ELSEVIER

Journal of Chromatography B, 703 (1997) 105–113

JOURNAL OF CHROMATOGRAPHY B

Quantitation of *o*-, *m*- and *p*-cresol and deuterated analogs in human urine by gas chromatography with electron capture detection

Russell L. Dills*, Garland M. Bellamy, David A. Kalman

Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Box 357234, Seattle, WA 98195-7234, USA

Received 13 March 1997; received in revised form 31 July 1997; accepted 31 July 1997

Abstract

A gas chromatographic method for the analysis of cresol metabolites of toluene and [$^2H_8$]toluene in urine was developed. Cresol glucuronides and sulfates in urine were hydrolyzed with β-glucuronidase and arylsulfatase. Following extraction with *tert*.-butyl methyl ether and solvent exchange into benzene, the cresols were derivatized with heptafluorobutyric anhydride to form the heptafluorobutyrate esters. The derivatives were analyzed by gas chromatography with electron capture detection. Chromatographic resolution was achieved between all cresol isomers and their $^2H_7$ analogs. Calibration ranged from 0.001 to 500 μg/ml. Recoveries were 55–97% and showed no trend with respect to analyte concentration. Within-day precision of analyses of benchmark urine samples had a coefficient of variation of less than 4%. The assay sensitivity was limited by chromatographic background but was sufficient for quantification of the unlabeled cresols in urine from men with only environmental exposure to toluene. Average levels in urine samples from 45 men were 0.023, 0.054 and 37 μg/ml for *o*-, *m*- and *p*-cresol, respectively. © 1997 Elsevier Science B.V.

Keywords: Urine; Cresol; Toluene

1. Introduction

Measurement of urinary metabolites of toluene has become an important tool for assessing occupational exposure to this common solvent. Hippuric acid (*N*-benzoylglycine) is the primary metabolite of toluene [1,2]. However, other sources of hippuric acid, such as endogenous metabolic pathways and intake of benzoic acid [3], compromise the use of this metabolite for biomonitoring of low level exposure to toluene [2,4]. Cresols are minor metabolites (1% of the absorbed dose) formed by the ring oxidation of toluene [2]; all three isomers are formed [5] and each has been examined to varying degrees as an alternative to hippuric acid as a biomonitoring tool. Most effort has been focused on *o*-cresol [4,6–8], but some studies indicate that *o*-cresol levels in urine may be elevated by smoking [9,10], making monitoring of this metabolite questionable in some cases. *p*-Cresol can be formed by microbial metabolism of tyrosine in the gut [11] and occurs in food [12] and, thus, has limited utility in biomonitoring. Studies of *m*-cresol, the cresol produced in the least amount during the metabolism of toluene, have been hampered by lack of sensitivity and of chromatographic resolution between it and the other cresols.

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00407-6

Correlation between *m*-cresol excretion and other biomarkers of toluene has been observed at a toluene exposure of 200 ppm [5] but not at a lower exposure of 55 ppm [13], possibly because the level of *m*-cresol was near or below the limit of quantitation.

Previous analyses of cresols in urine have used acid [7,11,13–30] or enzymes [31–33] to hydrolyze the glucuronide and sulfate conjugates of the cresols. The cresols were then extracted with solvent or in some cases were steam distilled [11,14,17,26,32]. The resulting solution has been analyzed without derivatization by packed-column gas chromatography (GC) with flame ionization detection (FID) [13,20,22,25,30], capillary column GC with FID [7,14,21,28], capillary column GC with mass spectrometric (MS) detection [29], high-performance liquid chromatography (HPLC) with fluorescence detection [11,18,19], HPLC with UV detection [17,27,31,33], HPLC with electrochemical detection [32], and MS with a silicone membrane source [24]. Various derivatives of cresols, i.e., acetate [15,26], pentafluoropropyl [23] and trimethylsilyl [34], have been formed and analyzed by packed [15] or capillary [23,26,34] column GC with FID. Heptafluorobutyryl derivatives have been used previously in the determination of phenols in water [35,36]. The analysis of phenols by GC and HPLC has been reviewed [37]. We present a capillary column GC method utilizing electron capture detection after derivatization of the enzymatically deconjugated cresols with heptafluorobutyric anhydride.

2. Experimental

2.1. Materials

Benzene was of distilled-in-glass grade (Omnisolv, EM Science, Gibbstown, NJ, USA). Toluene was not an adequate substitute for benzene, a known human carcinogen, because cresols were present in all grades of toluene available. Attempts at purifying toluene by basic extraction or chromatography on alumina, silica or cesium silicate did not reduce the cresols to non-interfering levels. *tert*.-Butyl methyl ether was of distilled-in-glass grade (Aldrich, Milwaukee, WI, USA). 4-Fluoro-2-methylphenol, *o*-cresol, *m*-cresol, *p*-cresol and 4-pyrrolidinopyridine were purchased from Aldrich. Sulfatase–glucuronidase (type H-1 from *Helix pomatia*) was purchased from Sigma (St. Louis, MO, USA). $^{2}H_{8}$-*o*-Cresol (d_{8}-*o*-cresol; 98 atom%) and $^{2}H_{8}$-*p*-cresol (d_{8}-*p*-cresol; 98 atom%) were bought from Cambridge Isotope Laboratories (Woburn, MA, USA). $^{2}H_{8}$-*m*-Cresol (d_{8}-*m*-cresol; 99 atom%) was purchased from MSD Isotopes (Point Claire, Canada). Heptafluorobutyric acid anhydride (HFBA) was obtained from Pierce (Rockford, IL, USA). Neomycin sulfate was of USP grade.

2.2. Synthesis of heptafluorobutyl esters of phenols for standards

Benzene (50 ml, glass distilled), a phenol (9 mmol), heptafluorobutyric anhydride (20 mmol) and triethylamine or pyridine (20 mmol) were added, in order, to a round-bottomed flask and refluxed for 1 h. The reaction mixture was washed with 2×50 ml of 1.0 *M* sodium phosphate (pH 6.0) and quickly washed with 2×50 ml 0.1 *M* NaOH. The benzene layer was dried with Na_2SO_4 and then evaporated. The HFB esters were then purified by vacuum distillation followed by silicic acid chromatography (toluene). Identities were confirmed by GC–MS. The cresol HFB esters had similar mass spectra. *o*-cresol: *m/z* 304 ($M^{+\cdot}$, 55%), 169 ($[M-C_3F_7]^+$, 21%), 107 ($[M-COC_3F_7]^+$, 100%) and 91 ($[M-OCOC_3F_7]^+$, 82%). *m*-Cresol: *m/z* 304 ($M^{+\cdot}$, 51%), 169 ($[M-C_3F_7]^+$, 18%), 107 ($[M-COC_3F_7]^+$, 50%) and 91 ($[M-OCOC_3F_7]^+$, 100%). *p*-Cresol: *m/z* 304 ($M^{+\cdot}$, 68%), 169 ($[M-C_3F_7]^+$, 17%), 107 ($[M-COC_3F_7]^+$, 100%) and 91 ($[M-OCOC_3F_7]^+$, 82%). d_7-*o*-Cresol: *m/z* 311 ($M^{+\cdot}$, 56%), 169 ($[M-C_3F_7]^+$, 5%), 114 ($[M-COC_3F_7]^+$, 100%) and 98 ($[M-OCOC_3F_7]^+$, 71%). d_7-*m*-Cresol: *m/z* 311 ($M^{+\cdot}$, 52%), 169 ($[M-C_3F_7]^+$, 13%), 114 ($[M-COC_3F_7]^+$, 57%) and 98 ($[M-OCOC_3F_7]^+$, 100%). d_7-*p*-Cresol: *m/z* 311 ($M^{+\cdot}$, 73%), 169 ($[M-C_3F_7]^+$, 17%), 114 ($[M-COC_3F_7]^+$, 100%) and 98 ($[M-OCOC_3F_7]^+$, 67%). 4-Fluoro-2-cresol: 322 ($M^{+\cdot}$, 46%), 169 ($[M-C_3F_7]^+$, 26%), 125 ($[M-COC_3F_7]^+$, 100%) and 109 ($[M-OCOC_3F_7]^+$, 72%). Purities were assessed by GC with FID and expressed as % peak area: d_7-*o*-Cresol, 94.7%; *o*-cresol, 96.2%; d_7-*m*-cresol, 86.0% (0.8% d_7-*p*-cresol); *m*-cresol, 97.0%; d_7-*p*-cresol, 89.9% (1.7% d_7-

o-cresol, 2.6% d_7-*m*-cresol); *p*-cresol, 93.5% (0.9% d_7-*m*-cresol) and 4-fluoro-2-cresol, 98.1%. Toluene was the major impurity (0.8–6.7%) and was difficult to remove without excessive loss of product.

2.3. Human exposure and urine collection protocol

Male human volunteers were exposed to 100 ppm of an equimolar mixture of $^2H_8$-labeled toluene and unlabeled toluene by inhalation for 2 h [38]. Urine samples were complete voids, collected once pre-exposure, separately at intervals chosen by the subject for the first 24 h post-exposure, and twice each day for the next three days. Urine samples were stored at −20°C.

2.4. Sample preparation

To 1.0 ml of urine, 0.39 ml of buffer (0.1 *M* sodium phosphate buffer, pH 5.0), 0.10 ml of neomycin sulfate (5.0 mg/ml, in buffer), 0.100 ml of internal standard solution (100 μg/ml 4-fluoro-2-cresol in acetonitrile) and 0.6 ml of sulfatase–glucuronidase (350 units/ml sulfatase and approximately 6000 units/ml glucuronidase activity in buffer) were added. After gentle mixing, the mixture was incubated at 37°C for 16 h in a PTFE-lined screw-cap test tube.

The hydrolysate was adjusted to pH 1.5 by the addition of 0.15 ml of 2.0 *M* hydrochloric acid. Solid sodium chloride was added to saturate the solution. Samples were extracted with 2.5 ml of *tert.*-butyl methyl ether by vortex-mixing for 2 min and then with 2.0 ml of the ether. The organic layers were combined and dried with approximately 0.5 g of anhydrous Na_2SO_4 for 20 min. After centrifugation to pellet the Na_2SO_4, the organic phase was placed in a 5-ml conical, microreaction vial. 4-Pyrrolidinopyridine was added (0.10 ml, 18 mg/ml in benzene). Extracts were reduced in volume to approximately 10 μl in a heated vacuum centrifuge, (6 min at 400–660 Pa; model SVC100H, Savant Instruments, Farmingdale, NY, USA).

The residue was dissolved in 1.2 ml of benzene, 0.2 ml of HFBA was added and the mixture was heated for 60 min at 80°C after sealing the vial with a PTFE-lined cap. The samples were briefly vortex-mixed after 15 min of heating, to assist dissolution of the derivatizing agents. The derivatization mixture was transferred to a test tube and extracted twice with 1.5 ml of 1.0 *M* sodium phosphate buffer (pH 6.0) by vortex-mixing for 2 min to remove excess derivatizing reagent. The organic layer was centrifuged briefly and transferred to GC vials.

With each set of samples, a benchmark urine sample and a procedural blank (using water instead of urine) were processed in duplicate or triplicate in an identical manner to that for the samples. The benchmark was made by spiking urine from a person without occupational exposure to toluene with deuterated and non-deuterated cresols. In the benchmark, *o*- and *m*-cresols had a nominal concentration of 1 μg/ml, d_7-*p*-cresol was 5 μg/ml and *p*-cresol was approximately 60 μg/ml. These levels mimic those seen in urine from exposed subjects.

2.5. Sample analysis

The gas chromatograph (HP 5880, Hewlett-Packard, Avondale, PA, USA) was equipped with an electron capture detector (ECD) and an autosampler (HP 7673A). A capillary column (DB-5, 30 m×0.25 mm I.D., 1.0 μm film thickness; J&W Scientific, Folsom, CA, USA) was used with hydrogen carrier gas (50 cm/s linear velocity at 90°C). The injector temperature was 200°C. Split injections (1 μl) were made at a split ratio of 1:24. The quartz injection liner and quartz wool plug were silanized in place with an injection mixture of trimethylchlorosilane and hexamethyldisilazane [39]. The oven temperature program was 90°C for 15 min followed by a 20°C/min ramp to 280°C, which was held for 2 min. The detector temperature was set at 300°C and supplied with nitrogen make-up gas at 50 ml/min.

Peak areas were determined by the valley-to-valley mode of integration. The internal standard method of quantitation was used. Background area ratios, as assessed by the procedural blanks, were subtracted from those of the samples prior to computation of concentration. A combined standard solution containing analytes and internal standard was serially diluted with a constant internal standard concentration to generate calibrant solutions. Typical ranges for the calibrants were: *o*- and *m*-cresols, 0.001–10 μg/ml; d_7-*p*-cresol, 0.01–100 μg/ml and *p*-cresol, 0.1–500 μg/ml. Calibration curves were fit to the

equation of the form, $y=ax^{1.2}+bx+c$, by weighted least-squares regression, where concentration is y and the area ratio is x. The weighting factor was the reciprocal of the square of the concentration.

GC–MS analysis, for confirmation of the identity of synthesized standards, was performed on an HP5890 gas chromatograph coupled to a Finnigan 4000 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) operated in the electron impact mode at 70 eV. A capillary column (DB-5, 30 m× 0.25 mm I.D., 0.25 μm film thickness; J&W Scientific) was used with helium as the carrier gas (25 cm/s linear velocity at 90°C). The temperature program was the same as listed for the GC–ECD analysis.

3. Results and discussion

3.1. Method development

We chose enzymatic hydrolysis of the cresol conjugates with arylsulfatase and β-glucuronidase rather than acid hydrolysis to reduce artifact formation and allow the assay to be generalized to phenolic metabolites of other aromatic solvents. Phenolic glucuronides are resistant to complete hydrolysis by acid [40–42]. The high acid concentrations and heat needed for complete hydrolysis may covert phenolic acids into phenols [40]. In addition, more sensitive phenols, such as vinyl phenol, may be oxidized and, thus, limit the possible applications of the assay.

The enzyme concentration necessary to hydrolyze urine samples overnight was determined from the time course of hydrolysis at different enzyme concentrations. For this experiment, a composite sample from ten volunteers exposed to d_8-labeled toluene and toluene was representative of samples with the highest expected concentration of cresols (approximately 25 μg/ml). Enzyme concentrations of 1800 units of β-glucuronidase per ml and 107 units of arylsulfatase per ml gave responses that reached maxima for all analytes within 14 h. These enzyme concentrations were doubled in the assay to allow for subject variation in cresol excretion and toluene dosage.

Substitution of *tert*.-butyl methyl ether for the commonly used [37] but peroxidizable extraction solvents, diethyl or diisopropyl ether, avoided potential safety hazards and losses of analytes by reaction with peroxides. Experiments in which dry extracts were derivatized in *tert*.-butyl methyl ether showed massive ECD responses in reagent blanks. Both the oxygen in the ether and dissolved derivatization reagent contributed to the elevated baseline. Poor precision in spiked samples (C.V.> 30%) was a consequence of the baseline disturbances. Solvent exchange into benzene prior to derivatization eliminated this general interference and improved the precision. Solvent exchange greatly reduced the concentrations of *tert*.-butyl methyl ether and derivatization reagent. However, loss of up to 30% of the analyte occurred in this step due to volatilization of the HFB esters. Benzene was chosen because it was polar enough to solubilize the reagents, and did not have an ECD response. Toluene was an unacceptable substitute because it contains cresols.

Combinations of various electrophoric derivatization reagents (e.g. pentafluoropropionic acid anhydride and heptafluorobutylimadazole) and organic bases (e.g. triethylamine), were tried in attempts to eliminate low level interferences with the chromatographic peaks of the analytes. HFBA and 4-pyrrolidinopyridine were the combination that had the least interference. 4-Pyrrolidinopyridine also acted as a trapping agent for the cresols during reduction of solvent volume.

3.2. Method validation

The isomers of the cresols and the deuterated analogs were resolved (Fig. 1D). Water blanks frequently had small peaks at the retention times of the analytes (Table 1); small peaks at the retention times of *o*-cresol and *p*-cresol appear in the chromatogram of a water blank in Fig. 1A. To correct for the presence of this background, water blanks were processed in duplicate or triplicate and the area ratios of peaks at the retention times of the analytes to the internal standard were subtracted from those of samples. Although the calibrants are quantifiable to lower levels, the blanks set the lower quantitation limits. The concentrations of the small peaks in the water blanks were too low to determine their identity

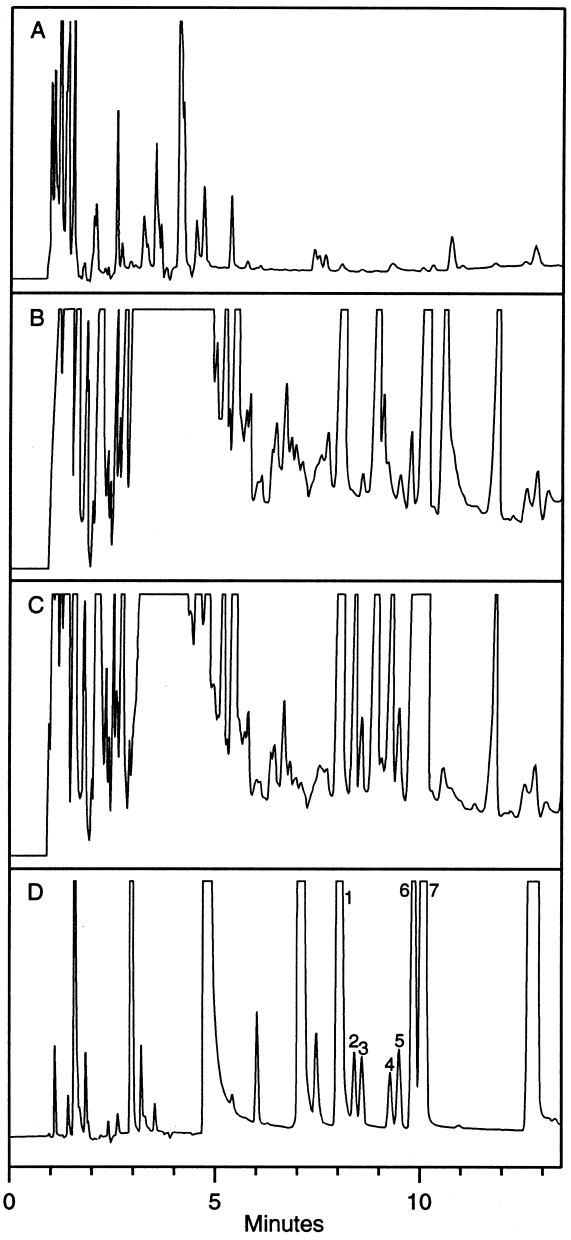

Fig. 1. Gas chromatograms of assay blank (A), pre-exposure (background) urine sample (B), urine sample taken immediately after exposure (C) and standard (D). The assay blank was water that was processed identically to urine. Peak identities and retention times (min): 1, 4-fluoro-*o*-cresol, 7.88; 2, d_7-*o*-cresol, 8.13; 3, *o*-cresol, 8.35; 4, d_7-*m*-cresol, 9.15; 5, *m*-cresol, 9.38; 6, d_7-*p*-cresol, 9.73; 7, *p*-cresol, 9.97. Nominal concentrations for the standard shown are: *o*- and *m*-cresols, 0.1 μg/ml; d_7-*p*-cresol, 1.5 μg/ml; *p*-cresol, 8.5 μg/ml and 4-fluoro-*o*-cresol (ISTD), 26 μg/ml.

by mass spectrometry. Other cases of interference were infrequently seen in chromatographic profiles of urine from particular individuals; incomplete resolution between unknown components and the analyte peaks, *o*-cresol, d_7-*o*-cresol and d_7-*p*-cresol, occurred with a frequency of less than 7%.

Quantitation by absolute calibration was based on the response of standard solutions prepared from synthesized cresol HFB esters. Area ratios of analytes to internal standard were slightly curved over the three order-of-magnitude range of calibration. Over smaller ranges (1–2 orders of magnitude), the calibration curves appeared to be linear. An extended range of calibrants (four orders of magnitude) showed that the curve was sigmoidal. The lower half was our typical range of calibration and was found to best fit equations of the form $y=ax^d+bx+c$, where d was found to be 1.2 for all analytes.

Within-day precision for analyses of benchmark samples had C.V.s ≤3% for all analytes (Table 2). Between-day C.V.s were approximately 16% (Table 2). Recoveries were calculated from urine samples spiked with the deuterated analytes and the internal standard. Recoveries were 55–97% and showed no trend with respect to analyte concentration (Table 3). Extensive recovery experiments were not performed with unlabeled cresols because of their occurrence in urine. Preliminary experiments in water demonstrated equivalent behavior of deuterated and non-deuterated cresols (data not shown). Evaporation of the extract during the course of solvent exchange was the primary cause of lowered recoveries. Recoveries were lowered when prolonged evaporation took place; timing of the evaporation was critical for high recoveries. Phenol, a compound with higher volatility than that of the cresols, had uniformly lower recoveries than the cresols. The internal standard, a phenol of approximately the same volatility as that of the analytes, appeared to track the losses of the analytes and thus allowed for correction of the recoveries.

We examined the stability of GC-ready samples at different storage temperatures. The cresols' HFB esters in prepared samples are stable for at least ten days at room temperature or at −80°C. The HFB esters did hydrolyze slowly even at −80°C; storage for six months produced significant losses (>50%). Deuterated and non-deuterated *m*- and *p*-cresol HFB

Table 1
Occurrence of low level peaks at retention times of analytes in procedural blank samples (water; $n=40$) and concentrations of analytes in pre-exposure urine samples ($n=45$)

	Water blanks		Pre-exposure urine samples	
Analyte	Observed frequency (%)	Concentration Mean[a]±S.D. (μg/ml)	Observed frequency (%)	Concentration median (μg/ml)
d_7-*o*-Cresol	27	0.007±0.004	0	
o-Cresol	76	0.013±0.011	89	0.015
d_7-*m*-Cresol	80	0.004±0.003	0	
m-Cresol	56	0.003±0.002	96	0.036
d_7-*p*-Cresol	46	0.007±0.012	0	
p-Cresol	1	0.002	100	29

[a]Median or mean are of samples or blanks with concentrations >0 μg/ml.

Table 2
Precision for analyses of benchmark samples [C.V. (%)]

Analyte	Within-day[a]	Between-day
4-Fluoro-*o*-cresol	3	18
d_7-*o*-Cresol	2	16
o-Cresol	1	14
d_7-*m*-Cresol	3	16
m-Cresol	3	16
d_7-*p*-Cresol	3	16
p-Cresol	2	15

[a]Data are from duplicate analyses performed on sixteen days.

esters decreased in concentration by approximately 70% after storage for ten days at approximately −5°C. This may be due to condensation of water vapor inside the GC vial and subsequent hydrolysis of the esters. The addition of small amounts of water to the GC-ready samples was found to increase the hydrolysis of the esters at −5°C. Prompt analysis of GC-ready samples is recommended. Analysis of benchmark urine samples demonstrated no loss of analytes in urine when stored for seven months at −20°C. Others have shown that cresol concentrations are stable for at least several months in unprocessed urine samples that are stored at −20°C [14].

3.3. Method application

Subjects in our study were non-smokers and the concentration of unlabeled cresols present in the pre-exposure urine samples are comparable to those found in other studies (Table 4). Concentrations of urinary *p*-cresol in smokers are likely to be higher than those found in our study of non-smokers because of its presence in inhaled smoke. Only one previous study has published values for *m*-cresol in the urine of unexposed subjects [13].

The assay was sensitive enough to detect background levels of non-deuterated cresols in urine from subjects without workplace or experimental exposure to toluene (Fig. 1B, Table 1, Fig. 2) and had sufficient dynamic range to quantitate cresols in urine after inhalation exposure (2 h) to an equimolar mixture of 100 ppm toluene and d_8-labeled toluene (Fig. 1C). Exposure to d_8-labeled toluene allowed

Table 3
Recovery of deuterated analytes from urine ($n=4$)

4-Fluoro-*o*-cresol		d_7-*o*-Cresol		d_7-*m*-Cresol		d_7-*p*-Cresol	
Concentration (μg/ml)	Recovery Mean±S.D. (%)	Concentration (μg/ml)	Recovery Mean±S.D. (%)	Concentration (μg/ml)	Recovery Mean±S.D. (%)	Concentration (μg/ml)	Recovery Mean±S.D. (%)
42	55±1	2.8	70±2	2.8	81±2	37	72±1
10	61±1	0.69	73±1	0.71	90±1	9.3	75±1
2.6	62±4	0.17	75±3	0.18	97±7	2.3	85±6
		0.011	91±30	0.011	70±5	0.15	86±8

Table 4
Previous analyses for the cresols in urine

Urine concentration (μg/ml)			Population	Reference	Reference for method
o-Cresol Mean±S.D. (*n*)	*m*-Cresol Mean±S.D. (*n*)	*p*-Cresol Mean±S.D. (*n*)			
<0.05–0.16	NA, NR with *p*-	NA, NR with *m*-		[7]	[7]
ND	NR with *p*-	38.3±28.8 (28)		[6]	[44]
ND	NR with *p*-	46.8±33.3 (19)	Nonsmokers	[15]	[15]
ND	NR with *p*-	109±80 (11)	Smokers	[15]	[15]
ND	ND	31±28 (55)		[17]	[17]
0.55±0.043 (18)	NA	NA	Men	[45]	[18]
0.09	NA	NA	Smokers and non smokers	[9]	[19]
0.066±0.0048[a] (35)	NA	NA	Chinese men	[46]	[20]
0.036±0.023 (68)	NA	NA	Korean women	[47]	[20]
ND	ND	12.1±4.5[a] (428)	Japanese men	[22]	[22]
0.250±0.290 (33)	0.080±0.120 (33)	21±21 (33)		[13]	[13]
ND	ND	15 (34)		[11]	[11]
ND	ND	11±1		[24]	[24]
ND	ND	29±22 (23)	Men	[28]	[28]
0.159±0.11 (18)	ND	31.2±18 (18)	Men	[5]	[28]
0.042±0.007[a] (246)	NA	NA	Chinese men	[10]	[20]
0.023±0.006[a] (271)	NA	NA	Chinese women	[10]	[20]
0.023±0.003[a] (175)	NA, NR with *p*-	NA, NR with *m*-	Nonsmokers	[29]	[20]
0.063±0.002[a] (176)	NA, NR with *p*-	NA, NR with *m*-	Smokers	[29]	[29]
0.075±0.002[a] (24)	NA	28.2±1.5 (26)		[48]	[16]
NA	NA, NR with *p*-	89 *m*- and *p*-		[30]	[30]

[a]Geometric mean and S.D.
NR=isomers not chromatographically resolved. NA=not analyzed. ND=not detected.

accurate assessment of the toxicokinetics of toluene in the presence of uncontrolled environmental exposure to toluene during the experimental period [43]. The profiles of urinary excretion rates for the deuterated and non-deuterated cresols clearly show divergence caused by non-experimental exposure to toluene and by other sources for the non-deuterated cresols, particularly *p*-cresol (Fig. 3). Urinary excretion of *m*-cresol did not parallel that of *o*-cresol in a previous study [13], probably because a less sensitive method of detection (FID) was used for low concentration exposure (55 ppm). In contrast, our study confirmed that washout is observable for *m*- and *o*-cresol as shown previously in a study with a higher (200 ppm) and more prolonged (i.e., 4 h) exposure [5]. Measurement of urinary deuterated cresols allowed controlled exposure assessment uncomplicated by environmental exposure.

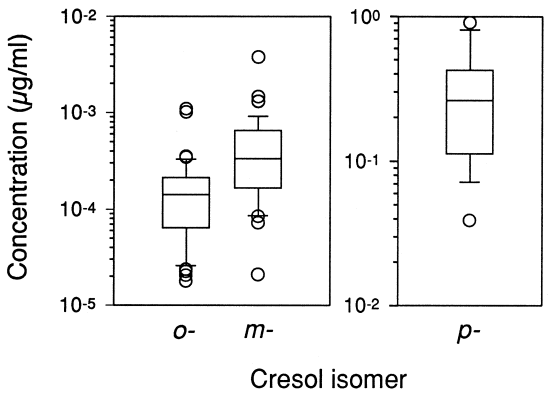

Fig. 2. Box plots of cresols in pre-exposure urine samples (n=45). The centerline of the box is the median, the upper and lower bounds are the 25 and 75 percentiles and the bars are the 10 and 90 percentiles.

Acknowledgements

The authors are indebted to Reneé McCormick and Louis Korff for their valuable technical assistance. This work was supported by a grant from the

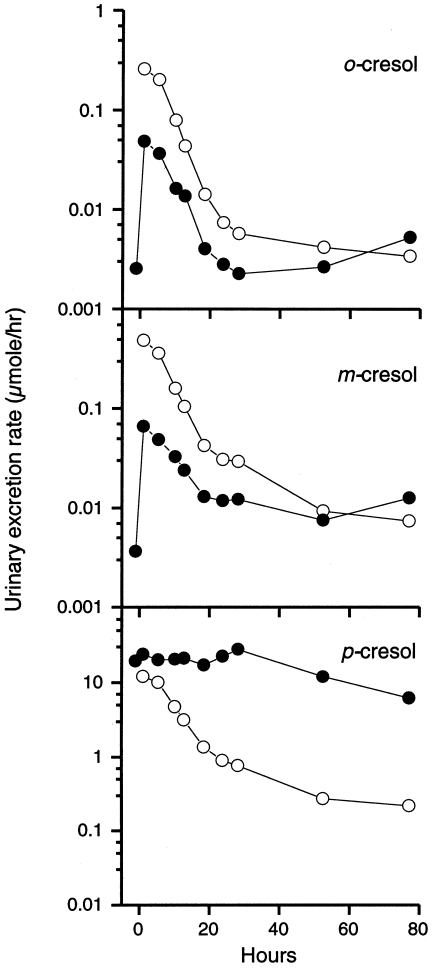

Fig. 3. The urinary excretion rate for a subject followed for 80 h from the start of a 2 h-exposure to an equimolar mixture of d_8-labeled toluene and toluene at 100 ppm. (●) Non-deuterated cresols. (○) Deuterated cresols.

National Institute of Environmental Health Sciences (NIEHS ES 04696).

References

[1] American Conference of Governmental Industrial Hygienists, Documentation of the Threshold Limit Values and Biological Exposure Indices, Vol. III, 6th Ed., American Conference of Governmental Industrial Hygienists, Inc., Cincinnati, 1991, p. BEI-169.
[2] K.-H. Cohr, J. Stokholm, Scand. J. Work Environ. Health 5 (1979) 71.
[3] D. Szadkowski, A. Borkamp, G. Lehnert, Int. Arch. Occup. Environ. Health 45 (1980) 141.
[4] R. Andersson, A. Carlsson, M.B. Nordqvist, J. Sollenberg, Int. Arch. Occup. Environ. Health 53 (1983) 101.
[5] W. Woiwode, K. Drysch, Br. J. Ind. Med. 38 (1981) 194.
[6] J. Angerer, Int. Arch. Occup. Environ. Health 43 (1979) 63.
[7] P. Pfäffli, H. Savolainen, P.-L. Kallimoäki, P. Kalliokoski, Scand. J. Work Environ. Health 5 (1979) 286.
[8] P. Apostoli, F. Brugnone, L. Perbellini, V. Cocheo, M.L. Bellomo, R. Silvestri, Int. Arch. Occup. Environ. Health 50 (1982) 153.
[9] M. Døssing, J. Bælum, S.H. Hansen, G.R. Lundqvist, N.T. Andersen, Br. J. Ind. Med. 40 (1983) 470.
[10] O. Inoue, K. Seiji, T. Watanabe, Z. Chen, M.-Y. Huang, X.-P. Xu, X. Qioa, M. Ikeda, Am. J. Ind. Med. 25 (1994) 697.
[11] K.E. Murray, R.F. Adams, J. Chromatogr. 431 (1988) 143.
[12] Agency for Toxic Substances and Disease Registry, Public Health Service, U.S. Department of Health and Human Services, Toxicological Profile for Cresols, ATSDR/TP-91/11, 1992, p. 73.
[13] K. Kono, Y. Yoshida, H. Yamagata, M. Watanabe, Y. Takeda, M. Murao, K. Doi, M. Takatsu, Jpn. J. Ind. Health 23 (1985) 37.
[14] J. Angerer, IARC Sci. Pub. 85 (Environmental Carcinogens: Methods of Analytical Exposure Measurement) 10 (1988) 293.
[15] M. Balíková, J. Kohlíček, J. Chromatogr. 497 (1989) 159.
[16] G. Bieniek, T. Wilczok, Br. J. Ind. Med. 43 (1986) 570.
[17] M. Buratti, O. Pellegrino, G. Caravelli, D. Xaiz, C. Valla, A. Colombi, Med. Lav. 80 (1989) 254.
[18] E. De Rosa, F. Brugnone, G.B. Bartolucci, L. Perbellini, M.L. Bellomo, G.P. Gori, M. Sigon, P. Chiesura Corona, Int. Arch. Occup. Environ. Health 56 (1985) 135.
[19] S.H. Hansen, M. Døssing, J. Chromatogr. 229 (1982) 141.
[20] K. Hasegawa, S. Shiojima, A. Koizumi, M. Ikeda, Int. Arch. Occup. Environ. Health 52 (1983) 197.
[21] R. Heinrich, J. Angerer, Fresenius' Z. Anal. Chem. 322 (1985) 766.
[22] T. Kawai, S. Horiguchi, Osaka City Med. J. 26 (1980) 135.
[23] M. Korn, R. Wodarz, K. Drysch, F.W. Schmahl, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 313.
[24] A. Sturaro, L. Doretti, G. Parvoli, P. Traldi, Anal. Chim. Acta 224 (1989) 119.
[25] W. Tashkov, I. Benchev, N. Rizov, M. Kafedzhieva, A. Kolarska, Chromatographia 32 (1991) 466.
[26] L. Weber, J. Chromatogr. 574 (1992) 349.
[27] M. Yoshikawa, Y. Taguchi, K. Arashidani, Y. Kodama, J. Chromatogr. 362 (1986) 425.
[28] W. Woiwode, R. Wodarz, K. Drysch, H. Weichardt, Arch. Toxicol. 43 (1979) 93.
[29] T. Kawamoto, M. Koga, T. Oyama, Y. Kodama, Arch. Environ. Contam. Toxicol. 30 (1996) 114.
[30] A.B. van Haaften, S.T. Sie, Am. Ind. Hyg. Assoc. J. 26 (1965) 52.
[31] A. Brega, P. Prandini, C. Amaglio, E. Pafumi, J. Chromatogr. 535 (1990) 311.
[32] W.E. Schaltenbrand, S.P. Coburn, Clin. Chem. 31 (1985) 2042.

[33] J. Schlatter, A. Astier, Biomed. Chromatogr. 9 (1995) 302.
[34] F. Guneral, C. Bachmann, Clin. Chem. 40 (1994) 862.
[35] L.L. Lamparski, T.J. Nestrick, J. Chromatogr. 156 (1978) 143.
[36] G. Bengtsson, J. Chromatogr. Sci. 23 (1985) 397.
[37] E. Tesărová, V. Pacáková, Chromatographia 17 (1983) 269.
[38] M.S. Morgan, R.L. Dills, D.A. Kalman, Int. Arch. Occup. Environ. Health 65 (1993) S139.
[39] W. Jennings, Gas Chromatography with Glass Capillary Columns, 2nd Ed., Academic Press, New York, 1980, p. 69.
[40] O.M. Bakke, R.R. Scheline, Anal. Biochem. 27 (1969) 451.
[41] J.P. Buchet, IARC Sci. Pub. 85 (Environmental Carcinogens: Methods of Analytical Exposure Measurement) 10 (1988) 281.
[42] C.V. Eadsforth, P.C. Coveney, Analyst 109 (1984) 175.
[43] C.H. Pierce, R.L. Dills, M.S. Morgan, G.L. Nothstein, D.D. Shen, D.A. Kalman, Toxicol. Appl. Pharmacol. 139 (1996) 49.
[44] J. Angerer, K. Szadkowski, A. Mantz, R. Pett, G. Lehnert, Int. Arch. Arbeitsmed. 31 (1973) 1.
[45] E. De Rosa, G.B. Bartolucci, M. Sigon, R. Callegaro, L. Perbellini, F. Brugnone, Am. J. Ind. Med. 11 (1987) 529.
[46] O. Inoue, K. Seiji, T. Watanabe, M. Kasahara, H. Nakatsuka, S. Yin, G. Li, S. Cai, C. Jin, M. Ikeda, Int. Arch. Occup. Environ. Health 60 (1988) 15.
[47] O. Inoue, K. Seiji, H. Nakatsuka, M. Kasahara, T. Watanabe, B.-K. Lee, S.-H. Lee, K.-M. Lee, K.-S. Cho, M. Ikeda, Jpn. J. Ind. Health 26 (1988) 147.
[48] G. Bieniek, Occup. Environ. Med. 51 (1994) 354.