BUTYLTIN TRICHLORIDE in Urine 8320

Formula: $C_4H_9Cl_3Sn$ MW: 282.18 CAS: 1118-46-3 RTECS: WH6780000

METHOD: 8320, Issue 1 **EVALUATION:** FULL **Issue 1:** 10 January 2019

OSHA: N/A
NIOSH REL: N/A
OTHER OELs: Because exposure limits and guidelines may change over time, NIOSH recommends references [1-3] for updated limits and guidelines.

PROPERTIES: Colorless liquid; MP -63 ˚C; BP 93 ˚C @ 10 mmHg; Density - 1.693 g/mL @ 25 ˚C; VP 0.083 mmHg @ 20 ˚C [4]

SYNONYMS: butyltrichlorotin, butyltrichlorostannane, monobutyltin trichloride (MBTC)

SAMPLING

SPECIMEN: Spot urine specimens (before and after exposure; end of shift or end of work week) [5]

VOLUME: 15 mL minimum

PRESERVATIVE: None

SHIPMENT: Freeze urine: ship frozen in an insulated container

SAMPLE STABILITY: Stable at least 300 days at -20 ºC [6]

ACCURACY [6,7]

RANGE STUDIED: 0.12-58 µg/5-mL sample

BIAS: 0.063

OVERALL PRECISION ($\hat{S}_{rT}$): 0.041

ACCURACY: ± 13.0%

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY/MASS SPECTROMETRY-SELECTED ION MONITORING (GC/MS-SIM)

ANALYTE: Derivative of butyltin trichloride: butyltriethyltin

EXTRACTION: Liquid-liquid extraction

DERIVATIZATION: Grignard reaction with ethylmagnesium bromide

INJECTION VOLUME: 2 µL

TEMPERATURE:
- **INJECTION:** 230 ºC splitless for 0.75 min
- **DETECTOR:** 285 ºC
- **OVEN:** 75 ºC (1 min); 75 ºC to 225 ºC @ 12 ºC/min; hold 5 min

MASS SELECTIVE DETECTOR PARAMETERS:
- **SIM IONS (*m/z*):** 178.9, 234.9
- **DWELL TIME:** 200 msec per *m/z*

CARRIER GAS: Helium at 1.3 mL/min

COLUMN: Capillary, fused silica, phenyl arylene polymer virtually equivalent to (5% phenyl)-methylpolysiloxane (30 m x 0.32 mm ID, 0.5 µm film)

CALIBRATION: Spikes of known concentrations prepared in pooled urine

ESTIMATED LOD: 0.01 µg/5-mL sample [6]

APPLICABILITY: This method can be used for the Determination of butyltin trichloride in urine samples. It may also be useful for other butyltin chloride compounds. See Evaluation of Method section for more information.

INTERFERENCES: Because of the separation and mass-selective capabilities of GC/MS, interferences are not anticipated to have any impact on the analysis.

OTHER METHODS: None

REAGENTS:

1. Butyltin trichloride, ≥95% purity
2. Ethylmagnesium bromide, 1.0 M solution in tetrahydrofuran
3. Helium, UHP or Grade 5 (99.999%) or better
4. Sulfuric acid, 1 M: Dilute 5.6 mL conc. sulfuric acid to 100 mL deionized water
5. Citric acid/sodium citrate buffer (~pH 2.3): Dissolve 20.554 g citric acid monohydrate and 0.652 g sodium citrate dihydrate in 1 L deionized water
6. Toluene, ACS reagent grade or better
7. Tropolone extraction solution: 0.1% in toluene: Dissolve 100 mg tropolone (2-hydroxy-2, 4, 6-cycloheptatrienone, CAS# [533-75-5]) in 100 mL toluene
8. Alumina, acidic, Brockman activity I, 80-200 mesh
9. Sodium sulfate, granular, anhydrous; reagent grade or better

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Centrifuge tubes, 15-mL polypropylene with screw caps
2. Centrifuge, capable of at least 2400 RPM
3. Pipettes, Pasteur, 14.6 cm (5¾")
4. Culture tubes, glass, ~8-mL (13 mm x 100 mm), screw top with PTFE-lined caps
5. GC-MS capable of selected ion monitoring, column, autosampler and data collection system (p. 8320-1)
6. Microliter syringes
7. Volumetric flasks, glass, 5-mL
8. Pipettor, adjustable from 1-5 mL
9. Vortex mixer
10. Tumbler for centrifuge tubes, approximately 20 RPM
11. Bottles, polyethylene, 30- or 125-mL
12. Glass wool

SPECIAL PRECAUTIONS: Wear gloves, lab coat, and safety glasses while handling all chemicals and human urine products. Disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact urine should be placed in a biohazard bag. Standard precautions should always be used when handling bodily fluids and/or extracts of bodily fluids [8]. Handle urine samples and urine extracts using proper gloves. All work should be performed in a fume hood.

SAMPLING:

1. Collect urine in a 30-mL or 125-mL polyethylene bottle or other appropriate container.
   NOTE: Collect two urine specimens for each worker, one prior to exposure and one after exposure. The NIOSH Health Hazard Evaluation found no significant difference between end of shift and end of work week [5]. Also, collect and pool urine from unexposed subjects for use as a standard and quality control sample matrix.
2. Freeze the urine (-20 °C or lower) and ship frozen in an insulated container.

SAMPLE PREPARATION:

3. Allow urine to reach room temperature and mix thoroughly.
4. Place 5 mL of the urine specimen in a 15-mL polypropylene centrifuge tube using a pipettor.
5. To the urine specimen, add:
    a. 3 mL of ~pH 2.3 citric acid/sodium citrate buffer
    b. about 350 mg anhydrous sodium sulfate (to break up emulsions)
    c. 1.5 mL 0.1% tropolone in toluene solution
6. Cap and tumble for one hour at ~20 RPM.
7. Centrifuge for at least 10 min at 2400 RPM (1050 G).
8. Transfer the upper toluene layer to an 8-mL glass culture tube with a PTFE-lined cap using a Pasteur pipette.
9. Repeat steps 5c through 8 and combine the extracts.
10. Add about 350 mg anhydrous sodium sulfate to the combined extracts to remove any of the aqueous phase that may have been inadvertently transferred.
11. Decant the dried sample from step 10 into a second 8-mL glass culture tube with a PTFE-lined cap.
12. Rinse the sodium sulfate residue in the first tube twice with a small (~300 µL) quantity of toluene which is then combined with the dried extract in the second tube.
13. Add 250 µL of the ethylmagnesium bromide solution to the extract, mix, and allow the mixture to react for 15-20 minutes.
14. Add 1 mL of 1 M sulfuric acid (to react with the remaining ethylmagnesium bromide) and vortex the tube for approximately 30 seconds.
15. Prepare a clean-up column in a 14.6 cm disposable Pasteur pipette by adding, in order, 1) a glass wool plug, 2) alumina, acidic, Brockmann activity I, 80-200 mesh, enough to form a column of approximately 1 cm, and 3) ~100 mg of anhydrous sodium sulfate.
16. After the layers separate, elute the top toluene layer through this clean-up column.
17. Collect the eluate in a 5-mL volumetric flask.
18. Rinse the tube with the sulfuric acid solution (Step 14) several times (a minimum of two) with small amounts (~300 µL) of toluene and elute the rinses through the clean-up column.
19. Flush the remaining sample from the column by adding small portions (~300 µL, a minimum of twice) of toluene to the top of the column.
20. Adjust the final volume to 5.0 mL with toluene as needed.
21. Transfer an aliquot of the extract to a GC vial for analysis.

CALIBRATION AND QUALITY CONTROL:

22. Calibrate daily with at least six working standards covering the expected concentration range of the samples. Suggested working standard concentration range is 0.007-150 µg/5 mL [6].
    a. Prepare each working standard by spiking a known amount of the butyltin trichloride stock solution into enough pooled urine to make a total of 5.0 mL.
    b. Prepare at least one pooled urine blank by transferring 5.0 mL of pooled urine (the same pooled urine used for creating the working standards) into a culture tube.
    c. Prepare quality control (QC) samples by spiking known amounts of a separately prepared butyltin trichloride stock solution into pooled urine (the same pooled urine used for creating the working standards and blanks). It is recommended that two QC levels be prepared; a lower level at 3-5X the limit of quantitation (LOQ) and a higher level nearer the top end of the calibration curve. Run enough QC samples to be greater than 5% of the sample set. QC values should normally be within ±20% of the spiked values. If not, the batch is considered out of control, the batch data discarded, and corrective actions taken before more specimens are analyzed.
23. Process each working standard, blank, and QC sample using the same procedure as for the samples (steps 3 through 21).

24. Prepare calibration curves by plotting peak area (or peak height, although area is recommended) for the standards on the y-axis vs. concentration of analyte on the x-axis. The standard curve should have a coefficient of determination (r2) of equal to or greater than 0.98 to be acceptable for use. Furthermore, when each standard is plugged back into the calibration equation, the value should be within ±20% of the expected.

MEASUREMENT:

25. Set the gas chromatograph according to manufacturer's recommendations and to conditions given on the front page of the method. With the chromatographic conditions outlined on page 1, the retention time of the butyltin trichloride derivative (butyltriethyltin) was 7.3 min. A sample chromatogram is shown in Figure 1.
26. Set the mass spectrometer according to manufacturer's recommendations and to conditions given on the front page of the method. The mass spectrum for the butyltriethyltin can be found in the backup data report [6]. The most abundant isotopic mass of this compound is 264. The monitored ions are M-85 and M-29.
27. Inject each sample, working standard, blank, and QC sample.
28. Measure peak area or peak height in each chromatogram.
    NOTE: If the sample peak area or height is greater than that of the highest standard, dilute the sample extract with toluene and reanalyze. Apply the appropriate dilution factor in the calculations.

CALCULATION OF ANALYTE PER SAMPLE:

29. Determine mass of analyte in µg per 5-mL sample using the calibration curve prepared in step 24. Apply any dilution factor if needed.
    NOTE: If the creatinine value of the sample was obtained, the concentration could be reported as µg/g creatinine if desired. The mass of analyte in µg/L can be calculated by multiplying the result by 200. The result can also be expressed in µg tin/volume by multiplying the µg per 5-mL sample or µg/L by the butyltin trichloride conversion factor (%tin/mole) of 0.4207.

EVALUATION OF METHOD:

The first procedure considered for this method was a modification of the Chau, *et al.* protocol using *in situ* derivatization of the alkyltin compounds by sodium tetraethylborate [9]. While this procedure was found to be generally acceptable, recoveries were found both by Chau and DataChem Laboratories (DCL) to be inferior to the tropolone extraction/ethylmagnesium bromide derivatization. A composite procedure made up of modifications of elements of both Chau's protocol and a procedure used by Meinema [10] was subsequently used at DCL. Since tin concentrations were expected to be low, a GC/MS-SIM analysis was chosen over the GC/FPD procedure. This method was evaluated over the following range: butyltin trichloride: 0.12-4.0 µg/5 mL sample. This range represents from 3X the estimated LOQ to 100X the estimated LOQ. Six replicates were analyzed at each level. The average recovery at the various levels ranged from 103% at 3X LOQ to 110% at 30X LOQ for butyltin trichloride [6]. Recoveries were determined by quantitating against spiked and derivatized standards in urine. The analytical range of the method was extended during a secondary laboratory evaluation step (known as a User Check). Two higher concentration levels were evaluated (12.5 and 60.5 µg/5 mL) by analyzing five replicates at each level. These levels showed recoveries of 90.2 and 92.7% with relative standard deviations of 4.0 and 3.6% respectively [6]. The Limit of Detection (LOD) and LOQ were determined by analyzing a series of derivatized spiked standards with the data fitted to a quadratic or linear curve, then estimated according to the Burkart Method [11]. The LOD was determined to be 0.01 µg/5 mL and the LOQ to be 0.04 µg/5 mL. A long-term storage study was carried out at the 10X LOQ level. Urine spiked with the analyte was stored at -20 °C for 1, 7, 32, 137, and 315 days and then analyzed. No significant losses were observed over the entire period [6].

Additional compounds studied:

This method was originally developed to include two other butyltin chlorides: dibutyltin dichloride and tributyltin chloride, even though butyltin trichloride is the most occupationally relevant one as it is used in the glass industry. The analytical parameters obtained during method development for these two additional compounds were also acceptable [6]. However, the data obtained during the secondary laboratory evaluation for these additional compounds were not of such a quality that they could be considered fully evaluated. Both the method development data and secondary laboratory data can be found in the method backup data report [6]. Some of the pertinent analytical parameters for these two additional compounds are listed below as well as changes/additions to the written method needed for their analysis in case there is a desire to analyze for these compounds. The properties of these additional compounds are given in Table 1.

The ranges studied for the additional compounds were 0.18-6.0 µg/5-mL sample (with additional levels of 11.9 and 57.5 µg/5 mL added during the User Check) for dibutyltin dichloride and 0.15-5.0 µg/5-mL sample (with additional levels of 12.0 and 58.5 µg/5 mL added during the User Check) for tributyltin chloride.

NOTE: the additional levels added during the User Check were not used in the following accuracy, bias, and precision calculations. These values may all be found in the method backup data report [6].

ACCURACY:	Dibutyltin dichloride: ± 13.5% Tributyltin chloride: ± 8.62%
BIAS:	Dibutyltin dichloride: -0.091 Tributyltin chloride: -0.051
OVERALL PRECISION ($\hat{S}_{rt}$):	Dibutyltin dichloride: 0.0266 Tributyltin chloride: 0.0215
ESTIMATED LOD:	Dibutyltin dichloride: 0.02 µg/5 mL Tributyltin chloride: 0.01 µg/5 mL

NOTE: The tributyltin chloride LOD experiment had an interfering ion that caused this number to be more approximate than that of the other two compounds. See backup data report [6] for more information.

Recoveries ranged from 88.1% (at 30X LOQ) to 93.9% (at 10X LOQ) for dibutyltin dichloride and from 94.2% (at 10X LOQ) to 95.9% (at 100X LOQ) for tributyltin chloride [6].

Changes and additions to the method needed to incorporate these two additional compounds:

The mass spectrometer parameters should be changed to: SIM ions (*m/z*): 4-9.6 min: 178.9, 234.9, 262.9, 290.9; 9.6 min to end of run: 234.9, 262.9, 290.9.
Additional reagents required: dibutyltin dichloride, ≥95% purity and tributyltin chloride, ≥95% purity.
Retention times: dibutyltin dichloride derivative (dibutyldiethyltin), 9.2 min; tributyltin chloride derivative (tributylethyltin), 10.7 min (these peaks are shown in Figure 1.)
Conversion factors (%tin/mole) to be used if results are expressed in µg tin/volume are 0.3907 for dibutyltin dichloride and 0.3647 for tributyltin chloride.

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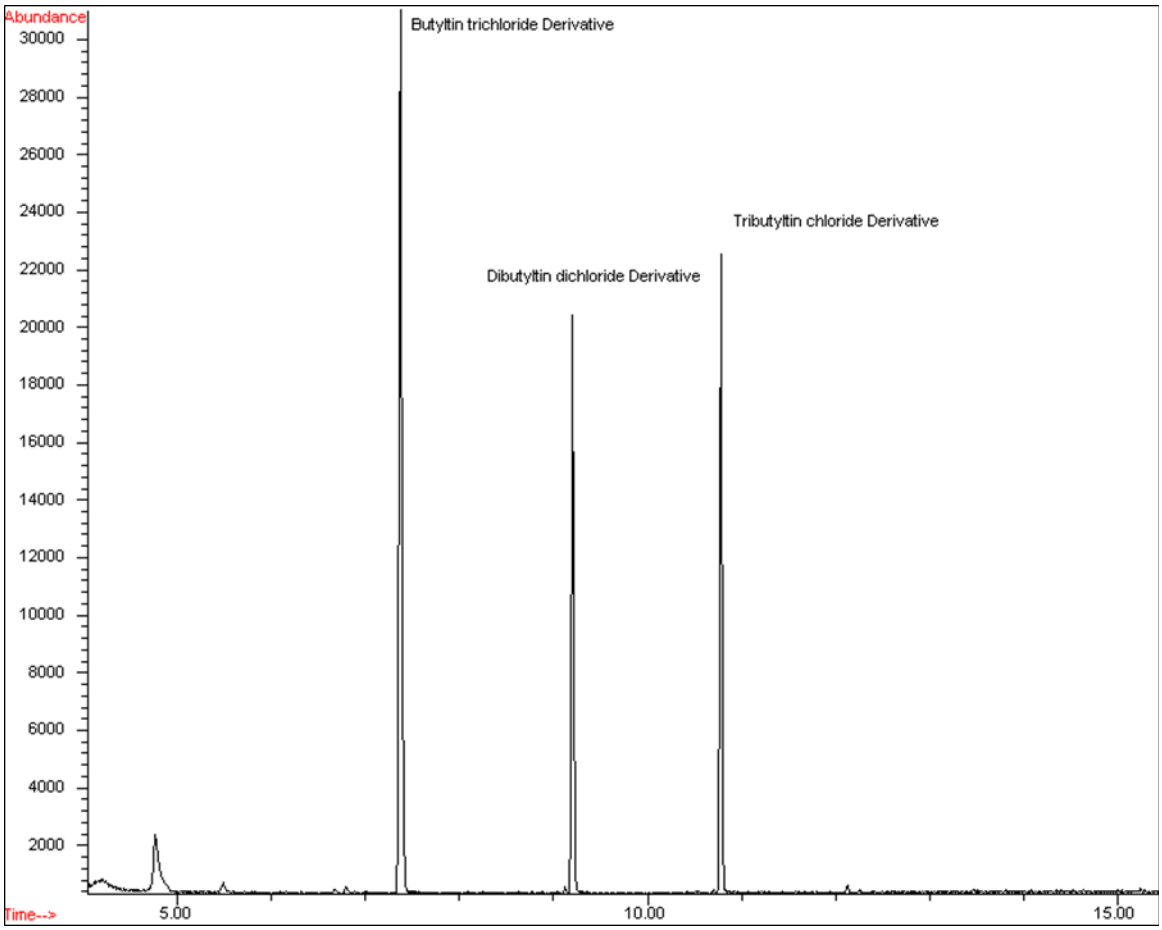
TABLE 1. GENERAL INFORMATION for additional compounds [4]

	Dibutyltin dichloride	**Tributyltin chloride**
Formula	$C_8H_{18}Cl_2Sn$	$C_{12}H_{27}ClSn$
M.W.	303.84	325.51
CAS#	[683-18-1]	[1461-22-9]
RTECS	WH7100000	WH6820000
Synonyms	Dibutyldichlorotin dibutyldichlorostannane	chlorotributyltin tributylchlorotin TBTC Pestanal®
MP	37-40 °C	-9 °C [12] -19 °C [13]
BP @ mmHg	135 °C @10mm	171-173 °C @ 25mm
Density	1.36 g/cm^3	1.20 g/mL
Appearance	Beige or colorless solid	Clear, light yellow liquid
Vapor pressure	0.0012 mmHg @ 25 °C	<0.01 mmHg @ 25 °C

FIGURE 1. Chromatogram of the three butyltin derivatives: butyltriethyltin (the derivative of butyltin trichloride), dibutyldiethyltin (the derivative of dibutyltin dichloride), and tributylethyltin (the derivative of tributyltin chloride)



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