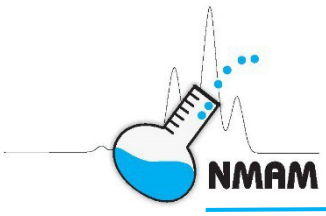
NIOSH Manual of Analytical Methods (NMAM) 5th Edition



BACKUP DATA REPORT
NIOSH Method No. 8321

Title: *o*-Cresol in Urine

Analyte: *o*-Cresol

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NOTE: Method developed under contract CDC-200-2001-08000

o-CRESOL in URINE: Backup Data Report

INTRODUCTION AND BACKGROUND

The National Institute for Occupational Safety and Health (NIOSH) requested the development of a method for the determination of *o*-cresol in urine. The presence of *o*-cresol in urine can be the product from two sources of exposure. One of them is direct exposure to *o*-cresol, and the second, no less important, is exposure to toluene.

o-Cresol

In 90% of the uses, cresols are organic intermediates in the manufacturing of phenolic and epoxy resins and plasticizers, herbicides, rubber and plastic antioxidants, dyes, deodorizing and odor-enhancing compounds, and pharmaceuticals. Additional industrial uses of *o*-cresol or mixtures of cresols include antiseptics and disinfectants, cleaning compounds, degreasers, paint strippers and paints, adhesive and connected products, additives to phenol-formaldehyde resins, wood preservatives, photography, ore flotation agent, and cutting oils [1].

Symptoms of exposure to *o*-cresol may include skin eruptions, as well as liver and kidney damage [2]. *o*-Cresol may cause burns to the eyes, headache, dizziness, nausea, vomiting, stomach pain, exhaustion, and possibly coma [3]. It may also cause painless blanching or erythema, profuse sweating, intense thirst, diarrhea, cyanosis from methemoglobinemia, hemolysis, convulsions, pulmonary edema, death from respiratory failure, jaundice, oliguria, and anuria [4]. When *o*-cresol comes into contact with the skin, it may cause prickling, intensive burning, loss of feeling, wrinkling, white discoloration, softening, and later, gangrene. Other symptoms include extensive damage to the eyes, blindness, weakness of muscles, dimness of vision, ringing of ears, rapid breathing, mental confusion and loss of consciousness [5]. *o*-Cresol

may cause skin burns, central nervous system depression and gastroenteric disturbances [6, 7]. It may also cause skin and eye irritation [8].

The United States Environmental Protection Agency has classified *o*-cresol as a possible human carcinogen [9]. *o*-Cresol is not included for classification of cancer risk by the International Agency for Research on Cancer [10].

Toluene

o-Cresol is a metabolite of toluene, accounting for a small percentage of the absorbed toluene dose. When absorbed, toluene is primarily oxidized to benzoic acid, which is then conjugated with glycine to produce hippuric acid. A small quantity of the toluene absorbed (less than 1%) is oxidized at the aromatic ring and excreted in urine as cresols [11].

Toluene is one of the most extensively used industrial solvents. Because a high number of workers are exposed annually and because of its effects on the central nervous system, a reliable and unambiguous method for toluene exposure assessment is needed. Hippuric acid is the most frequently used biomarker in the biological monitoring of occupational exposure to toluene. This product of solvent biotransformation may be also found in the urine of individuals who have not been exposed to this solvent. Hippuric acid is a normal component of urine and its concentration is typically increased with increased consumption of phenolic compounds (e.g., tea, wine, fruit juices). These phenols are converted to benzoic acid, which is then converted to hippuric acid and excreted in the urine. A smaller fraction of the absorbed toluene is oxidized to aromatic compounds, including *ortho*-cresol, which is not found significantly in the urine of nonexposed individuals. The concentration of hippuric acid in the urine of individuals exposed to a low toluene concentration does not differ from that of individuals not exposed to the solvent [12]. This has led to the conclusion that hippuric acid should not be utilized in thebiological

monitoring of occupational exposure to low levels of toluene in the air. Toluene can be measured in blood [13], but collecting blood is more invasive to the individual.

Toluene is a clear, colorless liquid with a distinctive smell. It is added to gasoline along with benzene and xylene. Toluene occurs naturally in crude oil and in the tolu tree. It is produced in the process of making gasoline and other fuels from crude oil, in making coke from coal, and as a by-product in the manufacture of styrene. Toluene is used in making paints, paint thinners, fingernail polish, lacquers, adhesives, and rubber. It is also used in printing and leather tanning processes. It is disposed of at hazardous waste sites as used solvent or at landfills where it is present in discarded paints, paint thinners, and fingernail polish [14]. The odor of toluene can be detected in air at a concentration of 8 parts of toluene per million parts of air (ppm). Toluene can be tasted in water at a concentration of 0.04–1 ppm. People who work with gasoline, kerosene, heating oil, paints, and lacquers are at the greatest risk of exposure. Because toluene is a common solvent and is found in many consumer products, people can be exposed to toluene at home and outdoors while using gasoline, nail polish, cosmetics, rubber cement, paints, paintbrush cleaners, stain removers, fabric dyes, inks, and adhesives. Smokers are exposed to small amounts of toluene from cigarette smoke. Low-to-moderate, day-after-day exposure in the workplace can cause tiredness, confusion, weakness, drunken-type actions, memory loss, nausea, and loss of appetite. These symptoms usually disappear when exposure is stopped. Researchers do not know if long-term exposure to low levels of toluene will cause any permanent effects on the brain or body after many years [14]. Further research on the biological effects of toluene exposure is needed to determine exposure levels that lead to irreversible neurobehavioral changes [11].

Analysis

Numerous methods for the determination of cresols have appeared in the literature, based on different analytical techniques, e.g., high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [15], gas chromatography with flame ionization detection (GC-FID) [16], and GC with mass spectrometric (MS) detection [17]. The methodology presented in this report is based on the preparation of urine extracts and the subsequent analysis by GC-MS. The use of the highly selective MS has a great advantage over other detectors as it eliminates many of the interferences of other organic compounds that may be present in the samples.

The isomers of cresol are excreted in urine as their glucuronides and sulfates. To analyze for cresols directly, they must be first separated from the biological conjugate and matrix. This is usually accomplished by heating a urine sample with a concentrated mineral acid (e.g., hydrochloric acid, sulfuric acid) for 30 minutes to 1 hour. The transfer of cresol from the aqueous hydrolysate to an organic solvent is accomplished by simple extraction with a volatile organic solvent such as methylene chloride or ethyl ether [18].

In the present method, the sample preparation technique is an adaptation of the procedure used in NIOSH method 8305 [19]. The acid used was concentrated hydrochloric acid and the organic extraction solvent was methyl tert-butyl ether (MTBE). Nitrobenzene was used as an internal standard to normalize the peak areas of *o*-cresol detected in the samples [19]. The extracts were quantified by operating the mass spectrometer in the selected-ion monitoring (SIM) mode, which provides higher sensitivity than the full scan mode.

REAGENTS AND MATERIALS

Presented in Table 1 is the list of reagents and solvents used for this method and its evaluation. Urine used for the study was provided by volunteer donation and stored at 4 ºC.

TABLE 1. LIST OF CHEMICALS

Chemical	Vendor	CAS #	Purity	Lot #
o-Cresol	Chem Service	95-48-7	99.5%	225-31A
Hydrochloric acid	Fisher	7647-01-0	35-38%	4101080-07549
Methanol	Burdick & Jackson	67-56-1	GC[2]	BW298 / BT477
Methyl-tert-butyl ether	Omnisolv	1634-04-4	99.96%	42086
Nitrobenzene	Chem Service	98-95-3	99.5%	219-78A
Sodium sulfate anhydrous	Mallinckrodt	7757-82-6	AR	8024X12612
Water	DataChem Laboratories	7732-18-5	ASTM Type II	N/A

Preparation of Primary Stock and Internal Standard Solutions

The nitrobenzene (internal standard) solution and *o*-cresol primary stock solutions were prepared as noted in Table 2. The solutions were stored at -10 ºC.

TABLE 2. PRIMARY STOCK AND INTERNAL STANDARD SOLUTION PREPARATION

ID	Description	Conc	Preparation
IS	Internal Standard	508	0.1276 g nitrobenzene (99.5% pure) in 250 mL methanol
PSS	Primary Stock Standard	10,680	0.1073 g *o*-cresol (99.5% pure) in 10 mL methanol
ISS1	Intermediate Stock Standard 1	1,068	0.5 mL PSS diluted to 5 mL with methanol
ISS2	Intermediate Stock Standard 2	106.8	0.05 mL PSS diluted to 5 mL with methanol

SAMPLE PREPARATION AND INSTRUMENT CONDITIONS

Sample Preparation

The same preparation method was used consistently for all the samples and standards throughout the study. Aliquots of 5 mL of human urine were dispensed into 15 mL graduated polypropylene centrifuge tubes. The working standards were spiked with specific amounts of *o*-cresol. All samples and standards were acidified with 1 mL of concentrated hydrochloric acid.

The tubes were capped, shaken vigorously for about one minute, and immersed in a water bath at 95 ºC for 1.5 hours. After this, they were removed from the bath and allowed to cool to room temperature.

Aliquots of 0.250 mL nitrobenzene internal standard solution (see Table 2) were added to each tube, then water (ASTM Type II) was added to the 10 mL calibration mark, and finally 2 mL of MTBE were added. The tubes were capped again and shaken vigorously for about 2 minutes.

After the phases separated, the top organic phase was transferred to 2-mL GC amber vials containing around 0.2 g of sodium sulfate (to absorb any water present). The vials were capped and stored at 4 ºC until ready for analysis.

Instrument Conditions

- Instrument: Hewlett Packard (HP) Model 5890 gas chromatograph equipped with an HP Model 5972 mass spectrometer and an HP Model 7673 autosampler
- Column: fused silica capillary column (DB-5ms, 30 m x 0.32 mm I.D., 0.5 µm film)
- Carrier gas: helium
- Gas flow: 1.30 mL/min
- Pressure: constant
- Oven Program: 50 °C for two minutes; 10 °C/min, up to 150 °C; 20 °C/min, up to 310 °C; hold for 5 minutes
- Injector temperature: 265 ºC
- MS detector temperature: 285 ºC
- Injection volume: 2 µL
- Injection mode: splitless
- MS Detector: set to monitor ions *m/z* 108, 107, 77, and 123 in SIM mode with a dwell time of 30 msec. Ions *m/z* 108 and 123 were the quantifying ions for *o*-cresol and nitrobenzene, respectively. Ions *m/z* 107 and 77 were used for qualitative purposes to confirm *o*-cresol and nitrobenzene, respectively.

Calibration

Nine working standards ranging from 0.043 µg/mL to 64.1 µg/mL were prepared by spiking into 5 mL of urine the amounts shown in Table 3.

TABLE 3. PREPARATION OF WORKING STANDARDS

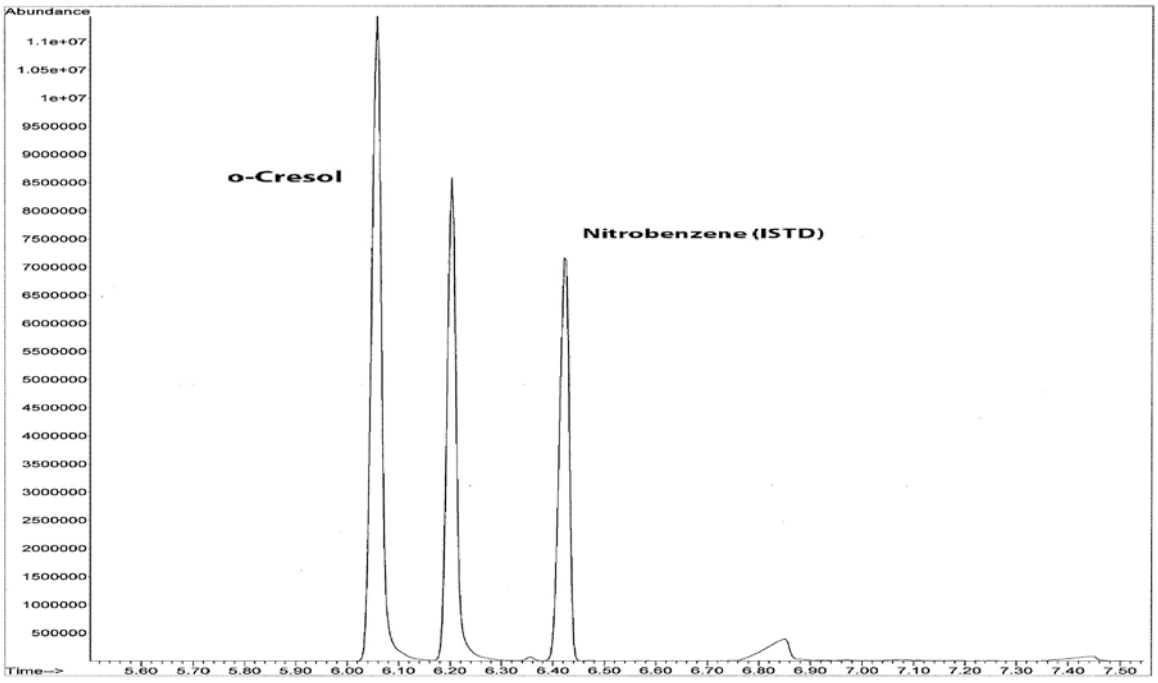
Standard #	Stock Solution	Volume Taken (mL)	Final Dilution Volume (mL)	*o*-Cresol in Urine (µg/mL)
W$1	ISS2	0.002	5.0	0.043
W$2	ISS2	0.005	5.0	0.107
W$3	ISS2	0.010	5.0	0.214
W$4	ISS2	0.050	5.0	1.07
W$5	ISS2	0.100	5.0	2.14
W$6	PSS	0.005	5.0	10.7
W$7	PSS	0.010	5.0	21.4
W$8	PSS	0.020	5.0	42.7
W$9	PSS	0.030	5.0	64.1
Blank	-	0	5.0	0
QC	ISS2	0.005-0.025	5.0	0.1- 0.5

The calibration curve was made by plotting response ratios (Y axis) vs. concentration ratios (X axis) of *o*-cresol to nitrobenzene, using a quadratic model fitting.

Chromatographic Analysis

Figure 1 displays a representative chromatogram when the previously described conditions are employed. This chromatogram is from a 40 µg/mL standard prepared in the urine pool used for the calibration standards utilized in the User Check evaluation (Appendix 2.) The unlabeled peak in the center appears in nearly every individual urine or urine pool. Full-scan mass spectra determined this peak to be *p*-cresol, possibly co-eluting with *m*-cresol. The mass spectra of these compounds are very similar to *o*-cresol. While adequate separation is obtained under method conditions, it would be possible to misidentify this peak and caution should be used. The breakdown of proteins in the body, specifically tyrosine and phenylalanine, lead to a “normal” endogenous appearance of *p*-cresol in the urine [16].

FIGURE 1. Chromatogram of a 40 µg/mL standard prepared in urine



The conditions applied extend the run time considerably beyond the elution of the last peak. This is necessary to bake out any higher-molecular-weight compounds that may be present in the sample extract.

LIMITS OF DETECTION AND QUANTITATION STUDY

For the limit of detection (LOD) and limit of quantitation (LOQ) study, urine standards ranging from 0.0107 µg/mL to 64.1 µg/mL were analyzed. The four lower concentrations were prepared in triplicate, as shown in Table 4.

TABLE 4. LOD/LOQ STANDARDS

STD #	*o*-Cresol (µg/mL urine)
$1	0.0107
$2	0.0107
$3	0.0107
$4	0.0214
$5	0.0214
$6	0.0214
$7	0.0427
$8	0.0427
$9	0.0427
$10	0.107
$11	0.107
$12	0.107
$13	0.214
$14	1.07
$15	2.14
$16	10.7
$17	21.4
$18	64.1

The LOD and LOQ calculated by fitting the data from the analysis of these standards to a quadratic curve and applying Burkart's Method [21] are shown in Table 5.

TABLE 5. LOD/LOQ STUDY RESULTS

R^2	LOD	LOQ
0.9998	0.009 µg/mL urine	0.03 µg/mL urine

Since the LOD estimated by this method is lower than the lowest standard used in the study, the value of the lowest standard becomes the new LOD**.** The final LOD and LOQ for the method are shown in Table 6.

TABLE 6. METHOD LOD/LOQ

LOD	LOQ
0.01 µg/mL urine	0.03 µg/mL urine

LONG-TERM STABILITY STUDY

This study was directed to evaluate the behavior of *o*-cresol in urine samples over a period of time and under two different typically-used storage temperatures. A set of thirty-nine tubes containing 5 mL of urine from the same source were spiked with *o*-cresol at a concentration of approximately 30 x LOQ. Twelve of these tubes were left at room temperature. The other twenty-seven tubes were stored in the refrigerator at 4 ºC. All the specimens were protected from light.

The recoveries of *o*-cresol obtained for the stability study are presented in Table 7.

TABLE 7. RESULTS FOR LONG-TERM STABILITY STUDY

Initial Concentration = 0.854 µg/mL
Replicates Recoveries (µg/mL)

Day	Temperature	R1	R2	R3	R4	R5	R6	Average Recovery %	STD. DEV.
1	4 ºC	0.846	0.915	0.951	0.947	0.921	1.16	112	12.4
7	4 ºC	0.847	0.857	0.806	0.817	0.827	0.822	97.1	2.2
10	4 ºC	0.808	0.954	0.872	-	-	-	103	6.1
14	4 ºC	0.842	0.739	0.779	-	-	-	92.1	6.1
21	4 ºC	0.753	0.828	0.883	-	-	-	96.2	7.6
30	4 ºC	1.01	0.845	0.837	0.749	0.920	0.945	104	10.7
1	~22 ºC	0.881	0.855	1.15	0.829	0.809	0.722	102	16.9
7	~22 ºC	0.767	0.712	0.767	0.814	0.831	0.704	89.7	6.0

The average recoveries for the samples stored at 4 ºC ranged from 92.1 to 112 %, illustrating that refrigeration at 4 ºC is effective for analyte preservation. On the other hand, the samples stored at room temperature began to show analyte loss after 7 days, as seen by comparing the average value obtained for the set left at room temperature (89.7%) with the average value obtained for the set stored at 4 ºC after 7 days (97.1%). It is recommended to keep

the samples refrigerated to minimize the amount of bacterial growth and possible analyte degradation.

PRECISION, BIAS, AND ACCURACY STUDY

The study was performed to evaluate the precision, bias, and accuracy of the method against the acceptance criteria. The precision reflects the ability of the method to replicate measurement results. It is assumed to be constant or homogeneous over all concentrations tested for the method evaluation. The accuracy criterion requires that a method give a result that is within ±25% of the true concentration with a probability of 0.95 for an individual observation (i.e., that the accuracy of an acceptable method is no greater than 25%). Bias, as the uncorrectable relative discrepancy between the mean of the distribution of measurements from a method and the true concentration being measured, must not be greater than 10% [22].

The five concentration levels considered for the study ranged from 1 x LOQ to 100 x LOQ. Each level was evaluated with six replicates. Three blank samples were included in the study, with non-detectable amounts of *o*-cresol present in the results. The samples were prepared according to the method. The recoveries obtained and the average for each level are presented in Table 8.

TABLE 8. *o*-CRESOL RECOVERIES

Level	~1 x LOQ	~3 x LOQ	~10 x LOQ	~30 x LOQ	~100 x LOQ
***o*-Cresol µg/mL urine**	0.0214	0.0854	0.214	0.854	2.14
Recovery *o*-Cresol (%)	122 136 126 108 108 112	96.0 93.7 90.2 89.0 86.7 80.8	78.0 85.5 90.7 88.3 88.3 89.7	91.6 98.1 102 91.2 94.3 97.7	95.7 95.9 77.2 78.9 85.9 106
Average Recovery (%)	118	89.4	86.8	95.8	89.9
Precision CV	0.0956	0.0601	0.0532	0.0446	0.124

Bartlett's test for homogeneity of the individual coefficients of variation for all test levels gave a Chi squared value of 6.98, which was acceptable for a 95% distribution, allowing the data to be pooled from all 5 levels. There were no Grubb's outliers at any level; however, the F′ test for homogeneity of bias failed for a 95% distribution. After omitting the lowest level, 1 x LOQ, both tests, Bartlett's and F′, passed for a 95% distribution.

The accuracy was obtained using a nomogram relating accuracy to precision and bias. The ordinate axis of the graph is method precision while the abscissa is method bias. Accuracy is marked by the curved lines on the graph [22]. The results of the study are shown in Table 9.

TABLE 9. PRECISION, BIAS, AND ACCURACY

Compound	Range Studied (µg/mL urine)	Precision Overall S_{rT}	Average Bias	Bias Range	Accuracy (%)
o-Cresol in Urine	0.0214 to 2.14	0.0771	-0.0952	-0.132 to -0.0416	21

The detailed data used in calculating the precision, bias, and accuracy are included in Appendix 1.

SUMMARY

The data generated during the development of this method met NIOSH criteria for precision, bias, and accuracy in all studies performed [22] and proved to be adequate for the purpose it was developed. The method also meets guidelines for bioanalytical method development and evaluation [23].

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APPENDIX 1
RECOVERY DATA FOR o-CRESOL IN URINE
Part 1 (n=6)

Level	~1 x LOQ	~1 x LOQ	~3 x LOQ	~3 x LOQ	~10 x LOQ	~10 x LOQ	~30 x LOQ	~30 x LOQ	~100 x LOQ	~100 x LOQ
o-Cresol Applied	0.0214 µg/mL	0.0214 µg/mL	0.0854 µg/mL	0.0854 µg/mL	0.214 µg/mL	0.214 µg/mL	0.854 µg/mL	0.854 µg/mL	2.14 µg/mL	2.14 µg/mL
o-Cresol Recovered	µg/mL	%	µg/mL	%	µg/mL	%	µg/mL	%	µg/mL	%
Sample 1	0.026	121.5	0.082	96.0	0.167	78.0	0.782	91.6	2.048	95.7
Sample 2	0.029	135.5	0.080	93.7	0.183	85.5	0.838	98.1	2.053	95.9
Sample 3	0.027	126.2	0.077	90.2	0.194	90.7	0.873	102.2	1.653	77.2
Sample 4	0.023	107.5	0.076	89.0	0.189	88.3	0.779	91.2	1.689	78.9
Sample 5	0.023	107.5	0.074	86.7	0.189	88.3	0.805	94.3	1.839	85.9
Sample 6	0.024	112.1	0.069	80.8	0.192	89.7	0.834	97.7	2.264	105.8
Average	0.025	118.4	0.076	89.4	0.186	86.8	0.819	95.8	1.924	89.9
Std Dev	0.0024	11.32	0.0046	5.37	0.0099	4.61	0.0365	4.28	0.2381	11.13
CV2	0.09561	0.09561	0.06013	0.06013	0.05317	0.05317	0.04461	0.04461	0.12374	0.12374

RECOVERY DATA FOR o-CRESOL IN URINE
Part 2

Statistical Element	Value
CV2 pooled (all 5 levels included)	0.08110
CV2 pooled (omitting lowest concentration level)	0.07705
o-cresol recovered weighted average (all 5 levels included)	96.06 %
o-cresol recovered weighted average (omitting lowest concentration level)	90.48 %
chi squared (all 5 levels included)	$\chi^2 = 6.9846$
chi squared (lowest level omitted)	$\chi^2 = 6.5082$
Bartlett's criteria for 5 concentration levels (5% of significance level)	$9.49 \geq \chi^{2<}$, $\chi^2 = 6.9846 \Leftrightarrow$ "PASS"
Bartlett's criteria for 4 concentration levels (5% of significance level)	$7.81 \geq \chi^{2<}$, $\chi^2 = 6.5082 \Leftrightarrow$ "PASS"
F′ criteria (at 0.05) for 5 concentration levels	F′ = 2.7587
F′ criteria (at 0.05) for 4 concentration levels	F′ = 3.0984
F′ method for 5 concentration levels	FM′ = 12.0796 > F′⇔ "DO NOT PASS"
F′ method for 4 concentration levels	FM′ = 1.2075 < F′ ⇔ "PASS"

BIAS CALCULATION FOR o-CRESOL IN URINE

$$B = \left(\frac{o - \text{cresol recovered}}{o - \text{cresol applied}}\right) - 1$$

Test Concentration Levels	1 x LOQ	3 x LOQ	10 x LOQ	30 x LOQ	100 x LOQ
1	0.21495	-0.03981	-0.21963	-0.08431	-0.04299
2	0.35514	-0.06323	-0.14486	-0.01874	-0.04065
3	0.26168	-0.09836	-0.09346	0.02225	-0.22757
4	0.07477	-0.11007	-0.11682	-0.08782	-0.21075
5	0.07477	-0.13349	-0.11682	-0.05738	-0.14065
6	0.12150	-0.19204	-0.10280	-0.02342	0.05794
Average Bias	0.183801	-0.106167	-0.132399	-0.041569	-0.100779
Standard Deviation	0.11318	0.05375	0.04613	0.04275	0.11127
N	6	6	6	6	6

Weighted Average for Bias = -0.09523
Average Standard Deviation = 0.06348

Appendix 2
Review of User Check for NIOSH Method 8321 (*o*-Cresol in urine)

User check samples were prepared by a NIOSH researcher (Dr. Clayton B'Hymer, BHAB) to be analyzed by ALS Environmental using draft NIOSH Method 8321. A total of 25 urine samples were prepared. The urine was obtained from volunteers at NIOSH and then combined and mixed in the BHAB labs 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 the analyte at each of the following levels: 0.253 µg/mL, 0.506 µg/mL, 5.06 µg/mL, and 30.35 µg/mL. The samples were shipped frozen to ALS Environmental on July 8, 2014 and arrived there the next day. The samples were analyzed on July 25, 2014. No significant deviations from the analytical procedure in NIOSH Method 8321 were noted.

For this analysis, the Reporting Limit (RL, which is equivalent to the limit of quantitation, LOQ) was determined by ALS to be 0.070 µg/mL. As mentioned above, the spike levels ranged from 0.253 to 30.35 µg/mL, which is 4 to 430 times the RL and fall within the range of the calibration curve. The calibration curve covers concentrations from 0.040 to 60.0 µg/mL.

o-Cresol was detected in the blank urines, which is to be expected, so blank correction is required for this set of samples. The average concentration found in the blank samples was 0.047 µg/mL with a relative standard deviation (RSD) of 2.3%. While this value is below the reporting limit set by ALS, it is above the lowest point of the calibration curve and was measured with high precision, so it is appropriate to add the blank value to the known spiked amount to give a "true" target value. A summary table for each level is shown below and a table of data for all samples can be found at the end of this report.

Table 2.1 Recovery results

True target conc (µg/mL)	Average Recovery (%)	Standard Deviation	% RSD
0.300	96.7	0.76	0.79
0.553	98.4	0.61	0.62
5.11	100.3	0.63	0.63
30.40	92.8	1.30	1.40
Overall	97.1	2.95	3.03

RSD=relative standard deviation

The recovery accuracy at every level is within +/- 8% of the true value, which is well within the +/- 15% accuracy required 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 0.6 to 1.4 per cent, which is also well within acceptable limits. The contract lab reported no difficulties understanding the draft method nor in setting it up or analyzing the samples. The User Check laboratory followed the procedure and the method has been shown to have adequate precision and accuracy. It is recommended that the method, NIOSH Method 8321 (*o*-Cresol in urine) be approved and accepted for inclusion in the NIOSH Manual of Analytical Methods.

Table 2.2 Recovery data table

Spike ID	Target conc (µg/mL)	Found conc* (µg/mL)	Target + blank (µg/mL)	Found conc (µg/mL)	Recovery (%)
4	0	0.048	0	0.048	
7	0	0.046	0	0.046	
14	0	0.048	0	0.048	
16	0	0.048	0	0.048	
25	0	0.046	0	0.046	
1	0.253	0.287	0.300	0.287	95.7
9	0.253	0.289	0.300	0.289	96.3
12	0.253	0.291	0.300	0.291	97.0
19	0.253	0.293	0.300	0.293	97.7
21	0.253	0.291	0.300	0.291	97.0
3	0.506	0.549	0.553	0.549	99.3
8	0.506	0.543	0.553	0.543	98.2
15	0.506	0.544	0.553	0.544	98.4
17	0.506	0.546	0.553	0.546	98.7
24	0.506	0.540	0.553	0.540	97.6
5	5.06	5.12	5.11	5.12	100.3
10	5.06	5.11	5.11	5.11	100.1
11	5.06	5.18	5.11	5.18	101.4
20	5.06	5.10	5.11	5.10	99.9
22	5.06	5.11	5.11	5.11	100.1
2	30.35	28.6	30.40	28.6	94.1
6	30.35	27.7	30.40	27.7	91.1
13	30.35	28.4	30.40	28.4	93.4
18	30.35	27.9	30.40	27.9	91.8
23	30.35	28.5	30.40	28.5	93.8

*Ave blank=0.047
Ave = Average; SD = standard deviation; RSD = relative standard deviation.
SD and RSD are calculated using the recovery values.

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[1] FDA [2001]. Guidance for industry: Bioanalytical method validation.
http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf