The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) Study

Extracting and Preparing Urine Samples for Analysis of 3,5,6-Trichloro-2-Pyridinol

Battelle
Columbus, OH 43201
Contract No. 68-D-99-011

Standard Operating Procedure **CTEPP-SOP-5.22**

Title: Extracting and Preparing Urine Samples for Analysis of 3,5,6-Trichloro-2-Pyridinol

Source: Battelle

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Exposure Measurements & Analysis Branch

STANDARD OPERATING PROCEDURE (SOP) FOR EXTRACTING AND PREPARING URINE SAMPLES FOR ANALYSIS OF 3,5,6-TRICHLORO-2-PYRIDINOL

Prepared by: _____ Date: _____

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1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for extracting and preparing urine samples for analysis of 3,5,6-trichloro-2-pyridinol (3,5,6-TCP).

2.0 Summary of Method

The method for extracting and preparing urine samples for analysis of 3,5,6-TCP is summarized in this SOP. The method covers the hydrolysis, extraction and derivatization of the sample that is to be analyzed by gas chromatography/mass spectrometry. *Note: This method is also used to extract and prepare urine samples for the analysis of 2-isopropyl-4-methyl-6-hydroxypyrimidine but the recoveries are usually less than 10%.*

3.0 Definition

3.1 TCP: 3,5,6- Trichloro-2-pyridinol, the target analyte.

3.2 IMP: 2-Isopropyl-4-methyl-6-hydroxypyrimidine, the target analyte.

3.3 Internal Standard (IS): The compounds that are added to sample extracts just prior to GC/MS analysis. The ratio of the detector signal of the native analyte to the detector signal of the corresponding IS is compared to ratios obtained for calibration curve solutions where the IS level remains fixed and the native analyte levels vary. The IS is used to correct for minor run-to-run differences in GC injection, chromatographic behavior, and MS ionization efficiency.

4.0 Cautions

Standard laboratory protective clothing, gloves, and eye covering is required.

5.0 Responsibilities

5.1 The project staff who performs the sample extractions will be responsible for obtaining samples from the sample coordinator, entering relevant information in the extraction/preparation laboratory record books, and sending final extracts for analyses.

5.2 The CTEPP Laboratory Team Leader (LTL), the QA Officer, and Task Order Leader (TOL) will oversee the sample extraction operation and ensure that SOPs are followed by all project staff.

6.0 Apparatus, Materials, and Reagents

6.1 Apparatus and materials

6.1.1 Microliter syringes in volumes of 1000, 100, 50, and 10 uL

6.1.2 Eppendorf Reference adjustable pipettors in ranges of 10 to 100 and 100 to 1000 uL

6.1.3 Graduated cylinders of volume 10 ml

6.1.4 Digital laboratory timer

6.1.5 Thermometers to read at range of 50° to 100° degrees C

6.1.6 Sample vials with Teflon-lined screw caps; muffled

6.1.7 Oven (Blue-M or comparable)

6.1.8 Disposal centrifuge tubes

6.1.9 Vortex mixer (American Scientific Products)

6.1.10 Pasteur glass pipettes (muffled and stored in clean glass jars)

6.1.11 Silylated GC vials, standard HP autosampler vials with Teflon septa

6.1.12 Glass funnels (muffled)

6.1.13 Centrifuges

6.2 Reagents

6.2.1 Hydrochloric acid, 36-38%, J.T. Baker analytical reagent grade

6..2.2 Labeled TCP, Carbon-13 and Nitrogen-15 isotopes. Synthesized by Dow-Elanco, reference number B-844-167A

6.2.3 Internal Standard Spiking Solution ($^{13}C^{15}N$-TCP)

6.2.4 Matrix Standard Spiking Solution (3,5,6-TCP and IMP)

6.2.5 Sodium Chloride, 20% solution

6.2.6 Sodium Sulfate anhydride, J.T. Baker analytical reagent grade

6.2.7 Chlorobutane

6.2.9 N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), Pierce Chemical company

7.0 Procedure

7.1 Sample Preparation.

7.1.1 Samples are removed from the freezer and thawed to room temperature.

7.1.2 An Eppendorf Reference Pipettor is set at 1000 µL and fitted with a disposable tip.

7.1.3 A 1000 µL aliquot of the sample is transferred with the Eppendorf to a 2-dram vial. For matrix spike sample, a 50 µL aliquot of matrix spike standard solution is spiked into the urine sample.

7.1.4 Any unused sample is returned to the custodian freezer.

7.2 Hydrolysis of Samples.

7.2.1 An Eppendorf Reference Pipettor is set at 100 µL and fitted with a disposable tip.

7.2.2 Each sample vial is spiked with 100 µL of concentrated hydrochloric acid using the Eppendorf Pipettor.

7.2.3 The reaction vial is tightly capped, placed in a metal rack, and the rack placed in the constant temperature cabinet at about 80°C. The exact temperature and starting time are noted in a laboratory record book (LRB).

7.2.4 The rack and vials are removed after one hour heating time has elapsed. The exact temperature and ending time are noted in the LRB.

7.3 Preparing Sample for GC/MS analysis.

7.3.1 The reaction vials are allowed to cool to room temperature.

7.3.2 Allow the sample to cool to room temperature, add 1 mL of a 20% aqueous solution of sodium chloride, 10 µL of the internal standard solution, and 1 mL of chlorobutane to each vial.

7.3.3 Vortex each vial for approximately 10 minutes and centrifuge the sample vial for approximately 10 minutes.

7.3.4 Transfer approximately 800 µL of chlorobutane extract to a centrifuge tube or a clean vial and add small amount (~0.5 g) of sodium sulfate anhydride to the chlorobutane extract, vortex the samples for about 30 seconds and transfer the chlorobutane extract to a GC vial .

7.3.5 Spike 100 µL of MTBSTFA into each sample and seal the sample vial with a Teflon septum.

7.3.6 Heat the samples in an oven at about 70°C for one hour. The exact temperature and starting time are noted in the LRB.

7.3.7 The GC vial is removed from the heater after one hour time has elapsed. The exact temperature and ending time are noted in the LRB.

7.3.8 The GC vial is stored in freezer at $\leq$-10°C until ready for GC/MS analysis.

8.0 Records

8.1 Records of the field blank levels and field spike recovery values will be retained in a project laboratory record book that is kept in the extraction laboratory. This record book will serve as a continuing file for reference on expected performance of the methods and likely contaminant levels that will arise as a result of field handling. These samples will be identified in the laboratory record book by field sample ID and the assigned laboratory analysis number (a unique number that combines the 5 digit laboratory book number-2 digit page number-2 digit line number), the date of extraction, the lot number of solvents used for extraction, and surrogate recovery values.

8.2 The record of the extraction of samples will be maintained in a project laboratory notebook that is kept in the extraction laboratory. This record book will contain the field sample ID, the assigned laboratory analysis number (see above), the date of extraction, and the lot number of solvent used for extraction. The record book will be retained in the

laboratory where these operations are performed until the conclusion of the study and will be archived in a secure room for three years after completion of the study.

9.0 Quality Control and Quality Assurance

9.1 A field blank and laboratory method blank consists of a urine sample vial that will be extracted together with the field samples. The field blank analyses are performed to verify that minimal contamination occurs through sample handling during shipping and field operations. The laboratory method blank analyses are performed to verify that minimal contamination occurs through sample preparation. The laboratory fortified blank analyses are performed to verify the recoveries of analytes preparation procedures.

9.2 Field crews will be reminded to wear clean clothing and shoes, to remove all pesticide products from their residences that may contain these analytes, and to refrain from using these materials while part of the field crew. Field crews will also be reminded to obtain clean clothing after visiting a home where they know or suspect that these pesticides have been applied within the previous week. Cigarette smoking is not permitted during the field sampling. Field crews should store the samples in a clean environment away from any known combustion sources.

9.3 Matrix spike recovery values of 50-150% for 3,5,6-TCP in blanks, and actual samples will be deemed acceptable, and no correction to the data will be made. *Note: The recovery values for IMP are expected to be low and usually ranged from 0 to 10%.* For recoveries less than 50% or greater than 150%, the data will be flagged. For recoveries greater than 130%, the concentration of the matrix spiking solution will be checked against a calibration curve to determine whether inadvertent solvent loss has resulted in higher spike levels. If this has occurred, the matrix spiking solution will be re-prepared and re-analyzed.

9.4 One laboratory method blank that is analyzed as a sample concurrently with a field sample set will be analyzed for every 50 samples processed. If significant target analyte levels (>0.01 μg) are found in the field blanks, the source of contamination must be identified and more laboratory blanks, together with additional field blanks, trip blanks, and storage blanks, will be analyzed.

10.0 Reference

10.1 J. C. Chuang, M. Brinkman, and Y. L. Chou, "Analysis of Multimedia Samples and Food Data." Final Report for Contract No. 68-D4-0023, Work Assignment 3-11, 1998.

10.2 J. C. Chuang, J. M. Durnford, M. A. Pollard, D. B. Davis, R. Jones, C. Hines, and J. K. Finegold, "Development and Evaluation of Immunochemical Methods for Environmental Monitoring and Human Exposure Assessment" Final Report for Contract No. 68-D4-0023, Work Assignment 3-03, 1998.