QUALITY ASSURANCE AND QUALITY CONTROL FOR SERUM DIOXIN ANALYSIS ON TRICHLOROPHENOL WORKERS IN NEW PLYMOUTH

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Introduction

The Dow Chemical Company and University of Otago conducted a study on the serum levels of polychlorinated dibenzodioxins (PCDD), dibenzofurans (PCDF), biphenyls (PCB) and lipids in workers from the Dow AgroSciences New Zealand site in New Plymouth. A portion of these workers had potential exposure to trichlorophenol. The serum draw occurred in September of 2005, July of 2006 and May 2007. The study was accompanied by a multi-step QA/QC program for the determination of PCDD/F, PCB and lipids which exceeded the requirements of the EPA methods chosen for the analytical program.

Materials and Methods

In total 348 serum samples were collected and analyzed in this study. The analysis of the halogenorganic compounds was performed by AsureQuality (formerly AgriQuality), Lower Hutt, NZ; Aotea Pathology Ltd (formerly Wellington Pathology), NZ determined the lipid content. In general the analytical protocol followed US EPA methods 8290/1613b for PCDD/F and method 1668 for PCB. The lipid content was calculated from the average total cholesterol and triglyceride level with the formula used by the CDC for the lipid determination in human serum[1]. It included the determination of all 17 2378-substituted PCDD/F congeners, the 12 WHO-PCB and PCB-138, PCB-153 and PCB-180 as indicator components.

A key part of the study was the development and application of an intensive QA/QC program which is based on experiences from other studies. The Dow Chemical Company had recently conducted [2] a large serum study on chlorophenol workers in the United States. This led to some additional QA/QC steps compared to the CDC-method, in particular the use of the corresponding ^{13}C-labelled standards of all target analytes as internal standards. Furthermore the potential impact of shipping, handling and storage on the lipid concentration was investigated. Most of these QA/QC-measures were included in the original study protocol, but some were added in the course of the study.

We obtained pooled human serum samples from the blood bank in New Zealand which served as control samples for all initial and ongoing quality tests of trichlorophenol and referent workers. We used this serum also as a base for fortified reference samples (fortification on 2 different levels). This paper will describe various steps and factors taken into consideration for the testing program to ensure the highest levels of quality assurance, control, data integrity and comparability.

Results and Discussion

Prior to the main study we conducted a pilot study with 27 serum samples to test the logistics for serum drawing, storage, shipment, and analysis of the samples. In this phase we also conducted an audit of the laboratory and performed a rigorous cleaning of the area which was exclusively designated for the sample preparation of our serum samples. Furthermore, new glassware and equipment was used for this study. This step ensured that serum samples which have levels of the target analytes at the background level range are free of any cross-contamination. This phase also included tests about the stability of the lipid content in serum samples under various storage and shipment conditions and a comparison between the lipid determination method chosen for this study and the method used for an earlier serum study of non-occupationally exposed New Zealand residents which included the determination of the phospholipids[3]. We used a pooled human serum sample from the blood bank in New Zealand for this evaluation. For both methods of lipid evaluation, the standard deviation was less than 5 % indicating excellent reproducibility. As there was virtually no difference between the 2 methods for the lipid determination, we decided to use the CDC method for our study. This was confirmed in the analysis of the

27 serum samples of the pilot phase whose lipid content was also determined with both methods. The results differed by less than 0.2 % on average.

Table 1: Reproducibility and comparison of different lipid determination methods in a pooled serum sample

	NZ method Total Lipid [g/l]	CDC method Total Lipid [g/l]	Difference in %
Pool QA1	6.12	6.16	0.66 %
Pool QA2	6.29	6.34	0.80 %
Pool QA3	6.09	6.16	1.28 %
Pool QA4	5.86	5.72	-2.33 %
Average	6.10	6.09	0.10 %
Std dev.	2.9 %	4.3%	

NZ-method: Total lipid = 1.677*(Total Cholesterol [g/l]- Free Cholesterol) + Free Cholesterol [g/l] + Triglycerides [g/l] + Phospholipid [g/l]
CDC-method: Total lipid = 2.27*Total Cholesterol [g/l] + Triglycerides [g/l] + 0.623

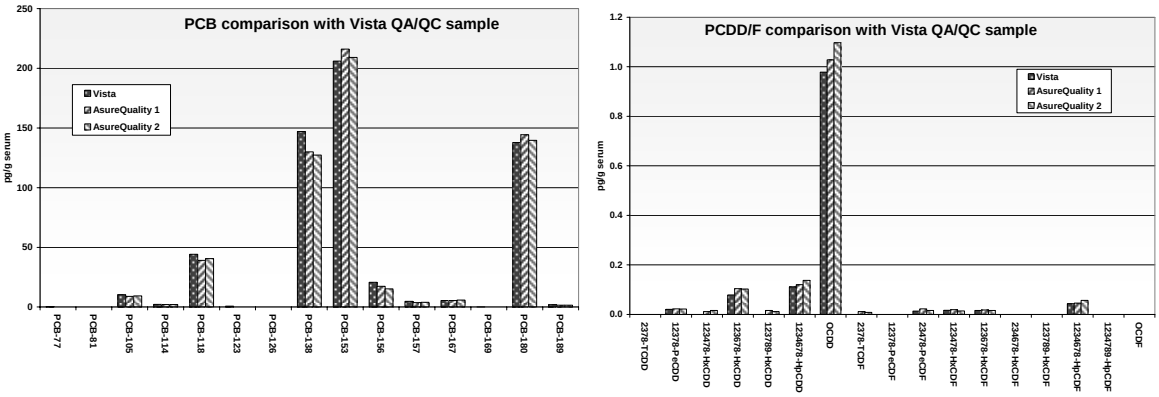
Due to recent increased security measures, most, if not all, parcels are X-rayed for air shipment. We were concerned that this X-ray process could have an impact on the sample integrity. We analyzed the lipid content of the pooled serum sample after passing the sample 1, 5 and 10 times respectively through the X-Ray scanner for hand luggage at Wellington International Airport to investigate the potential impact.

Table 2: Determination of the lipid content in a pooled serum sample after X-Ray treatment

	% lipid
X-Ray 1x	0.575
X-Ray 5x	0.549
X-Ray 10x	0.584
Control 1	0.592
Control 2	0.584

We found no major impact on the lipid content after X-ray treatment, however, a small impact cannot be completely ruled out.
In the pilot phase of the study, AsureQuality also analyzed a pooled serum sample from Vista (formerly Alta) Analytical, El Dorado Hills, CA, USA which served as QA/QC sample in the Michigan Trichlorophenol worker serum study[1].

Figure 1: Comparison of the analysis of a QA/QC sample for PCDD/F and PCB in 2 different laboratories



This analysis demonstrated that the data are in excellent agreement in both laboratories.

For the second phase of our study, we created a new QA/QC sample, again based on a pooled serum sample from the blood bank which served as an ongoing control sample. In order to cover a wider concentration range we fortified a portion of this serum with all PCDD/F and PCB target analytes (QA1). A portion of this sample was additionally fortified with 2378-TCDD (QA2) so all other fortified target analytes had the same concentration as in QA1. At least 2 of the 3 QA/QC samples together with a Method blank were included in each batch of samples for analysis.

Further tests were made with the pooled serum sample (QA0) to investigate the reproducibility and the effect of storage conditions on the lipid content to both refrigerator and ambient temperatures. Compared to frozen control samples there was no discernable effect of storage for different storage conditions up to about 1 week.

Table 3: Impact of different storage conditions on lipid content

Refrigeration		Ambient air		Reproducibility	
Test	% lipid	Test	% lipid	Test	% lipid
Refrigerated 1 day	0.498	Ambient 2 days	0.507	QA 0	0.498
Refrigerated 2 days	0.489	Ambient 4 days	0.481	QA 0	0.498
Refrigerated 3 days	0.506	Ambient 6 days	0.498	QA 0	0.498
Refrigerated 4 days	0.498	Ambient 8 days	0.524	QA 0	0.507
Refrigerated 5 days	0.498	QA 0 (Control)	0.507		
Refrigerated 6 days	0.481	QA 0 (Control)	0.489		
Refrigerated 7 days	0.498	QA 0 (Control)	0.498		

In addition each of the QA samples were analyzed by Vista prior to the analysis of the serum samples of this study. The results listed in table 4 compare these data with the average of AsureQuality's data and also show excellent agreement between both laboratories.

Table 4: Comparison of the PCDD/F analysis of the QA/QC sample by Vista and AsureQuality

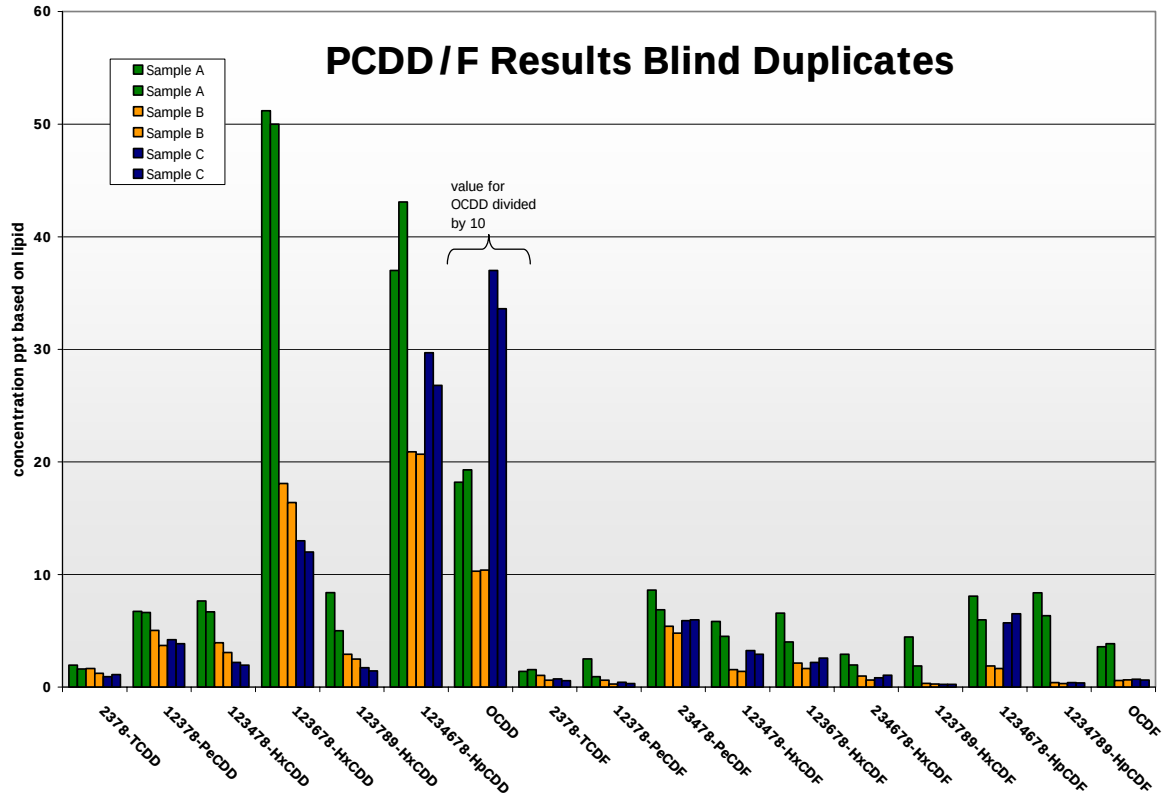
	AsureQuality Average	Vista	AsureQuality Average	Vista	AsureQuality Average	Vista
	QA 0	QA 0	QA 1	QA 1	QA 2	QA 2
2378-TCDD	ND	0.87	2.22	ND	92.7	97.4
12378-PeCDD	2.23	1.8	5.73	5.28	5.83	4.41
123478-HxCDD	1.53	ND	4.31	ND	4.09	5.62
123678-HxCDD	6.41	7.06	10.5	7.6	10.5	10.1
123789-HxCDD	ND	ND	5.05	ND	5.09	5.79
1234678-HpCDD	12.6	11.7	16.9	12.8	16.1	15.7
OCDD	105	112	118	139	112	120
2378-TCDF	1.43	ND	2.64	ND	2.47	1.76
12378-PeCDF	ND	ND	4.51	ND	5.12	3.97
23478-PeCDF	3.04	1.97	6.46	5.65	6.53	5.36
123478-HxCDF	1.5	1.26	4.85	ND	4.93	4.59
123678-HxCDF	1.39	1.17	5.58	ND	5.19	4.98
234678-HxCDF	0.7	ND	4.44	ND	4.33	4.1
123789-HxCDF	ND	ND	4.47	ND	3.81	3.23
1234678-HpCDF	4.18	3.22	7.92	5.29	7.51	7.01
1234789-HpCDF	ND	ND	4.49	ND	4.55	3.87
OCDF	ND	ND	9.17	ND	9.09	7.41

As a final QA/QC step in our study, we collected 3 serum samples from volunteers who were willing to donate double the amount of blood, 160 ml, as blind duplicates to the laboratory. The samples were labeled as regular samples. The results for the PCDD/F are shown in figure 3. Table 5 contains the TEQ data for all analytes.

Table 5: Results of 3 blind duplicate samples [PCDD/F and PCB in ng WHO-TEQ 2005/kg lipid]

	Sample A			Sample B			Sample C		
	Result 1	Result 2	Var.	Result 1	Result 2	Var.	Result 1	Result 2	Var.
Lipids [%]	0.571	0.571	0 %	0.626	0.639	1 %	0.772	0.755	1 %
PCDD/F only	15.2	17.1	6 %	11.4	8.6	16 %	9.08	8.60	3 %
PCB only	2.73	2.97	4 %	2.53	2.21	7 %	2.97	2.48	10 %
PCDD/F + PCB	17.9	20.1	5 %	13.9	10.8	14 %	12.1	11.1	4 %

Figure 3: PCDD/F data in 3 blind duplicate samples



In conclusion, our QA/QC program for this study provides evidence for the validity and reliability of the dioxins, furans, and PCB evaluations done at AsureQuality.

References

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