B. INTRODUCTION

The Chloropicrin Manufacturers' Task Force contracted PTRL West to develop and validate a method to determine chloropicrin in air. The validated method is intended for use in ambient air monitoring for chloropicrin. The study was conducted under the US EPA guideline OCCSP 860.1340 and in compliance with Good Laboratory Practices (GLP) as stated in FIFRA 40 CFR Part 160. For the Study Protocol and Protocol Amendments 1, 2 and 3, see Appendix A, (Section H), of this report.

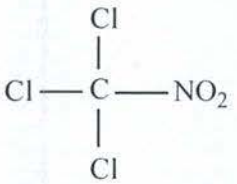
C. MATERIALS AND METHODS

The analytical method described below was used for the analysis of chloropicrin trapped from air using Xad-4 resin air sampling sorbent tubes. The method described traps chloropicrin from the vapor phase for a 48 hour trapping period with a flow rate of ~ 100 mL/min at ambient laboratory conditions. The method developed was an adaptation of that described in Reference 1, to convert the analysis detection technique from electron capture detection to mass spectroscopy as well as to increase the trapping capacity and lower the limit of quantitation.

C.1. Test Substance

Reference Item:	chloropicrin
Supplier:	Trinity Manufacturing, Inc.
Lot Number:	012-093A
PTRL West Inventory Number:	2269W-001
Purity:	99.59%
Expiration Date:	August 24th, 2016
Date Received:	July 19, 2012
Storage Location:	Room Temperature Cabinet
Storage Temp.:	Room Temperature

Structure:



Chloropicrin

For a Certificate of Analysis, See Appendix B (Section H of this report).

C.2. Solvents and Reagents

Ethyl acetate (EtOAc) was used as the extraction solvent, (HPLC grade or similar).

C.3. Equipment

Microliter syringes (Hamilton, various volumes)

Pipetors (Pipetman, Eppendorf), models capable of distributing volumes ranging from 20 µL to 200 µL and 0.10 mL to 1.0 mL

SKC personal air sampling pumps (model 224-44XR or 224-PPCXR4)

SKC DryCal DC-Lite (Bios) flowmeter

Tweezers

Tygon® tubing

Temperature/Relative Humidity Logger, (Dickson TP125)

Vials, 16 mL amber glass with PTFE lined screw caps

Vials, 12 mL amber glass with PTFE lined septum caps

Vials, 8 mL amber glass with PTFE lined septum caps

Volumetric Flasks (various volumes, class A)

Vortex mixer

Xad-4 sampling tubes (SKC, Inc., cat. No. 226-175, 8 mm O.D. x 150 mm L, 400 mg/200mg sorbent beads)

Xad-4 sampling tubes (SKC, Inc., cat. No. 092314-002, 10 mm O.D. x 156 mm L, 1400mg/250mg sorbent beads)

Glass manifold, capable of connecting at least 3 sorbent tubes to a single sampling pump

C.4. Preparation of Analytical Standard Solutions

C.4.1. Stock Solutions

Duplicate stock solutions of chloropicrin were made from the reference standard 2269W-001. For each stock solution, approximately 600 mL of chloropicrin was aliquoted into a tared 4 mL amber glass vial, with a septa cap, containing approximately 2 mL ethyl acetate (EtOAc). The vial was then re-weighed to determine the amount of chloropicrin added to the vial. The EtOAc was then transferred to a 10 mL volumetric flask by rinsing with

EtOAc and making to the mark. The weights were then purity corrected so as to calculate the correct concentration of the solution in mg's chloropicrin per mL of EtOAc. These solutions were designated stock solutions "A" and "B", respectively.

Stock Solution	Weight Standard Added (mg)	Purity	Final Volume (mL)	Concentration (mg/mL)
A	994.08	0.9959	10.0	99.00
B	998.69	0.9959	10.0	99.46

Stock A was used to create calibration standard solutions ranging in concentration from 0.50 ng/mL to 50 ng/mL in EtOAc as well as the fortifying solutions and stock B was used to create a check standard solution to confirm stock solution preparation accuracy. Stock solutions were re-prepared after 75 days with no apparent stability concern.

C.4.2. Fortification Solutions

A high level fortification solution was prepared by diluting the stock solution to a concentration of 1.0 mg/mL. In a 10 mL volumetric flask, 0.101 mL of the stock solution was added and brought to volume with EtOAc.

A mid level fortification solution was prepared by dilution of the high level fortification solution. In a 10 mL volumetric flask, 0.10 mL of the high fortification solution (1.0 mg/mL) was added and brought to volume with EtOAc. The resulting concentration was 10 µg/mL.

A low level fortification solution was prepared by dilution of the mid level fortification solution. In a 10 mL volumetric flask, 1.0 mL of the mid fortification solution (10 µg/mL) was added and brought to volume with EtOAc. The resulting concentration was 1.0 µg/mL.

The second stock solution "B" was diluted to a concentration within the calibration curve. In a 10 mL volumetric flask, 0.1005 mL of the stock solution, 99.46 mg/mL, was added and brought to volume with EtOAc to give a concentration of 1.0 mg/mL. This solution was diluted to 10 µg/mL by adding 0.10 mL to a 10 mL volumetric flask and making to the mark with EtOAc. Next, 0.10 mL was further diluted to 100 ng/mL by adding 0.10 mL to a 10 mL volumetric flask and bringing to the mark with ethyl acetate. Finally, a 10ng/mL solution was made by diluting 0.10 mL and adding 0.90 mL EtOAc in an autosampler vial with vortexing to mix.

Fortification solutions were stored in amber vials in a freezer at < 0°C when not in use.

C.4.3 Calibration standards

An intermediate standard solution of chloropicrin at a concentration of 100 ng/mL was prepared from the mid level fortification solution described in section C.4.2. In a 10 mL volumetric flask, 0.10 mL of the mid level fortification solution was added and brought to volume with EtOAc.

Calibration standards were prepared by diluting the low level fortification solution and the intermediate standard solutions or subsequent calibration solutions using EtOAc. Microliter syringes were used for transfers and volumetric flasks were used for measuring final volumes. Calibration standard solutions of chloropicrin were made as follows:

Intermediate Standard or Calibrant solution used	Volume Taken	Final Volume	Final Concentration
10 µg/mL	0.05 mL	10.0 mL	50 ng/mL
10 µg/mL	0.04 mL	10.0 mL	40 ng/mL
10 µg/mL	0.03 mL	10.0 mL	30 ng/mL
1.0 µg/mL	0.20 mL	10.0 mL	20 ng/mL
1.0 µg/mL	0.10 mL	10.0 mL	10 ng/mL
1.0 µg/mL	0.05 mL	10.0 mL	5.0 ng/mL
100 ng/mL	0.20 mL	10.0 mL	2.0 ng/mL
100 ng/mL	0.10 mL	10.0 mL	1.0 ng/mL
100 ng/mL	0.05 mL	10.0 mL	0.50 ng/mL
5.0 ng/mL	0.20 mL	10.0 mL	0.10 ng/mL
5.0 ng/mL	0.10 mL	10.0 mL	0.05 ng/mL

Calibration standard solutions were stored in amber glass vials, in a freezer, when not in use. Calibrant and fortification solutions were stable for at least 66 days of freezer storage as seen by consistent results from the extract stability assay, where day zero results using the first set of calibrants were comparable to the 2 month results using a new set of calibrants (made 46 days after the first set). The test substance solutions were protected from light as much as possible.

C.5. Sample Tube Preparation and Fortification – Method Validation

Trapping of chloropicrin from the vapor phase was accomplished using XAD-4 sorbent tubes (1400mg/250mg) in an air sampling train as shown in Figure 1. The trapping duration was for ~48 hours. The temperature and relative humidity was monitored during the 48 hour sampling time. Details of the trapping procedure follows:

1. Attached an SKC 224-44XR or SKC 224-PPCXR4 sample pump via Tygon® tubing to a glass manifold with 3 open ports (or to a "Y" connector for two control tubes).

2. For method validation, four pumps, three glass manifolds and one "Y" connector were used as follows:

   Two (2) control samples were connected to a pump with "Y" connector.

   Three (3) low level fortification sample tubes (30 ng chloropicrin) were connected to a pump with glass manifold.

   Three (3) mid level fortification sample tubes (300 ng chloropicrin) were connected to a pump with glass manifold.

   Three (3) high level fortification sample tubes (15,000 ng chloropicrin) were connected to a pump with glass manifold.

3. Xad-4 resin tubes were opened at each end of the glass tubes. Each tube contained 1400 mg of resin beads in front portion and 250 mg in the back portion as provided by SKC, Inc. as a custom order. The tubes were attached to the manifold ports (or the dual ports of a "Y" connector for controls) with Tygon® tubing. The tubes were aligned such that the smaller portion of resin beads (back portion) were closest to the glass manifold (ie the air flows through the front portion beads first). A flow adjustment needle valve was placed between the manifold and the resin tube to allow for fine adjustment of the airflow through the tube. The front portion of the sorbent tube was attached to a 500 mL flask for introduction of chloropicrin via a glass microliter syringe. Any ports on the manifolds that are not needed are closed with a plastic plug. Electrical tape was used to secure all connections. See also Figure 1.

4. The air sample pumps were turned on when fortifying the setup. The airflow to each tube was adjusted to ~100 mL/minute using the flow adjustment needle valve. A DryCal DC Lite flow meter was used to monitor the flow with the flow meter attached to the 500 mL flask at the flask inlet.

5. The flow rate was recorded (beginning) for each tube and the flow meter was disconnected.

6. While the pump was running, a known mass of chloropicrin (in either 15 or 30 µL of EtOAc solvent) was introduced to the apparatus by microliter syringe by injecting the solution into the trapping flask inlet. Tube fortifications for method validation were conducted as follows:

Fortification Level	Concentration of Soln. Used	Volume spiked	chloropicrin added to tube (µg)
LOQ (30 ng)	1.0 µg/mL	30 µL	30 ng
10X LOQ (300 ng)	10 µg/mL	30 µL	300 ng
500X LOQ (15,000 ng)	1.0 mg/mL	15 µL	15,000 ng

7. Air was drawn through the tube for ~ 48 hours. The air flow was measured at the end of each day and the beginning of each day and record and adjusted as necessary.

8. Just prior to the end of the sampling period, the air flow rates through each tube were measured and recorded as before.

9. After the appropriate trapping interval, the pumps were turned off and the sorbent tubes removed for extraction of the sorbent beads. Extraction was done on the same day as the end of the trapping period.

C.6. Sample Tube Preparation and Fortification – Storage Stability

The storage stability samples were prepared by spiking the sorbent tubes directly. Air was drawn through the tubes while spiking directly onto the front sorbent bed. After a brief drying period (approximately 1-2 minutes), the sorbent tubes were removed from the air flow, capped with plastic caps and stored in a freezer.

Set-up instructions for sample tubes:

1. Attached an SKC 224-44XR or SKC 224-PPCXR4 sample pump via Tygon® tubing to a glass manifold with 2 open ports for two storage stability tubes.

2. For storage stability, one control and two fortification tubes were set up for each time point plus one additional time point, (0 day, 8 day, 14 day, 28 day) as follows:

   For control tubes, the ends were opened then capped and stored frozen until needed for extraction.

   The storage stability tubes are fortified at the mid fortification level (300 ng chloropicrin per tube).

3. The Xad-4 resin tubes (SKC, Inc, Xad-4 1400mg front/250 mg back) were opened at each end. The tubes were attached to the manifold ports with Tygon® tubing. The tubes were aligned such that air flow goes through the front portion (1400 mg) of Xad-4 resin first. A flow adjustment needle valve was placed between the manifold and the resin tube to allow for fine adjustment of the airflow through the tube. Any ports on the manifolds that were not needed are closed with a plastic plug. Electrical tape was used to secure all connections.

4. The air sample pumps were turned on. The airflow to each tube was adjusted to ~100 mL/minute using the flow adjustment needle valve and a DryCal DC Lite flow meter to monitor the air flow.

5. While the pump was running, 300 ng of chloropicrin (in 30 µL of EtOAc solvent) was added by microliter syringe by injecting the solution into the front portion of the sorbent media of the sample tube. Tube fortifications for storage stability were conducted as follows:

Fortification Level	Concentration of Soln. Used	Volume spiked	chloropicrin added to tube (µg)
Control level	0	0	0
10X LOQ (300 ng)	10 µg/mL	30 µL	300 ng

6. Air was drawn through the tube for 2-3 minutes.

7. After the brief drying period (approximately 1-2 minutes), the tubes were removed from the setup and the ends capped with the provided plastic caps. The tubes were then stored in a freezer until needed for extraction.

C.7. Extraction of chloropicrin from Xad-4 Sorbent Tubes

1. 16 mL, 12 mL and 8 mL amber glass vials were filled with 10 mL, 10 mL and 5 mL of EtOAc, respectively, and then chilled in a refrigerator.
2. The caps on a sorbent tube were removed and the front-end glass wool was transferred to the chilled 16 mL amber glass vial containing 10 mL EtOAc followed by the front side sorbent beads. The vial was designated as the "front" extract. Set controls and forts used the 12 mL vials from above and were extracted as above.
3. The back side glass wool was removed and discarded. The back side sorbent beads were transferred to the chilled 8 mL amber glass vial containing 5 mL EtOAc. The vial was designated as the "back" extract.
4. The vials were Capped immediately with Teflon® lined caps and vortexed to mix.
5. The vials were shaken on a wrist action shaker for ~1 hour.
6. Supernatants were transferred to 8 mL amber glass storage vials and designated the "final extract" (front or back as appropriate). The final extracts were aliquoted to the GC autosampler vials for analysis directly (controls and low level forts, and all back sample extracts) or after dilution with EtOAc. Dilutions were as follows:

   Low level front extracts: no dilution required

   Mid level front extracts: 10X (0.10 mL extract + 0.90 mL EtOAc) or 5X (0.20 mL extract + 0.80 mL EtOAc)

   High level front extracts: 500X (serial dilution; 0.1 mL diluted to 10 mL, followed by 0.20 mL of dilution + 0.80 mL EtOAc)

7. Sample "final extracts" were stored in the 8 mL amber glass storage vials in a freezer.

Analyze samples by GC-MS/MS.

Sample extracts may be stored in a freezer prior to analysis for at least 66 days, the length of time between initial extraction and the 2 month re-analysis.

C.8. Concurrent Fortification Samples (Storage Stability Sets)

With the storage stability sample sets, two concurrent fortification samples (also referred to as "set forts" in the spreadsheets) plus a set control were prepared. These samples consisted of the front portion of Xad-4 sample tubes placed in 16 mL extraction vials, containing 10 mL of chilled EtOAc, spiked with 30 µL of a 10 µg/mL chloropicrin solution, and extracted as described in section C.7. The concurrent fortification samples represent 10X LOQ samples without the air trapping component, or extraction efficiency samples. After extraction the supernatant was diluted as in section C.7, step 6, and stored in a freezer.

C.9. GC-MS/MS Analytical Method

Gas Chromatography with Triple Quadrapole Mass Spectrometer (GC – QQQ #1)

C.9.1. Components

GC	Agilent series 7890A Gas-Liquid Chromatograph
Detector	Agilent series 7000B GC-MS/MS triple quad mass spectrometer
Autosampler	ATLAS Combi-PAL auto sampler
Data System	Agilent Technologies MassHunter software (Data acquisition B.06.01.13.12, Quantitative Analysis version B.05.02)

C.9.2. Parameters

Column	DB-624 (30m x 0.25 mm x 1.40µm)
Injector Temperature	150°C
Transfer Temperature	200°C
Carrier Gas	Helium
Column Head Pressure	8.07 psi
He Carrier Gas	2.25 mL/minute
N_2 Collision Gas	1.5 mL/minute
Temperature program	Initial Temperature: 50°C hold for 1 min Ramp 15°C/minute to 140°C, no hold, Ramp 30°C/minute to 200°C
Injection volume	2 µL
Injection mode	pulsed splitless (25 psi, 1 minute)
GC liner	Double gooseneck (inert)

Ionization Source	Electron impact (+), 30 ms dwell, 25 volt collision energy
Multiple Reaction Monitoring (MRM)	Quantitation transition: m/z 117 to 82 Confirmation transition: m/z 119 to 84
Source Temperature	230°C
Retention Time	Approximately 6.2 minutes
Run time	9 minutes

A typical injection sequence included a solvent blank (EtOAc), followed by the calibration standard solutions (0.50 to 20 or 50 ng/mL). Following the calibration standard solutions were an EtOAc blank, a 5.0 ng/mL QC standard, followed with the two set control samples and two control sample, front and back extracts. The controls were followed by a 5.0 ng/mL QC standard then the five low set fortification repetitions and the low fortification sample repetitions (front extracts only). The low fort samples were followed by another 5.0 ng/mL QC standard. The sequence continued similarly with the mid fortification samples then the high fortification samples, with 5.0 ng/mL QC standard injections following each fortification level. Finally the low, mid and high fortification sample back extracts were injected with a closing QC as the final injection. The QC injections were not used in establishing the linearity curve or to quantify the samples, but were included to assess the stability of the detector response over the course of the injection sequence.

C.10. Methods of Calculation

C.10.1. Standards

$$\text{Volume of solvent (mL)} = \frac{(W)}{(FC)} \times \frac{P}{100}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

C.10.2. Recoveries

The recoveries of chloropicrin from fortified Xad-4 sample tubes were calculated as follows:

Quadratic equation formula from calibration curve $y = ax^2 + bx + c$ for chloropicrin with 1/x weighting factor (generated with MassHunter® software)

where x = concentration (ng/mL)

y = Sample peak area

a = quadratic coefficient ($a \neq 0$)

b = linear coefficient

c = constant (y-intercept)

ng chloropicrin Detected = calc. conc.(ng/mL) x dilution factor x volume of extraction solvent (mL)

Percent Recovery = [ng chloropicrin detected ÷ Fortification Level (ng)] x 100

Two ion transitions were monitored by MRM (multiple reaction monitoring). Each ion transition was used separately to determine recoveries of chloropicrin, and each was used separately to quantify the recoveries from the method validation. The transitions were from parent ion (chloropicrin of m/z of 164) losing its nitro group to leave a positive ion of m/z of 117 (or 119 for chlorine isotope C_{37}). These ions then fragmented further to either m/z of 82 or 84, respectively (loss of chlorine).

C.11. Statistical Methods

The statistical methods employed in this study include: averages, standard deviations, relative standard deviations, and quadratic equations with 1/x weighting factors.

C.12. Time Required for Completion of a Sample Set

A sample set consisted of a reagent blank, two controls, and 9 fortified samples (3 repetitions at each of three fortification levels). Time required from sorbent tube extraction until the completion of instrumental analysis and data evaluation was as follows:

- Sample preparation, one anayst approximately 6 hours
- GC/MS/MS analysis and data processing (two MS/MS transitions) approximately 6 hours (includes overnight automated sample analysis).
- Additional time was required for air trapping portion (~2 days).

TOTAL = approximately 12 hours for one analyst to complete a set (approximately one a one half calendar days) or 24 hours (2 calendar days) including overnight automated instrument analysis.

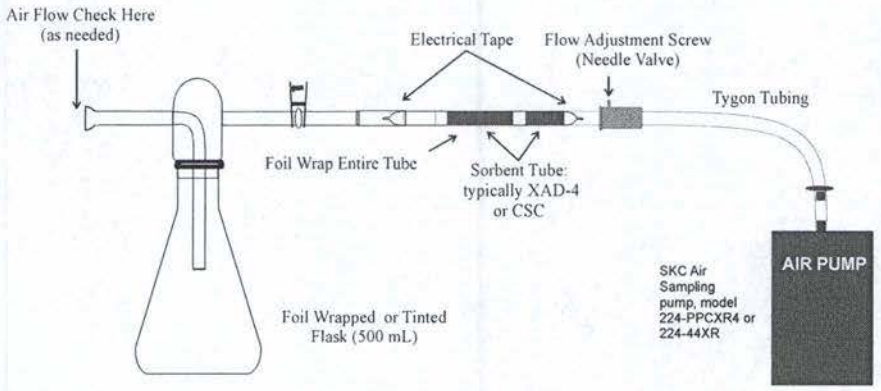
Additional time, ~ 48 hours, was required when preparing trapping efficiency samples prior to sorbent tube extraction.

C.13. Protocol Amendments

The protocol was amended three times for this study. The first amendment identified the test/reference substance to be used and added GLP certification to be conducted at PTRL West. The second amendment allowed for larger capacity sorbent tubes, extended the trapping interval to 48 hours and set the limit of quantitation to 30 ng. The third amendment removes the vapor phase trapping efficiency from the experiment, allowing for a more direct assessment of the stability of the analyte on the sorbent media. The protocol and protocol amendments are provided in Appendix A.

G. FIGURES

Figure 1. Air Trapping Apparatus



Note: a manifold is placed between needle valve and air pump to allow for multiple sample trapping trains ($n \leq 3$) on a single pump.