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Interlaboratory Comparison Study to Support the Deepwater Horizon Natural Resource Damage Assessment: Description and Results for QA11Blood01- PAHs, PAH Metabolites, and DOSS in Solution and Blood

John R. Kucklick
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John R. Kucklick
Michele M. Schantz
Analytical Chemistry Division
Material Measurement Laboratory
National Institute of Standards and Technology
Gaithersburg, MD 20899 and Charleston, SC 29412

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ABSTRACT

To support natural resource damage assessment (NRDA) in response to the Deepwater Horizon (DWH) oil spill in the Gulf of Mexico, a large number of samples were collected from protected species including marine mammals and sea turtles. Analysis of these samples will continue for the foreseeable future. To support NRDA sample analyses, NOAA will require analytical laboratories in addition to the commercial laboratories that are currently listed as providing support to NRDA. To compare the data among these laboratories, interlaboratory comparison studies have been initiated, and the results from the fourth exercise, QA11Blood01, are reported here. In this exercise, selected polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs, hydroxylated PAH metabolites and dioctyl sodium sulfosuccinate (DOSS) were determined in the exercise material, which consisted of a whole blood and plasma collected from American alligators (*Alligator mississippiensis*) amended with the test compounds. A solution containing known amounts of the test compounds was also distributed to help gauge analyses conducted on a matrix-free sample. The results from this fourth exercise are reported along with a summary of the analytical methods used.

INTRODUCTION

The National Institute of Standards and Technology (NIST) has conducted three interlaboratory comparison exercises to support the natural resource damage assessment (NRDA) in response to the Deepwater Horizon (DWH) oil spill in the Gulf of Mexico with marine sediment [1], crude oil [2], and mussel tissue [3] as the matrices of interest. The exercises were initiated to provide information on the comparability of data from different laboratories for PAHs (polycyclic aromatic hydrocarbons), alkylated PAHs, and petroleum biomarkers (hopanes and steranes). A large number of coastal sediment and tissue samples (e.g., oysters) have been collected outside of the spill zone to define baseline environmental conditions prior to being exposed to oil. For the foreseeable future, subsequent analysis of oiled sediments and oil-exposed oysters will be required as well. To support these efforts, additional analytical laboratories are being used to provide greater capacity for the NRDA evaluation.

The present exercise was designed to evaluate measurements of wildlife blood for selected PAHs, alkylated PAHs, hydroxylated PAH metabolites, and dioctyl sodium sulfosuccinate (DOSS). Blood was a matrix of interest for NRDA as this tissue was commonly collected from wildlife for assessment of hydrocarbon exposure. The study was performance-based; hence, laboratories were free to use the methods of their choosing. It is important to note that this exercise was not designed nor intended to reflect method performance for past results reported by the laboratories but rather to provide the laboratories the opportunity to assess methods of interest. The analytes chosen for this study were those commonly found in oil and/or used for source apportionment studies, or are metabolites of aromatic hydrocarbon exposure.

The data received from six laboratories for the exercise are summarized in this report along with summaries of the analytical methods used by each laboratory. For this exercise, laboratory performance was based on p-scores that help gauge laboratory precision. Due to

the small number of laboratories participating, z-scores, which are indicative of agreement to a consensus value, were not calculated. Among laboratory agreement is thus shown graphically.

SOURCE OF MATERIAL

There were four materials sent out to participants (Table 1): (1) a mixture of the PAH-related compounds in isooctane, (2) DOSS in methanol, (3) the target compounds added to pooled alligator plasma, and (4) the target compounds added to pooled alligator whole blood. The compounds used to prepare the samples above were purchased in either neat form or solution from Accustandard (New Haven, CT), Chiron (Trondheim, Norway) or Sigma (St. Louis, MO). Individual stock solutions of compounds were used to prepare a mixed spiking solution in ethanol that was added to the blood materials, a mixed PAH solution in isooctane, and a DOSS solution in methanol. Alligator plasma and whole blood were pooled from wild alligators, which were sampled during capture-release health assessments, and captive alligators. The blood (approximately 275 g of plasma or whole blood) was added to a 500 mL Erlenmeyer flask, spiked with approximately 0.8 g of the mixed analyte spiking solution, and stirred for 4 hours in the dark before aliquotting into 4.5 mL cryovials. Cryovials were then immediately frozen at -80 ^{o}C.

Table 1: Summary of samples and target compounds added to the QA11blood01 materials. Blood material values are pg/g while solution is ng/g.

	Material			
Compound	QA11Plasma	QA11WholeBlood	QA11Solution#1-PAH	QA11Solution#2-DOSS
Fluorene	5007	5029	16.6	
Phenanthrene	2925	2938	9.06	
Pyrene	1992	2001	6.17	
Benzo[a]pyrene	1745	1752	5.34	
2 methylnaphthalene	21387	21480	68.8	
2,6-dimethyl naphthalene	8516	8553	26.5	
1,7-dimethylphenanthrene	543	546	1.71	
9-methylphenanthrene	1982	1990	6.11	
3-methylphenanathrene	4785	4806	14.8	
1-naphthol	1644	1651	5.04	
2,3-dihydroxynaphthalene	7220	7252	22.1	
2-methyl-1-naphthol	337	338	1.13	
2-hyrdoxyfluorene	5320	5343	16.5	
1-hydroxypyrene	846	850	2.67	
Dioctylsodium succinate (DOSS)	29965	30095		110

SAMPLE DISTRIBUTION

Four cryovials of QA11Plasma or QA11WholeBlood each containing 4 mL of frozen material were sent to seven laboratories the week of October 10, 2011. Each laboratory was requested to analyze three samples of each blood material and three measurements of QA11Solution#1-PAH and QA11Solution#2-DOSS with their laboratory's and/or program's current analytical protocols being used for the determination of the concentrations (mass/mass) of the parent PAH compounds, alkylated PAH compounds, hydroxylated PAH compounds and DOSS.

The instructions including the list of target analytes sent to participants are given in Appendix A.

EVALUATION OF EXERCISE RESULTS

Establishment of the Assigned Values

Laboratory data submission: Each participating laboratory was asked to submit data from three replicate determinations of the "unknown" material QA11Plasma and QA11WholeBlood and was requested to report results of concurrent analyses of QA11Solution#1-PAH and QA11Solution#2-DOSS. The solution materials were used because NIST does not have a reference material for PAHs in blood, and the solutions provided information on laboratory measurements made on a matrix-free sample. Laboratories were requested to report these results to at least three significant figures and to provide brief descriptions of their cleanup and analytical procedures.

Determination of laboratory analyte means: For each laboratory, the laboratory analyte mean of the three sample results (S1, S2, and S3) was calculated for each analyte. Non-numerical data were treated as follows: A mean "<value" was used when three "<values" were reported; NA (not analyzed/determined) was used for three reported NAs; and, if the reported results were of mixed type, e.g., S1 and S2 were numerical values and S3 was reported as "<value", the two similar "types" were used to either determine the mean or to set a non-numerical descriptor.

Determination of assigned values: In prior exercises, the inclusion of a laboratory's data in the consensus value was based on their performance on the reference material. If values were within 30% of the assigned value for the reference material, their measurements on the unknown were included in the calculation of the consensus value for the unknown. This approach was not possible in the present exercise as there was no reference material included in the exercises. Consequently, the consensus values for this exercise are simply the median of all values submitted by all laboratories. No values were excluded due to the small number of laboratories submitting data.

REPORTED RESULTS

Laboratories were assigned numerical identification codes in order of receipt of their data with the exception of the two NIST data sets, which are designated Laboratory 1 and 2. The laboratory mean replicate data are shown in Tables 2, 3 and 4 for QA11Solution#1-PAH and QA11Solution#2-DOSS, QA11Plasma, and QA11Wholeblood, respectively. Included in the tables are the by-compound mean and standard deviation for the three measurements made on each matrix by each laboratory and the exercise assigned median values plus standard deviation. The gravimetric concentration that was based on the original weighing of the target compounds to make the exercise materials is also provided. Summaries of the methods used by each laboratory are in Appendix B. A second set of plasma and whole blood samples was distributed to laboratory 5 in February 2012 for an additional DOSS determination. Laboratory 5 therefore has two sets of DOSS values, those from the initial determination in 2011 and from the second set of samples analyzed in 2012.

Performance Scores

The exercise coordinators recognize that different environmental monitoring programs have different data quality objectives and needs. The acceptability of the results submitted by a particular laboratory will be decided by the individual program(s) for which the laboratory provides data. Typically, each program will use these exercise results in conjunction with the laboratory's performance in the analysis of certified reference materials and/or control materials, and of other quality assurance samples. These exercise results are exhibited in a number of ways in this report to facilitate their use by most environmental monitoring programs in their acceptability assessments.

Precision Assessment (p-score)

$$\text{p-score} = \sigma_{lab} / \sigma_{target}$$

For the calculation of p-scores [4-5] for this program, the σ values used are coefficients of variation (CV calculated as relative standard deviations) with the current target σ (CV) for the three replicates being 15 %. The value of 15 % was chosen for use interlaboratory comparison exercises of this type run by NIST. P-scores are only available for analytes where three measurements were made by the laboratory. Tables 2 through 4 summarize the relative standard deviations (RSDs) calculated from the three concentrations reported by the laboratory for each analyte quantified. Table 5 gives the p-scores (15 %). A p-score of 1 indicates that the laboratory's CV was 15 %, and a p-score of 2 indicates that the laboratory's CV was 30 %.

RESULTS and DISCUSSION

NOAA's NRDA office requested that NIST coordinate an interlaboratory comparison exercise for PAHs, PAH metabolites, and DOSS in blood as this tissue had been collected from several wildlife species following the spill. With the help of NOAA and NRDA, NIST requested that five laboratories outside of NIST participate in an interlaboratory comparison exercise using blood-based samples. Of these five laboratories, four submitted data sets with two additional data sets submitted by NIST. Laboratory 5 submitted two data sets for DOSS (see above). The listing of participating laboratories is given in Appendix C.

Tables 2 through 4 summarize the laboratory means and exercise assigned values for the PAHs, alkylated PAHs, hydroxylated PAHs and DOSS in the solution, plasma, and whole blood materials, respectively. The consensus values for the materials were simply the median concentrations of the submitted results. Given the small number of values reported, no data were excluded from the derivation of the consensus value. The gravimetric values are given in the tables for guidance. For most compounds, the gravimetric value was within the range of data reported back by the laboratories especially for the solution material. This was also true for the blood materials with the exception of the methylnaphthalenes that had higher gravimetric values than the reported values. The most likely explanation for this discrepancy is that the methylnaphthalenes that were added to the blood were partially lost through evaporation at some point in the preparation of the blood materials. There were few reported values for the hydroxylated PAHs so it is difficult to gauge the accuracy of the gravimetric values relative to these compounds. Prior experience with hydroxylated compounds has shown that concentrations can change in solution either through degradation, conjugation, or possibly sorption to container walls. DOSS was measured by three laboratories (Labs 3, 5, and 6). The median of measured values for DOSS agreed very well with the gravimetric concentration (Tables 2-4). The CV for the three labs was higher for DOSS in the blood materials (ca. 40 %) than for the solution (ca. 5 %).

As expected, the agreement among laboratories for the compounds in the matrix-free solution was better than that observed in the blood materials. The coefficients of variation (standard deviation/median) were 17 %, 53 %, and 70 % for the solution, plasma, and whole blood, respectively. Excluding the high fluorene and pyrene values reported by Lab 6 reduced the CVs to 33 % for plasma and 48 % for whole blood. This indicates that reported values in plasma are less variable among laboratories than those made on whole blood. This result is reasonable as the plasma is largely free of cellular material relative to whole blood and therefore should be less subject to matrix effects during analysis.

The precision or "p" scores, which are a measure of within laboratory repeatability, are given in Table 5. Again, a p-score of ≤1 indicates that a laboratory's reported values are within 15 % of their mean reported value. P-scores were only calculated on the blood materials and were generally <0.5 for most analytes in most laboratories. 1,7-dimethylphenanthrene had a higher p-score than other compounds probably due to the low concentration of this compound relative to other compounds in the samples (Tables 2-4).

The methods used to analyze the samples varied among laboratories (Appendix B Tables B1-B7). Extraction methods for the PAHs included microwave extraction (Lab 1), liquid:liquid extraction (Labs 2, 4, and 5), and QuECHERS (Quick Easy Cheap Effective Rugged Safe, essentially liquid:liquid extraction with acetonitrile following the addition of salts; Labs 3 and 6). DOSS was extracted by QuECHERS (Labs 3 and 6) or by liquid:liquid extraction using acetonitrile (Lab 5). Cleanup of samples prior to PAH analysis included size exclusion followed by solid phase extraction (Labs 1, 3, and 4) and no cleanup (labs 2, 5, and 6). Prior experience in our laboratory has shown cleanup of blood extracts is required for the analysis of persistent organic pollutants by GC/MS [4], hence it was surprising that several laboratories opted for no cleanup. Labs did not clean up extracts prior to DOSS analysis. For the analysis of the parent PAHs, five laboratories used GC/MS with 5% methylpolysiloxane-phase column (Labs 2 through 5) or a 50% methylpolysiloxane-phase column (Lab 1). Lab 6 used UPLC/FLR/MS/MS with a C18 column. For alkylated PAHs, all labs used GC/MS with columns noted in Table B5; although, Lab 6 used GC/MS/MS. All labs used LC/MS/MS for the analysis of DOSS. Hydroxylated PAHs were determined by Labs 1 and 2 using GC/MS following derivatization with MSTFA and Lab 6 performed the analysis by UPLC/FLR/MS/MS. Additional details are given in Appendix B.

In conclusion, results demonstrated that it is possible to measure a suite of parent and alkylated PAHs in blood with reasonable agreement among laboratories. The three laboratories that measured the oil dispersant compound, DOSS, were also able to provide a consensus value that was very close to the nominal concentration of DOSS in the samples (Tables 3 and 4). PAH metabolites were a challenge for laboratories with very few results being submitted by the group. While there are several methods for PAH metabolites in urine published in the literature, this is not the case for PAHs in blood which may explain the low number of values submitted by laboratories. Based on preliminary work done by NIST, hydroxylated PAHs also might not achieve stable concentrations when spiked in blood. Further studies are needed to determine the feasibility of providing a reference material for hydroxylated PAHs in blood and to provide reliable methods for hydroxylated PAH analysis in blood samples.

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Disclaimer

Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

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Table 2: Tabular results from the analysis of QA11Solution#1-PAH and QA11Solution#2-DOSS. All values are ng/g. Gravimetric refers to concentration expected based on the mass of compound added to the solution.

	Laboratory														
	1		2		3		4		5		6				
Compound	**mean**	**RSD (%)**	**mean**	**RSD (%)**	**mean**	**RSD (%)**	**mean**	**RSD (%)**	**mean**	**RSD (%)**	**mean**	**RSD (%)**	**median**	**SD**	**Gravimetric**
fluorene	15.9	2.0	16.7	7.4	14.4	NA	11.1	3.4	12.2	3.7	9.07	7.9	13.3	2.9	16.6
phenanthrene	9.75	0.7	9.27	5.0	7.07	NA	8.08	1.7	7.35	3.8	<LOD	NA	8.08	1.2	9.06
pyrene	6.15	3.1	5.09	6.5	5.43	NA	5.24	12	5.05	5.1	7.61	5.6	5.33	1.0	6.17
benzo[a]pyrene	4.75	3.7	6.86	9.0	4.51	NA	4.13	9.9	4.00	0.8	7.30	10	4.63	1.4	5.34
1-methylnaphthalene	<LOD	NA	NA	<LOD	<1.6	<LOD	<1.5	NA	NA	NA	NA	NA			
2-methylnaphthalene	58.7	0.8	65.2	6.6	78.2	NA	62.9	2.4	50.5	2.6	55.1	4.6	60.8	9.6	68.8
2,6-dimethylnaphthalene	23.2	1.8	19.9	9.9	24.3	NA	22.6	8.6	20.6	6.7	24.4	2.8	22.9	1.9	26.5
1,7-dimethylphenanthrene	2.26	7.0	1.79	10	1.37	NA	2.07	13	NA	NA	NA	NA	1.93	0.4	1.71
9-methylphenanthrene	7.44	1.6	6.16	12	5.42	NA	5.26	2.2	4.81	2.6	5.52	1.5	5.47	0.9	6.11
3-methylphenanthrene	15.2	1.8	16.4	8.2	12.4	NA	11.6	7.4	12.1	5.6	14.9	10.6	13.7	2.0	14.8
1-naphthol	<LOD	NA	6.86	9.0	NA	NA	NA	NA	NA	NA	5.14	4.4	6.00		5.04
2,3-dihydroxynaphthalene	<LOD	NA	<1	NA	NA	NA	NA	NA	NA	NA	<LOD	NA			22.1
2-methyl-1-naphthol	<LOD	NA	<1	NA	NA	NA	NA	NA	NA	NA	<LOD	NA			1.13
2-hydroxyfluorene	7.80	2.3	NA	NA	NA	NA	NA	NA	NA	NA	12.7	1.6	10.2		16.5
1-hydroxypyrene	2.06	0.3	other	NA	NA	NA	NA	NA	NA	NA	<LOD	NA			2.67
DOSS (ion)	NA	NA	NA	NA	116	1.0	NA	NA	110	7.7	121	1.3	116	5.7	110

Table 3: Tabular results from the analysis of QA11Plasma. All values are pg/g. Gravimetric refers to concentration expected based on the mass of compound added to the blood.

	Laboratory															
	1		2		3		4		5a		5b		6			
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	median	SD	Gravimetric
fluorene	4897	NA	4333	5.3	4290	3.6	4310	5.0	3480	2.6			10646	12.2	4322	2645	5029
phenanthrene	3285	NA	6693	3.0	3120	0.6	2733	1.5	2200	4.5			<LOD	NA	3120	1775	2938
pyrene	2406	NA	1900	4.6	2420	0.7	1890	3.2	1623	2.8			11078	7.9	2153	3700	2001
benzo[a]pyrene	NA	NA	1620	3.8	1807	1.7	1353	1.7	1180	0.8			<LOD	NA	1487	278	1752
1-methylnaphthalene	<LOD	NA	NA	NA	422	6.6	<300	NA	NA	NA			NA	NA			
2-methylnaphthalene	30838	NA	9140	3.7	14100	18.4	17700	3.4	14167	1.1			11671	11.5	14133	7686	21480
2,6-dimethylnaphthalene	14335	NA	4690	4.2	5090	12.1	6523	3.2	5773	1.8			3002	18.7	5432	3985	8553
1,7-dimethylphenanthrene	<LOD	NA	608	13.2	646	2.3	508	16.2	NA	NA			<LOD	NA	608	71	546
9-methylphenanthrene	1864	NA	2313	10.7	2350	1.1	1820	4.7	1657	10.7			944	NA	1842	514	1990
3-methylphenanthrene	4451	NA	4897	3.7	5130	1.2	4273	3.5	3947	2.8			7442	27.0	4674	1259	4806
1-naphthol	NA	NA	1963	5.4	NA	NA	NA	NA	NA	NA			<LOD	NA			1651
2,3-dihydroxynaphthalene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			26227	NA			7252
2-methyl-1-naphthol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			338
2-hydroxyfluorene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			3444	15.3			5343
1-hydroxypyrene	NA	NA	other	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			850
DOSS (ion)	NA	NA	NA	NA	29300	1.2	NA	NA	49300	19.2	30300	NA	33041	14.0	31671	9345	30095

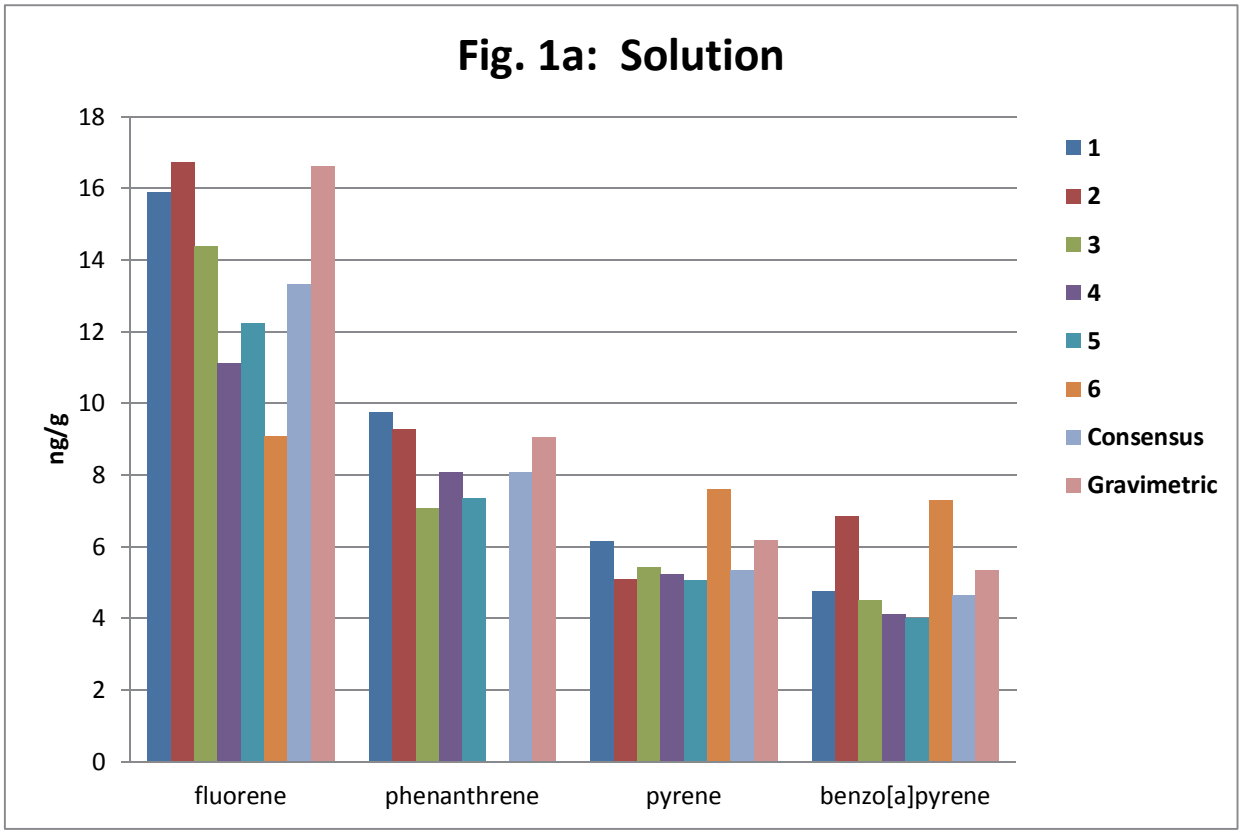
Table 4: Tabular results from the analysis of QA11WholeBlood. All values are pg/g. Gravimetric refers to concentration expected based on the mass of compound added to the blood.

	Laboratory																
	1		2		3		4		5a		5b		6				
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	median	SD	Gravimetric
fluorene	5178	5.6	4217	7.9	3030	4.0	1237	4.1	2333	1.6			12051	19.1	3623	3870	5007
phenanthrene	3228	5.1	7723	3.5	3040	1.4	2517	13.2	2013	5.2			<LOD	NA	3040	2296	2925
pyrene	2285	7.4	1747	3.2	2367	2.2	1840	5.2	1447	6.9			11859	11.4	2063	4065	1992
benzo[a]pyrene	NA	NA	1627	10.5	1123	12.7	678	10.3	555	17.1			<LOD	NA	901	486	1745
1-methylnaphthalene	<LOD	NA			356	4.2	<300	NA	NA	NA			NA	NA			
2-methylnaphthalene	22377	2.6	9730	3.9	8390	12.8	15767	11	8720	74.1			5853	54.9	9225	6138	21387
2,6-dimethylnaphthalene	10286	NA 3.9	NA 4620	8.4	4017	7.9	5860	14	5150	2.5			2167	21.3	4885	2724	8516
1,7-dimethylphenanthrene	<LOD	NA	858	6.6	632	1.4	402	6.5	NA	NA			NA	NA	632	228	543
9-methylphenanthrene	1697	2.1	2473	5.9	2330	0.9	1663	7.2	1623	10.5			450	NA	1680	716	1982
3-methylphenanthrene	4228	2.2	5613	1.0	5123	0.6	3990	11.8	3360	6.0			4985	8.7	4607	835	4785
1-naphthol	NA	NA	2100	5.7	NA	NA	NA	NA	NA	NA			10789	NA			1644
2,3-dihydroxynaphthalene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			7220
2-methyl-1-naphthol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			337
2-hydroxyfluorene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			5320
1-hydroxypyrene	NA	NA	other	NA	NA	NA	NA	NA	NA	NA			<LOD	NA			846
DOSS (ion)	NA	NA	NA	NA	27567	3.7	NA	NA	55367	3.7	27200	NA	28236	3.6	27901	13856	29965

Table 5: p scores based on a relative standard deviation of 15% for the blood materials.

	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5a		Lab 6	
	plasma	whole blood	plasma	whole blood	plasma	whole blood	plasma	whole blood	plasma	whole blood	plasma	whole blood
fluorene	NA	0.37	0.35	0.53	0.24	0.27	0.33	0.27	0.17	0.11	0.81	1.27
phenanthrene	NA	0.34	0.20	0.23	0.04	0.10	0.10	0.88	0.30	0.35	NA	NA
pyrene	NA	0.49	0.31	0.21	0.05	0.14	0.21	0.35	0.19	0.46	0.53	0.76
benzo[a]pyrene	NA	NA	0.25	0.70	0.11	0.85	0.11	0.69	0.06	1.14	NA	NA
1-methylnaphthalene	NA	NA	NA	NA	0.44	0.28	NA	NA	NA	NA	NA	NA
2-methylnaphthalene	NA	0.58	0.25	0.26	1.23	0.85	0.23	0.72	0.07	4.94	0.77	3.66
2,6-dimethylnaphthalene	NA	0.62	0.28	0.56	0.81	0.53	0.22	0.95	0.12	0.17	1.24	1.42
1,7-dimethylphenanthrene	NA	NA	0.88	0.44	0.16	0.10	1.08	0.43	NA	NA	NA	NA
9-methylphenanthrene	NA	0.14	0.72	0.39	0.08	0.06	0.31	0.48	0.72	0.70	NA	NA
3-methylphenanthrene	NA	0.14	0.25	0.07	0.08	0.04	0.23	0.79	0.19	0.40	1.80	0.58
1-naphthol	NA	NA	0.36	0.38	NA	NA	NA	NA	NA	NA	NA	NA
2,3-dihydroxynaphthalene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2-methyl-1-naphthol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2-hydroxyfluorene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.02	NA
1-hydroxypyrene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
DOSS (ion)	NA	NA	NA	NA	0.08	0.25	NA	NA	1.28	0.24	0.93	0.24

Figures 1a and 1b: QA11Solution#1-PAH. Consensus is the median of all values.



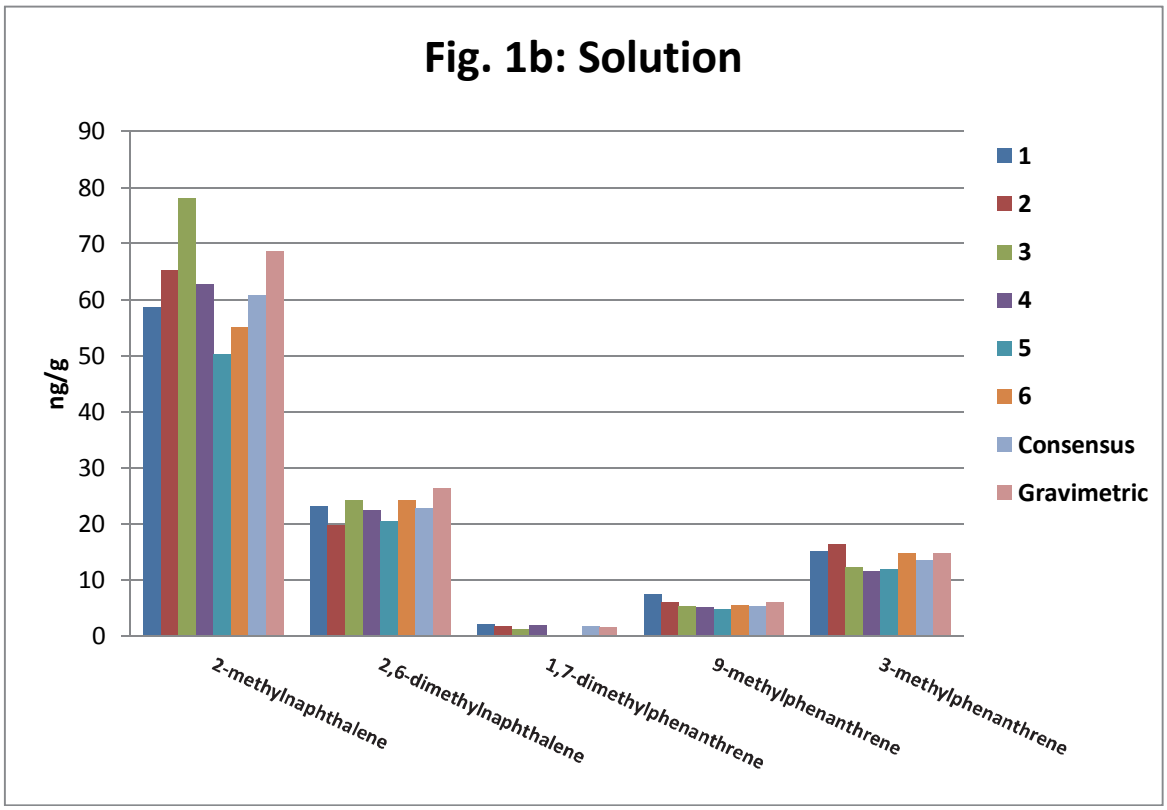
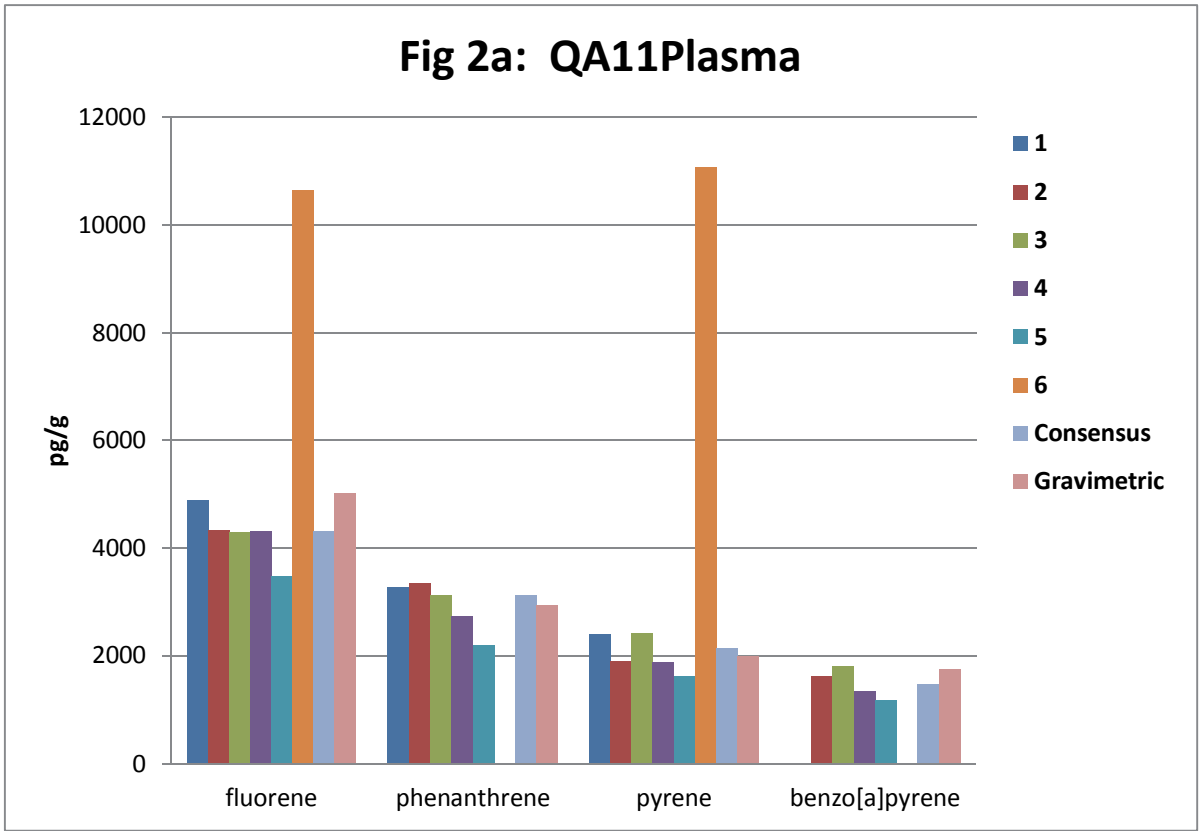


Fig. 1b: Solution
ng/g
90
80
70
60
50
40
30
20
10
0
2-methylnaphthalene
2,6-dimethylnaphthalene
1,7-dimethylphenanthrene
9-methylphenanthrene
3-methylphenanthrene
1
2
3
4
5
6
Consensus
Gravimetric

Figures 2a and 2b: Graphical results from QA11Plasma. Consensus is the median of all values.



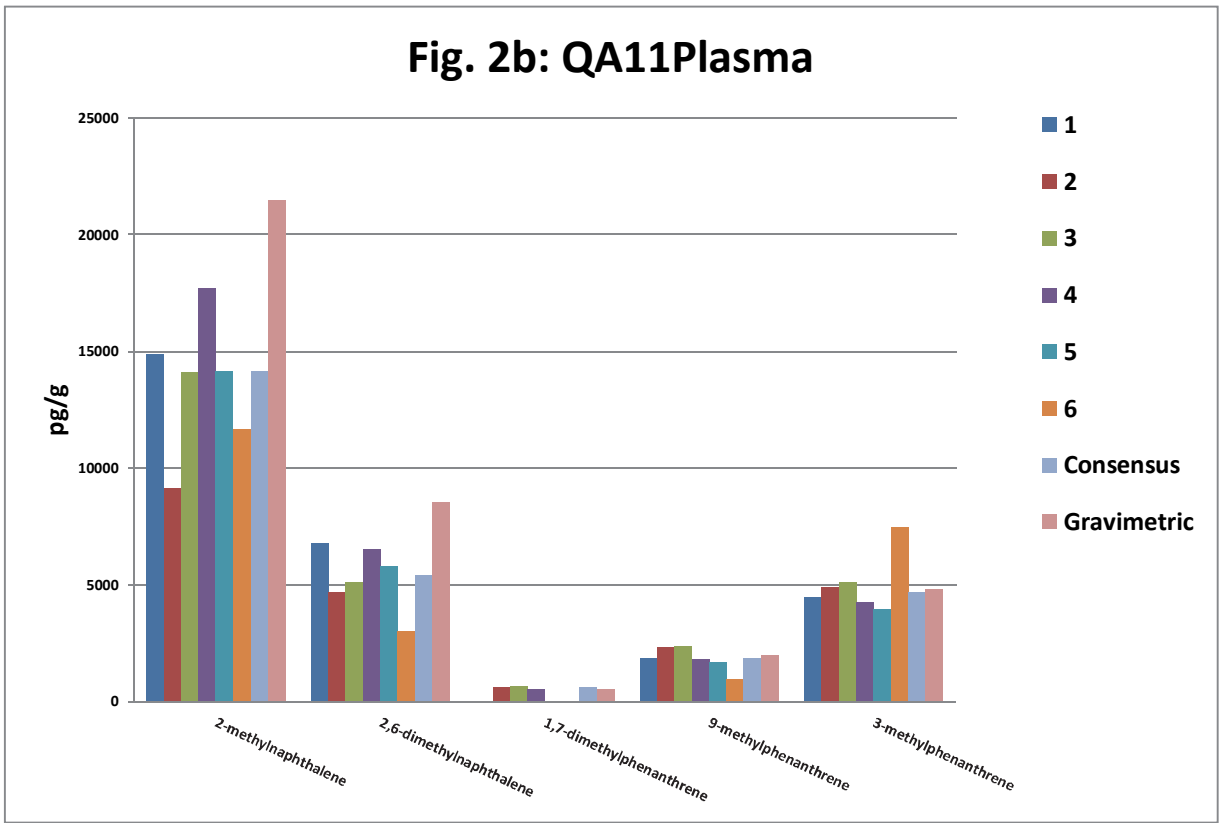
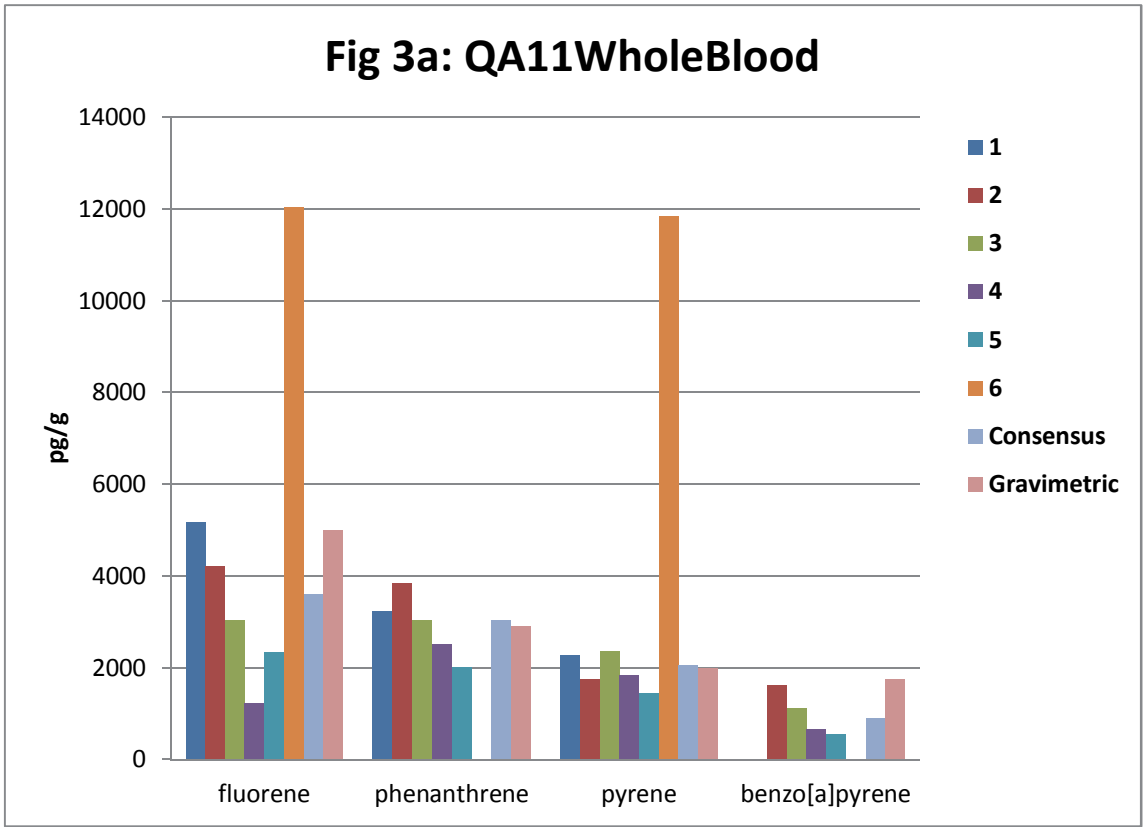


Fig. 2b: QA11Plasma
pg/g
25000
20000
15000
10000
5000
0
2-methylnaphthalene
2,6-dimethylnaphthalene
1,7-dimethylphenanthrene
9-methylphenanthrene
3-methylphenanthrene
1
2
3
4
5
6
Consensus
Gravimetric

Figures 3a and 3b: Graphical results from QA11WholeBlood. Consensus is the median of all values.



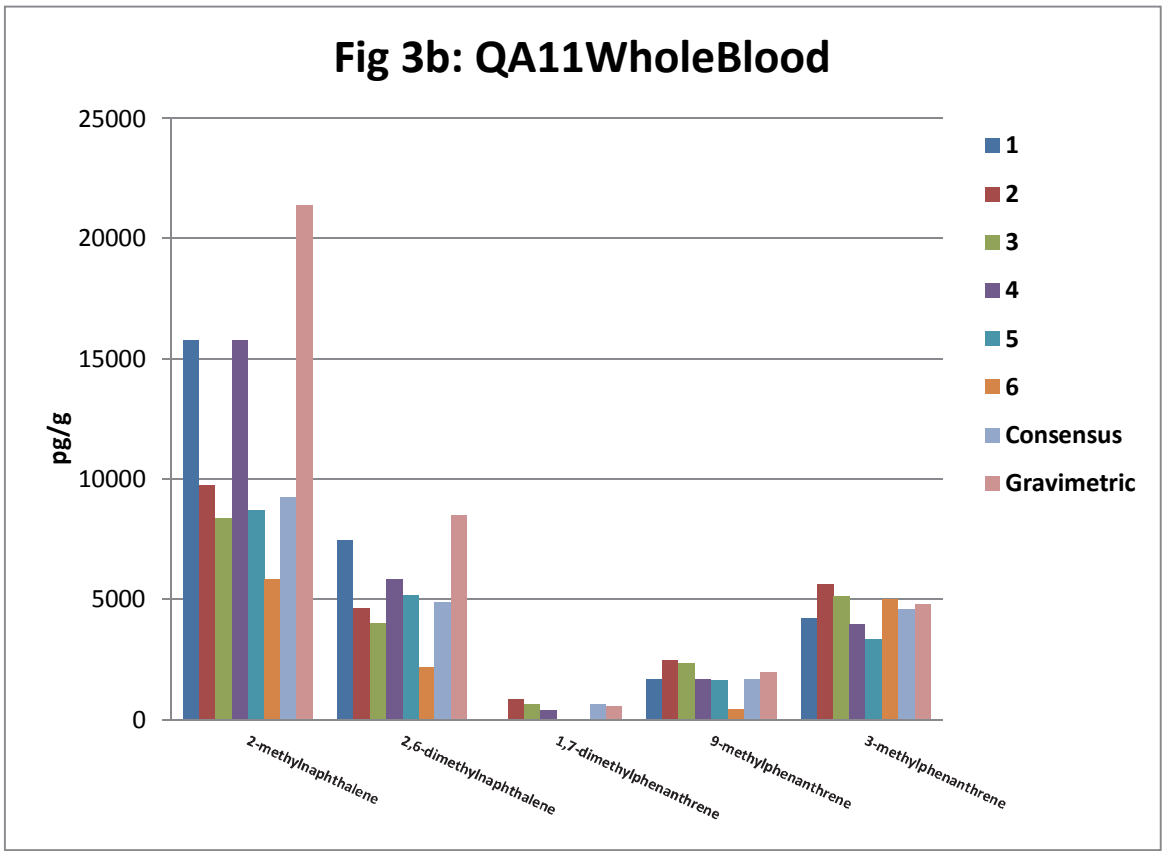


Fig 3b: QA11WholeBlood
25000
20000
15000
10000
5000
0
pg/g
1
2
3
4
5
6
Consensus
Gravimetric
2-methylnaphthalene
2,6-dimethylnaphthalene
1,7-dimethylphenanthrene
9-methylphenanthrene
3-methylphenanthrene

Appendix A: Instructions for the Exercise

Interlaboratory Analytical Comparison Study to Support Deepwater Horizon Natural Resource Damage Assessment

Intercomparison Exercise: Blood QA1101 Description of Materials and Instructions

Intercomparison Exercise Materials:

There are three materials to be used for the exercise. The plasma and whole blood (QA11Plasma and QA11WholeBlood) samples are 4.5 mL aliquots of plasma and whole blood in collected non-lethally from American alligators. Both materials have been amended with the parent PAHs, alkylated PAHs and PAH metabolites listed in Table 1 and dioctyl sodium sulfosuccinate (DOSS). Concentrations range from <1 ng/mL up to approximately 20 ng/mL. Four vials per material are supplied; three are to be analyzed for the exercise and an extra vial is supplied as a backup.

Two additional solutions are also supplied. The first solution contains parent, alkylated PAHs and PAH metabolites in isooctane (QA11Solution#1-PAH) and the second solution is DOSS in methanol (QA11Solution#2-DOSS).

Storage of Materials:
The blood materials should be stored at a minimum of -20 ^{o}C in the dark. Blood can be stored in a -80 ^{o}C freezer as well (preferred). The two solutions (QA11Solutions #1 and #2) should be stored between of -4 ^{o}C and -20 ^{o}C in the dark until use.

Instructions for Use:
You are to analyze all materials using **your** laboratory's and/or program's analytical protocols, for the concentrations (mass/mass) of the parent PAH compounds, alkylated PAH compounds, PAH metabolites and DOSS. A target list of compounds are presented in Table 1; however, participants do not need to quantify all of these compounds and can add additional compounds when reporting the data.

The amount of blood used for each analysis should correspond to the amount of blood that you would typically analyze as prescribed in your protocols. Prior to opening the blood cryovials you should vortex the vial to mix the contents. Samples for analysis should be withdrawn immediately after opening the cryovial and should be processed without delay. Unused material can be stored frozen in the cryovials.

The PAH and DOSS solutions (QA11Solutions #1 and #2) should be analyzed as is without cleanup. The mass of each vial (solution, cap and vial) was taken just prior to shipment. Weight each vial prior to use and use the supplied tare mass (vial plus cap) to adjust for solvent evaporation.

You should analyze three samples of QA11Plasma and QA11WholeBlood and make three measurements each of the QA11Solution#1-PAH and QA11Solution#2-DOSS. If time allows, we are asking that you analyze the blood materials in separate batches; i.e., separate extractions, cleanup, and calibration curves. This will allow a more realistic assessment of laboratory precision over a longer term than the assessment obtained when a laboratory places all three samples in the same batch and the resulting extracts are analyzed using the same calibration curve, etc. Likewise, if time allows, make three separate measurements of QA11Solution#1-PAH and QA11Solutions#2-DOSS using a separate calibration curve for each measurement.

Reporting of Results:
Please report one result, as if three figures were significant, for each of the analytes quantified in each of the three replicates of the QA11WholeBlood and QA11Plasma and the determination of compounds in QA11Solution#1-PAH and QA11Solution#2-DOSS. Report results in units of pg/g. Report the date of measurement of each sample in the requested m/d/y format.

If you know that a target or non-target compound is interfering (coeluting) with the determination of a target analyte, please identify this issue by qualifying the data and note the data qualifier used at the bottom of your table of results. Please note that any changes you make to the column or row headings **within** the tables will **not** be seen by the coordinators because only the table entries and comments at the bottom of the tables are automatically transferred to the exercise database.

We prefer that concentration values be reported for each analyte determined. If the measured concentration is below your typical reporting concentration for an analyte in a particular matrix, you can report the number and list the appropriate detection limit, quantification limit, etc. at the bottom of the data table. However, if you need to report non-numerical data please use the following conventions:

NA	"Not analyzed", "not determined"
<"value"	"Less than specified concentration", e.g., <8 pg/g
Other	"Other"; add note of explanation at end of data table, e.g., interference
DL	"Below detection limit" may be used, however, <"value" is preferable

Do not use negative numbers or parentheses to indicate "less than detection limits".

The attached file is an EXCEL file, QA11Blood01.xls. If you have any software/hardware conversion problems, please contact John Kucklick. The data file templates also include places for you to list the surrogate/internal standards and type of calibration curve used, and to provide a brief description of the analyses. Please **do not** add spaces before entering numbers in the table cells and enter them as "numbers" not as "labels". Please **do not** insert any columns or rows **within** the table in the data file. If you wish to include additional data and/or other information or comments, you may add it to the bottom of the data table in the attached file.

Submit your results by **November 28, 2011** as an attached file via e-mail to: **John.kucklick@nist.gov**

Further Information:

If you need further information, please contact John at the following address or phone numbers:

John Kucklick
NIST/Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, SC 29412
Phone:843-725-4816

Table 1: List of Analytes of Interest in the Interlaboratory Analytical Comparison Study to Support Deepwater Horizon Natural Resource Damage Assessment

Parent PAHs:
Benzo[a]pyrene
Fluorene
Pyrene
Phenanthrene

Alkylated PAHs:
2,6-dimethylnaphthalene
2-methylnaphthalene
1,7-dimethylphenanthrene
9-methylphenanthrene
3-methyl phenanthrene

PAH Metabolites:
2,3-dihydroxynaphthalene
1-naphthol
2-hyrdoxyfluorene
2-methyl-1-naphthol
1-hydroxypyrene

Oil Dispersant Indicator Compound:
dioctyl sodium sulfosuccinate (DOSS)

Appendix B: Summary of Methods Used

Table B1: Methods used for solutions.

PAHs

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
1	GC/MS	Rxi-17sil	60 m x 0.25 mm x 0.25 µm	PTV	6	1600 ng/mL to 0.32 ng/mL
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 160 ng/g
3	Agilent 7890/Agilent 5975 MSD	DB-5	60 m x 0.25 mm x 0.25 µm	on-column	7	0.001 - 1.0 ng/µL
4	GC/MS	Restek Rtx-5	30 m x 0.25 mm x 0.25 µm	split/splitless	5	50-5000 ng/mL
5	GC/MS	5% Diphenyl, 95% Dimethy polysiloxane	30 m x 0.25 mm x 0.25 µm	split/splitless	10	2-2000 ng/mL
6	Waters Acquity UPLC/FLR/MS/MS	Waters CSH C18	0.1 m x 2.1 mm x 1.7 µm	NA	6	0.5-500 ng/mL

Alkylated PAHs

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
1	GC/MS	Rxi-17sil	60 m x 0.25 mm x 0.25 µm	PTV	6	300 ng/mL to 0.3 ng/mL
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 25 ng/g
3	Agilent 7890/Agilent 5975 MSD	DB-5	60 m x 0.25 mm x 0.25 µm	on-column	7	0.001 - 1.0 ng/µL
4	GC/MS	Restek Rtx-5	30 m x 0.25 mm x 0.25 µm	split/splitless	5	50-5000 ng/mL
5	GC/MS	5% Diphenyl, 95% Dimethy polysiloxane	30 m x 0.25 mm x 0.25 µm	split/splitless	10	2-2000 ng/mL
6	Waters Quattro Micro GC/MS/MS	Restek 5% diphenyl/ 95% dimethyl polysiloxane	31 m x 0.25 mm x 0.25 µm	splitless	5	0.5-200 ng/ml

Hydroxylated PAHs

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
1	GC/MS	Rxi-17sil	60 m x 0.25 mm x 0.25 µm	PTV	6	188 ng/mL to 0.2 ng/mL
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 25 ng/g
6	Waters Acquity UPLC/FLR/MS/MS	Waters CSH C18	0.1 m x 2.1 mm x 1.7 µm	NA	6	0.5-500 ng/mL

DOSS

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
3	Waters AcquityUPLC/AB Sciex QTrap 5500	C 18	0.05 m x 2.1 mm x 1.8 µm (particle size)	on-column	6	0.5 - 312.5 ng/mL
5	HPLC/MS/MS	C8	150 mm x 3 mm x 3 µm (particle size)	NA	7	1-1000 ng/mL
6	Waters Acquity UPLC/MS/MS	Waters BEH Shielded RP18	0.05 m x 2.1 mm x 1.7 µm (particle size)	NA	6	0.5-200 ng/mL

Table B2: Internal standards used for each compound for the analysis of solutions

	Lab					
Compound	**1**	**2**	**3**	**4**	**5**	**6**
fluorene	naphthalene-d8	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10	Acenaphthene-d10	chrysene-d12/naphthalene-d8
phenanthrene	naphthalene-d8	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10	phenanthrene-d10	chrysene-d12/naphthalene-d8
pyrene	pyrene-d10	fluoranthene-d10	benzo[a]pyrene-d12	fluoranthene-d10	chrysene-d12	chrysene-d12/naphthalene-d8
benzo[a]pyrene	benzo[a]pyrene-d12	benzo[a]pyrene-d12	benzo[a]pyrene-d12	benzo[a]pyrene-d12	perylene-d12	chrysene-d12/naphthalene-d8
1-methylnaphthalene	naphthalene-d8		naphthalene-d8	2-methylnaphthalene-d10	NA	chrysene-d12/naphthalene-d8
2-methylnaphthalene	naphthalene-d8	naphthalene-d8	naphthalene-d8	2-methylnaphthalene-d10	naphthalene-d8	chrysene-d12/naphthalene-d8
2,6-dimethylnaphthalene	naphthalene-d8	naphthalene-d8	acenaphthene-d10	2,6-dimethylnaphthalene-d12	naphthalene-d8	chrysene-d12/naphthalene-d8
1,7-dimethylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10	fluoranthene-d10	NA	chrysene-d12/naphthalene-d8
9-methylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10*	phenanthrene-d10	phenanthrene-d10	chrysene-d12/naphthalene-d8
3-methylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10	phenanthrene-d10	chrysene-d12/naphthalene-d8
1-naphthol	^{13}C pentachlorophenol	naphthol-d7				chrysene-d12/naphthalene-d8
2,3-dihydroxynaphthalene	^{13}C pentachlorophenol	naphthol-d7				chrysene-d12/naphthalene-d8
2-methyl-1-naphthol	^{13}C pentachlorophenol	naphthol-d7				chrysene-d12/naphthalene-d8
2-hydroxyfluorene	^{13}C 6-hydroxy chrysene	naphthol-d7				chrysene-d12/naphthalene-d8
1-hydroxypyrene	^{13}C 6-hydroxy chrysene	naphthol-d7				chrysene-d12/naphthalene-d8
DOSS			DOSS-d34		DOSS-d34	SDS-d25/SDS-d1

*the response factor for 1-methylphenanthrene was used to quantify 9-methyl phenanthrene

Table B3: Methods used for the analysis of QA11Plasma and QA11WholeBlood

Lab #	Reported	g extracted QA11Plasama	g extracted QA11WhloeBlood	Extraction Method	Extraction Solvent	Extraction Time	Extraction Other
1	12/1/2011	4	4	Focused Microwave Extraction	5 mL sample + 5 mL formic acid + 6 mL 20% dichloromethane:hexane (V:V), repeat once	250 W, 10 min ramp, 3 min hold, 90 ^{o}C, max pressure was 1.72 MPa with stirring and PowerMax on	
2	12/5/2011	4	4	Liquid:liquid	Hexane:MTBE (1:1) 1st time followed by hexane:toluene (1:1) twice	overnight in refrigerator for 1st solvent; then 10 to 15 min for other 2 solvent mixes	formic acid added just prior to first solvent addition
3	11/28/2011	2	2	PAHs - ASE; DOSS - QuEChERs	PAHs - methylene chloride; DOSS - acetonitrile	PAHs - 24 min @ 2000 psi and 100 deg C; DOSS - n/a	
4	12/12/2011	4	4	liquid-liquid extraction	Hexane	30 minutes.	
5	11/28/2011	1 for PAH 0.1 for DOSS	1 for PAH 0.1 for DOSS	Solvent partition	Acetonitrile for DOSS, Hexane for PAH	1 min	
6	12/12/2011	0.5 and 1.0	0.5 and 1.0	QuEChERS/LLE	Acetonitrile/Hexane	40 min	

Table B4: Methods used for the analysis of QA11Plasma and QA11WholeBlood

		Method used for quantification			
Lab#	Sample extract cleanup method	PAH	Alkyl PAH	Hydroxylated PAH	DOSS
1	Size exclusion chromatography (PlGel 300 mm x 7.5 mm) followed by alumina SPE	IS	IS	NA	NA
2	none	IS	IS	IS	
3	PAHs - silica/alumina columns and HPLC-SEC; DOSS - none	IS	IS		IS
4	Chromatographic clean-up using Biobead, 5% Silica and 2% Alumina columns	IS	IS		
5	None	IS	IS		Isotope Dilution
6	N/A	IS	IS	IS	IS

Table B5: Methods used for the analysis of QA11Plasma and QA11WholeBlood

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
1	GC/MS	Rxi-17sil	60 m x 0.25 mm x 0.25 µm	PTV	6	1600 ng/mL to 0.32 ng/mL
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 160 ng/g
3	Agilent 7890/Agilent 5975 MSD	DB-5	60 m x 0.25 mm x 0.25 µm	on-colµmn	7	0.001 - 1.0 ng/µL
4	GC/MS	Restek Rtx-5	30 m x 0.25 mm x 0.25 µm	split/splitless	5	50-5000 ng/mL
5	GC/MS	5% Diphenyl, 95% Dimethy polysiloxane	30 m x 0.25 mm x 0.25 µm	split/splitless	10	2-2000 ng/mL
6	Waters Acquity UPLC/FLR/MS/MS	Waters CSH C18	0.1 m x 2.1 mm x 1.7 µm	NA	6	0.5-500 ng/mL

Alkylated PAHs

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
1	GC/MS	Rxi-17sil	60 m x 0.25 mm x 0.25 µm	PTV	6	300 ng/mL to 0.3 ng/mL
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 25 ng/g
3	Agilent 7890/Agilent 5975 MSD	DB-5	60 m x 0.25 mm x 0.25 µm	on-column	7	0.001 - 1.0 ng/µL
4	GC/MS	Restek Rtx-5	30 m x 0.25 mm x 0.25 µm	split/splitless	5	50-5000 ng/mL
5	GC/MS	5% Diphenyl, 95% Dimethy polysiloxane	30 m x 0.25 mm x 0.25 µm	split/splitless	10	2-2000 ng/mL
6	Waters Quattro Micro GC/MS/MS	Restek 5% diphenyl/ 95% dimethyl polysiloxane	31 m x 0.25 mm x 0.25 µm	splitless	5	0.5-200 ng/ml

Hydroxylated PAHs

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
2	GC/MS	HP5-MS	30 m x 0.25 mm x 0.25 µm	splitless	4	0.5 ng/g to 25 ng/g
6	Waters Acquity UPLC/FLR/MS/MS	Waters CSH C18	0.1 m x 2.1 mm x 1.7 µm	NA	6	0.5-500 ng/mL

DOSS

Lab #	Instrument	Phase	Dimensions	Mode of injection	Calibration curve # points	Range
3	Waters AcquityUPLC/AB Sciex QTrap 5500	C 18	0.05 m x 2.1 mm x 1.8 µm (particle size)	on column	6	0.5 - 312.5 ng/mL
5	HPLC/MS/MS		150 mm x 3 mm x 3 µm (particle size)	NA	7	1-1000 ng/mL
6	Waters Acquity UPLC/MS/MS	Waters BEH Shielded RP18	0.05 m x 2.1 mm x 1.7 µm (particle size)	NA	6	0.5-200 ng/mL

Table B6: Methods used for the analysis of QA11Plasma and QA11WholeBlood

		PAHs		
Lab #	IS/surrogate added prior to extraction	Used?	Added prior to analysis	Used?
1	napthalane-d8; pyrene-d10; benzo[a]pyrene-d12; phenanthrene-d10	Yes		
2	naphthalene-d8; phenanthrene-d10; fluoranthene-d10; B[a]P-d12	Yes		
3	naphthalene-d8, acenaphthene-d10, Benzo[a]pyrene-d12	Yes	phenanthrene-d10; prior to cleanup	*
4	phenanthrene-d10; fluoranthene-d10, benzo(a)pyrene-d12, 2-methylnaphthalene-d10 and 2,6-Dimethylnaphthalene-d12	Yes	acenaphthene-d10, pyrene-d10 and benzo(e)pyrene-d12	
5	fluorene-d10, fluoranthene-d10, terphenyl-d14	yes	naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, perylene-d12	yes
6	naphthalene-d8	Yes	chrysene-d12	Yes
		Alkylated PAHs		
Lab #	IS/surrogate added prior to extraction	Used?	Added prior to analysis	Used?
1	napthalane-d8; pyrene-d10; benzo[a]pyrene-d12; phenanthrene-d10	Yes		
2	naphthalene-d8; phenanthrene-d10; fluoranthene-d10; B[a]P-d12	Yes		
3	naphthalene-d8, acenaphthene-d10, benzo[a]pyrene-d12	Yes	phenanthrene-d10; prior to cleanup	*
4	phenanthrene-d10; fluoranthene-d10, benzo[a]pyrene-d12, 2-methylnaphthalene-d10 and 2,6-dimethylnaphthalene-d12	Yes	d10-acenaphthene, d10-pyrene and d12-benzo(e)pyrene	
5	fluorene-d10, fluoranthene-d10, terphenyl-d14	yes	naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, perylene-d12	yes
6	naphthalene-d8	Yes	chrysene-d12	yes
		Hydroxylated PAHs		
Lab #	IS/surrogate added prior to extraction	Used?	Added prior to analysis	Used?
2	naphthol-d7	Yes		
6	naphthalene-d8		chrysene-d12	
		DOSS		
Lab #	IS/surrogate added prior to extraction	Used?	Added prior to analysis	Used?
3	DOSS-d34	Yes	Sodium dodecyl sulfate-d25	*
5	DOSS-d34	Yes	DOSS-C13	Yes
6	Sodium dodecyl sulfate-d1 (SDS-d1)	Yes	Sodium dodecyl sulfate-d25 (SDS-d25)	Yes
	*used to calculate surrogate recovery			

Table B7: Internal standards used for each compound for the analysis of QA11Plasma and QA11WholeBlood

	Lab					
	1	**2**	**3**	**4**	**5**	**6**
			PAHs			
fluorene	naphthalene-d8	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10*	Acenaphthene-d10	chrysene-d12/naphthalene-d8
phenanthrene	naphthalene-d8	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10*	phenanthrene-d10	chrysene-d12/naphthalene-d8
pyrene	pyrene-d10	fluoranthene-d10	benzo[a]pyrene-d12	fluoranthene-d10*	chrysene-d12	chrysene-d12/naphthalene-d8
benzo[a]pyrene	benzo[a]pyrene-d12	benzo[a]pyrene-d12	benzo[a]pyrene-d12	benzo[a]pyrene-d12*	perylene-d12	chrysene-d12/naphthalene-d8
			Alkylated PAHs			
1-methylnaphthalene	naphthalene-d8		naphthalene-d8	2-methylnaphthalene-d10**	NA	chrysene-d12/naphthalene-d8
2-methylnaphthalene	naphthalene-d8	naphthalene-d8	naphthalene-d8	2-methylnaphthalene-d10**	naphthalene-d8	chrysene-d12/naphthalene-d8
2,6-dimethylnaphthalene	naphthalene-d8	naphthalene-d8	acenaphthene-d10	2,6-dimethylnaphthalene-d12**	naphthalene-d8	chrysene-d12/naphthalene-d8
1,7-dimethylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10	fluoranthene-d10*	NA	chrysene-d12/naphthalene-d8
9-methylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10*	phenanthrene-d10	chrysene-d12/naphthalene-d8
3-methylphenanthrene	phenanthrene-d10	phenanthrene-d10	acenaphthene-d10	phenanthrene-d10*	phenanthrene-d10	chrysene-d12/naphthalene-d8
			PAH Metabolites			
1-naphthol		naphthol-d7				chrysene-d12
2,3-dihydroxynaphthalene		naphthol-d7				chrysene-d13
2-methyl-1-naphthol		naphthol-d7				chrysene-d14
2-hydroxyfluorene		naphthol-d7				chrysene-d15
1-hydroxypyrene		naphthol-d7				chrysene-d16
			DOSS			
DOSS			DOSS-d34		DOSS-d34	SDS-d25/SDS-d1
notes:			acceptable range 60 % to 130 % *the response factor for 1-methylphenanthrene was used to quantify 9-methyl phenanthrene	*acceptable range 30 % to 130 % **acceptable range 20 % to 130 %	acceptable range 70 % to 130 %	acceptable range 50 % to 130 %

Appendix C: Participating Laboratories

Participating Laboratories

Axys Analytical Services Ltd., Sidney, BC Canada

Columbia Analytical Services Inc., Kelso, WA USA

National Institute of Standards and Technology, Charleston, SC USA

National Institute of Standards and Technology, Gaithersburg, MD USA

National Oceanic and Atmospheric Administration, Northwest Fisheries Science Center, Seattle, WA USA

University of Connecticut, Center for Environmental Sciences and Engineering, Storrs, CT USA