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INTERLABORATORY QUALITY CONTROL STUDY FOR THE ANALYSIS OF CHLOROPHENOLS IN FISH AND RELATED MATERIALS

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MANAGEMENT PERSPECTIVE

The constant use of large quantities of pentachlorophenol (PCP) and 2,3,4,6-tetrachlorophenol as wood preservatives by the lumber industries in the lower mainland area of British Columbia has created a threat to the water quality in the Fraser River Estuary. Other studies have already indicated that these as well as other chlorophenols are entering the Fraser River and are being accumulated in the biota. In order to maintain water quality and preserve fishery in that area, routine monitoring of environmental samples for chlorophenols has been required. This interlaboratory QA study establishes the degree of comparability of phenol results among the laboratories involved in analyzing samples from Fraser River Estuary.

PERSPECTIVE-GESTION

L'utilisation régulière de grandes quantités de pentachlorophénol (PCP) et de 2,3,4,6-tétrachlorophénol à titre d'agents de conservation dans l'industrie du bois du sud de la Colombie-Britannique met en danger la qualité de l'eau de l'estuaire du Fraser. D'autres études ont déjà montré que ces substances ainsi que d'autres chlorophénols sont déversés dans le Fraser et s'accumulent dans le biote. Afin de préserver la qualité de l'eau et des poissons dans cette région, il est nécessaire d'exercer une surveillance régulière en analysant la teneur en chlorophénols d'échantillons environnementaux. La présente étude inter-laboratoire d'assurance de la qualité permettra d'établir le degré de comparabilité des résultats entre les divers laboratoires qui fournissent actuellement des données obtenues de analyse d'échantillons provenant de l'estuaire du Fraser.

ABSTRACT

An interlaboratory study for the analysis of chlorophenols in fish and related samples was set up for the laboratories involved in analyzing samples from the Fraser River Estuary. Participants were requested to analyze five chlorophenols including PCP and 2,3,4,6-tetrachlorophenol (=TeCP) in six fortified or natural samples. To simulate real life situations, a homogenate of naturally contaminated starry flounders caught in the Fraser River was used to evaluate the analytical performance of participants. Comparable and reproducible results were obtained for PCP and 2,3,4,6-TeCP in the fish samples. After rejection of outliers, the range of interlab results did not exceed a factor of two for the above two phenols. The interlaboratory medians for PCP and 2,3,4,6-TeCP were within ±20% of the design values determined by the quality control lab. Presumably due to lower levels present in the fish samples, interlab results for 2,4,6- and 2,3,6-trichlorophenols were more divergent. Since the standard solution samples had similar or slightly worse accuracy than the fish samples, erratic in-house standard solutions rather than extraction, cleanup, and derivatization procedures were more likely to be the major source of error in this study.

RESUME

Une étude inter-laboratoire visant à déterminer la présence de chlorophénols dans les poissons et autres substances a été mise sur pied pour les laboratoires qui analysent les échantillons provenant de l'estuaire du Fraser. Les participants ont dû analyser cinq chlorophénols, y compris le PCP et le 2,3,4,6-tétrachlorophénol (-TeCP) dans six échantillons enrichis ou naturels. Afin de simuler les conditions réelles, on a utilisé un échantillon homogène de plies étoilées contaminées naturellement et provenant du Fraser pour évaluer la performance des participants. Des résultats comparables et reproductibles ont été obtenus pour le PCP et le 2,3,4,6-TeCP dans les échantillons de poissons. Après l'élimination des valeurs extrêmes, l'écart des résultats des divers laboratoires n'était pas superiéur à un facteur de 2 pour les deux phénols dont il vient d'être question. Les médicanes inter-laboratoires pour le PCP et le 2,3,4,6-TeCP se situaient dans une marge de $\pm$ 20% des valeurs prévues déterminées par le laboratoire de contrôle de la qualité. Probablement à cause des teneurs plus faibles pour ces phénols dans les échantillons de poissons, les résultats inter-laboratoires pour le 2,3,4,6- et le 2,3,6-trichlorophénol présentaient une plus grande variabilité. Étant donné que les échantillons de solution standard affichaient une précision semblable ou légèrement inférieure à celle des échantillons de poissons, la qualité de la solution standard du laboratoire, plutôt que les méthodes d'extraction, de nettoyage et de dérivation, est probablement la principale source d'erreur dans cette étude.

INTRODUCTION

Chlorophenols are a class of industrial chemicals which are present in many water, sediment, and biota samples at analytically significant levels. Large quantities of pentachlorophenol (PCP) and 2,3,4,6-tetrachlorophenol (TeCP) are used annually in the lower mainland area of British Columbia as a result of heavy pulp and paper as well as wood preserving activities. Several reports regarding the occurrence and distribution of chlorophenols in the Fraser River Estuary system have been published (1-4). The results in these studies indicate that the chlorophenols used in the lumber industries are entering the Fraser River and are being accumulated in the biota. In order to maintain the water quality and preserve the fisheries and wildlife, a Fraser River Estuary Management Program (FREMP) was launched in 1985. Routine monitoring of environmental samples for chlorophenols has been carried out by various parties in recent years.

In response to a request from Water Quality Branch, Pacific and Yukon Region, regarding the quality of chlorophenol data in fish samples, an interlaboratory QA study was set up for a group of laboratories which are contributing such data to the above program. The primary objective of this study is to establish the comparability of chlorophenol results among the laboratories involved.

STUDY DESIGN

Nine government and contract laboratories in the Vancouver and Burlington area that are currently involved in analyzing samples from Fraser River Estuary were invited and agreed to participate in this study. A list of participants is given in the Appendix.

The participants were requested to analyze the following five chlorophenols in six test samples (Table 1): 3,4-dichlorophenol, 2,3,6- and 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and PCP. These phenols were chosen for this study because they were found in many recent Fraser River biota samples (1). Samples 1 and 2 in sealed glass ampuls were standard solutions of all five chlorophenols in methanol (Table 2). Sample 2 was a 1 to 4 dilution of sample 1 in methanol. Samples 3 and 4 were fish oil fortified with the same five chlorophenols. The original oil was found to be free of the five chlorophenols, therefore fortified oil samples were prepared by spiking a known amount of a chlorophenol mixture in acetone into a known weight of oil. The oil was then mixed and the acetone was evaporated using a three-stage Snyder column and a warm water bath. The oil was mixed again before it was subsampled and sealed in glass ampuls. Note that sample 3 has chlorophenol concentrations four times those of sample 4. Samples 5 and 6 were duplicates of fish homogenate. They were prepared from about 40 finger-lengths, naturally contaminated starry flounders with their heads, tails, and fins removed. The fish were caught in June 1986 in the Fraser River.

Homogenization of fish tissue was done in a 3.8 L stainless steel Waring blender until it was of smooth, homogeneous consistency. The blended tissue was immediately subsampled into clean jars in 5 g portions with the actual weight recorded on the label of each jar. Tissue samples were stored frozen at -20°C. Except for the Burlington laboratories, all test samples were delivered to the participants in insulated containers packed with freezer packs by air courier on 7 July.

The participants were requested to analyze all six samples for the above five phenols using their in-house standards and procedures. To avoid inhomogeneity of the fish samples caused by separation of lipid from the tissue after subsampling, the participants were asked to use the entire jar contents for analysis.

ANALYSIS OF FISH SAMPLES

The following procedure was used in our laboratory to generate reference values of chlorophenols in the fish samples.

After the weight was recorded, the fish tissue was quantitatively transferred to a mortar and ground wih equal weight of precleaned anhydrous sodium sulfate. The mixture was then soxhlet extracted for eight hours with 350 mL of a 60+40 mixture of acetone and hexane. The organic extract was evaporated down using a three-stage Snyder column and the solvent replaced by a 1+1 dichloromethane (DCM)/cyclohexane mixture. Lipid and oil in the concentrated sample

extract was removed by a Bio-Beads S-X3 column using the above DCM/cyclohexane mixture as eluant and a flow rate of 5.0 mL/min. The first 145 mL were discarded and the next 165 mL containing the phenols were collected. The phenols were then back-extracted by three successive partitionings using a total of 100 mL of 2% $KHCO_3$. The acetate derivatives of chlorophenols were formed by previously published procedures (5, 6). Briefly, phenols in $KHCO_3$ solution were stirred with 1 mL of triple-distilled acetic anhydride. The acetates were removed from the aqueous layer by petroleum ether which was then evaporated down to a small volume. The acetates were cleaned up on a miniature 5% deactivated silica gel column before GC-ECD analysis was conducted. The chlorophenol results for samples 5 and 6 (shown in Table 2) were the average five analyses obtained by our laboratory.

RESULTS AND DISCUSSIONS

The participants were requested to submit their results along with a brief description of their analytical methodology by 15 September 1986. Only six out of the nine laboratories provided results as of 31 October. A preliminary data summary was prepared and distributed to the data contributors on 15 October.

The analytical procedures used by the participants in this study are presented in Table 3. Various extraction methods such as column extraction, soxhlet apparatus, shaking, or polytron were used by different participants in the extraction of fish tissue. In some

cases, fish samples were acidified before they were extracted with solvents such as dichloromethane (DCM), acetone, petroleum ether (PE), o mixtures of DCM and diethyl ether as well as benzene and hexane. Lipids in the extracts were usually removed by gel permeation chromatography (GPC). In general, the chlorophenol fraction was evaporated to a small volume and reacted with diazomethane to yield the chloroanisoles. Alternatively, phenols were back-extracted into a $KHCO_3$ solution and reacted with acetic anhydride to form the acetate derivatives. Final analysis was performed by GC-ECD with either packed or capillary columns. One laboratory used GC-MSD for quantitation. See Table 3 for more details.

All sample results reported by the participants were listed in Tables 4-1 to 4-5. Although all laboratories had the capability of analyzing all five chlorophenols and were requested to do so, some of them decided not to report results for the lower chlorophenols. Possible reasons were: (1) those compounds were not analyzed routinely, and/or (2) standards were not available.

Apart from the data supplied by laboratory B, results for sample 1 were satisfactory for tri-, tetra-, and penta-chlorophenols. Since only two sets of results were received for 3,4-dichlorophenol, those results were not evaluated. Laboratory B seemed to have a systematic error related to the accuracy of their in-house standards, as their chlorophenol results were mostly extremely high for both samples 1 and 2. Because of these outliers, the means and medians (Tables 4-1 through 4-5) of sample results were quite different.

Except for 2,4,6-trichlorphenol, the medians in sample 1 were about 25% lower than their corresponding design values. This is likely due to the small number of data sets available for this study and to the fact that additional sets of results can significantly change the median values. Participants were less accurate in analyzing sample 2 than sample 1, as many results were much lower than the design values. Laboratories B, C, and F did not come close to the 4:1 ratio when comparing results for samples 1 and 2 as anticipated since sample 2 was a 1:4 dilution of sample 1.

The interlaboratory results for both samples 3 and 4 (fortified fish oil) were satisfactory. The interlab medians for these oil samples were actually closer to the design values than those obtained for the standard solutions (samples 1 and 2). Also, the 4:1 ratio between samples 3 and 4 was established for all chlorophenol results. It is unexpected that more accurate results were obtained for the oil samples which required additional cleanup steps than the standard solution samples. Among the participants, laboratory B again had consistently higher chlorophenol results for both oil samples.

With a few exceptions, the in-house precision of duplicate analysis of the fish tissues was excellent as the individual chlorophenol results provided by the same laboratory for samples 5 and 6 were nearly identical. Only significant amounts of PCP and 2,3,4,6-tetrachlorophenol were present in the fish. For these two phenols, the overall comparability of results was very good since the interlaboratory relative standard deviations for these compounds were

less than 30% and the range of sample results never exceeded a factor of two. The interlaboratory medians for PCP and 2,3,4,6-TeCP in samples 5 and 6 further confirm our own design values (Table 2) since they were within ±20% of each other for each phenol. It should be noted that, although every effort has been made to preserve the samples, the stability of chlorophenols in fish tissues was never established for these samples. Fortunately, the study results suggested that chlorophenol stability after subsampling and during transportation was not a problem. Interlaboratory results for the two trichlorophenols, which were present at less than 2 ng/g in the tissues, were not as comparable. The reported results had a range larger than a factor of 10. The reported detection limits for chlorophenols in fish varied from 5 to less than 0.5 ng/g, depending on the participant and parameter.

In conclusion, the results in this study indicated that all participants have the capability of performing sensitive and isomer specific analysis of chlorophenols in fish samples. They generated comparable and reproducible results for PCP and 2,3,4,6-tetrachlorophenol in naturally contaminated starry flounders caught in the Fraser River. Presumably, because of the lower levels present in the tissue samples, results for the two trichlorophenols were much more divergent. At least one laboratory can benefit from more accurate standard solutions and/or more stringent in-house quality assurance.

ACKNOWLEDGEMENTS

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LIST OF PARTICIPANTS

B.C. Ministry of Environment and Parks, Vancouver

Environment Canada, National Water Research Institute, Environmental Contaminants Division, Burlington

Environment Canada, Water Quality Branch, Pacific and Yukon Region

Environment Canada, Water Quality National Laboratory, Burlington

EPS/DFO Laboratory Services, Vancouver

Department of Fisheries and Oceans, Fisheries Research Branch, West Vancouver Laboratory

The following laboratories received samples but did not submit results:

ASL Analytical Service Laboratories Ltd., Vancouver

Can Test Ltd., Vancouver

University of British Columbia, Environmental Engineering Laboratory, Vancouver

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TABLE 1 Samples distributed in this study.

Sample No.	Description
1	Standard solution of five chlorophenols in methanol
2	A 1:4 dilution of sample 1 in methanol
3	Fish oil fortified with five chlorophenols
4	Same as 3 except that chlorophenol levels are 25% of sample 3
5	Homogenate of naturally contaminated starry flounders caught in the Fraser River, B.C.
6	Duplicate of sample 5

TABLE 2 Reference chlorophenol values in the test samples.

Sample		Chlorophenol				
		3,4-	2,4,6-	2,3,6-	2,3,4,6-	PCP
1	ng/μL	9.70	5.20	5.45	2.00	2.12
2	ng/μL	2.43	1.30	1.36	0.50	0.53
3	μg/ g	9.70	5.20	5.45	2.00	2.12
4	μg/ g	2.43	1.30	1.36	0.50	0.53
5	ng/ g	<5	<2	<2	27.5±1.2*	52.7±2.5*
6	ng/ g	<5	<2	<2	27.5±1.2*	52.7±2.5*

*Replicate of 5.

E 3. Summary of analytical methodologies for ophenol samples.

Lab	Extraction	Cleanup/Derivatization	Analysis
A	- grind with Na_2SO_4 - extract with 350 mL DCM/diethyl ether (4:1) in glass column - rotary evaporate	- GPC with Bio-Beads S-X3 - diazomethane methylation - 5 g activated Florisil cleanup, collect 200 mL PE/diethyl ether (94.6)	- heptachlor added as ISTD in GC-EC analysis
B	- grind with Na_2SO_4 - soxhlet extraction with DCM	- GPC with Bio-Beads S-X2 - discard first 110 mL, collect next 110 mL - 0.1 M K_2CO_3 back-extraction (3 x 30 mL) of phenols - acetylate with acetic anhydride	- GC-ECD analysis on a DB-5 column - confirmation on a DB-17 column
C	- add 10 mL 20:80 ϕ/hexane and 25 mL 1:1 H_2SO_4/H_2O to tissue - digest overnight - extract acid phase with 50 mL hexane (3 times) - dry extract with Na_2SO_4 - rotary evaporate	- diazomethane methylation - treat wih conc. H_2SO_4 and Hg	- GC-ECD analysis
D	- add acetone and H_2SO_4 to tissue - homogenize with polytron (3 times) - rotary evaporate and substitute DCM for acetone - conc. to a small volume and make up to 10 mL with GPC solvent	- GPC - diazomethane methylation - 8 g activated Florisil column cleanup - collect the P.E. and P.E. + diethyl ether (94+6) fractions	- GC-ECD analysis of Florisil fractions
E	- add 10 mL conc. HCl and 10 mL P.E. to tissue - shake 30 min - extract P.E. phase with 0.1% NaOH - extract tissue three more times - acidify NaOH phase to pH 2 and extract with DCM (3x75 mL) - dry DCM with Na_2SO_4 - evaporate to ca. 10 mL	- diazomethane methylation - cleanup on a 2% deact. Florisil column - elute methyl ethers with 150 mL P.E.	- GC-ECD analysis on a OV-101/OV-210 column
F	- add 100 mL 1N HCl and 100 mL toluene and shake overnight - extract tissue twice again with 100 mL toluene with polytron	- GPC with Styragel column using $CHCl_3$ as eluant - back extract phenols with 0.05 M Na^3PO^4 (100 mL total in 3 fractions) - acetylate with acetic anhydride - silica gel column cleanup	- GC-MSD analysis

TABLE 4-1 Results for 3,4-dichlorophenol.

Lab	Sample Results					
	1 ng/uL	2 ng/uL	3 ug/g	4 ug/g	5 ng/g	6 ng/g
A	NA	NA	NA	NA	NA	NA
B	23.72	5.38	0.91	2.32	8.13	10.37
C	NA	NA	NA	NA	NA	NA
D	NA	NA	NA	NA	NA	NA
E	NA	NA	NA	NA	NA	NA
F	9.02	1.55	6.29	1.44	ND	ND
Design	9.70	2.43	9.70	2.43	<5	<5

NA = not analyzed

ND = none detected

TABLE 4-2 Results for 2,4,6-trichlorophenol.

Lab	Sample Results					
	1 ng/uL	2 ng/uL	3 ug/g	4 ug/g	5 ng/g	6 ng/g
A	7.08	1.38	5.20	1.72	ND	ND
B	14.66	1.30	7.16	1.61	1.30	1.03
C	5.61	0.459	3.15	0.90	4	3
D	NA	NA	NA	NA	NA	NA
E	4.40	0.96	4.95	1.29	16	20
F	3.46	0.34	3.36	0.77	10.2	9.5
Design	5.20	1.30	5.20	1.30	<2	<2
Median	5.61	0.96	4.95	1.29	7.1	6.3
Mean	7.04	0.89	4.76	1.26	7.88	8.38
S.D.	4.47	0.47	1.62	0.42	6.57	8.55

NA = not analyzed

ND = none detected

TABLE 4-3 Results for 2,3,6-trichlorophenol.

Lab	Sample Results					
	1 ng/uL	2 ng/uL	3 ug/g	4 ug/g	5 ng/g	6 ng/g
A	6.93	1.38	5.14	1.65	ND	ND
B	20.46	2.77	8.57	1.91	ND	ND
C	3.34	0.254	1.71	0.44	<1	<1
D	NA	NA	NA	NA	NA	NA
E	4.00	0.88	4.26	1.12	10	15
F	3.00	0.29	3.49	0.80	ND	ND
Design	5.45	1.36	5.45	1.36	<2	<2
Median	4.00	0.88	4.26	1.12	-	-
Mean	7.55	1.12	4.63	1.18	-	-
S.D.	7.38	1.04	2.54	0.60	-	-

NA = not analyzed

ND = none detected

TABLE 4-4 Results for 2,3,4,6-tetrachlorophenol.

Lab	Sample Results					
	1 ng/uL	2 ng/uL	3 ug/g	4 ug/g	5 ng/g	6 ng/g
A	1.54	0.33	1.35	0.38	30	24
B	10.79	1.72	3.82	0.69	32.28	24.29
C	1.66	0.24	2.39	0.60	46	45
D	NA	NA	NA	NA	NA	NA
E	1.57	0.33	1.60	0.40	36	38
F	2.14	0.25	1.58	0.38	29.5	30.0
Design	2.00	0.50	2.00	0.50	27.5	27.5
Median	1.66	0.33	1.60	0.40	32.3	30.0
Mean	3.54	0.57	2.15	0.49	34.8	32.3
S.D.	4.06	0.64	1.01	0.15	6.8	9.1

NA = not analyzed

ND = none detected

TABLE 4-5 Results for PCP.

Lab	Sample Results					
	1 ng/uL	2 ng/uL	3 ug/g	4 ug/g	5 ng/g	6 ng/g
A	1.90	0.49	2.05	0.54	39	35
B	10.92	2.23	3.89	0.77	72.46	54.18
C	0.828	0.126	2.49	0.70	53.3	54.5
D	1.321	0.360	2.165	0.496	46.3	47.0
E	1.24	0.30	1.54	0.38	43	51
F	1.81	0.26	1.65	0.40	38.5	38.8
Design	2.12	0.53	2.12	0.53	52.7	52.7
Median	1.57	0.33	2.11	0.52	44.7	49.0
Mean	3.00	0.63	2.30	0.55	48.8	46.8
S.D.	3.90	0.79	0.85	0.16	12.8	8.2

NA = not analyzed

ND = none detected

APPENDIX

The following results were received from another participant after the final report was typed and approved for distribution. These data are reproduced below for information only as they are not included and evaluated in this report.

RESULTS

Chlorophenols in Standard Solution s(ng/ul)

	1	2	Detection Limit
3,4-dichlorophenol	N/A	N/A	
2,4,6-trichlorophenol	N/A	N/A	
2,3,6-trichlorophenol	N/A	N/A	
2,3,4,6-tetrachlorophenol	3.06 ug/ Total Sample	0.47 ug/ Total Sample	0.001 ng/ul
pentachlorophenol	3.92 ug/ Total Sample	1.05 ug/ Total Samples	0.001 ng/ul

N/A = not analysed

RESULTS

Chlorophenols in Fish Oil (ug/g)

	3	4	Detection Limit
3,4-dichlorophenol	N/A	N/A	
2,4,6-trichlorophenol	N/A	N/A	
2,3,6-trichlorophenol	N/A	N/A	
2,3,4,6-tetrachlorophenol	2.99 ug/g	1.03 ug/g	0.10 ug/g
pentachlorophenol	3.04 ug/g	0.070 ug/g	0.10 ug/g

N/A = not analysed

RESULTS

Chlorophenols in Fish Tissue (ng/g)

	5	6	Detection Limit
3,4-dichlorophenol	N/A	N/A	
2,4,6-trichlorophenol	N/A	N/A	
2,3,6-trichlorophenol	N/A	N/A	
2,3,4,6-tetrachlorophenol	46. ng/g	21. ng/g	10. ng/g
pentachlorophenol	46. ng/g	35. ng/g	10. ng/g

N/A = not analysed