Development and Validation of the Method for the Detection of Tricresyl Phosphates by GC/MS

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16. Abstract

In this report, we present a summary of the method development and validation work performed in support of the Incident Monitoring and Reporting Project (Project 4). In the method development phase, the analytical procedure that is appropriate for the analysis of the three tricresyl phosphates (TCPs) was established and evaluated. The initial sampler was a van Netten (VN) Sampler configured to collect a 20-minute sample. The nominal flow rates ranged from 1 to 2 liters per minute (LPM). This sampler was modified so that it could sample air up to 4 hours with a nominal flow rate of 0.4 to 0.7 LPM. The report presents the results of the following tests: determination of *instrument detection limits* (IDL); recovery of spiked filters; determination of *method detection limits* (MDL); laboratory intercomparison of spiked filters; storage stability testing of spiked filters; and laboratory intercomparison of in-flight duplicate samples. We are able to detect TCP in loadings of greater than 0.4 ng/filter for three TCP isomers: T-*o*-CP, T-*m*-CP and T-*p*-CP. We recommend requiring an internal standard with this analytical method due to possible interference from other compounds collected on the sample filters that alter the retention time of the three TCP isomers. The storage stability testing indicates that TCPs sampled on filters are stable for a period of up to one month. It is recommended that the filters be stored cold after sampling to maximize the amount of analyte recovered. Trace amounts of TCP found in transportation blanks raised the LOD for air samples and introduced uncertainties in the interpretation of the results. TCP was detected in approximately 18% of the in-flight samples (actual samples and field blanks), but no TCP was detected in an equal number of pre-blank samples. The pre-blank samples were 2-hour laboratory air samples collected using the same VN sampler used in-flight. The filters in these lab blanks were removed from the sampler and analyzed to establish that the samples had no residual TCP prior to deployment in-flight. To limit these uncertainties at least five transportation (field) blanks should be included in each batch of samples. Inter-laboratory collaborations showed comparable results and also helped advance method development. Finally, the sampler tested proved convenient and unobtrusive for collecting onboard samples.

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ABBREVIATIONS

As used in this report, the following abbreviations/acronyms have the meanings indicated

ABBREVIATION	MEANING
CCAL	continuing calibration
DCM	dichloromethane
DL	detection limit
GC	gas chromatograph
HSPH	Harvard School of Public Health
ICAL	initial calibration
IDL	instrument detection limit
IS	internal standard
LOD	limit of detection
LPM	liters per minute
MCE	mixed-cellulose ester
MDL	method detection limit
MDM	minimum detectable mass
MS	mass spectrometer
ND	non-detects
ng	nanogram
PTV	programmed temperature vaporization
QC	quality control
RSD	relative standard deviation
SD	standard deviation
RT	retention time
SIM	select-ion monitoring
TCP	tricresyl phosphate
T-*m*-CP	tri-*m*-cresyl phosphate
T-*o*-CP	tri-*o*-cresyl phosphate
T-*p*-CP	tri-*p*-cresyl phosphate
UBC	University of British Columbia
VN Sampler	van Netten Sampler
µl	microliters

Contents

INTRODUCTION **1**

DETERMINATION OF INSTRUMENT DETECTION LIMITS (IDLs) 2
- Methods 2
- Results 2
- Summary 4

FILTER RECOVERY TESTING 4
- Methods 4
- Results 4
- Summary 5

METHOD DETECTION LIMITS (MDLs) DETERMINATION 5
- Methods 5
- Results 6
- Summary 6

LABORATORY INTERCOMPARISON 6
- Results 6
- Summary 7

STORAGE STABILITY TESTING 7
- Methods 7
- Results 7
- Summary 8

ONBOARD DUPLICATE STUDY 8
- Methods 8
- Results 8

CONCLUSIONS 12

APPENDIX A: Method for Detection of Tricresyl Phosphates by GC/MC A-1

DEVELOPMENT AND VALIDATION OF THE METHOD FOR THE DETECTION OF TRYCRYSLY PHOSPHATES BY GC/MS

INTRODUCTION

In this report, we present a summary of the method development and validation work performed in support of the Incident Monitoring and Reporting Project (Project 4). In the method development phase, the analytical procedure that is appropriate for the analysis of the three tricresyl phosphates (TCPs) was established and evaluated. The first step was to identify the target compounds so that conditions could be optimized for resolving the selected compounds.

Table 1 lists the three target compounds selected. Analysis using a gas chromatograph (GC) with a mass spectrometer (MS) detector was chosen to provide the required sensitivity, the necessary linearity, and the desired specificity. The initial sampler was a van Netten (VN) Sampler (Figure 1) configured to collect a 20-minute sample. The nominal flow rates ranged from 1 to 2 liters per minute (LPM). This sampler was modified so that it could sample air up to 4 hours with a nominal flow rate of 0.4 to 0.7 LPM.

Table 1. Target Analytes

Compound	Abbreviation	IDL ng/filter	Standard Source	CAS
tri-*o*-cresyl phosphate	T-*o*-CP	0.1	Accustandard	78-30-8
tri-*m*-cresyl phosphate	T-*m*-CP	0.1	Accustandard	563-04-2
tri-*p*-cresyl phosphate	T-*p*-CP	0.1	Accustandard	78-32-0

Figure 1: Side and top view of the van Netten sampler (on position), which is 9 cm in length, 5 cm in diameter.

As part of the method development process three different filter media were evaluated: Teflo (Pall Corporation, East Hills, NY), mixed cellulose ester (Pall Corporation, East Hills, NY),

and Quartz QM-A (Whatman, Florham Park, NJ). In addition, the method of extraction and extraction solvents were evaluated. The report presents the results of the following tests:

- Determination of *instrument detection limits* (IDLs)
- Recovery of spiked filters
- Determination of *method detection limits* (MDLs)
- Laboratory intercomparison of spiked filters
- Storage stability testing of spiked filters
- Laboratory intercomparison of in-flight duplicate samples

The final method is presented in Appendix A. The established method has an MDL of 0.4 ng/filter for each TCP isomer. From the results of the in-flight duplicate sampling described later in this report, we recommend the use of an internal standard with this method due to possible interference from compounds collected on the filter samples that alter the retention time of the three TCP isomers. An internal standard (IS) is a compound similar to the target analyte that is either not expected to be present in the samples or is labeled such as being deuterated. It is added to the final extract prior to analysis in order to monitor analyte retention time. Its response is also incorporated in the equation used to calculate the amounts in the sample, thereby taking into account run-to-run variation. There is evidence that TCP contamination may have occurred during sample handling, making detection of the low levels measured questionable. To control for this contamination, there should be at least five (5) transport blanks (field blanks) per batch. It is promising that in the laboratory intercomparison of in-flight samples TCP was detected only in in-flight samples and transport blanks, and no TCP was detected in any of the pre-blanks. Transport blanks, also known as field blanks, are taken on flights with actual samples but the sampler is not activated. The pre-blank was an actual two-hour sample of building air collected on each sampler before it was deployed in an in-flight situation. The purpose of the pre-blank was to identify gross TCP contamination of the sampler left over from a previous deployment of the sampler.

DETERMINATION OF INSTRUMENT DETECTION LIMITS (IDLs)

An IDL is the lowest value of analyte that the instrument can detect. It is determined on samples that have not gone through any sample preparation steps. An MDL is similar to an IDL, but is based on samples that have gone through the entire sample preparation scheme prior to analysis. The MDL gives the variation in instrument response at levels near the detection limit, from which 99% confidence limits are calculated from the standard deviation of the replicate blank values.

Detection limits (DLs) are estimates of concentrations at which we can be fairly certain that the compound is present in the sample. Concentrations below this limit may not be detected. Concentrations above this limit are almost certainly detected in the analysis. Using statistics, the certainty of detection can be quantitated as 99%. Samples below the detection limit may have target analytes present but in concentrations too low to be distinguished from background noise.

Methods

Determination of IDLs for GC/MS analysis of TCPs was performed by analyzing spiked solutions of 4 ng/ml and 1 ng/ml.

The IDL determination at the Harvard School of Public Health (HSPH) was performed using an Agilent 6890/5975 GC/MS. Our University of British Columbia (UBC) colleagues provided us with their insight and advice in regards to method parameters and choice of extraction solvent. After incorporating their suggestions, we improved instrument DLs for TCP.

Results

An initial calibration (ICAL) was established as follows in Table 2:

Table 2. Initial calibration range for target analytes

Analyte	Standard One ng/ml	Standard Two ng/ml	Standard Three ng/ml	Standard Four ng/ml
T-*o*-CP	40.0	10.0	4.0	1.0
T-*m*-CP	40.0	10.0	4.0	1.0
T-*p*-CP	40.0	10.0	4.0	1.0

We analyzed ten replicates at the two lowest levels (4 and 1 ng/ml) to establish our IDLs. The IDLs are calculated by multiplying the standard deviation of the replicate samples by the appropriate (based on the number of replicates) Student's t-value at the 99% confidence level (2.896 for nine replicates). We performed the IDL study at two low levels and since the standard deviation for the lowest level at 1ng/ml was acceptable, that became the reported IDL.

The linearity of the calibration curve for each target analyte calculated from the initial calibration standards is shown in Table 3. The relative standard deviation (RSD) is the standard deviation (SD) of the replicate measurements divided by average value of the measurements times 100 for each target analyte.

Table 3. Linearity for the ICAL

Analyte	Percent RSD
T-*o*-CP	13.1
T-*m*-CP	16.6
T-*p*-CP	8.0

The IDLs in Table 4 were obtained using the programmed temperature vaporization (PTV) inlet in solvent-vent mode. Injection volumes are 20 µl. With an expected sampled extract final volume then of 100 µl, the individual sample IDL should be 0.1 ng of TCPs. All later method development was done using a standard split/splitless inlet on the GC/MS due to difficulties maintaining consistent response with the PTV inlet system. The analytical method used is described in *Appendix A, Method for Detection of Tricresyl Phosphates by GC/MS.*

Table 4. IDL in ng/ml determination data, for 4 ng/ml and 1 ng/ml spikes

	Tri-*o*-cresyl Phosphate	Tri-*m*-cresyl Phosphate	Tri-*p*-cresyl Phosphate
Standard Amount ng/ml	1.0	1.0	1.0
1	0.65	0.61	0.73
2	0.69	0.73	0.96
3	0.67	0.88	0.73
4	0.99	1.11	1.28
5	1.01	0.89	0.85
6	0.79	0.63	0.64
7	0.86	0.99	0.85
8	0.69	0.88	0.82
9	0.73	0.67	0.75
10	0.60	0.60	0.63
avg	***0.77***	***0.80***	***0.84***
SD	***0.14***	***0.18***	***0.19***
IDL	***0.40***	***0.50***	***0.55***

Summary

IDLs for tri-*o*-cresyl phosphate, tri-*m*-cresyl phosphate and tri-*p*-cresyl phosphate were determined by analysis of two low levels, at 4.0 and 1.0 ng/ml, and the values for 1.0 ng/ml replicates were used for IDL calculations.

FILTER RECOVERY TESTING

Sample recovery is an assessment to determine the percentage of a known amount of target analyte that can be actually detected by the analytical method. Losses of analyte can occur during sample collection, storage, extraction and/or analysis. Determination of recovery of TCPs from the filters was performed by analyzing the filters spiked at 0.5 ng/filter and 5.0 ng/filter. Filter recovery was evaluated for three types of filters identified in the method: Teflo (Pall Life Sciences), mixedcellulose ester (MCE, [SKC, Inc.]) and quartz QMA filters.

Methods

Media Preparation

All filters were pre-cleaned by sonication in dichloromethane (DCM). Filters were covered with solvent and sonicated in a 100 ml wide-mouth jar with a teflon lined screw-cap. Extract was discarded and the process repeated two more times. Sonication period was 15 minutes each time. Filters were allowed to air dry.

Matrix Spiking

Two procedural blanks (PB) (unspiked clean filters) and six matrix spikes (spike of the target analyte) were analyzed for each filter type. The six matrix spikes were further divided into three each of a low and high levels. Spike solution had a concentration of 100.0 ng/ml for each TCP isomer. Spike volumes were 5.0 µl and 50.0 µl for the low and high spikes respectively. This yielded low spike amounts of 0.50 ng/filter and high spike amounts of 5.0 ng/filter.

Extraction

Extraction was performed by sonication. Filters were placed inside a clean 100 ml wide-mouth jar with a Teflon-lined screw cap and 6-8 ml of toluene was added, ensuring complete coverage of the filter. Jars were sonicated for 12 minutes. Extract was transferred by Pasteur pipette to a clean 24 ml vial. This was repeated two more times. The 18-24 ml of extract was placed under a gentle stream of nitrogen to reduce the extract volume. When a few drops remained, they were transferred to the vial which would go on the instrument. The 24 ml vial was rinsed with a small volume of toluene and this rinsate was added to the analytical vial. Then the extract volume was reduced to the final volume of 200 µl for the analysis.

Results

Continuing calibration (CCAL) standards were analyzed and evaluated successfully versus the ICAL. The ICAL standards are the first set of calibration standards analyzed in a run followed by the samples and blanks. Every eight samples in the run a second standard—known as the CCAL—is analyzed to monitor the instrument responses throughout the duration of the sequence. CCALs were acceptable. Acceptance criterion is a percent relative difference between the average response factor from the ICAL and the response factor from the CCAL of less than 25%. This would be the standard operating procedure for samples as well. We are currently investigating the use of internal standards (IS) for quantitation as opposed to the current method using External Calibration. Internal standards would alleviate run to run variations and give us a further check for individual runs.

Analysis was done by concentration-based calculations:

-The ICAL is the concentration of the final extract, measured in µl/ml

-The extract final volume is 200 µl.

-The mass of a target analyte on a given filter then would be calculated as:

sample (ng/ml) x 200 l x 1 ml/1000 µl = ng.

The percent recovery results for each sample are presented in Table 5.

Procedural blanks for all types of filters were clean of all target analytes.

Table 5. Percent Recovery (100 X Analyte recovered/analyte spiked)

	Spiked Amount	T-*o*-CP %	T-*m*-CP %	T-*p*-CP %
QMA-1	0.50 ng	92.8	112.0	119.8
QMA-2		106.2	129.6	140.0
QMA-3		99.2	113.8	119.4
Average		**99.4**	**118.5**	**126.4**
QMA-4	5.0 ng	110.9	123.9	130.3
QMA-5		117.4	127.7	138.4
QMA-6		110.0	121.3	128.6
Average		**112.8**	**124.3**	**132.4**
MCE-1	0.50 ng	85.0	103.4	106.6
MCE-2		95.2	106.8	116.0
MCE-3		96.6	119.0	125.4
Average		**92.3**	**109.7**	**116.0**
MCE-4	5.0 ng	100.8	114.1	124.5
MCE-5		110.5	123.9	133.3
MCE-6		110.5	122.7	125.9
Average		**107.3**	**120.2**	**127.9**
Teflo-1	0.50 ng	53.8	72.6	75.6
Teflo-2		60.2	75.6	82.0
Teflo-3		86.0	102.6	106.4
Average		**66.7**	**83.6**	**88.0**
Teflo-4	5.0 ng	69.4	81.4	91.5
Teflo-5		80.4	94.1	104.4
Teflo-6		101.9	115.9	134.5
Average		**83.9**	**97.1**	**110.1**

Summary

Thirty seven (37) mm quartz QMA filters and 37 mm Teflo, with ring, 2.0 µm pore PTFE membrane filters demonstrated the best recovery for all three target analytes. All three target compounds were successfully recovered at the 0.5 ng/filter level. Recoveries ranging from 80 to 120 percent are considered acceptable and no correction for recovery losses is required for recoveries that fall within that range.

METHOD DETECTION LIMITS (MDLs) DETERMINATION

MDL determination was performed by spiking filters with 1.0 ng of each of the target analytes, extracting them and analyzing them by GC/MS.

Methods

Solvent: DCM

Extraction is done by sonication. Filters are placed inside a clean 8 ml vial with a teflon lined screw cap and 5-6 mls of DCM are added, ensuring complete coverage of the filter. Vials are sonicated 30 minutes. Water in sonication bath is kept chilled with "blue-ice" packs. Extract is transferred by pasteur pipette to a clean 8 ml vial. The 5-6 mls of extract are placed under a gentle stream of nitrogen and reduced to dryness. A syringe is used to add 500 µL of Toluene to the vial and it is vortexed making sure the Toluene rinses the sides of the vial adequately. Then, an aliquot is withdrawn and placed into an insert in the analytical vial to be placed on the GC/MS instrument.

Matrix Spiking: Nine quartz QMA filters were spiked with a TCP standard mix such that the spike amount was 1 ng/filter for each TCP isomer.

Results

Table 6 presents the results of the MDL determination. Quartz QM-A filters were

prepared and treated in the same manner as described in the previous section on recovery testing. The MDL takes into account artifacts associated with sample extraction and preparation. The MDLs are calculated by multiplying the standard deviation of the replicate measurements by the appropriate (based on the number of replicates) Student's t-value at the 99% confidence level. (2.896 for nine replicates.)

Summary

MDLs for the three target analytes were determined. MDLs incorporate the extraction of the analyte from the filter so they are reported in ng/filter, while the IDLs are reported in ng/ml of solution since they only cover the analysis of a solution.

The IDLs were initially performed using the PTV inlet system, which was replaced by the split/splitless inlet (see the explanation in the IDL section above) that was used for the rest of the method development and the study. There was no need to repeat the IDL determination for the split/splitless inlet method because the MDLs using this method (Table 6) were even lower than the original IDLs using PTV inlet (Table 4).

LABORATORY INTERCOMPARISON

In March 2007, UBC and HSPH performed a round of laboratory intercomparison for the analysis of *o*, *m*, *p*-tricresyl phosphates. UBC prepared blind spiked samples and submitted them to both laboratories. Five sets of triplicate samples were prepared on mixed-cellulose ester filters. Samples were spiked with three levels of TCPs, and one was spiked with Mobil oil No. 291 and there was one blank set.

Results

Both laboratories produced comparable results. The results on the intercomparison are presented in Table 7.

Table 6. MDL Data in ng/filter

Sample Name	**T-*o*-CP**	**T-*m*-CP**	**T-*p*-CP**
(Amount spiked, 1. 0 ng/filter)	ng/filter	ng/filter	ng/filter
MDL-1	0.95	1.28	1.15
MDL-2	0.72	0.91	0.77
MDL-3	0.87	1.07	1.02
MDL-4	0.68	0.77	0.73
MDL-5	0.81	0.94	0.97
MDL-6	0.62	0.96	0.73
MDL-7	0.83	1.00	0.95
MDL-8	0.65	0.90	1.07
MDL-9	0.71	0.97	0.88
AVG	0.76	0.98	0.92
SD	0.11	0.14	0.15
MDL	**0.32**	**0.40**	**0.44**

Table 7. Results of the March 2007 laboratory inter comparison between UBC and HSPH for the analysis of TCP. Triplicate samples were spiked with 0, 5, 10, 15 ng of each TCP and approximately 3 ng (TCP) estimated from the reported TCP content in Mobil No.291 oil.

	5 ng TCP						10 ng TCP					
	Value	Value	Value	Mean	SD	%RE	Value	Value	Value	Mean	SD	%RE
HSPH T-o-CP	6.66	6.11	8.26	7.01	1.11	40.13	10.15	11.12	14.70	11.99	2.39	19.87
HSPH T-*m*-CP	6.57	6.44	6.42	6.47	0.08	29.47	10.32	11.41	11.71	11.14	0.73	11.43
HSPH T-*p*-CP	6.90	6.57	8.51	7.33	1.04	46.50	10.76	11.91	14.30	12.32	1.81	23.23
UBC T-*o*-CP	6.29	6.09	6.69	6.36	0.31	27.10	13.77	12.62	13	13.13	0.59	31.30
UBC T-*m*-CP	6.69	6.45	6.53	6.56	0.12	31.10	12.81	12.57	12.76	12.71	0.13	27.10
UBC T-*p*-CP	6.24	6.57	6.28	6.36	0.18	27.30	12.67	12.22	12.89	12.59		25.90
	15 ng TCP						0 ng TCP			Mobil oil no.291 (3 ng)		
	Value	Value	Value	Mean	SD	%RE	Value	Value	Value	Value	Value	Value
HSPH T-o-CP	17.28	19.03	18.99	18.43	1.00	22.88	0	0	0	0	0	0
HSPH T-m-CP	17.94	18.84	19.95	18.91	1.01	26.06	0	0	0	0	0	0
HSPH T-p-CP	17.03	18.08	18.15	17.75	0.63	18.33	0	0	0	0	0	0
UBC T-o-CP	18.67	20.02	19.58	19.42	0.69	29.50	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
UBC T-m-CP	18.36	19.89	19.77	19.34	0.85	28.90	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
UBC T-p-CP	18.46	20.17	19.7	19.44	0.88	29.60	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

% RE = (Measured - Expected) / Measured X 100, relative error is the measure of expected in terms of percentage bias of what is measured
Level at MDL is 5.0 ng/filter, 2 X MDL = 10 ng/filter and 3 X MDL = 15 ng/filter

Summary

The laboratory intercomparison results showed that both UBC and HSPH were able to detect the target analytes on spike filters. The analysis was performed on clean filters and not actual field samples, which would contain other material apart from the target analytes. The in-field duplicate testing described later in the report evaluates the laboratories' performance on actual environmental samples, which contain other compounds, which could interfere with the detection of the target analytes at these levels.

STORAGE STABILITY TESTING

Storage stability testing was performed to understand the stability of the target analytes on the sample media. The storage conditions tested were room temperature storage and refrigeration. These storage times were immediately after spiking, one week, two weeks and four weeks.

Methods

During the period from January 2007 through March 2007, HSPH conducted storage stability testing of samples spiked with *o*, *m*, *p*-tricresyl phosphates. There were seven sample sets, each with three samples spiked with 20 ng of the three TCP isomers. One set of samples was extracted immediately thereafter. Three of the remaining sets were refrigerated and the three other sets were stored at room temperature. One refrigerated set and one room temperature set were extracted after one week, two weeks and four weeks of storage. The storage stability test showed that samples can be stored at room temperature for a month after exposure and not suffer significant sample loss.

Results

The results of the storage stability are presented in Table 8.

Table 8. Results as % Recovery, of storage stability testing of MCE filters spiked with 20 ng of the three TCP isomers and extracted after 0, 1, 2 and 4 weeks. Half of the stored samples were stored at ambient temperature and half were stored under refrigeration.

		Day 0		Refrigerated, 1 Week		Ambient, 1 week		Refrigerated, 2 weeks		Ambient, 2 weeks		Refrigerated, 4 weeks		Ambient, 4 Weeks	
	Spike (ng)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T-o-CP	20	76.0	6.9	79.2	3.2	91.0	7.5	95.4	1.8	89.1	2.9	98.9	2.5	88.6	2.1
T-m-CP	20	77.3	9.5	84.8	2.1	96.0	5.1	100.4	2.6	92.9	3.1	103.0	1.9	85.2	1.3
T-p-CP	20	84.4	7.4	85.2	4.8	91.3	6.7	96.6	3.8	87.6	1.5	106.8	4.8	91.9	5.1

Summary

Spiked TCP analytes are stable (>85%) after one month at room temperature. However, recovery was slightly better with refrigeration at two and four weeks. Further, during actual sampling and shipping the samples may experience temperatures that deviate from typical room air conditions. Therefore, it is recommended that filters be shipped and stored under refrigeration.

ONBOARD DUPLICATE STUDY

The purpose of the onboard duplicate study was to determine the inter-laboratory variability of actual environmental samples. The sampling was conducted by team researchers. It began in February 2008 and was completed by April 2008.

Methods

The onboard duplicate study was administered through the University of Oregon. The goal of the project was to conduct an inter-laboratory analysis of actual in-flight samples. In the protocol, each sample would have its own pre-blank. A pre-blank was defined as a sample collected at the offices at the University of Oregon for two hours using the same sampler that was to be used for in-flight sampling, prior to be loaded with the actual in-flight sample filter. The purpose of these pre-blanking was to identify if any residual contamination remained in the sampler from previous use. The pre-blanks had 120 minutes of sampling, which means they were not actual blanks but samples collected outside of an onboard environment. The samplers were wiped down with alcohol wipes prior to pre-sampling and then again prior to being loaded with the in-flight sample. Samplers were sent in duplicate to researches (triplicate if a transportation blank was included) to take on prescheduled flights. The researchers collected the samples and returned them to the University of Oregon. Researchers were asked to keep a log indicating aircraft, sampling time, and to note any unusual events.

At the University of Oregon the duplicate samplers were split into two batches. One was sent to UBC and the other batch was sent to HSPH. HSPH sent 15 of its samples to the University of Medicine & Dentistry of New Jersey (UMDNJ) for analysis. These samples were sent blindly. The key to which sample was a pre-blank, actual sample or transportation blank was kept at the University of Oregon. The only notation on the sample was the sample number.

Results

Initial analysis of the duplicate samples HSPH did not detect any TCP isomers on any sample. Certain peaks that eluted at near the predicted retention time of the TCP isomers were observed. HSPH ran calibration standards (clean unused filters spiked with the three TCP isomers) at regular intervals which did not show the shift in retention times. Figure 2 and Figure 3 show a chromatogram of a sample with the retention time shift followed by a calibration standard showing no retention time shift.

Figure 2 and Figure 3 are chromatograms of a sample followed by a calibration standard showing the retention time shift between the actual samples and the associated calibration standards.

Figure 2. Sample 1156 (typical sample), QEDIT (peak quantifying software) view of T-*m*-CP's RT. Peak at 16.63 is too early. Red line is expected RT. The T-m-CP standard's retention time is 16.72.

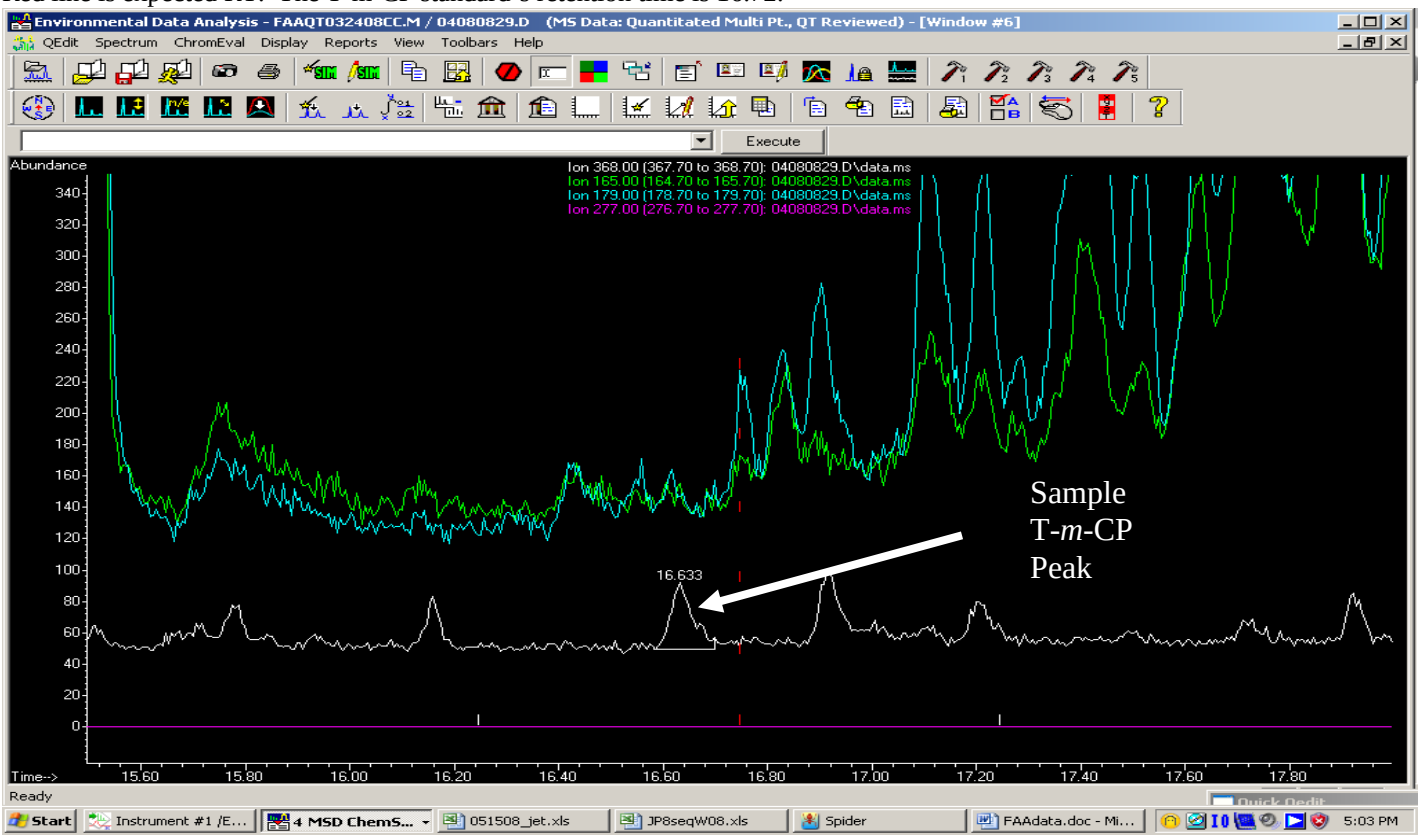
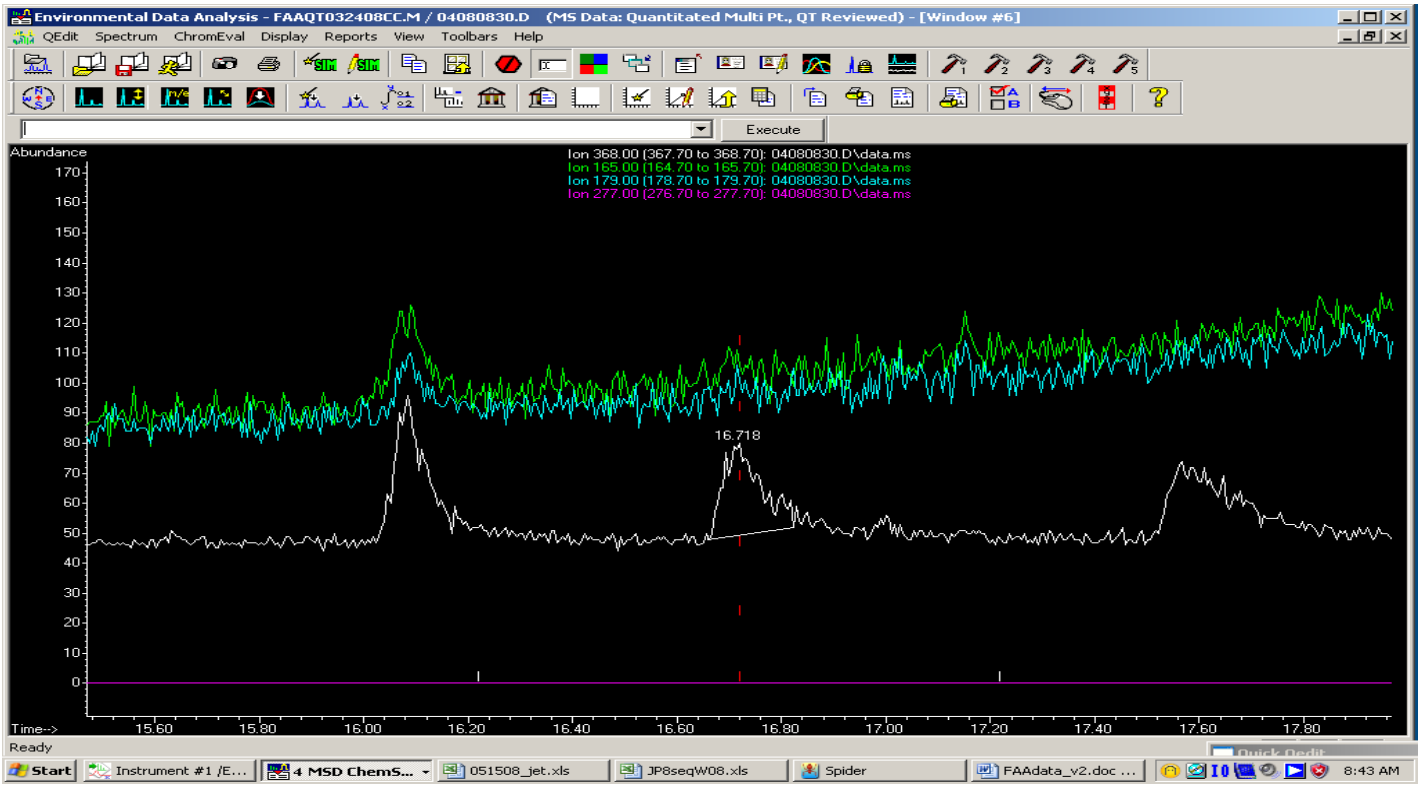


Figure 3. 2 ng/ml CCAL (next run after sample1156). QEDIT view of T-m-CP's RT. Peak's retention time is exactly at 16.72.



The analysis for TCP isomers is problematic in a number of ways. This resulted in the initial report of the duplicate study samples analyzed by HSPH to be non-detects (ND) for the three target isomers. After review and additional experimental analysis (see below), HSPH has determined that there were reportable amounts of some of the TCPs in some of the duplicate samples.

The initial report of NDs for all isomers in all samples stemmed from a slight shift in retention time (RT) in only the samples, not in any of the associated quality control (QC) runs. These QC runs included spike checks, extracted matrix spikes, and CCAL standards. In addition, in some samples it appeared that one isomer's RT shifted later and another isomer's RT shifted earlier. A RT shift of this nature is somewhat unusual and cannot be readily explained. So, though there were peaks close to the expected RTs, they were not quantitated because the CCALs throughout the sequence seemed to indicate the RTs for the target analytes were different.

Compounding the problem was the lack of qualifying ions in the spectra to confirm the target analytes. This appeared to be due to both the very small amount of TCP in the sample (very near the calculated MDL based on extraction of clean spiked filters) and the presence of large amounts of other organic compounds. The target ion for the TCPs has such low abundance that analysis was done in Select-Ion Monitoring (SIM) mode for maximum sensitivity. The target ion cannot be seen in full scan mode. However, scan mode does show the presence of other compounds such as phthalates in much greater amounts. These other compounds altered the retention time for the TCPs.

Standard addition was used to confirm the presence of TCPs in some of the samples. In standard addition, calibration standards are spiked onto actual samples rather than clean filters. The analysis of the standard addition samples for T-m-CP showed one peak with the slight RT shift (16.63) was observed rather than two peaks (16.63 and 16.72) as was observed if the standard and sample were analyzed separately. Also analyzed was another standard (92100 Fluka, Tricresyl phosphate technical, mixture of isomers), a mix of TCPs with two additional isomers. By comparing this standard to the samples, the pattern of the TCP isomer peaks was more easily recognizable and the RTs confirmed. This eliminated a confusion regarding RT shift for one peak early and another peak late.

To avoid these problems for future analyses, the use of an IS could be one option. A deuterium-labeled compound, spiked into each sample extract prior to analysis, should show RT shifts as well as take into account run-to-run instrument variability.

HSPH integrated all peaks that were discernable and reported values below the limit of detection (LOD); UBC only reported values above the LOD. In summary there were 31 usable sample pairs, i.e., samples in which UBC and HSPH each analyzed one sample of the pair. Here is a summary of our observations:

- No T-*p*-CP was detected in any sample by the two labs.
- UBC detected T-*o*-CP in every sample. This suggests contamination due to the high amounts detected in in-flight samples, transportation blanks and pre-blanks. The values for T-*o*-CP for UBC were voided because these levels appeared in both the control and the onboard samples in similar amounts, indicating contamination of the samples. The T-*o*-CP levels reported were 10 to 20 times higher than the detection limit. Once UBC had improved laboratory methods for a second round of sampling, none of the 71 blanks or actual samples reported T-*o*-CP.
- HSPH detected T-*o*-CP in one in-flight sample. This same in-flight sample had T-*m*-CP detected by UBC and not HSPH. The integration of the T-*m*-CP by HSPH on that sample was 0.39 ng which is just below the LOD.
- UBC and HSPH agreed that 23 of the sample pairs were below the limit of detection of 0.4 ng/filter for T-*m*-CP.
- UBC detected T-*m*-CP on 5 in-flight samples and 1 transport blank, of which 3 were also detected at or above 0.4 ng by HSPH. HSPH did not detect T-*m*-CP on the three other samples. HSPH did detect T-*m*-CP on 2 other in-flight samples, where UBC did not detect T-*m*-CP.

- UMDNJ did not detect TCP in any of their 15 samples. However, their samples were analyzed in scan mode which had detection limits higher than the amounts reported by the other two labs.

Detection Limits

The actual detection limit for a batch of samples is determined by the field blanks for that batch. Using the field blanks takes into account sampling artifacts associated with sample handling and transportation. To get the best estimate of the DL, each batch should have at least 5 field blanks and all blank peaks should be integrated (not truncated at the MDL), particularly in samples that were hand integrated as these samples were by both labs. The minimum detectable mass (MDM) for any sample would then be equal to the mean blank value plus 3 times the standard deviation of the blanks.

The individual blank sample correction that is applied to the filters using the pre-blank is used primarily in cases where the sample blank value increases predictably with temperature, as is the case with dinitrophenylhydrazine passive aldehyde samplers (where each sample, including blanks, is corrected by the laboratory by subtracting the value of an unexposed filter contained with in the housing of the sample). In all cases where this correction is performed on a per sample basis the MDM is still determined the same way as above:

For example J:

MDM(j) = Preblank(j) + Mean Transportation Blank value +3 Standard Deviation Blanks.

HSPH analyzed one transportation blank **(1)** in ng/filter and one sample **(2)** that was taken on a plane and not activated (equivalent to a transportation blank):

Sample No.	T-*o*-CP	T-*m*-CP	T-*p*-CP
(1) 1111	0	0.23	0
(2) 1093	0.345	0.345	0

UBC reported two transportation blanks [**(3)** and **(4)**]:

Sample No.	T-*o*-CP	T-*m*-CP	T-*p*-CP
(3) 1110	2.07	0.53	<0.40
(4) 1092	5.45	1.57	2.45

In addition, there are several samples 1171, 1172, 1173, 1176 and 1177 that are either transport (trip) blanks or samples that were taken on a plane and not activated. If the peaks associated with these samples are hand calculated, there should be a sufficient number of blanks from which to calculate the batch detection limit. We looked at the UBC data and some of these samples have visible peaks that were truncated.

Some T-*m*-CP was detected in some of the transport (trip) blanks and the highest amount of T-*m*-CP was detected in a transport (trip) blank (an unpaired sample analyzed by UBC only). Understanding the noise associated with the method is crucial to being able to distinguish between TCP in the cabin air and a sampling artifact. Even though the data suggests that some TCP was detected in the cabin air during duplicate sampling, without a detection limit calculated from the transportation blanks, it is difficult to quantify these low concentrations.

Table 9. Results of the in-flight duplicate sampling in ng per filter

UBC.desc	HSPH.desc	**UBC*, T-*o*-CP isomer**	**HSPH, T-*o*-CP isomer**	UBC, **T-*m*-CP** isomer	HSPH, **T-*m*-CP** isomer	**UBC, T-*p*-CP isomer**	**HSPH, T-*p*-CP isomer**
B757	B757	14.79	0.43	0.46	0.39	<0.40	0.00
pre blank to 1106	pre blank to 1107	2.56	0.14	<0.40	0.12	<0.40	0.00
B737-800	B737-800	3.25	0.00	0.79	0.62	<0.40	0.00
pre blank to 1108	pre blank to 1109	2.75	0.00	<0.40	0.14	<0.40	0.00
B737-800	B737-800	3.29	0.00	<0.40	0.18	<0.40	0.00
pre blank to 1110	pre blank to 1111	2.37	0.00	<0.40	0.23	<0.40	0.00
transport blank	transport blank	2.07	0.00	0.53	0.23	<0.40	0.00
pre blank to 1120	pre blank to 1121	2.85	0.00	<0.40	0.08	<0.40	0.00
B737-800	B737-800	3.57	0.00	0.7	0.40	<0.40	0.00
pre blank to 1122	pre blank to 1123	2	0.00	<0.40	0.00	<0.40	0.00
B737	B737	3.03	0.00	<0.40	0.14	<0.40	0.00
pre blank to 1124	pre blank to 1125	2.72	0.10	<0.40	0.24	<0.40	0.00
B737-800	B737-800	3.74	0.00	<0.40	0.15	<0.40	0.00
pre blank to 1126	pre blank to 1127	4	0.09	<0.40	0.38	<0.40	0.25
B737-800	B737-800	4.16	0.00	<0.40	0.43	<0.40	0.00
B757	B757	21	0.10	<0.40	0.22	<0.40	0.00
A319	A319	7.24	0.31	<0.40	0.54	<0.40	0.00
A320	A320	10.15	0.00	<0.40	0.12	<0.40	0.00
pre blank to 1141	pre blank to 1142	7.26	0.00	<0.40	0.00	<0.40	0.00
A320	A320	7.88	0.00	0.7	0.36	<0.40	0.00
Embraer 145	Embraer 145	7.5	0.14	<0.40	0.28	<0.40	0.00
Embraer 145	Embraer 145	7.14	0.10	1.16	1.01	<0.40	0.00
pre blank to 1157	pre blank to 1158	3.86	0.06	<0.40	0.13	<0.40	0.00
B737-300	B737-300	1.92	0.00	<0.40	0.28	<0.40	0.00
pre blank to 1159	pre blank to 1160	5.18	0.00	<0.40	0.14	<0.40	0.00
A319	A319	3.62	0.00	<0.40	0.19	<0.40	0.00
preblank to 1188	preblank to 1189	4.13	0.00	<0.40	0.12	<0.40	0.00
A320	A320	3.48	0.00	<0.40	0.13	<0.40	0.00
preblank to 1190	preblank to 1191	4.09	0.00	<0.40	0.08	<0.40	0.00
blank	blank	7.21	0.00	<0.40	0.00	<0.40	0.00
blank	blank	2.8	0.00	<0.40	0.00	<0.40	0.00

*These values were voided; contamination was suspected (see text).

CONCLUSIONS

Based on the results of the chemical analysis method reported here, we are able to detect TCP in loadings of greater than 0.4 ng/filter for three TCP isomers: T-*o*-CP, T-*m*-CP and T-*p*-CP. From our in-flight duplicate sampling results, we recommend requiring an internal standard with this analytical method, due to possible interference from other compounds collected on the sample filters that alter the retention time of the three TCP isomers. Our storage stability testing indicates TCPs sampled on filters are stable for periods of one month. It is recommended that the filters be stored cold after sampling to maximize the amount of analyte recovered. Trace amounts of TCP found in transportation blanks raises the LOD for air samples and introduces uncertainties in interpretation of the results. TCP was detected in approximately 18% of the in-flight samples (actual samples and field blanks), but no TCP was detected in an equal number of pre-blank samples. The pre-blank samples were 2-hour laboratory air samples collected using the same VN sampler

used in-flight. The filters in these lab blanks were removed from the sampler and analyzed to establish that the samples had no residual TCP prior to deployment in-flight. This suggests an aviation component to the TCP. To limit these uncertainties at least five transportation (field) blanks should be included in each batch of samples. Inter-laboratory collaborations showed comparable results and also helped advance method development. Finally the sampler tested proved convenient and unobtrusive for collecting onboard samples.

APPENDIX A
Method for the Detection of Tricresyl Phosphates by GC/MS

1. Sample Media

37 mm Mixed cellulose ester filters 0.8 μm pore
Pall GN-4 Metricel Membrane Filer P/N 64678
37 MM Teflo w/ring 2.0 μm pore PTFE Membrane, Gelman Laboratory (Pall) P/N R2PJ037
37 mm Quartz Filter, Whatman QM-A
Drain Disks (Whatman)

2. Sampling Instrument

Van-Netten Sampler
Flow rate 1 LPM (Calibration established in laboratory for each sampler using mass flow meter)

Sample Extraction

Solvent: Dichloromethane (DCM)

Extraction is done by sonication. Filters are placed inside a clean 8 ml vial with a teflon lined screw cap and 5-6 mls of (DCM are added, ensuring complete coverage of the filter. Vials are sonicated 30 minutes. Water in sonication bath is kept chilled with "blue-ice" packs. Extract is transferred by pasteur pipette to a clean 8 ml vial. The 5-6 mls of extract are placed under a gentle stream of nitrogen and reduced to dryness. A syringe is used to add 500 uls of Toluene to the vial and it is vortexed making sure the Toluene rinses the sides of the vial adequately. Then, an aliquot is withdrawn and placed into an insert in the analytical vial to be placed on the instrument.

4. Analytical Method

Samples are analyzed on an Agilent 6890N Gas Chromatograph with an attached 5975 Mass Spectrometer. The GC/MS is operated in Select Ion Monitoring (SIM) mode with Electron Impact Ionization (EI). Separation is by capillary column, an HP-5MS from Agilent. Column dimensions are 30m x 250 μm (id) x 0.25 μm (film thickness).

4.1 Control Information

Sample Inlet :	Agilent 6890 GC
Injection Source :	GC/ALS
Injection Location :	ALS
Detector :	MS Agilent 5975

4.2 Oven

Initial temp: 140 ºC (On) Maximum temp: 325 ºC
Initial time: 0.00 min Equilibration time: 0.50 min
Ramps:

#	Rate	Final temp	Final time
1	8.00	320 ºC	0.00
2	0.0(Off)		

Post temp: 0 ºC
Post time: 0.00 min
Run time: 22.50 min

REAR INLET (SPLIT/SPLITLESS)

Mode:	Splitless
Initial temp:	250 ºC (On)
Initial time:	0.00 min
Pressure:	12.78 psi
Purge flow:	50.0mL/min
Purge time:	2.00min
Total flow:	53.5 mL/min
Gas saver:	Off
Gas type:	Helium

COLUMN 1

Capillary Column	
Model Number:	Agilent 19091S-433
HP-5MS	5% Phenyl Methyl Siloxane
Max temperature:	325 ºC
Nominal length:	30.0 m
Nominal diameter:	250.00 µm
Nominal film thickness:	0.25 µm
Mode:	constant pressure
Pressure:	12.78 psi.
Nominal initial flow:	0.7 mL/min
Average velocity:	23 cm/sec
Inlet:	Back Inlet
Outlet:	MSD
Outlet pressure:	vacuum

THERMAL AUX 2

Use: MSD Transfer Line Heater
Description:
Initial temp: 280 ºC (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1		0.0 ºC	(Off)

POST RUN

Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

GC Injector

Back Injector:

Sample Washes	1
Sample Pumps	4
Injection Volume	5.00 microliters
Repeat Injection	4 times
Delay between repeats	0 seconds
Syringe Size	10.0 microliters
PreInj Solvent A Washes	1
PreInj Solvent B Washes	1
PostInj Solvent A Washes	2

PostInj Solvent B Washes	2
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes

4.3 Agilent 5975 MS Acquisition Parameters

4.3.1 General Information

Tune File:	atune.u
Acquistion Mode :	SIM

4.3.2 Agilent 5975 MS Information

Solvent Delay :	3.00 min
EM Absolute :	False
EM Offse:	0
Resulting EM Voltage :	2258.8

4.3.3 Agilent 5975 MS SIM Parameters

GROUP 1 TCP IONS

Group ID:	5
Resolution:	Low
Start Time:	16.80
Plot 1 Ion:	165.00
Ions/Dwell In Group	(Mass, Dwell) (Mass, Dwell)
	(165.00,100) (179.00,100)
	(277.00,100) (368.00,100)

4.3.4 Agilent 5975 MS Zones

MS Quad:	150 ºC maximum 200 ºC
MS Source :	230 ºC maximum 250 ºC

4.3.5 Agilent 5975 MS Tune Parameters

EMISSION:	34.610
ENERGY:	69.922
REPELLER:	34.814
IONFOCUS:	90.157
ENTRANCE_LE:	25.500
EMVOLTS:	1670.588
AMUGAIN:	1829.000
AMUOFFSET:	123.000
FILAMENT:	1.000
DCPOLARITY:	0.000
ENTLENSOFFS:	19.827
MASSGAIN:	-529.000
MASSOFFSET:	-40.000