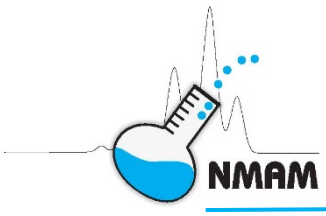


BACKUP DATA REPORT
NIOSH Method No. 8320

Title: Butyltin trichloride in urine

Analyte: Butyltin Trichloride

Author/developer:Don C. Wickman, James B. Perkins

Date: June 24, 2016

NOTE: Method developed under contract #CDC-200-2001-0800

BUTYLTIN TRICHLORIDE IN URINE

I. Introduction:

Butyltin compounds have been used extensively as antifouling coatings on ships and as a consequence, much work has been done on analyzing organotins in sediments, biota, and aqueous samples [1-13]. Included in this body of work are reviews of analytical methods [4, 10].

Of the three butyltin chlorides, monobutyl tin is the least toxic and tributyl tin the most. This trend is also true of other alkyl tins. A single alkyl chain compound is generally less toxic than the di- or tri-alkyltin. Of alkyl tins, the shorter the chain, e.g. ethyltins and methyltins, the more toxic the compound; the longer chain compounds are less toxic. A number of reviews of toxicological data on organotins are available [14-17].

There have been several reported incidences of human fatalities after exposure to organotin compounds. In France, in 1954, a pharmaceutical preparation called Stalinon caused a number of deaths. Stalinon was an oral capsule containing 15 mg of diethyltin diiodide prescribed for boils, staphylococcal skin infections, osteomyelitis, anthrax and acne. The main impurities were monoethyltin triiodide and triethyltin iodide. The triethyltin iodide was about 1.5 mg/capsule and was believed to be the main cause of the poisoning. Over 100 of the known 217 cases of poisoning died after an estimated dose of 3 g triethyltin iodide over 6-8 weeks. Triethyltin iodide as low as 70 mg over eight days appeared toxic in adults. Symptoms appeared after four days. If death occurred, it was often preceded by a coma or was during convulsions or from respiratory or cardiac failure [16].

In a separate incident, one worker out of six died twelve days after an industrial exposure to 50:50 dimethyltin and trimethyltin chloride vapor. Exposure was 1.5 hours total over three days. No exposure levels were given. Lethal doses for mono-organotins, however, ranging from 1500 -

6000 mg/kg for rodents have been reported suggesting these compounds have relatively low toxicity [15]. More recently, three died and over a thousand were poisoned by lard contaminated with tri- and di-methyltin in China [18].

Butyltin compounds are also used as stabilizers in plastics, catalysts, and biocides. Butyltin trichloride is increasingly used in the glass industry [19]. A thin coating of tin oxide is deposited on glass bottles by the decomposition of butyltin trichloride at elevated temperatures. Workers in this phase of bottle production may be exposed to the unreacted tin chloride. Because of the expanding industrial processes employing organotin compounds, a number of papers have recently quantified these compounds in urine, blood and air [18, 20-22]. While there are exposure limits for tin and organic tin compounds in air, there are currently no such exposure limits for tin or butyltin compounds in urine or blood.

Due to the organometallic nature of these compounds, a wide variety of analytical techniques can be employed to analyze these compounds. Atomic emission and absorption spectroscopy as well as inductively-coupled plasma mass spectrometry (ICP-MS) have been used to quantitate tin from an inorganic perspective [3, 5, 6, 9, 11, 18, 20, 21, 23, 24]. Because of the ionic behavior of the alkyltin halides, derivatization procedures such as Grignard reagents (which are alkyl, vinyl, or aryl-magnesium halides), sodium tetraethylborate or sodium borohydride have been used to convert the tin halides to pure alkyltin or alkyltin hydrides which are then chromatographically separated and detected often utilizing a flame photometric detector (FPD) or mass spectrometry [1, 3, 4, 12, 13, 18, 22, 25, 28, 29].

II. Reagents:

Reagent	Vendor	Purity (%)
Butyltin trichloride	Aldrich Chemical	95
Dibutyltin dichloride	Aldrich Chemical	96
Tributyltin chloride	Aldrich Chemical	96
Tetraethyl tin	Acros Chemical	97
Tetrabutyl tin	Acros Chemical	96
Sodium tetraethylborate	Aldrich Chemical	97
Ethylmagnesium bromide	Aldrich Chemical	1.0 M solution in tetrahydrofuran
Hexane	Burdick & Jackson	Pesticide Grade
Sulfuric acid	Fisher Scientific	95-98
Sodium acetate, acetic acid buffer, pH 4	Fisher Scientific	Reagent Grade
Sodium citrate dihydrate	J.T. Baker	99.6
Citric acid monohydrate	Mallinckrodt	100
Toluene	Burdick & Jackson	Pesticide Grade
Tropolone	Lancaster	98
Alumina, acid, Brockman activity 1, 80-200 mesh	Fisher Scientific	Chromatographic Grade
Sodium sulfate, anhydrous	Fisher Scientific	Reagent Grade
UriSub® synthetic urine	CST Technologies, Inc.	N/A
Florisil®	Sigma	Chromatographic Grade

III. Solutions Preparation:

1% Sodium tetraethylborate was prepared by weighing typically less than 350 mg sodium tetra-ethylborate into a 40-mL VOA glass vial with a PTFE septum. This weighing must be done in an inert atmosphere of nitrogen. Cap the vial and store in a freezer until use. For use, add sufficient water to prepare a 1% w/v solution by syringe through the septum. Once the solution is prepared, the cap can be removed. Prepare fresh daily.

To prepare the citric acid/sodium citrate buffer, dissolve 20.554 g citric acid and 0.652 g sodium citrate (~pH 2.3) in 1 L deionized water. Additional buffers at pH 3 and 4 were prepared for method development experiments. See Table 3 in section VI. B. for buffer composition.

0.1% tropolone in toluene was prepared by dissolving 100 mg tropolone in 100 mL toluene.

To prepare the 1 M sulfuric acid solution, dilute 5.6 mL concentrated sulfuric acid with water to 100 mL final volume.

IV. Materials:

- 15-mL Polypropylene centrifuge tubes with screw cap. VWR Cat. # 21008-089 or equivalent
- Centrifuge capable of at least 2400 RPM
- Disposable Pasteur pipettes, 14.6 cm (5¾ in) & 22.9 cm (9 in)
- ~8-mL Test or culture tubes, screw tops with PFTE-lined caps
- 40-mL VOA glass vials, caps to fit with holes and PFTE septa
- GC-MS capable of selected ion monitoring with autosampler and data collection system
- Microliter syringes for making standard solutions and GC injections
- 5-mL Glass volumetric flasks
- 1-5-mL Adjustable pipettor with tips
- Glass wool
- Vortex mixer
- Tumbler for centrifuge tubes, approximately 20 RPM

V. Analysis:

All of the method development was performed on an HP 5890 Gas Chromatograph (GC) coupled to an HP 5972 Mass Selective Detector and 7673B autosampler. The column used was 30 m x 0.32 mm ID with a 0.5 μm film DB-5ms. The mass spectrometer used electron impact ionization and was operated in the selected ion monitoring (SIM) mode. The following ions were monitored: 178.9, 234.9, 262.9, and 290.9. These were determined from the total ion scans of individual derivatized analytes (see Figures 1-3 below). The ions chosen were xxx.9 because tin and its isotopes have a negative mass defect of approximately 0.1 amu. Consequently, the masses (*m/z*) monitored have the units xxx.9 amu. For quantitation of the three analytes, all four ions were used to determine the peak in the chromatogram. Peak areas were used for quantitation.

Two microliters were injected in a 230 °C injector, splitless for 0.75 minutes. The initial oven temperature was 75 °C, held for one minute and then ramped to 225 °C at 12 °C/min. The final temperature was held for 5 minutes.

Experimentally obtained total ion spectra for the three butyltin derivatives are displayed in Figures 1-3. Following the spectra in Figure 4 is a typical chromatogram of all three derivatives in a mix.

Figure 1. Mass Spectrum of Butyltin Trichloride Derivative: Butyltriethyltin.

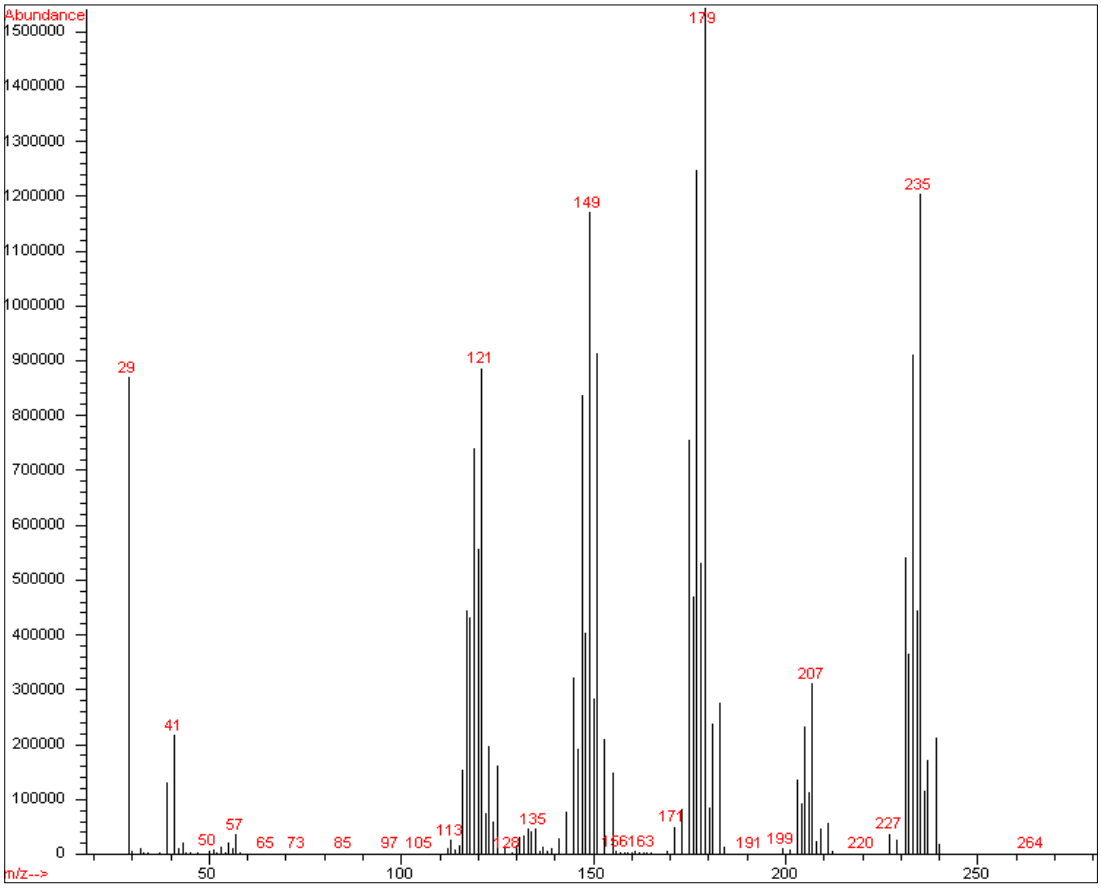


Figure 2. Mass Spectrum of Dibutyltin Dichloride Derivative: Dibutyldiethyltin.

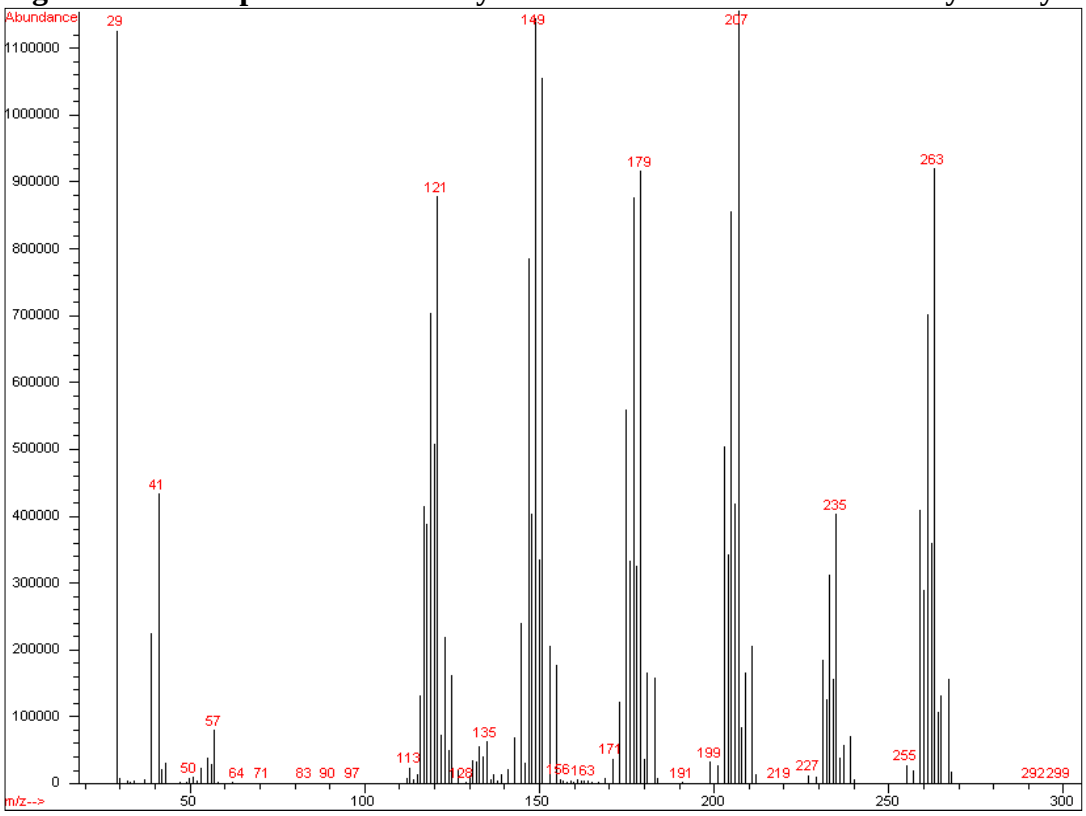


Figure 3. Mass Spectrum of Tributyltin Chloride Derivative: Tributylethyltin.

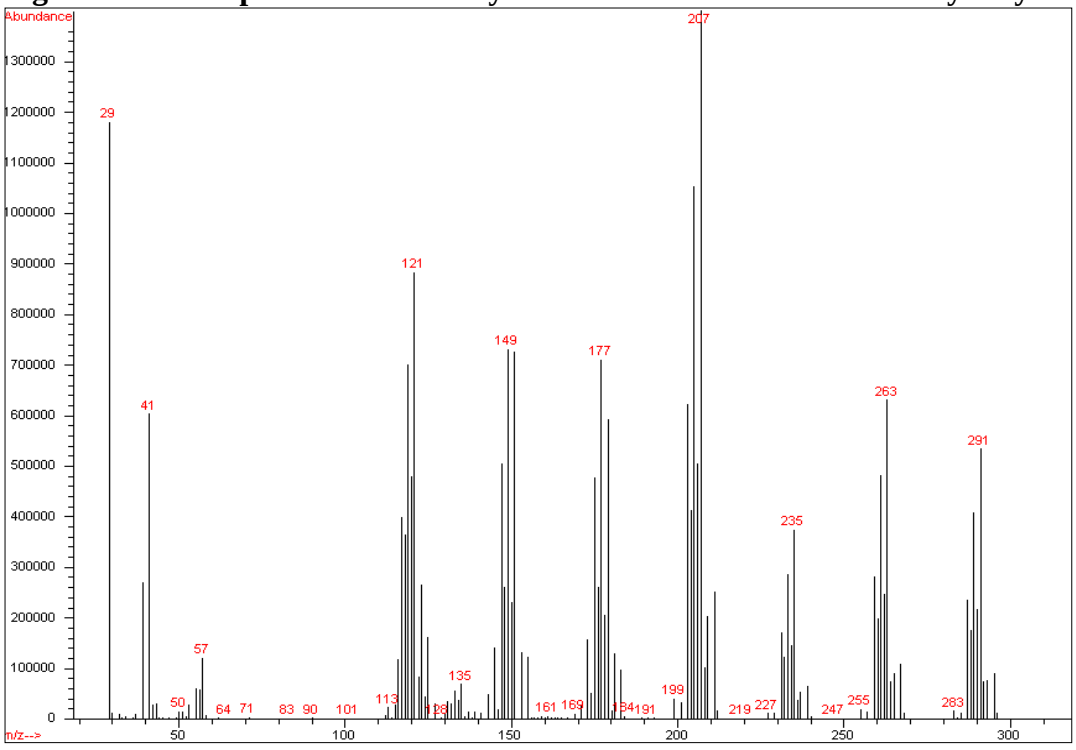
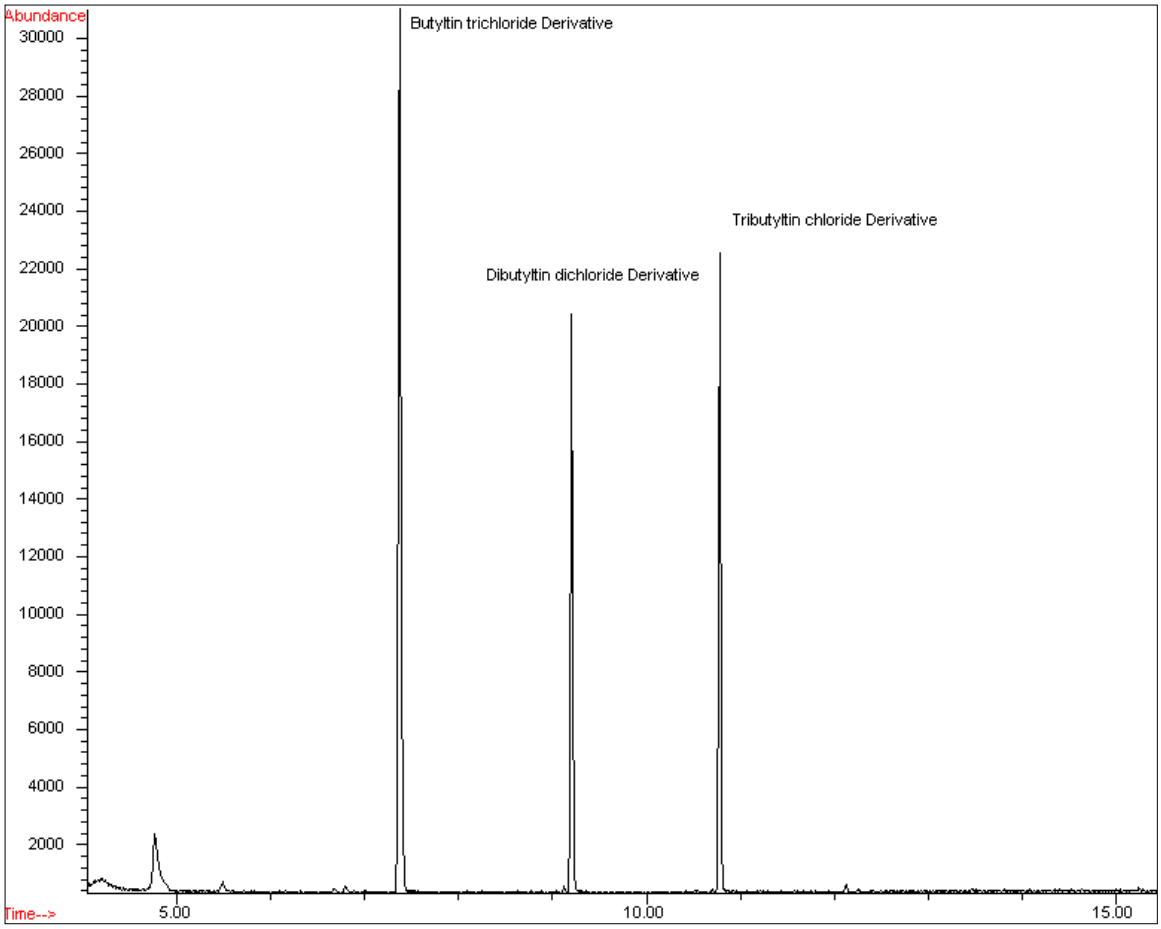


Figure 4. Chromatogram of the Three Butyltin Derivatives.



During method development, experimental conditions and parameters were evaluated that do not appear in the final version of the method. Data are presented from these experiments in the following sections. This chronological presentation has led to confusion on the part of some readers. To go directly to the experimental conditions and evaluation data found in the method, please click the appropriate following links: Final procedure, LOD/LOQ determination, Recoveries at various concentration levels, precision and accuracy, long-term storage.

VI. Method Development Experiments:

A. Sodium Tetraethylborate *in situ* Derivatization:

A sodium tetraethylborate derivatization protocol has been employed in methods for chlorinated alkyl tins in air [22, 28, 29] as well as by a number of researchers quantifying organotin compounds in sediment and biota [1, 3, 5-8, 10, 13]. Rapsomanikis *et al.* used an *in situ* aqueous ethylation to analyze lead and methyllead [23]. Since *in situ* procedures were successfully used to prepare lead and tin compounds for analysis in aqueous or biological samples using aqueous sodium tetraethylborate, this procedure was considered first. To improve recovery of the derivatized alkyl tin, the final extraction step was repeated. Due to the limited volume of sample available, a five mL aliquot was used for derivatization.

Five mL urine was placed in a 15-mL centrifuge tube. Three mL of a pH 4 buffer was added plus one mL of 1% sodium tetraethylborate solution in water. The tube was capped, mixed, and allowed to stand for at least 30 minutes. To extract the alkyl tin compounds, 1.2 mL hexane was added and vortexed for 30 seconds. The tube was then centrifuged for 10 minutes and the hexane layer withdrawn and placed in a 5-mL volumetric flask. The hexane extraction of the sample was repeated two more times with the extracts added to the 5-mL volumetric flask. After the last extraction, the sample was brought to volume with additional hexane and mixed.

Total tin had been analyzed in urine samples previously at DataChem Laboratories (DCL) by ICP-MS. Total tin concentrations were found to be quite low, typically in the 5-50 μg/L (as Sn) range. Analysis of butyltin trichloride in air samples at DCL on OVS-2 tubes using sodium tetraethylborate derivatization followed by quantification by GC-FPD in the tin mode with cool on-column injection gave a limit of detection (LOD) of 2-5 μg butyltin trichloride per 5-mL sample. This would compare to an LOD of approximately 200 μg/L as Sn, which would be too high for the GC-FPD to be the instrument of choice for the vast majority of samples. Since the cool on-column injector is not necessary for alkyltins other than to

increase the amount of sample on column, GC-MS in the SIM mode was used. With either derivatization reagent, sodium tetraethylborate or ethylmagnesium bromide, the compounds formed from the butyltin chlorides are the same, each chloride is replaced by an ethyl group. For example, the derivatization of butyltin trichloride would yield triethyl butyltin. No physical properties for these alkyltin derivatives could be located, nor were they commercially available for purchase. Consequently, the completeness of the reaction could not be determined.

Experiment 1. LOD/LOQ Determination:

Initial LOD/LOQ determinations were conducted by derivatizing a concentrated stock of the butyltin chlorides, then making serial dilutions followed by GC-MS analysis in the SIM mode. Peaks at the low end were manually re-integrated as needed. Peak areas were used to calculate the LOD and LOQ by Burkart's method [30]. Results were as follows:

Table 1. LOD/LOQ Determination Results

Analyte	µg/5 mL sample LOD	µg/5 mL sample LOQ	µg/L as Sn LOD	µg/L as Sn LOQ
Tributyltin chloride	0.03	0.1	6	20
Dibutyltin dichloride	0.02	0.08	5	20
Butyltin trichloride	0.04	0.1	8	30

Experiment 2. Derivatization of Spiked Urine and Aqueous Standards with Sodium Tetraethylborate; Recovery Study:

Five mL urine samples, each from a different subject and previously determined by ICP-MS analysis to have no tin, were spiked at approximately 10X LOQ and 30X LOQ. Six replicates at each level were prepared as were tin spikes in deionized water bracketing the urine spike range. The aqueous spikes were

to serve as standards for extraction recovery purposes. An additional spike was prepared in UriSub© synthetic urine. This spike was at the same level as the second highest standard. Samples were derivatized as described above. During the extraction step, it was noted that several urine samples had emulsions that would not clear. Some were so thick the tube could be upended and the emulsion acted as a cap on the liquid. Adding several hundred milligrams of sodium sulfate, mixing, and centrifuging the samples helped to clear these emulsions. Results are summarized below. Concentrations were determined using a calibration curve prepared from the aqueous spikes and concentrations were calculated using the response factor determined from the single spike in UriSub© synthetic urine.

Table 2a. Recovery Study Results (Results using the Aqueous Spike Calibration Curve) (n=6)

Analyte	10X LOQ Result Avg. % Recovery	10X LOQ Result % RSD	30X LOQ Result Avg. % Recovery	30X LOQ Result % RSD
Tributyltin chloride	83.6	7.6	84.0	4.2
Dibutyltin dichloride	85.4	8.1	84.7	4.2
Butyltin trichloride	82.5	10.8	85.4	3.3

Table 2b. Recovery Study Results (Results using the UriSub© Synthetic Urine Response Factor)

Analyte	10X LOQ Result Avg. % Recovery	10X LOQ Result % RSD	30X LOQ Result Avg. % Recovery	30X LOQ Result % RSD
Tributyltin chloride	85.6	8.5	94.2	4.5
Dibutyltin dichloride	90.7	9.1	98.5	4.4
Butyltin trichloride	90.2	11.1	98.4	3.6

Conclusions Concerning Sodium Tetraethylborate *in situ* Derivatization:

The % RSD at 10X LOQ was a factor of two to three times the % RSD at 30X LOQ. While experiments at 3X LOQ were not conducted, the concern was at that level the method would fail. Noted too was the fact that apparent recoveries were higher when quantitating using the synthetic urine response factor than when quantitating against standards prepared from aqueous spikes. It was decided to try the tropolone extraction protocol instead of the *in situ* derivatization to see if recoveries and precision at lower levels could be improved.

B. Derivatization with Ethylmagnesium Bromide Following Tropolone Extraction:

Tropolone (2-hydroxy-2,4,6-cycloheptatrienone) has been used by a number of researchers to complex butyltin chlorides in a variety of polar matrices to allow extraction into a non-polar solvent [5-9, 12, 18, 20, 21, 26, 27]. Derivatization is typically done by employing a Grignard reagent, although sodium tetraethylborate can be used [5-7]. Chau *et al.*, with an emphasis on extracting monobutyltin trichloride, felt toluene gave the best recoveries [9]. For the next series of experiments, tropolone in toluene was used to extract the butyltin chlorides followed by derivatization with ethylmagnesium bromide as the Grignard reagent. The procedure used was based on the procedures outlined in the journal articles.

A number of experiments were performed to test various aspects of the tentative protocol before the final procedure was settled on. These were either suggested by the literature or deemed necessary to provide the best recoveries.

In the experiments described below, 15-mL centrifuge tubes were prepared containing 5 mL urine previously determined at DCL to contain no tin. The source of the urine is described for each experiment and could be either pooled urine or urine from different subjects. The tubes were spiked with the previously prepared tin mix described above at a level of 10X LOQ.

Experiment 1. Buffers: Three urine samples, known to contain no tin, were pooled. From this supply, six spiked tubes and a blank were prepared. These tubes tested the effectiveness of different buffers. With the sodium tetraethylborate derivatization, the reaction was carried out at pH 4.0 - 4.5. A pH of about 2 was recommended for Grignard reactions. Both the pH 4 acetate buffer and newly prepared citrate buffers were tried at different pHs.

Three citric acid/sodium citrate buffers were prepared. Amounts listed in Table 3 below are grams added per liter of water.

Table 3. Buffer Composition

Buffer	**~pH 2**	**~pH 3**	**~pH 4**
Citric acid monohydrate	20.544	17.96	13.115
Sodium citrate dihydrate	0.652	4.265	11.051

In all cases, 3 mL buffer was added to the 5 mL spiked urine. Samples were then extracted and derivatized with Grignard reagent as described above. Peak areas are tabulated in Table 4.

Table 4: Results for Buffers

Sample type	**Peak area Butyltin Trichloride**	**Peak area Dibutyltin Dichloride**	**Peak area Tributyltin Chloride**
pH 4 Acetate buffer - tropolone extract	7728	4642	6440
pH 4 Citrate buffer - tropolone extract	20866	9486	12061
pH 3 Citrate buffer - tropolone extract	21916	9625	12492
pH 2 Citrate buffer - tropolone extract	21389	9284	12520

The highest peak areas were obtained for both the pH 2 & pH 3citrate buffers. The pH 2 buffer was selected for use in the method. No tin compounds were observed in the blank.

Experiment 2. Reproducibility Experiment: Three sets of three spiked replicate samples were

prepared from three different NIOSH supplied urine samples. These three sets were prepared and analyzed the same way to see how reproducible results are. Additionally, a set of three replicate samples containing pooled urine from the buffer experiment above were analyzed. Peak areas for the four sets of samples are averaged and presented in Table 5.

Table 5. Results for Reproducibility Experiment

Urine source	Butyltin Trichloride avg peak area	Butyltin Trichloride %RSD	Dibutyltin dichloride avg peak area	Dibutyltin dichloride %RSD	Tributyltin chloride avg peak area	Tributyltin chloride %RSD
NIOSH 12-6-B	24654	0.45	10198	0.90	14189	3.31
NIOSH 12-5-B	26832	0.78	9127	2.46	14055	0.55
NIOSH 6-5-B	24656	2.62	10044	1.82	13901	1.21
Pooled 2al	22216	3.23	9724	4.06	12831	2.54

Similar peak areas were observed for all sets of samples with the largest variation in average areas with dibutyltin dichloride, approximately 9%. Overall, this was not viewed as a significant problem.

Experiment 3. Extraction and Cleanup Experiments: The amount of tin extracted from the urine matrix by the tropolone-toluene extraction solution was tested. The first tropolone extract from four spiked samples was combined, derivatized and analyzed; the second extraction was similarly prepared and analyzed.

A number of journal articles recommended a cleanup procedure before analysis [2, 4, 6, 8, 9, 12, 18, 21]. Florisil and alumina cleanups were tried on aliquots of the combined first and second extractions.

Extraction and Cleanup Experiment Results: The efficiency of the first extraction was determined by dividing the peak area of the first extract analysis by the sum of the areas for both extracts. For the cleanup experiment, 1.5 mL aliquots of the first and second extracts were each passed through a 1-

cm column of either florisil, alumina, or sodium sulfate in a 14.6 cm (5¾ in) Pasteur pipette prior to analysis. The column was rinsed with small portions of toluene and the volume brought to 2 mL. Peak areas for each analyte are tabulated with the cleanup procedure used.

Table 6. Second Extraction and Sample Cleanup Results

Sample type/Cleanup Step	Butyltin Trichloride	Dibutyltin Dichloride	Tributyltin Chloride
First extract as % of total area (average)	87%	89%	82%
First extract peak area-florisil	51077	16418	26148
First extract peak area - alumina	57462	18861	28385
First extract peak area - sodium sulfate	60634	19900	29599
Second extract peak area - florisil	8117	2217	6323
Second extract peak area - alumina	8652	2249	5410
Second extract peak area - sodium sulfate	8737	2219	6781

Extraction and Cleanup Experiment Conclusions: The efficiency of the first extraction was overall approximately 86%. The second extraction was believed necessary because of the low butyltin concentrations expected in the field samples. Alumina appeared to be a better cleanup media than florisil as evidenced by the slightly higher peak areas for the alumina aliquot. To eliminate any traces of water prior to analysis, a small quantity of sodium sulfate will be used in addition. The sodium sulfate did not seem to have a detrimental effect of the analysis results.

Experiment 4. Derivatization Kinetics: Fifteen mL of toluene-tropolone was spiked with butyltin chlorides and mixed thoroughly. To this, 1.25 mL ethylmagnesium bromide solution was added and vortexed. At timed intervals, 3-mL aliquots were removed and transferred to another tube containing one mL sulfuric acid solution to quench the reaction. The alkyltins were subsequently analyzed.

Derivatization Kinetics Results: Peak areas vs. time of reaction quenching are shown in graphic form in Figure 5 and in tabular form in Table 7.

Figure 5. Butyltin Kinetics

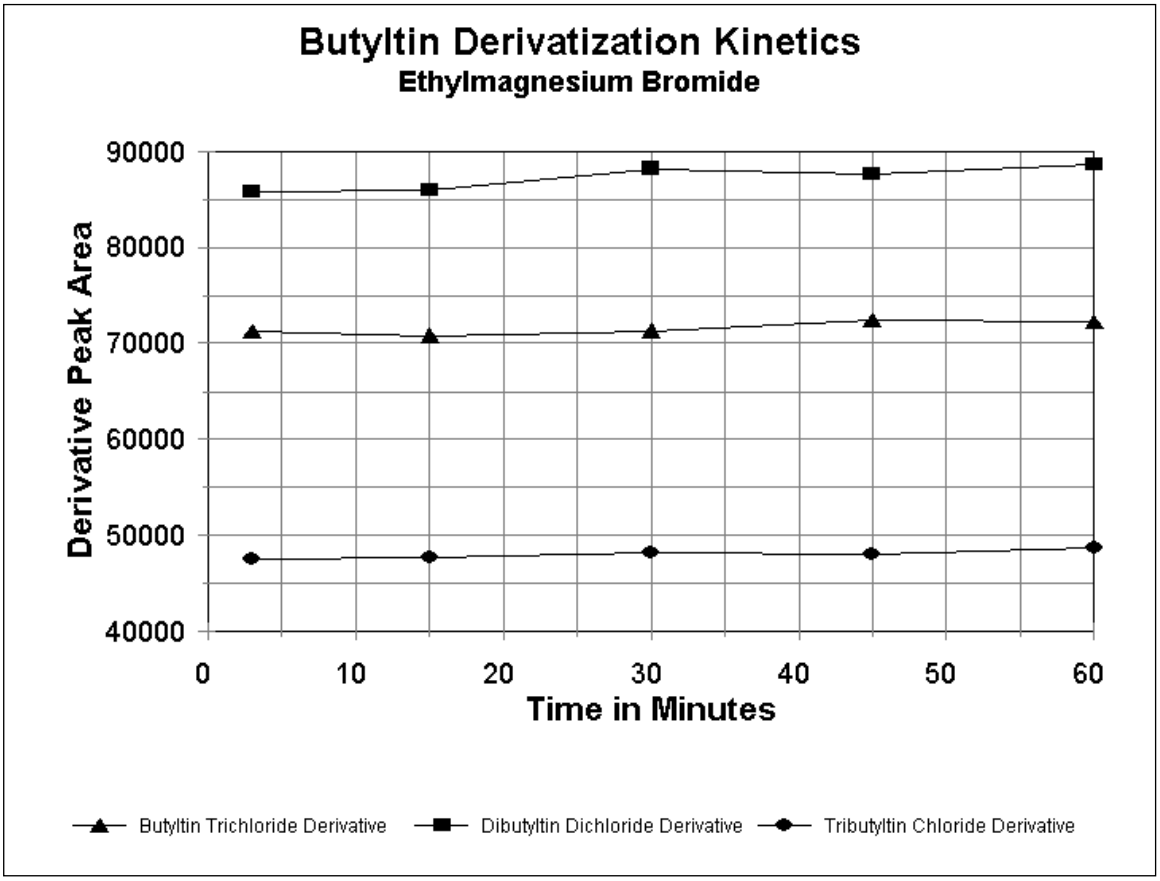


Table 7. Tabulated Peak Areas for Reaction Kinetics

Time (minutes)	Peak area Butyltin Trichloride	Peak area Dibutyltin Dichloride	Peak area Tributyltin Chloride
3	71304	85909	47553
15	70850	85947	47670
30	71345	88264	48258
45	72396	87604	48034
60	72353	88762	48682

Derivatization Kinetics Conclusions:

The reaction occurs very quickly and the reaction products appear stable in the sulfuric acid environment. A derivatization time of 15-20 minutes at a minimum will be adequate.
Longer times are not a detriment.

VII. Final Procedure:

Incorporating the results of these experiments, the final experimental protocol for the remainder of the method development and field sample analysis was as follows.

Thaw the urine samples. Place five mL of the urine sample in a 15-mL polypropylene centrifuge tube. Add three mL of ~pH 2.3 citric acid-sodium citrate buffer, 1.5 mL 0.1 % tropolone in toluene solution, and about 350 mg anhydrous sodium sulfate. Some urine samples can form emulsions during tumbling that are difficult to break down. For this reason, the samples should have the sodium sulfate added prior to tumbling at about 20 rpm for one hour. This is followed by centrifuging for at least 10 minutes at 2400 rpm (1050 G). The upper toluene layer is transferred to an 8-mL glass culture tube with a PTFE-lined cap. An additional tropolone extraction procedure is conducted on the urine sample and the extracts combined. To the combined extracts, 350 mg anhydrous sodium sulfate is added to remove any of the aqueous phase that may have been inadvertently transferred and to dry the toluene. The dried sample is decanted into a second 8-mL glass culture tube with a PTFE-lined cap. The sodium sulfate residue in the first tube is rinsed twice with a small (~300 µL) quantity of toluene which is then combined with the dried extract in the second tube. A 250 µL quantity of the ethylmagnesium bromide solution is added to the toluene, mixed, and the mixture allowed to react for 15-20 minutes at a minimum. After the allotted reaction time, 1 mL of 1 M sulfuric acid is added to quench the remaining ethylmagnesium bromide and the tube is vortexed for approximately 30 seconds. After the layers separate, the top toluene layer is eluted through a clean-up column that is prepared in a 14.6 cm (5¾") disposable Pasteur pipette. The pipette

contains, in order from bottom to top: 1) a glass wool plug; 2) alumina, acid, Brockman activity I, 80-200 mesh, enough to form a column of approximately 1 cm and; 3) ~100 mg of anhydrous sodium sulfate. The eluate is collected in a 5-mL volumetric flask. The tube with the sulfuric acid solution is rinsed several times with small amounts of toluene and the rinses eluted through the clean-up column. The remaining sample is flushed from the column by adding small portions of toluene to the top of the column. The final volume is adjusted to 5.0 mL with toluene as needed. Analysis is by the instrument conditions described previously.

VIII. Limit of Detection and Limit of Quantitation Determination for Method Spiking Levels:

The LOD and LOQ were determined by spiking 5 mL of urine in 15-mL centrifuge tubes with known concentrations of the three butyltin chlorides. A mixed standard was prepared as follows; the solvent used in all cases was 1% acetic acid in acetonitrile. For tributyltin chloride (96%, d = 1.200 mg/µL), 10 µL were diluted in 10 mL to give a concentration of 1.152 µg/µL. For dibutyltin dichloride (96%), 16.05 mg were dissolved in 10 mL giving a concentration of 1.54 µg/µL. For butyltin trichloride (95%, d = 1.693 mg/µL), 10 µL were diluted in 10 mL to give a concentration of 1.608 µg/µL.

In previous work with these particular chemicals, it was found that dibutyltin dichloride was contaminated with butyltin trichloride. The concentration of butyltin trichloride was determined in the dibutyltin dichloride by peak area determination against a separate butyltin trichloride series of standards and found to be 0.04987 µg butyltin trichloride per 1 µg dibutyltin dichloride.

Table 8. Stock Standard Mix Concentrations

Analyte	µg/µL	Volume (µL)	Final Volume (mL)	Final Conc. (µg/µL)
Tributyltin chloride	1.152	17.5	5	0.004032
Dibutyltin dichloride	1.54	14	5	0.004312
Butyltin trichloride	1.608	10	5	0.003431*

* includes contribution by dibutyltin contaminant.

Microliter amounts of the solution were spiked into 5 mL urine according to the following schedule:

Table 9. Working Standard Spiking Schedule (Concentration in µg/ 5 mL Sample)

Standard	Spiked Volume (µL)	Butyltin Trichloride Concentration	Dibutyltin Dichloride Concentration	Tributyltin Chloride Concentration
1	250	0.85775	1.078	1.008
2	175	0.600425	0.7546	0.7056
3	100	0.3431	0.4312	0.4032
4	50	0.1716	0.2156	0.2016
5	24	0.085775	0.1078	0.1008
6	10	0.03431	0.04312	0.04032
7	5	0.01716	0.02156	0.02016
8	2	0.006862	0.008624	0.008064

Spiked samples were extracted with toluene-tropolone and then derivatized with ethylmagnesium bromide followed by the column cleanup as described in the previously described procedure. An aliquot of each sample was transferred to a GC vial for analysis by GC-MS in the SIM mode. Peak areas for butyltin and dibutyltin were tabulated and the LOD/LOQ determined by Burkart's method [30]. An interfering peak was observed which prevented accurate quantitation of the tributyltin peak. This peak was composed of the *m/z* 179 ion. This had not been noticed previously because the LOD/LOQ was determined by a derivatization of a high standard followed by serial dilution of this stock. Additionally, sodium tetraethylborate was used instead of the ethylmagnesium bromide. It is unknown if this had an effect or not. Monitoring the *m/z* 179 ion was stopped prior to the retention time of the tributyltin peak. This seemed to solve the problem and allow good quantitation of the tributyltin peak in later analyses. This would have also required the reanalysis of the LOD/LOQ standards to determine the LOD and LOQ of tributyltin chloride. For further method development purposes, the LOD and LOQ were estimated for this compound. It should be noted that LODs/LOQs for this procedure are lower by about half than LOQs for the sodium tetraethylborate derivatization. The LODs were determined to be 0.01 µg/5 mL for tributyltin chloride

(estimated), 0.02 µg/5 mL for dibutyltin dichloride, and 0.01 µg/5 mL for butyltin trichloride. Spiking levels for the method development were then determined to be:

Table 10. Method Development Urine Spiking Levels (Spiking Levels in µg/5 mL Sample)

Analyte	LOQ	3X LOQ	10X LOQ	30X LOQ	100X LOQ
Tributyltin chloride	0.05*	0.15	0.5	1.5	5
Dibutyltin dichloride	0.06	0.18	0.6	1.8	6
Butyltin trichloride	0.04	0.12	0.4	1.2	4

*Estimated LOQ

IX. Extraction Efficiency at 3X, 10X, 30X, and 100X LOQ:

A new mix of analytes based on the previously determined LOD/LOQ was prepared from the concentrated stocks as described below:

Table 11. Extraction Efficiency Spiking Mix

Analyte	µg/µL	Volume (µL)	Final Volume (mL)	Final Conc. (µg/µL)
Tributyltin chloride	1.152	110	5	0.025344
Dibutyltin dichloride	1.54	98	5	0.030184
Butyltin trichloride	1.608	58	5	0.020158*

* includes contribution by dibutyltin contaminant.

Aliquots of this solution were spiked into 5 mL urine in 15-mL centrifuge tubes at the four spiking levels. Six replicate samples were prepared at each level.

Table 12. Target Concentrations (Concentration Levels in µg/ 5 mL Sample)

LOQ level	Spiked Volume (µL)	Butyltin Trichloride Concentration	Dibutyltin Dichloride Concentration	Tributyltin Chloride Concentration
100 X	200	4.03156	6.0368	5.0688
30X	60	1.20947	1.8110	1.5206
10X	20	0.40316	0.6037	0.5069
3X	6	0.12095	031811	0.1521

Samples were refrigerated overnight and extracted the following day. An aliquot of each derivatized sample was transferred to a GC vial for analysis by GC-MS. Peak areas for all analytes were tabulated and concentrations determined using calibration curves made from analytes in spiked urine. A summary of the recovery data is shown in Table 13.

Table 13. Recovery Summary

LOQ Level	Butyltin Trichloride avg recovery	Butyltin Trichloride %RSD	Dibutyltin dichloride avg recovery	Dibutyltin dichloride %RSD	Tributyltin chloride avg recovery	Tributyltin chloride %RSD
100X	106.1	1.75	91.6	1.24	95.9	0.97
30X	109.6	6.28	88.1	2.55	94.5	2.78
10X	105.9	4.22	93.9	1.61	94.2	1.96
3X	102.8	4.14	90.1	3.5	95.1	2.01

All data are presented in Table 14.

Table 14. Extraction Efficiency at 3X, 10X, 30X, and 100X LOQ

	Butyltin Trichloride				Dibutyltin Dichloride			Tributyltin Chloride		
	Result		Target		Result		Target	Result		Target
100X LOQ										
1	4.3799	4.032	108.63		5.5764	6.037	92.37	4.8559	5.069	95.80
2	4.3133	4.032	106.98		5.4592	6.037	90.43	4.8696	5.069	96.07
3	4.3146	4.032	107.01		5.5593	6.037	92.09	4.9100	5.069	96.86
4	4.2196	4.032	104.65		5.4355	6.037	90.04	4.7906	5.069	94.51
5	4.2399	4.032	105.16		5.5089	6.037	91.25	4.8215	5.069	95.12
6	4.1937	4.032	104.01		5.6322	6.037	93.29	4.9165	5.069	96.99
		average	106.07				91.58			95.89
		Std Dev	1.75				1.24			0.97
30X LOQ										
1	1.1779	1.209	97.43		1.6794	1.811	92.73	1.4451	1.521	95.01
2	1.3286	1.209	109.89		1.5385	1.811	84.95	1.4091	1.521	92.64
3	1.3263	1.209	109.70		1.5977	1.811	88.22	1.4029	1.521	92.24
4	1.3770	1.209	113.90		1.5852	1.811	87.53	1.4566	1.521	95.77
5	1.3715	1.209	113.44		1.5818	1.811	87.34	1.3994	1.521	92.01
6	1.3726	1.209	113.53		1.5876	1.811	87.66	1.5088	1.521	99.20
		average	109.65				88.07			94.48
		Std Dev	6.28				2.55			2.78
10X LOQ										
1	0.4065	0.403	100.87		0.5633	0.604	93.26	0.4616	0.507	91.05
2	0.4311	0.403	106.97		0.5834	0.604	96.59	0.4829	0.507	95.25
3	0.4273	0.403	106.03		0.5586	0.604	92.48	0.4695	0.507	92.60
4	0.4228	0.403	104.91		0.5736	0.604	94.97	0.4789	0.507	94.46
5	0.4568	0.403	113.35		0.5588	0.604	92.52	0.4858	0.507	95.82
6	0.4168	0.403	103.42		0.5644	0.604	93.44	0.4863	0.507	95.92
		average	105.93				93.88			94.18
		Std Dev	4.22				1.61			1.96
3X LOQ										
1	0.1216	0.121	100.50		0.1664	0.181	91.93	0.1432	0.152	94.21
2	0.1244	0.121	102.81		0.1742	0.181	96.24	0.1460	0.152	96.05
3	0.1176	0.121	97.19		0.1575	0.181	87.02	0.1410	0.152	92.76
4	0.1281	0.121	105.87		0.1582	0.181	87.40	0.1430	0.152	94.08
5	0.1301	0.121	107.52		0.1609	0.181	88.90	0.1498	0.152	98.55
6	0.2672	0.121	220.83	*	0.1606	0.181	88.73	0.1445	0.152	95.07
		average	102.78				90.04			95.12
		Std Dev	4.14				3.50			2.01

* outlier, not included in recovery calculations

X. Precision and Accuracy: [31]

Butyltin Trichloride:

When all values are used, the Chi^2 value was found to be 5.9304, the overall precision ($\hat{S}_{rt}$) was 0.04114 and the bias was 0.06251 (see Appendix 1 detailing results for all cases). Accuracy was calculated to be 13.02% from these values.

Dibutyltin Dichloride:

When all values are used, the Chi^2 value was found to be 6.1235, the overall precision ($\hat{S}_{rt}$) was 0.02656 and the bias was -0.09108 (see Appendix 2 detailing results for all cases). Accuracy was calculated to be 13.48% from these values.

Tributyltin Chloride:

When all values are used, the Chi^2 value was found to be 4.6069, the overall precision ($\hat{S}_{rt}$) was 0.02150 and the bias was -0.05083 (see Appendix 3 detailing results for all cases). Accuracy was calculated to be 8.62% from these values.

XI. Long Term Storage:

A long term storage study was done by spiking 5 mL aliquots of urine with all three analytes at 10X LOQ. That level was chosen because most of the butyltin trichloride found in urine samples collected for a NIOSH study was close to this concentration. Since the urine samples had been stored frozen for an extended period of time, the storage study was conducted for ten months with the samples frozen at -20 °C. During the course of the study, samples were removed, thawed, extracted, derivatized, and analyzed against freshly spiked urine samples. The unspiked urine used for standards had been frozen with the spiked samples and was spiked prior to sample preparation.

To determine the effects, if any, on the butyltin recoveries due to the use of preserving agents, eight tubes spiked at 10X LOQ were prepared with: 1) ascorbic acid, 100 mg/5 mL aliquot; four

tubes and 2) nitric acid, 200 µL/5 mL aliquot; four tubes. Two each of the two preserved sets were prepared and analyzed with the 127 and 315 day storage samples.

Tables 15a-c give a summary of the storage study. Tables 16-18 give the complete data set for each of the three analytes. Figure 6 is a graphical representation of the data for all three analytes while no preservative was being used.

Table 15a. Long Term Storage* Recovery Summaries (recoveries as % ± std deviation)

Analyte	Target (µg/5mL)	Day 1	Day 7	Day 32	Day 127	Day 315
Tributyltin chloride	0.5069	94.5±3.7	98.1±2.4	93.5±1.0	111.6±3.6	97.4±8.5
Dibutyltin dichloride	0.5982	99.6±2.6	103.6±0.5	100.3±1.2	107.8±2.0	92.9±1.9
Butyltin trichloride	0.3977	95.8±2.3	104.0±1.5	97.7±1.6	103.6±3.6	96.1±3.6

*samples unpreserved

Table 15b. Long Term Storage* Recovery Summaries (recoveries as %)

Analyte	Target (µg/5mL)	Day 127	Day 315
Tributyltin chloride	0.5069	115.6	97.4
Dibutyltin dichloride	0.5982	103.6	92.9
Butyltin trichloride	0.3977	110.6	96.1

*samples ascorbic acid preserved

Table 15c. Long Term Storage* Recovery Summaries (recoveries as %)

Analyte	Target (µg/5mL)	Day 127	Day 315
Tributyltin chloride	0.5069	115.1	87.5
Dibutyltin dichloride	0.5982	120	91.8
Butyltin trichloride	0.3977	116.3	90.2

*samples nitric acid preserved

Table 16. Long Term Storage: All Data; Butyltin Trichloride

Butyltin Trichloride

		Result	Target	% Recovery
Day 1				
	1	0.3944	0.3977	99.17
	2	0.3669	0.3977	92.26
	3	0.3858	0.3977	97.01
	4	0.3805	0.3977	95.68
	5	0.3768	0.3977	94.74
	6	0.3820	0.3977	96.05
		Average		95.82
		Rel. Std Dev		2.31
Day 7				
	1	0.4153	0.3977	104.43
	2	0.4179	0.3977	105.08
	3	0.4068	0.3977	102.29
	4	0.4184	0.3977	105.20
	5	0.4052	0.3977	101.89
	6	0.4176	0.3977	105.00
		Average		103.98
		Rel. Std Dev		1.50
Day 32				
	1	0.3941	0.3977	99.09
	2	0.3787	0.3977	95.22
	3	0.3931	0.3977	98.84
	4	0.3946	0.3977	99.22
	5	0.3838	0.3977	96.50
	6	0.3879	0.3977	97.54
		Average		97.74
		Rel. Std Dev		1.62
Day 127				
	1	0.4028	0.3977	101.28
	2	0.4059	0.3977	102.06
	3	0.4289	0.3977	107.85
	4	0.4062	0.3977	102.14
	5	0.4081	0.3977	102.62
	6	0.4363	0.3977	109.71
		Average		104.27
		Rel. Std Dev		3.56
Day 315				
	1	0.3811	0.3977	95.83
	2	0.3936	0.3977	98.97
	3	0.3595	0.3977	90.39
	4	0.4009	0.3977	100.80
	5	0.3835	0.3977	96.43
	6	0.3747	0.3977	94.22
		Average		96.11
		Rel. Std Dev		3.65

Table 17. Long Term Storage: All Data; Dibutyltin Dichloride

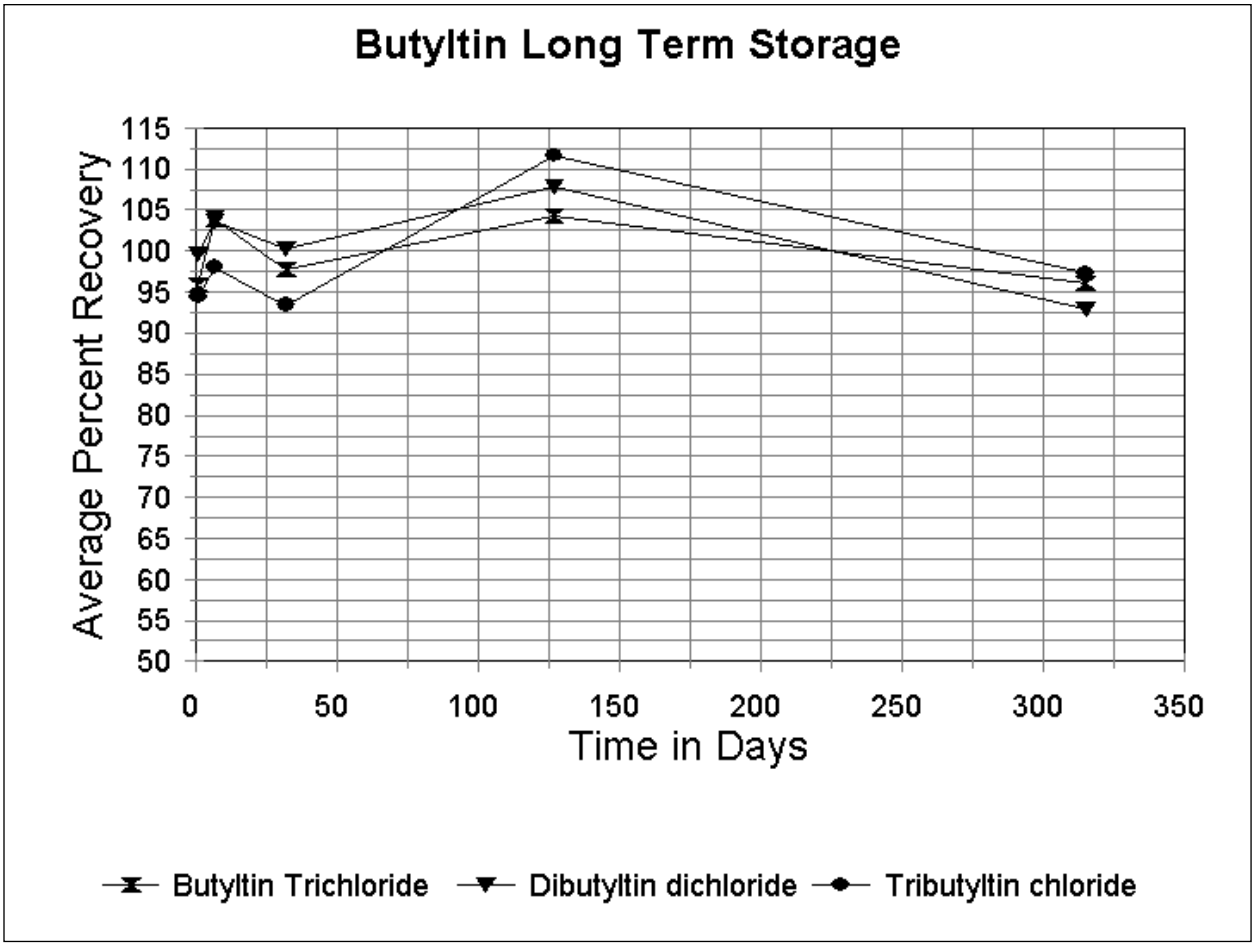
Dibutyltin Dichloride

		Result	Target	% Recovery
Day 1				
	1	0.6148	0.5982	102.77
	2	0.5776	0.5982	96.56
	3	0.6141	0.5982	102.66
	4	0.5867	0.5982	98.08
	5	0.5965	0.5982	99.72
	6	0.5870	0.5982	98.13
		Average		99.65
		Rel. Std Dev		2.58
Day 7				
	1	0.6198	0.5982	103.61
	2	0.6173	0.5982	103.19
	3	0.6194	0.5982	103.54
	4	0.6250	0.5982	104.48
	5	0.6161	0.5982	102.99
	6	0.6211	0.5982	103.83
		Average		103.61
		Rel. Std Dev		0.52
Day 32				
	1	0.6017	0.5982	100.59
	2	0.5909	0.5982	98.78
	3	0.6088	0.5982	101.77
	4	0.6043	0.5982	101.02
	5	0.5920	0.5982	98.96
	6	0.6032	0.5982	100.84
		Average		100.33
		Rel. Std Dev		1.20
Day 127				
	1	0.6353	0.5982	106.20
	2	0.6358	0.5982	106.29
	3	0.6498	0.5982	108.63
	4	0.6367	0.5982	106.44
	5	0.6470	0.5982	108.16
	6	0.6662	0.5982	111.37
		Average		107.85
		Rel. Std Dev		2.01
Day 315				
	1	0.5659	0.5982	94.60
	2	0.5390	0.5982	90.10
	3	0.5688	0.5982	95.09
	4	0.5592	0.5982	93.48
	5	0.5497	0.5982	91.89
	6	0.5509	0.5982	92.09
		Average		92.88
		Rel. Std Dev		1.87

Table 18. Long Term Storage: All Data; Tributyltin Chloride

Tributyltin Chloride								
		Result	Target	% Recovery		Result	Target	% Recovery
Day 1					**Day 7**			
	1	0.4954	0.5069	97.73	1	0.4910	0.5069	96.86
	2	0.4657	0.5069	91.87	2	0.4939	0.5069	97.44
	3	0.4853	0.5069	95.74	3	0.4888	0.5069	96.43
	4	0.4905	0.5069	96.76	4	0.5059	0.5069	99.80
	5	0.4889	0.5069	96.45	5	0.5173	0.5069	102.05
	6	0.4470	0.5069	88.18	6	0.4860	0.5069	95.88
		Average		94.46		Average		98.08
		Rel. Std Dev		3.68		Rel. Std Dev		2.38
Day 32					**Day 127**			
	1	0.4765	0.5069	94.00	1	0.5322	0.5069	104.99
	2	0.4667	0.5069	92.07	2	0.5608	0.5069	110.63
	3	0.4710	0.5069	92.92	3	0.5773	0.5069	113.89
	4	0.4811	0.5069	94.91	4	0.5662	0.5069	111.70
	5	0.4715	0.5069	93.02	5	0.5827	0.5069	114.95
	6	0.4766	0.5069	94.02	6	0.5755	0.5069	113.53
		Average		93.49		Average		111.62
		Rel. Std Dev		1.01		Rel. Std Dev		3.60
Day 315								
	1	0.5497	0.5069	108.44				
	2	0.4399	0.5069	86.78				
	3	0.5014	0.5069	98.91				
	4	0.5025	0.5069	99.13				
	5	0.4451	0.5069	87.81				
	6	0.5225	0.5069	103.08				
		Average		97.36				
		Rel. Std Dev		8.53				

Figure 6. Butyltin Long-Term Storage Results



XII. Conclusions:

The butyltin chloride analysis protocol involving complexation and extraction by tropolone-toluene followed by derivatization with ethylmagnesium bromide and GC-MS in the SIM mode has been demonstrated to be both a precise and accurate [31] method of determining butyltins in urine, with limits of detection low enough to detect alkyltin concentrations in urine that would be typically found following exposure to butyltin chlorides in an industrial setting. The analytes have been found to be stable for at least 315 days, frozen. No sample preservation other than freezing was deemed necessary.

XIII. References:

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Appendix 1. Precision and Accuracy for Butyltin Trichloride

TABLE 1. RECOVERY FROM SPIKED URINE

ANALYTE: **Butyltin Trichloride** Method = Extraction-Derivatization
MEDIA: **Urine** Instrument: GC-MS
INT STD = **none** Derivatizing agent = Ethylmagnesium bromide

			Concentration Level							
			3X LOQ		10X LOQ		30X LOQ		100X LOQ	
			Spike in ug/5-mL 0.121		Spike in ug/5-mL 0.403		Spike in ug/5-mL 1.209		Spike in ug/5-mL 4.032	
Replicate			Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery
1			0.1216	100.50	0.4065	100.87	1.1779	97.43	4.3799	108.63
2			0.1244	102.81	0.4311	106.97	1.3286	109.89	4.3133	106.98
3			0.1176	97.19	0.4273	106.03	1.3263	109.70	4.3146	107.01
4			0.1281	105.87	0.4228	104.91	1.3770	113.90	4.2196	104.65
5			0.1301	107.52	0.4568	113.35	1.3715	113.44	4.2399	105.16
6			none	none	0.4168	103.42	1.3726	113.53	4.1937	104.01
7			none	none	none	none	none	none	none	none
average =			0.1244	102.78	0.4269	105.93	1.3257	109.65	4.2768	106.07
std dev =			0.00501	4.1374	0.01701	4.2210	0.07587	6.2756	0.07061	1.7513
CV 2i=			0.04026	ok	0.03985	ok	0.05723	ok	0.01651	inlier CV
Bias i =			0.02777	ok	0.05926	ok	0.09648	ok	0.06072	ok
N =			5		6		6		6	

TABLE 2. CALCULATION of ACCURACY, OVERALL PRECISION, and MEAN BIAS

Analyte = Butyltin Trichloride Instrument = GC-MS
Medium = Urine Derivatizing agent = Ethylmagnesium bromide
Int Std = none Range studied = 0.121 to 4.032 ug/5-mL

Section 1

		LEVELS OMITTED for BARTLETT'S TEST	LEVELS OMITTED for F' TEST	FINAL OVERALL VALUES					Continued below in
				Pooled CV = **Overall Srt**	Calculated, not using nomogram **Accuracy**	**MEAN BIAS**	**Range of Bias** From =	To =	Section 2 on LINE:
		none	none	**0.04114**	**13.02**	**0.06251**	0.02777	0.09648	A
		3X LOQ	3X LOQ	**0.04138**	**14.02**	**0.07216**	0.05926	0.09648	B
		10X LOQ	10X LOQ	**0.04160**	**13.21**	**0.06365**	0.02777	0.09648	C
		30X LOQ	30X LOQ	**0.03358**	**10.58**	**0.05052**	0.02777	0.06072	D
		100X LOQ	100X LOQ	**0.04690**	**14.03**	**0.06314**	0.02777	0.09648	E

Section 2

	Homogeneity of individual group CVs.						**Homogeneity of individual group biases.**					
		Bartlett's Criteria for 4, 5, or 6 conc. levels			**Pass Bartlett's?**			F' Theoretical for 4, 5, or 6 conc. levels			**PASS F' test?** Is F' < theoretical?	
		Percentile of X^2 dist.		df	Percentile of X^2 dist.					df		
LINE:	**Chi sq'd**	0.95	0.975		0.95	0.975	**F' =**	at a=0.05	at a=0.025		at a=0.05	at a=0.025
A	5.9304	7.81	9.35	3	**YES**	**YES**	1.00126	3.12735	3.90343	3	**YES**	**YES**
B	5.8038	5.99	7.38	2	**YES**	**YES**	0.46302	3.68232	4.76505	2	**YES**	**YES**
C	5.7560	5.99	7.38	2	**YES**	**YES**	1.16801	3.73889	4.85670	2	**YES**	**YES**
D	3.5895	5.99	7.38	2	**YES**	**YES**	0.38431	3.73889	4.85670	2	**YES**	**YES**
E	0.7868	5.99	7.38	2	**YES**	**YES**	1.07161	3.73889	4.85670	2	**YES**	**YES**

Appendix 2. Precision and Accuracy for Dibutyltin Dichloride

TABLE 1. RECOVERY FROM SPIKED URINE

ANALYTE: **Dibutyltin Dichloride** | Method = Extraction-Derivatization
MEDIA: **Urine** | Instrument: GC-MS
INT STD = **none** | Derivatizing agent = Ethylmagnesium bromide

			Concentration Level							
			3X LOQ		10X LOQ		30X LOQ		100X LOQ	
			Spike in ug/5-mL 0.181		Spike in ug/5-mL 0.604		Spike in ug/5-mL 1.811		Spike in ug/5-mL 6.037	
Replicate			Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery
1			0.1664	91.93	0.5633	93.26	1.6794	92.73	5.5764	92.37
2			0.1742	96.24	0.5834	96.59	1.5385	84.95	5.4592	90.43
3			0.1575	87.02	0.5586	92.48	1.5977	88.22	5.5593	92.09
4			0.1582	87.40	0.5736	94.97	1.5852	87.53	5.4355	90.04
5			0.1609	88.90	0.5588	92.52	1.5818	87.34	5.5089	91.25
6			0.1606	88.73	0.5644	93.44	1.5876	87.66	5.6322	93.29
7			none	none	none	none	none	none	none	none
average =			0.1630	90.04	0.5670	93.88	1.5950	88.07	5.5286	91.58
std dev =			0.00633	3.4982	0.00970	1.6065	0.04615	2.5482	0.07461	1.2359
CV 2i=			0.03885	ok	0.01711	inlier CV	0.02893	ok	0.01350	inlier CV
Bias i =			-0.09963	ok	-0.06123	ok	-0.11925	>10%	-0.08422	ok
N =			6		6		6		6	

TABLE 2. CALCULATION of ACCURACY, OVERALL PRECISION, and MEAN BIAS

Analyte = Dibutyltin Dichloride | Instrument = GC-MS
Medium = Urine | Derivatizing agent = Ethylmagnesium bromide
Int Std = none | Range studied = 0.181 to 6.037 ug/5-mL

Section 1		LEVELS OMITTED for BARTLETT'S TEST	LEVELS OMITTED for F' TEST	FINAL OVERALL VALUES Pooled CV = **Overall Srt**	Calculated, not using nomogram **Accuracy**	**MEAN BIAS**	**Range of Bias** From =	To =	Continued below in Section 2 on LINE:
		none	none	**0.02656**	**13.48**	**-0.09108**	-0.11925	-0.06123	A
		3X LOQ	3X LOQ	**0.02091**	**12.26**	**-0.08823**	-0.11925	-0.06123	B
		10X LOQ	10X LOQ	**0.02903**	**14.88**	**-0.10103**	-0.11925	-0.08422	C
		30X LOQ	30X LOQ	**0.02572**	**12.40**	**-0.08169**	-0.09963	-0.06123	D
		100X LOQ	100X LOQ	**0.02966**	**14.22**	**-0.09337**	-0.11925	-0.06123	E

Section 2	**Homogeneity of individual group CVs.**						**Homogeneity of individual group biases.**					
		Bartlett's Criteria for 4, 5, or 6 conc. levels			**Pass Bartlett's?**			F' Theoretical for 4, 5, or 6 conc. levels			**PASS F' test?** Is F' < theoretical?	
		Percentile of X^2 dist.		df	Percentile of X^2 dist.							
LINE:	**Chi sq'd**	0.95	0.975		0.95	0.975	**F' =**	at a=0.05	at a=0.025	df	at a=0.05	at a=0.025
A	6.1235	7.81	9.35	3	**YES**	**YES**	1.17547	3.09839	3.85870	3	**YES**	**YES**
B	2.8834	5.99	7.38	2	**YES**	**YES**	1.24834	3.68232	4.76505	2	**YES**	**YES**
C	4.3919	5.99	7.38	2	**YES**	**YES**	0.41808	3.68232	4.76505	2	**YES**	**YES**
D	5.8760	5.99	7.38	2	**YES**	**YES**	0.52170	3.68232	4.76505	2	**YES**	**YES**
E	2.8011	5.99	7.38	2	**YES**	**YES**	1.17169	3.68232	4.76505	2	**YES**	**YES**

Appendix 3. Precision and Accuracy for Tributyltin Chloride

TABLE RECOVERY FROM SPIKED URINE

ANALYTE: **Tributyltin Chloride**
MEDIA: **Urine**
INT STD = **none**

Method = Extraction-Derivatization
Instrument: GC-MS
Derivatizing agent = Ethylmagnesium bromide

	Concentration Level							
			3X LOQ		10X LOQ		30X LOQ		100X LOQ	
			Spike in ug/5-mL 0.152		Spike in ug/5-mL 0.507		Spike in ug/5-mL 1.521		Spike in ug/5-mL 5.069	
Replicate			Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery	Found in ug/5-mL	Percent Recovery
1			0.1432	94.21	0.4616	91.05	1.4451	95.01	4.8559	95.80
2			0.1460	96.05	0.4829	95.25	1.4091	92.64	4.8696	96.07
3			0.1410	92.76	0.4695	92.60	1.4029	92.24	4.9100	96.86
4			0.1430	94.08	0.4789	94.46	1.4566	95.77	4.7906	94.51
5			0.1498	98.55	0.4858	95.82	1.3994	92.01	4.8215	95.12
6			0.1445	95.07	0.4863	95.92	1.5088	99.20	4.9165	96.99
7			none	none	none	none	none	none	none	none
average =			0.1446	95.12	0.4775	94.18	1.4370	94.48	4.8607	95.89
std dev =			0.00305	2.0061	0.00994	1.9610	0.04233	2.7832	0.04919	0.9704
CV 2i=			0.02109	ok	0.02082	ok	0.02946	ok	0.01012	inlier CV
Bias i =			-0.04879	ok	-0.05819	ok	-0.05524	ok	-0.04110	ok
N =			6		6		6		6	

TABLE 2. CALCULATION of ACCURACY, OVERALL PRECISION, and MEAN BIAS

Analyte = Tributyltin Chloride
Medium = Urine
Int Std = none

Instrument = GC-MS
Derivatizing agent = Ethylmagnesium bromide
Range studied = 0.152 to 5.069 ug/5-mL

Section 1		LEVELS OMITTED for BARTLETT'S TEST	LEVELS OMITTED for F' TEST	FINAL OVERALL VALUES					Continued below in Section 2 on LINE:
				Pooled CV = **Overall Srt**	Calculated, not using nomogram **Accuracy**	**MEAN BIAS**	**Range of Bias** From =	To =	
		none	none	**0.02150**	**8.62**	**-0.05083**	-0.05819	-0.04110	A
		3X LOQ	3X LOQ	**0.02163**	**8.71**	**-0.05151**	-0.05819	-0.04110	B
		10X LOQ	10X LOQ	**0.02172**	**8.41**	**-0.04838**	-0.05524	-0.04110	C
		30X LOQ	30X LOQ	**0.01808**	**7.91**	**-0.04936**	-0.05819	-0.04110	D
		100X LOQ	100X LOQ	**0.02413**	**9.38**	**-0.05407**	-0.05819	-0.04879	E

Section 2	Homogeneity of individual group CVs.						Homogeneity of individual group biases.					
		Bartlett's Criteria for 4, 5, or 6 conc. levels			**Pass Bartlett's?**			F' Theoretical for 4, 5, or 6 conc. levels			**PASS F' test?** Is F' < theoretical?	
		Percentile of X^2 dist.		df	Percentile of X^2 dist.							
LINE:	**Chi sq'd**	0.95	0.975		0.95	0.975	**F' =**	at a=0.05	at a=0.025	df	at a=0.05	at a=0.025
A	4.6069	7.81	9.35	3	**YES**	**YES**	0.11834	3.09839	3.85870	3	**YES**	**YES**
B	4.4909	5.99	7.38	2	**YES**	**YES**	0.12014	3.68232	4.76505	2	**YES**	**YES**
C	4.4832	5.99	7.38	2	**YES**	**YES**	0.07206	3.68232	4.76505	2	**YES**	**YES**
D	2.6199	5.99	7.38	2	**YES**	**YES**	0.10869	3.68232	4.76505	2	**YES**	**YES**
E	0.7536	5.99	7.38	2	**YES**	**YES**	0.03241	3.68232	4.76505	2	**YES**	**YES**

Appendix 4

Review of User Check for NMAM Method 8320 (Butyltin trichloride in urine)

User check samples were prepared by a NIOSH researcher (Dr. Clayton B'Hymer) to be analyzed by ALS Environmental using draft NIOSH Method 8320. A total of 25 urine samples were prepared. The urine was obtained from volunteers at NIOSH and then combined and mixed into a single pool of urine from which all samples were prepared. The final volume of each sample was 20 mL. Five samples were left blank. Five samples were prepared containing each of the analytes at the levels shown in the following table.

Compound	**Level 1 (µg/5 mL)**	**Level 2 (µg/5 mL)**	**Level 3 (µg/5 mL)**	**Level 4 (µg/5 mL)**
Butyltin trichloride	0.128	0.250	12.5	60.5
Dibutyltin dichloride	0.121	0.237	11.85	57.5
Tributyltin chloride	0.123	0.240	12.0	58.5

The samples were shipped frozen to ALS Environmental on October 27, 2015 and arrived there the next day. The samples were analyzed on November 16, 2015. No significant deviations from the analytical procedure in NIOSH Method 8320 were noted.

For this analysis, the Reporting Limit (RL, which is equivalent to the limit of quantitation, LOQ) was determined by ALS to be 0.012 µg/5 mL for all three compounds. As mentioned above, the spike levels ranged from 0.121 to 60.5 µg/5 mL, which is 10 to 5000 times the RL and fall within the range of the calibration curve. The calibration curve covers concentrations from 0.012 to 120.0 µg/5 mL.

None of the butyltin chloride compounds was detected in the blank urines, which is to be expected. Summary tables for each analyte at each level are shown below and tables of data for all the samples can be found at the end of this report.

Compound	**Target conc. (µg/5 mL)**	**Average Recovery (%)**	**RSD (%)**
Butyltin trichloride	0.128	79.2	4.9
Butyltin trichloride	0.250	83.5	5.8
Butyltin trichloride	12.5	90.2	4.0
Butyltin trichloride	60.5	92.7	3.8
Overall		86.4	7.6

Compound	Target conc. (µg/5 mL)	Average Recovery (%)	RSD (%)
Dibutyltin dichloride	0.121	68.8	7.1
Dibutyltin dichloride	0.237	74.3	1.9
Dibutyltin dichloride	11.85	80.7	6.1
Dibutyltin dichloride	57.5	76.0	5.7
Overall		74.9	7.7

Compound	Target conc. (µg/5 mL)	Average Recovery (%)	RSD (%)
Tributyltin chloride	0.123	54.2	8.9
Tributyltin chloride	0.240	56.1	5.0
Tributyltin chloride	12.0	72.0	5.3
Tributyltin chloride	58.5	78.3	3.0
Overall		65.1	17.0

The average recovery for butyltin trichloride is very near the ± 15% accuracy (± 20% at the lowest level) recommended for bioanalytical methods by the US Food and Drug Administration [1]. The relative standard deviation (RSD, which is a measure of precision) for all levels ranged from 3.8 to 5.8 per cent, which is also well within acceptable limits. The average recoveries for dibutyltin dichloride and tributyltin chloride are outside of the recommended criteria. In the case of tributyltin chloride, values of roughly 50% recovery were found at the lower two levels. The contract lab reported no difficulties understanding the draft method nor in setting it up or analyzing the User Check samples. The User Check laboratory followed the procedure and the method has been shown to have adequate precision and accuracy for butyltin trichloride while showing less than adequate precision and accuracy for dibutyltin dichloride and tributyltin chloride. Therefore, the method was rewritten as a single analyte method. Fortunately, butyltin trichloride is the most commonly used and occupationally relevant of these three butyltin chloride compounds. It is recommended that the method, NIOSH Method 8320 (Butyltin trichloride in urine) be approved and accepted for inclusion in the NIOSH Manual of Analytical Methods.

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Research Chemist
June 24, 2016

[1] U.S. Food and Drug Administration. "Guidance for Industry: Bioanalytical Method Validation." US FDA, May 2001.

Data table for butyltin trichloride

Spike ID	Target conc (µg/5 mL)	Analyzed conc (µg/5 mL)	Recovery (%)	Avg recovery	Std. Dev.	RSD
6	0	ND				
9	0	ND				
12	0	ND				
19	0	ND				
24	0	ND				
4	0.128	0.10	78.91			
5	0.128	0.10	77.34			
10	0.128	0.11	85.94			
11	0.128	0.10	77.34			
20	0.128	0.10	76.56	79.22	3.85	4.86
3	0.25	0.23	91.20			
7	0.25	0.20	81.60			
8	0.25	0.20	80.40			
17	0.25	0.21	85.20			
22	0.25	0.20	79.20	83.52	4.84	5.80
2	12.5	11.8	94.40			
14	12.5	10.6	84.80			
18	12.5	11.4	91.20			
21	12.5	11.5	92.00			
23	12.5	11.1	88.80	90.24	3.64	4.03
1	60.5	57.6	95.21			
13	60.5	53.2	87.93			
15	60.5	57.4	94.88			
16	60.5	54.7	90.41			
25	60.5	57.4	94.88	92.66	3.30	3.57
	Overall average		86.41			
	Std. Dev.		6.57			
	RSD		7.60			

Data table for dibutyltin dichloride

Spike ID	Target conc (µg/5 mL)	Analyzed conc (µg/5 mL)	Recovery (%)	Avg recovery	Std. Dev.	RSD
6	0	ND				
9	0	ND				
12	0	ND				
19	0	ND				
24	0	ND				
4	0.121	0.08	63.64			
5	0.121	0.08	64.46			
10	0.121	0.09	75.21			
11	0.121	0.08	68.60			
20	0.121	0.09	71.90	68.76	4.90	7.13
3	0.237	0.18	75.53			
7	0.237	0.17	72.57			
8	0.237	0.17	73.00			
17	0.237	0.18	74.68			
22	0.237	0.18	75.53	74.26	1.40	1.88
2	11.85	9.4	79.24			
14	11.85	8.6	72.83			
18	11.85	9.8	82.70			
21	11.85	10.0	84.39			
23	11.85	10.0	84.39	80.71	4.88	6.05
1	57.5	41.7	72.52			
13	57.5	42.0	73.04			
15	57.5	46.5	80.87			
16	57.5	42.0	73.04			
25	57.5	46.3	80.52	76.00	4.29	5.65
	Overall average		74.93			
	Std. Dev.		5.80			
	RSD		7.74			

Data table for tributyltin chloride

Spike ID	Target conc (µg/5 mL)	Analyzed conc (µg/5 mL)	Recovery (%)	Avg recovery	Std. Dev.	RSD
6	0	ND				
9	0	ND				
12	0	ND				
19	0	ND				
24	0	ND				
4	0.123	0.06	46.34			
5	0.123	0.07	54.47			
10	0.123	0.07	57.72			
11	0.123	0.07	58.54			
20	0.123	0.07	53.66	54.15	4.83	8.92
3	0.24	0.14	56.25			
7	0.24	0.13	55.00			
8	0.24	0.13	53.75			
17	0.24	0.15	60.83			
22	0.24	0.13	54.58	56.08	2.80	5.00
2	12	8.5	71.00			
14	12	7.9	65.83			
18	12	9.0	75.33			
21	12	8.9	74.33			
23	12	8.8	73.67	72.03	3.82	5.30
1	58.5	46.0	78.63			
13	58.5	43.5	74.36			
15	58.5	47.2	80.68			
16	58.5	45.9	78.46			
25	58.5	46.3	79.15	78.26	2.35	3.00
	Overall average	65.13				
	Std. Dev.		11.04			
	RSD		16.96			