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UPPER GREAT LAKES CONNECTING CHANNELS INTERLABORATORY PERFORMANCE EVALUATION STUDY QM-13: CHLOROPHENOLS IN AMPULES, FISH OILS AND TISSUES FINAL REPORT

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and the Quality Management Work Group

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UPPER GREAT LAKES CONNECTING CHANNELS

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sent to the QMWG for review and approval

MANAGEMENT PERSPECTIVE

The Upper Great Lakes Connecting Channels (UGLCC) have been designated as "Areas of Concern" by the International Joint Commission. A Canada - U.S. binational study, involving the identification and assessment of the environmental impacts of toxic substances, in those areas was initiated in 1984. In order to assist analytical laboratories, which are contributing data to the UGLCC study, to generate reliable and accurate data during the study, a Quality Management Work Group was formed and 13 interlaboratory performance evaluation studies were implemented.

This report summarizes and evaluates the results from the final interlaboratory performance evaluation study, QM-13, which consisted of the analysis of chlorophenols in ampules, fish oils and tissues. Results were received from two Canadian laboratories out of five participants. Overall 74% of the data reported was comparable and satisfactory.

Dr. J. Lawrence
Director
Research and Applications Branch

PERSPECTIVE-GESTION

La Commission mixte internationale a désigné comme étant "zones de problèmes potentiels" les chenaux reliant les Grands Lacs d'amont. Une étude canado-américaine, comportant l'identification et l'évaluation des incidences environnementales de substances toxiques dans ces régions a été entreprise en 1984. Afin d'appuyer les laboratoires d'analyse qui contribuent des données à cette étude et d'obtenir des données fiables et précises, un groupe de travail sur la gestion de la qualité a été formé et 13 études d'évaluation de la performance interlaboratoire ont été mises en marche.

Le présent article résume et évalue les résultats obtenus dans le cadre de l'étude finale de l'évaluation de la performance interlaboratoire, QM-13, comportant l'analyse de chlorophénols dans des ampoules, des huiles de poissons et des tissus. Les résultats de deux laboratoires canadiens sur cinq ont été reçus. Globalement, 74 % des données rapportées étaient comparables et satisfaisantes.

J. Lawrence
Directeur
Direction de la recherche et des applications

ABSTRACT

The Upper Great Lakes Connecting Channels (UGLCC) Study recognizes Quality Assurance/Quality Control (QA/QC) aspects as crucial elements to the overall utility of study results. As part of the QA/QC program, thirteen interlaboratory performance evaluation studies were designed and conducted by the Quality Management Work Group.

This report describes the results from the thirteenth interlaboratory performance evaluation study, QM-13, which consisted of the analysis of chlorophenols in ampules, fish oils and tissues. Results were received from two out of five participating laboratories (both Canadian). In general, results generated by these two laboratories were comparable and satisfactory for two standard solutions in ampules and two fish oils in most cases. However, results for duplicate fish tissues were more divergent. Lab U001 produced very accurate and precise results for 2,4,5,6-TeCP and PCP, but less satisfactory results for 2,4,6-TCP. While Lab U057 produced extremely high results for 2,4,6-TCP and PCP in both fish tissues.

RÉSUMÉ

Le groupe d'étude de l'Upper Great Lakes Connecting Channels (chenaux reliant les Grands Lacs d'amont), reconnaît comme étant cruciaux à l'utilité des résultats d'une étude, la gestion et le contrôle de la qualité. Dans le cadre du programme de gestion et de contrôle de la qualité, le groupe de travail sur la gestion de la qualité a entrepris et conçu 13 études d'évaluation de la performance interlaboratoire.

Le présent article décrit les résultats de la treizième étude d'évaluation de la performance interlaboratoire (QM-13) qui comportait l'analyse de chlorophénols dans des ampoules, des huiles de poissons et des tissus. Deux laboratoires (tous deux canadiens) parmi les cinq laboratoires participants ont fait parvenir leurs résultats. En général, ces résultats étaient comparables et satisfaisants pour deux solutions normales dans des ampoules et deux huiles de poisson dans la plupart des cas. Dans le cas des tissus, les résultats sont cependant plus divergents. Le laboratoire U001 a produit des résultats très précis dans le cas du 2,4,5,6-tétrachlorophénol et du pentachlorophénol mais des résultats moins satisfaisants pour le 2,4,6-trichlorophénol tandis que le laboratoire U057 a produit des résultats extrêmement élevés pour le 2,4,6-trichlorophénol et le pentachlorophénol dans les deux tissus de poissons.

1.0 INTRODUCTION

The Upper Great Lakes Connecting Channels (UGLCC) have been designated as "Areas of Concern" by the International Joint Commission (IJC). To identify and deal with the environmental problems, a three year, binational study was started in 1984, involving Canadian and U.S. environmental and resource agencies, to study the St. Marys, St. Clair and Detroit Rivers, and Lake St. Clair. The study involves identifying, quantifying and determining the environmental impacts of conventional and toxic substances from various sources.

The UGLCCS recognizes Quality Assurance/Quality Control (QA/QC) aspects as crucial elements to the overall utility of study results. As part of the QA/QC program, thirteen interlaboratory performance evaluation (QC) studies were designed and conducted by the Quality Management Work Group. The goal of these QC studies is to assist analytical laboratories, which are producing data for the UGLCC study, to generate reliable, accurate data and to assess their overall performance during this study. A total of some 100 parameters (organic, inorganic and physical properties) in three types of matrices (water, sediment and biota), will be assessed.

This thirteenth interlaboratory study, QM-13, was initiated on May 9, 1986. It involved the analysis of chlorophenols in ampules, fish oils and fish tissues. The original deadline for reporting results was set for August 1, 1986. There were no results submitted by any participants, so the study was not closed until October 17, 1986. However, since only one laboratory submitted their results, the deadline was further extended to October 31, 1987.

2.0 STUDY PROFILE

From the returned questionnaires, the following five laboratories affirmed that they would participate in this study: U001, U057, U009, U049 and U063. By the time the study was closed the last

three laboratories had not sent back any results. See the list of participants at the end of this report.

Each laboratory was provided with six test samples for analyzing the following five chlorophenols: 3,4-dichlorophenol (DCP), 2,4,6- and 2,3,6-trichlorophenols (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP). Samples 1301 and 1302, in sealed glass ampules, were standard solutions containing all five chlorophenols in methanol. Sample 1302 was a 1 to 4 dilution of sample 1301 in methanol. Samples 1303 and 1304 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. Details in the preparation of these fortified fish oils were described in another report (1). Samples 1305 and 1306 were blind duplicates of a starry flounder homogenate which contained native chlorophenols. The homogenate was subsampled into 5 g portions with the actual weight recorded on the label of each jar. Tissue samples were stored frozen at -20°C. See Table 1 for the detailed descriptions of test samples. The design values of chlorophenols in test samples are presented in Table 2. The design values were verified based on in-house analysis.

Participants were asked to analyze samples 1301 through 1306 for the above five chlorophenols, using their in-house procedures and standards. To avoid inhomogeneity of fish samples caused by separation of lipid from the tissue after subsampling, the participants were asked to use the entire jar contents for analysis. Tissue samples were distributed to the participants in insulated containers packed with freezer packs. Participants were asked to keep the tissues at -20°C until analysis. Chlorophenols in fish tissues were shown to be stable for at least 15 weeks at -20°C in another study (2). Thus the integrity of the test samples was assured.

3.0 RESULTS AND DISCUSSION

3.1 Analytical Methodology

The following procedure (1,2) was used in our laboratory to generate reference values of chlorophenols in the fish tissues.

After the weight was recorded, the fish tissue (ca. 5.0g) was quantitatively transferred to a mortar and ground with 10.0 g weight of precleaned anhydrous sodium sulfate. The mixture was then soxhlet extracted for eight hours with 350 mL of 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 were removed by a Bio-Beads S-X3 column using the above DCM/cyclohexane mixture as eluant at a flow rate of 5.0 mL/min. The first 145 mL were discarded and the next 165 mL containing the chlorophenols were collected. The chlorophenols were then back-extracted by three successive partitionings using a total of 100 mL of 1% K_2CO_3. The acetate derivatives of chlorophenols were formed by previously published procedures (3). Briefly, chlorophenols in K_2CO_3 solution were stirred with 2 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 1305 and 1306, as shown in Table 2, were the average five analyses obtained by our laboratory.

Since only two laboratories submitted results for the present QM-13 interlaboratory study, the complete analytical methods submitted by these two laboratories are given below.

U001

Chlorophenols Analysis in Fish Tissue

Fish tissue was acidified and extracted thrice with toluene at room temperature. After GPC, the chloroform extract was base partitioned with 2% potassium bicarbonate. The basic extract was acetylated and cleaned up on a silica gel (5%) column. Quantitation of the acetyl-chlorophenols was done by GC-MSD analysis. GC conditions are given below:

Column Head Pressure:	12.5 psi
Column Temperature Initial:	70°C; hold for 0.5 min.
Programming Rate 1:	10°C/min. (70° to 120°C) hold at 120°C for 5.0 min.
Programming Rate 2:	2°C/min. (120° to 160°C) hold at 160°C for 1.0 min.
Column Post-Run Final Temperature:	200°C for 10 min.

U057

Free Chlorophenols

A few drops of HCl were added and the samples were extracted with acetone:hexane (6:4). The hexane layer was separated and extracted with a 0.1 M aqueous solution of K_2CO_3. Acetylation was accomplished by the addition of acetic anhydride to the carbonate solution. The chlorophenol acetates were then extracted with hexane. Cleanup of the hexane extract was done using a micro-column packed with silica gel. Toluene was used as the eluting solvent. Analysis was performed using a Varian 3500 gas chromatograph with a nickel-63 electron capture detector. A J&W DB-5 capillary column (30 meter, 0.25 mm I.D., 0.25 micron film thickness) was used.

Fish Oil and Fish Tissue

A few drops of HCl were added and the samples were extracted with acetone:hexane (6:4) using a Polytron tissue homogenizer. The solvent was evaporated and the oil residues were redissolved in hexane. Extraction was carried out using a 0.1 M aqueous solution of the K_2CO_3. The procedure from this point on was identical to that used for the free chlorophenols.

3.2 Data Evaluation

All results submitted by the participants are listed in the data summary (Appendix II). The detection limits of five chlorophenols in test samples are tabulated in Appendix III. Since only two laboratories reported results for this interlaboratory study and laboratory U057 reported only those parameters which were US EPA priority pollutants (2,4,6-TCP and PCP), none of the ranking, flagging and other statistical techniques were used for data evaluation. To evaluate the accuracy of the chlorophenol results, the percent recovery based on the design value were calculated for each laboratory and tabulated in Table 3.

To provide a semi-quantitative evaluation of the results, the results were designated as very low, low, high and very high, based on the reported results as a % of the design value as shown below:

$\geq$176%	very high
151-175%	high
150-50%	satisfactory
49-25%	low
$\leq$24%	very low

See Table 4 for a summary of each laboratory's results.

4.0 GENERAL COMMENTS

Only one of two reporting laboratories submitted their results by the second closing date (October 17, 1986). However, laboratory U057 submitted their results on June 1, 1987. The following laboratories had not sent back any results: Laboratory U009 sent a letter on March 19, 1987 to decline participation in QM-13 due to reorganization of their lab personnel. Although laboratories U049 and U063 were allowed more time for reporting their results, both laboratories also declined their participation in this study due to other commitments to their clients.

Analysis of chlorophenols especially in fish oils and tissues required a very tedious procedure. It involved solvent extraction, concentration, GPC cleanup for oils and lipids, back extraction with base, acetylation and silica-gel cleanup before detection by GC/ECD or GC/MSD. Thus the chlorophenol results were evaluated as satisfactory when the recoveries were within ±50% of the design values as shown on Page 5, instead of ±25% used for evaluation of other organic parameters in the previous interlaboratory studies.

The interlaboratory results for two standard solutions (Samples 1301 and 1302) were satisfactory in most cases except for the 2,4,6-TCP, 2,3,6-TCP and PCP results reported by laboratory U001 on Sample 1302 which were less than 50% recovered based on the design values. In general, the accuracy of the in-house standards from these two reporting laboratories, U001 and U0057, were satisfactory and comparable.

The interlaboratory results for two fortified fish oils (Samples 1303 and 1304) were satisfactory from both laboratories except the 2,4,6-TCP result of lab U057 for sample 1304 was higher than 150%. It is unexpected that rather accurate results were obtained for the oil samples which required an additional cleanup step to that of the standard solutions. This observation was also found in the other interlaboratory study (1) which contained the identical samples.

The interlaboratory results for fish tissues (Samples 1305 and 1306) were less accurate in some cases. Only 2,3,4,6-TeCP and PCP were present in the fish in significant amounts (ng/g level). Lab U001 provided very accurate results for 2,3,4,6-TeCP and PCP in both samples and the precision of duplicate samples was excellent with RSD better than ±5%. However, their 2,4,6-TCP results were about five times higher than the concentration estimated by our laboratory. The erratic results could be attributed to the level of 2,4,6-TCP in the samples since they were close to their detection limit. Whereas the chlorophenol results reported by lab U057 were less satisfactory for both fish samples since the recoveries for 2,4,6-TCP and PCP were very high. This problem was suggestive of contamination during the sample preparation (especially in the extraction step) because the precision of duplicate samples was not good and the result for sample 1305 was higher than sample 1306. The other possible reason was due to interferences since this lab did not use GPC cleanup for removing lipids from sample extracts.

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REFERENCES

1. Lee, H.B., Szawiola, R and Chau, A.S.Y. Interlaboratory Analysis of Chlorophenols in Fish and Related Materials. NWRI Contribution 86-172, 1986.

2. Li, W.C., Lee, H.B. and Chau, A.S.Y. Preservation of Organics, Part III: Stability of Chlorophenols in Preserved Fish Homogenates. NWRI Contribution No. 897-125, 1987.

3. Lee, H.B., Weng, L.D. and Chau, A.S.Y. Chemical Derivatization Analysis of Pesticide Residues, VIII. Analysis of 15 Chlorophenols in Natural Water by In-Situ Acetylation. J. Assoc. Anal. Chem. 67, 789-794, 1984.

LIST OF PARTICIPANTS

Barringer Magenta Ltd., Rexdale, Ontario
National Water Quality Laboratory, Burlington, Ontario

The following laboratories were given samples, but did not submit any results:

Beak Analytical Services, Mississauga, Ontario
Ontario Ministry of the Environment, Trace Organic Section, Rexdale, Ontario
Zenon Environmental Inc., Burlington, Ontario

TABLE 1

Samples Distributed in this Study

Sample	Description
1301	Standard solution of five chlorophenols in methanol
1302	a 1:4 dilution of sample 1301 in methanol
1303	Fish oil fortified with five chlorophenols
1304	Same as 1303 except that chlorphenol levels are 25% of sample 1303
1305	Homogenate of naturally contaminated starry flounders caught in the Fraser River, B.C.
1306	Duplicate of sample 1305

TABLE 2

Reference Chlorophenol Values in the Test Samples

Sample		Chlorophenol				
		3,4-	2,4,6-	2,3,6-	2,3,4,6-	PCP
1301	ng/µL	9.70	5.20	5.45	2.00	2.12
1302	ng/µL	2.43	1.30	1.36	0.50	0.53
1303	µg/g	9.70	5.20	5.45	2.00	2.12
1304	µg/g	2.43	1.30	1.36	0.50	0.53
1305	ng/g	<5	<2	<2	27.5 ± 1.2*	52.7 ± 2.5*
1306	ng/g	<5	<2	<2	27.5 ± 1.2*	52.7 ± 2.5*

* Replicate of 5.

TABLE 3

Percent Recovery Calculated from the Design Values for Chlorophenols

Lab Number: U001

Parameter	1301	1302	1303	1304	1305	1306
			%			
3,4-DCP	93.0	63.8	64.8	59.3	ND	ND
2,4,6-TCP	66.5	26.2	64.6	59.2	510(E)	460(E)
2,3,6-TCP	55.0	21.3	64.0	58.8	ND	ND
2,3,4,6-TeCP	107	50.0	79.0	76.0	107	109
PCP	85.4	49.1	77.8	75.5	73.1	73.6

* See Appendix I for explanation of glossary of terms.

TABLE 3

Percent Recovery Calculated from the Design Values for Chlorophenols

Lab Number: U057

Parameter	1301	1302	1303	1304	1305	1306
			%			
3,4-DCP	NA	NA	NA	NA	NA	NA
2,4,6-TCP	74.2	126	88.8	178	9400(E)	7400(E)
2,3,6-TCP	NA	NA	NA	NA	NA	NA
2,3,4,6-TeCP	NA	NA	NA	NA	NA	NA
PCP	65.1	126	137	115	380	277

* See Appendix I for explanation of glossary of terms.

TABLE 4

Summary of Laboratory Results Based on the % Recovery of the Design Values (see page 5)

Lab No.	Parameter	Comments
U001		
	2,4,6-TCP	Sample 1302 - low; Samples 1305 and 1306 - v. high
	2,3,6-TCP	Sample 1302 - v. low
	PCP	Sample 1302 - low
U057	2,4,6-TCP	Samples 1304, 1305 and 1306 - v. high
	PCP	Samples 1305 and 1306 - v. high

APPENDIX I

Glossary of Terms

NA:	not analyzed
N or ND:	not detected
E:	estimate value

APPENDIX II

UGLCC Interlaboratory Performance Evaluation Study
QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Final Data Summary

DATA SUMMARY

QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Printout prepared: 87/11/12
Parameter: 3,4-dichlorophenol

Lab No.	1301 (ng/µL)	1302 (ng/µL)	1303 (µg/g)	1304 (µg/g)	1305 (ng/g)	1306 (ng/g)
U001	9.02	1.55	6.29	1.44	ND	ND
U057	NA	NA	NA	NA	NA	NA
Design Value	9.70	2.43	9.70	2.43	<5	<5

DATA SUMMARY

QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Printout prepared: 87/11/12
Parameter: 2,4,6-trichlorophenol

Lab No.	1301 (ng/µL)	1302 (ng/µL)	1303 (µg/g)	1304 (µg/g)	1305 (ng/g)	1306 (ng/g)
U001	3.46	0.34	3.36	0.77	10.2	9.5
U057	3.86	1.64	4.62	2.32	188	148
Design Value	5.20	1.30	5.20	1.30	<2	<2

DATA SUMMARY

QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Printout prepared: 87/11/12
Parameter: 2,3,6-trichlorophenol

Lab No.	1301 (ng/µL)	1302 (ng/µL)	1303 (µg/g)	1304 (µg/g)	1305 (ng/g)	1306 (ng/g)
U001	3.00	0.29	3.49	0.80	ND	ND
U057	NA	NA	NA	NA	NA	NA
Design Value	5.45	1.36	5.45	1.36	<2	<2

DATA SUMMARY

QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Printout prepared: 87/11/12
Parameter: 2,3,4,6-tetrachlorophenol

Lab No.	1301 (ng/µL)	1302 (ng/µL)	1303 (µg/g)	1304 (µg/g)	1305 (ng/g)	1306 (ng/g)
U001	2.14	0.25	1.58	0.38	29.5	30.0
U057	NA	NA	NA	NA	NA	NA
Design Value	2.00	0.50	2.00	0.50	27.5±1.2	27.5±1.2

DATA SUMMARY

QM-13 Chlorophenols in Ampules, Fish Oils and Tissues

Printout prepared: 87/11/12
Parameter: PCP

Lab No.	1301 (ng/µL)	1302 (ng/µL)	1303 (µg/g)	1304 (µg/g)	1305 (ng/g)	1306 (ng/g)
U001	1.81	0.26	1.65	0.40	38.5	38.8
U057	1.38	0.67	2.91	0.61	200	146
Design Value	2.12	0.53	2.12	0.53	52.7±2.5	52.7±2.5

APPENDIX III

The Detection Limits of the Five Chlorophenols in the Test Samples

Sample		Chlorophenol				
		3,4-	2,4,6-	2,3,6-	2,3,4,6-	PCP
U001						
1301 & 1302	ng/μL	0.025	0.025	0.025	0.025	0.025
1303 & 1304	ug/g	0.025	0.025	0.025	0.025	0.025
1305 & 1306	ng/g	5.0	5.0	5.0	5.0	5.0
U057						
1301 & 1302	ng/μL	NA	0.002	NA	NA	0.001
1303 & 1304	μg/g	NA	0.002	NA	NA	0.001
1305 & 1306	ng/g	NA	10.0	NA	NA	5.0