0.510	-3%	-0.1
PEL24	n/a			n/a			n/a			n/a		
PEL98	0.360	-7%	-0.3	1.920	-21%	-0.8	1.51	21%	0.8	0.810	54%	2.2
PEL 99	0.360	-7%	-0.3	2.35	-3%	-0.1	1.26	1%	0.0	0.487	-7%	-0.3
mean	0.389			2.42			1.25			0.524		
RSD	12%			15%			16%			32%		

n/a = method used was unsuccessful, no results reported
%DA = percent deviation from consensus value
Z = Z-score

Appendix 4: Result tables (continued).

Results for the four selected expert labs

Biomarker	TCPy					
Control material	R2A (=C)			R2B (=D)		
Conc. hom. test (ng/ml)	0.417			3.59		
Assigned value (ng/ml)	*			4.304		
Rel. uncertainty	26%			5%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	0.447	*	*	4.50	5%	0.2
PEL2	0.589	*	*	4.217	-2%	-0.1
PEL3	0.401	*	*	3.710	-14%	-0.6
PEL24	1.11	*	*	4.79	11%	0.5
mean	0.637			4.30		
RSD	51%			11%		

***** no assigned value because the uncertainty of the mean is too high
%DA = percent deviation from consensus value
Z = Z-score

Appendix 4: Result tables (continued).

Results for the four selected expert labs

Biomarker	3-PBA						4-F-3-PBA					
Control material	R2A (=C)			R2B (=D)			R2A (=C)			R2B (=D)		
Conc. hom. test (ng/ml)	0.253			1.429			1.21			0.0967		
Assigned value (ng/ml)	0.279			1.756			1.561			0.102		
Rel. uncertainty	14%			12%			16%			6%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	0.21	-25%	-1.0	1.57	-11%	-0.4	1.27	-19%	-0.7	0.114	12%	0.5
PEL2	0.389	39%	1.6	2.373	35%	1.4	1.487	-5%	-0.2	0.527	417%	17
PEL3	0.247	-11%	-0.5	1.500	-15%	-0.6	1.180	-24%	-1.0	0.097	-5%	-0.2
PEL24	0.270	-3%	-0.1	1.580	-10%	-0.4	2.308	48%	1.9	0.095	-7%	-0.3
mean	0.279			1.756			1.561			0.102		
RSD	28%			24%			33%			10%		

R2B 4-F-3-PBA: result for PEL2 was identified as Grubbs' outlier and excluded for calculation of the mean and assigned value

%DA = percent deviation from consensus value

Z = Z-score

Appendix 4: Result tables (continued).

Results for the four selected expert labs

Biomarker	cis-DBCA						cis-DCCA					
Control material	R2A (=C)			R2B (=D)			R2A (=C)			R2B (=D)		
Conc. hom. test (ng/ml)	0.571			0.539			0.158			1.201		
Assigned value (ng/ml)	*			0.627			*			1.361		
Rel. uncertainty	35%			8%			22%			9%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	too high matrix effect			too high matrix effect			0.058	*	*	1.68	23%	0.9
PEL2	0.608	*	*	0.709	13%	0.5	0.169	*	*	1.410	4%	0.1
PEL3	0.598	*	*	0.538	-14%	-0.6	0.145	*	*	1.140	-16%	-0.6
PEL24	1.567	*	*	0.633	1%	0.0	0.206	*	*	1.213	-11%	-0.4
mean	0.924			0.627			0.145			1.361		
RSD	60%			14%			44%			18%		

***** no assigned value because the uncertainty of the mean is too high
%DA = percent deviation from consensus value
Z = Z-score

Appendix 4: Result tables (continued).

Results for the four selected expert labs

Biomarker	trans-DCCA						ClF3CA					
Control material	R2A (=C)			R2B (=D)			R2A (=C)			R2B (=D)		
Conc. hom. test (ng/ml)	1.227			0.109			1.101			0.283		
Assigned value (ng/ml)	*			0.126			*			0.418		
Rel. uncertainty	25%			16%			28%			14%		
Lab code	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z	ng/ml	%DA	Z
PEL1	1.36	*	*	<0.05	*-60%*	*-2.4*	problems w. analysis			problems w. analysis		
PEL2	1.038	*	*	0.167	33%	1.3	1.305	*	*	0.514	23%	0.9
PEL3	1.240	*	*	0.107	-15%	-0.6	1.110	*	*	0.312	-25%	-1.0
PEL24	2.760	*	*	0.104	-17%	-0.7	2.606	*	*	0.429	3%	0.1
mean	1.600			0.126			1.674			0.418		
RSD	49%			28%			49%			24%		

* no assigned value because the uncertainty of the mean is too high
%DA = percent deviation from consensus value
Z = Z-score
PEL1, R2B trans-DCCA: %DA and Z-score for information only (calculated using 0.05 ng/ml as result)

Appendix 5: Method details Glyphosate and AMPA

		PRETREATMENT				EXTRACTION & CLEANUP		
Lab	LOQ (ng/ml)	Pretreatment	urine aliquot used (ml)	pH adjustment (provide buffer and pH)	Deconjugation	Technique	specify SPE column or LLE solvent	Derivatisation
GLYPHOSATE								
PEL1	0.1	none	1	pH 9 tetraborate	none	LLE	diethyl-ether	FMOC
PEL2	0.1	none	0.05		none	Evaporation to dryness		TFAA/TFE
PEL3	0.1	none	1	no	none	SPE (off-line)	strata SAX	FMOC
PEL24	N/A*	none	1	yes (1% NH4OH)	none	SPE (off-line)	Oasis MAX	none
PEL98	0.05	none	0.05	N/A	none	Dilution with ACN, vaccum drying		TFAA/TFE
PEL 99	0.26		0.1	acetone: acetonit	none	LLE (after derivatisation)	MTBE	PFBBr
AMPA								
PEL1	0.5	none	1	pH 9 tetraborate	none	LLE	diethyl-ether	FMOC
PEL2	0.1	none	0.05		none	Evaporation to dryness		TFAA/TFE
PEL3	0.2	none	1	no	none			FMOC
PEL24	N/A*	none	1	yes (1% NH4OH)	none	SPE (off-line)	Oasis MAX	none
PEL98	0.05	none	0.05	N/A	none	Dilution with ACN, vaccum drying		TFAA/TFE
PEL 99	0.29		0.1	acetone: acetonit	none	LLE (after derivatisation)	MTBE	PFBBr

	INSTRUMENTAL ANALYSIS						
Lab	Separation technique	injection volume (µl)	Column	Detection	MS instrument/type used	for MS(/MS): ionisation	Quantifier transition/ion (m/z x>y)
GLYPHOSATE							
PEL1	(U)HPLC	10	Kinetex C18	MS/MS	Shimadzu 8060	ESI neg	390 > 63
PEL2	GC	1	Agilent HP Innowax	MS/MS	Thermo TSQ9000	NCI	370 -> 245
PEL3	(U)HPLC	20	Cortecs, 100x2.1 mm, 2.7 µm	MS/MS	Sciex API5500	ESI neg	390>150
PEL24	(U)HPLC	10	Dionex Ion Pac AG11-HC	MS/MS	QTRAP 6500plus	ESI neg	168/63
PEL98	GC	1	ZB-WAX, 30m, 0.25 mm, 0.25 µm	MS/MS	Agilent 7000 A	NCI	370>245
PEL 99	(U)HPLC	10	BEH Phenyl 100x2.1 mm; 1.7 µm	MS/MS	Xevo TQ-XS Waters	ESI pos	710.30>448.20
AMPA							
PEL1	(U)HPLC	10	Kinetex C18	MS/MS	Shimadzu 8060	ESI neg	332 > 110
PEL2	GC	1	Agilent HP Innowax	MS/MS	Thermo TSQ9000	NCI	351 -> 268
PEL3	(U)HPLC	20	Cortecs, 100x2.1 mm, 2.7 µm	MS/MS	Sciex API5500	ESI neg	332>110
PEL24	(U)HPLC	10	Dionex Ion Pac AG11-HC	MS/MS	QTRAP 6500plus	ESI neg	110/63
PEL98	GC	1	ZB-WAX, 30m, 0.25 mm, 0.25 µm	MS/MS	Agilent 7000 A	NCI	351>268
PEL 99	(U)HPLC	10	BEH Phenyl 100x2.1 mm; 1.7 µm	MS/MS	Xevo TQ-XS Waters	ESI pos	652.30>390.20

	CALIBRATION & QUANTIFICATION		
Lab	specify for each compound which internal standard you used for quantification	moment of addition of internal standard?	Preparation of calibration standards
GLYPHOSATE			
PEL1	Glyphosate 13C2-15N	before derivatisation	cal stds prepared in blank urine processed as samples
PEL2	1,2-13C215N-Glyphosate	before extraction	cal stds prepared in blank urine processed as samples
PEL3	13C2-15N-Glyphosate	before extraction	cal stds prepared in solvent/eluent
PEL24	isotopically labelled	before extraction	cal stds prepared in solvent/eluent
PEL98	Glyphosate-d2	before dilution	water
PEL 99	Glyphosate-13C15N	before derivatisation	cal stds prepared in blank urine processed as samples
AMPA			
PEL1	Glyphosate 13C2-15N	before derivatisation	cal stds prepared in blank urine processed as samples
PEL2	13C-15N-AMPA	before extraction	cal stds prepared in blank urine processed as samples
PEL3	13C-15N-AMPA	before extraction	cal stds prepared in solvent/eluent
PEL24	isotopically labelled	before extraction	cal stds prepared in solvent/eluent
PEL98	AMPA-13C15N	before dilution	water
PEL 99	AMPA-13C15N-d2	before derivatisation	cal stds prepared in blank urine processed as samples

Appendix 5 continued. Method details: TCPy and pyrethroid biomarkers (1/2)

	PRETREATMENT					EXTRACTION & CLEANUP		
Lab	Pretreatment	urine aliquot used (ml)	pH adjustment (provide buffer and pH)	Deconjugation	time(hrs) / temp (°C)	Technique	specify SPE column or LLE solvent	Derivatisation
TCPy								
PEL1	none	2.5	pH 4.8 acetate buffer	Helix Pomatia	16 hrs / 37 °C	LLE	hexane	none
PEL2	none	1	pH 5 acetate buffer	ß-Glucuronidase/Arylsulfatase (Helix Pomatia)	16 h / 37 °C	SPE (off-line)	Isolute 101	MTBSTFA
PEL3	none	5	pH 4.5, acetate buffer	ß-Glucuronidase/Arylsulfatase (Helix Pomatia)	overnight, 37°C	SPE (off-line)	strata X C18	none
PEL24	none	1	acetate (pH=5)	Helix Pomatia	12 h/37 °C	SPE (off-line)	Oasis HLB	none
PYRETHROID BIOMARKERS								
PEL1	none	2.5	pH 4,8 acetate buffer	Helix Pomatia	16 hrs / 37 °C	LLE	hexane	none
PEL2	none	4.5		acid hydrolysis	1 h / 100 °C	LLE, base/acid partitioning/back extraction.	Hexane	MTBSTFA
PEL3	none	5	pH 4.5, acetate buffer	Helix Pomatia, ß-Glucuronidase/Arylsulfatase	overnight, 37°C	SPE (off-line)	strata X C18	
PEL24	none	1	acetate (pH=5)	Helix Pomatia	12 h/37 °C	SPE (off-line)	Oasis HLB, ac	none

	INSTRUMENTAL ANALYSIS					CALIBRATION & QUANTIFICATION	
Lab	Separation technique	injection volume (µl)	Column	Detection technique	MS instrument/ type used	moment of addition of internal standard?	Preparation of calibration standards
TCPy							
PEL1	(U)HPLC	10	Atlantis T3	MS/MS	Sciex 5500	before extraction	cal stds prepared in blank urine processed as samples
PEL2	GC	1	Agilent HP-5ms-UI	MS/MS	Agilent Technologies	before deconjugation	cal stds prepared in blank urine processed as samples
PEL3	(U)HPLC	50	Acquity HSS T3, 2.1x100 mm, 1.7 µm	MS/MS	Sciex 6500+	before deconjugation	cal stds prepared in solvent/eluent
PEL24	(U)HPLC	10	Acquity BEH HSST3	MS/MS	Agilent 4595 QQQ	to final extract	standard addition
PYRETHROID BIOMARKERS							
PEL1	(U)HPLC	10	Atlantis T3	MS/MS	Sciex 5500	before extraction	cal stds prepared in blank urine processed as samples
PEL2	GC	1.2	Agilent DB35MS	MS (single)	HP 6890	before deconjugation	cal stds prepared in blank urine processed as samples
PEL3	(U)HPLC	50	Acquity HSS T3, 2.1x100 mm, 1.7 µm	MS/MS	Sciex 6500+	before deconjugation	cal stds prepared in solvent/eluent
PEL24	(U)HPLC	10	Acquity BEH HSST3	MS/MS	Agilent 4595 QQQ	to final extract	standard addition

Appendix 5 continued. Method details: TCPy and pyrethroid biomarkers (2/2)

			INSTRUMENTAL ANALYSIS				CALIBRATION & QUANTIFICATION
Lab	Biomarker	LOQ (ng/ml)	Separation technique	Detection technique	for MS(/MS): ionisation	Quantifier transition/ion (m/z x>y)	specify for each compound which internal standard you used for quantification
PEL1	TCPy	0.1	(U)HPLC	MS/MS	ESI neg	196 > 35	trans-DCCA d6
PEL2	TCPy	0.5	GC	MS/MS	EI	254 → 219	TCPy-13C3
PEL3	TCPy	0.1	(U)HPLC	MS/MS	ESI neg	195.8>35	13C3-TCPy
PEL24	TCPy	0.01	(U)HPLC	MS/MS	ESI neg	196/35	nicarbazin
PEL1	3-PBA	0.05	(U)HPLC	MS/MS	ESI neg	213 > 93	3-PBA C13
PEL2	3-PBA	0.1	GC	MS (single)	EI	271	13C6-3-PBA
PEL3	3-PBA	0.1	(U)HPLC	MS/MS	ESI neg	213>93	13C6-3-PBA
PEL24	3-PBA	0.01	(U)HPLC	MS/MS	ESI neg	213/93	nicarbazin
PEL1	4-F-3-PBA	0.05	(U)HPLC	MS/MS	ESI neg	231 > 93	3-PBA C13
PEL2	4-F-3-PBA	0.1	GC	MS (single)	EI	289	13C6-4-F-PBA
PEL3	4-F-3-PBA	0.1	(U)HPLC	MS/MS	ESI neg	230.8>93	13C6-4-F-3-PBA
PEL24	4-F-3-PBA	0.05	(U)HPLC	MS/MS	ESI neg	231/93	nicarbazin
PEL1	cis-DBCA	N/A					
PEL2	cis-DBCA	0.1	GC	MS (single)	EI	355	2-PBA
PEL3	cis-DBCA	0.1	(U)HPLC	MS/MS	ESI neg	297>79	13C2-D-cis-DBCA
PEL24	cis-DBCA	0.25	(U)HPLC	MS/MS	ESI neg	294/79	nicarbazin
PEL1	cis-DCCA	0.05	(U)HPLC	MS/MS	ESI neg	209 > 37	trans-DCCA d6
PEL2	cis-DCCA	0.1	GC	MS (single)	EI	265	13C2-cis-DCCA
PEL3	cis-DCCA	0.1	(U)HPLC	MS/MS	ESI neg	207>35	13C2-D-cis-DCCA
PEL24	cis-DCCA	0.25	(U)HPLC	MS/MS	ESI neg	207/35	nicarbazin
PEL1	trans-DCCA	0.05	(U)HPLC	MS/MS	ESI neg	209 > 37	trans-DCCA d6
PEL2	trans-DCCA	0.1	GC	MS (single)	EI	265	13C4-d3-trans-Cl2CA
PEL3	trans-DCCA	0.1	(U)HPLC	MS/MS	ESI neg	207>35	D6-trans-DCCA
PEL24	trans-DCCA	0.025	(U)HPLC	MS/MS	ESI neg	207/35	nicarbazin
PEL1	ClF3CA	N/A					
PEL2	ClF3CA	0.1	GC	MS (single)	EI	299	13C4-d3-trans-Cl2CA
PEL3	ClF3CA	0.1	(U)HPLC	MS/MS	ESI neg	241.2>35	-
PEL24	ClF3CA	0.05	(U)HPLC	MS/MS	ESI neg	241/35	nicarbazin