Experimental exposure to toluene: further consideration of cresol formation in man*

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ABSTRACT In two separate experiments 10 healthy men each were exposed at rest in an exposure chamber to about 200 ppm toluene in the air. Hippuric acid, o-, m-, p-cresol, and phenol in urine were detected by capillary gas chromatography at the beginning and at the end of exposure, and at variable times after the cessation of exposure. In addition toluene in blood was determined at the same intervals. The results indicate that in addition to hippuric acid, o-, m-, and p-cresol are metabolites of toluene; the detoxication lasting 24 hours at least.

Formation of cresols after exposure to toluene was first suggested by Laham.¹ Using elaborate analytical methods, o- and p-cresol were detected in printing workers exposed to toluene.²⁻⁴ In a further investigation o-, m-, and p-cresol were found in an occupationally exposed group.⁵ Elucidation of a complete metabolic pattern is important in any discussion concerning the toxicity of toluene and the present investigation was set up to study this process further. Male volunteers were exposed to toluene experimentally in a chamber, and urinary metabolites were detected at intervals by means of capillary gas chromatography. This procedure has the advantage that environmental conditions may be standardised, and interference with other pollutants does not occur.

Materials and methods

EXPOSURE CHAMBER

To allow experimental exposures, a climatic chamber (Type 21, 5E/+2JV/10DV-EX, Weiss Company, Giessen) was established in our institute and specially modified in co-operation with the maker, the university building authorities, safety engineers, technicians, the insurance authorities, and the users. This comprehensive collaboration was necessary to ensure the detailed safety specification required for human experimental exposure. The degree of explosion protection was classed "EX-G3."⁶ The interior has an area of 8.96 m² and a volume of 21.5 m³, and the chamber is isolated from adjacent rooms by a sluice.

*Dedicated to Professor Dr H Weichardt on his 65th birthday.

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To minimise adsorption effects, the inner walls consist of stainless steel. The air conditioning has an operating temperature range from +10°C to 40°C ($\pm 1.5^\circ\text{C}$) and a humidity range from 30% to 80% rel ($\pm 5\%$). For the experiments, 22°C and 45% rel humidity were set up. Feeding-, outgoing- and circulating air are led in isolated circuits. All ventilators are linked up with an emergency plant.

DOSE-REGULATING EQUIPMENT

The dose-regulating equipment had been recently constructed in our laboratory. The pollutant—for instance, an organic solvent—is nebulised directly into the circulating air and is passed through a sieve plate ceiling into the chamber. This homogenous dispersion gives a concentration profile, which is stable for hours. A concentration range from 5 to 1000 ppm at solvent boiling points up to 160°C is available, and it is also possible to introduce stable concentrations of vapour mixtures and to make regular alterations to the concentration.

MEASUREMENT OF THE VAPOUR CONCENTRATION

The measurement of vapour concentrations was carried out using three methods.

Charcoal adsorption

Charcoal adsorption of airborne toluene was performed over periods of one hour each, followed by gas-chromatographic head space analysis.⁷

Apparatus—Perkin-Elmer F-40 gas chromatograph designed for head space analysis.

Column—15% Carbowax 1500 + 0.5% Triton X 305 on Chromosorb P AW, 80/100 mesh, stainless

steel 2 m $\times$ $\frac{1}{8}$ " od coupled with 5% SE 52 on Chromosorb G/AW, 80/100 mesh, stainless steel 2 m $\times$ $\frac{1}{8}$ " od. All reagents (analytical grade) purchased from Merck, D-Darmstadt.

Carrier—N₂, pressure 1.8 bar (24 ml/min).

Temperature—oven 110°C isothermal, flame ionisation detector 160°C, injector 160°C. One thousand microlitres injected automatically; water bath 60°C, injection line 170°C.

Control by detection tubes⁸

Spot checks were carried out using detection tubes "toluene 5a" purchased from Dräger, D-Lübeck. As shown previously,¹⁷ this method is highly accurate if critically applied.

Infrared spectrometry

Direct control monitoring was achieved by infrared spectrometry. A standard infrared spectrometer was equipped with a gas cell variable in optical path length from 0.75 to 20.25 m. The ambient air to be analysed was dried by passage through magnesium perchlorate and sucked through the detector cell by means of a membrane pump with a flow rate of about 15 l/hr. With a scale expansion device and an auxiliary recorder a measuring range from 10⁻² to 10⁴ ppm is yielded. For calibration known amounts of the pollutants are injected directly into the gas cell with a microlitre syringe. The accuracy of repeated detector signals is better than 10%.

Apparatus—Infrared spectrometer Acculab II^{TM}, Beckman; 20 meter multipass gas cell, Wilks; auxiliary recorder, Servogor; membrane pump AL 17, Neuberger; magnesium perchlorate, analytical grade, Merck.

Measuring parameters for toluene—analytical wavelength 13.7 μ = 730 cm⁻¹; pathlength 5.25 m, scale expansion $\times$ 1.

The concentration profile of one experimental exposure and further experimental details are given in fig 1.

Fig 1 Concentration profile of toluene during one experimental exposure.

EXPERIMENTAL EXPOSURE TO TOLUENE

According to the methods outlined above, atmospheres containing 200 ppm toluene were set up and controlled. In two experiments 10 healthy male volunteers each were exposed at rest for four hours. Blood and urine spot samples were taken before starting the experiment, at the end of a four-hour exposure, and after a further four, 20, and 44 hours. The subjects were allowed to drink during exposure.

DETECTION OF METABOLITES IN URINE

Density of the urine samples was checked pyknometrically. o-, m-, p-cresol and phenol were determined by capillary gas chromatography as described previously.⁵ For the analysis of hippuric acid, m-methyl-hippuric acid as an internal standard was added to 1 ml urine. The pH was set to 2 with hydrochloric acid, and the sample was extracted twice with 2 ml ethyl acetate. The organic layer was separated by centrifugation and dried over sodium sulphate and the solvent removed under a slow stream of nitrogen. After the addition of 1 ml 3 M hydrogen chloride solution in isopropanol, esterification was carried out at 110°C for one hour. The solvent was again removed and the sample redissolved in 200 μ l dichloromethane. One microlitre of the final solution was injected for gas-chromatographic analysis.

During sample preparation and derivatisation, both hippuric acid and m-methyl-hippuric acid behave similarly. Before the experiment began we ensured that m-methyl-hippuric acid does not occur in normal people or in people exposed to toluene.

Apparatus—Carlo Erba Gas-Chromatograph 2150 equipped with flame ionisation detector (FID) and Spectra Physics Integrator System I. Glass capillary: 25 m $\times$ 0.3 cm id, coated with Silicone OV 225 purchased from Merck. Carrier: hydrogen, pressure 0.6 bar. Temperature of injector and FID: 275°C. 1 μ l (about 5 nano moles) is injected at a split rate of 1:30. Analysis was carried out in isothermal mode at 200°C.

DETECTION OF TOLUENE IN BLOOD

Toluene in blood was analysed by a gas-chromatographic head space technique.⁷ About 1 ml whole blood was heparinised and analysed according to the method given above for head space analysis of charcoal adsorbates.

Results and discussion

Experimental exposure to toluene at the MAK-value of 200 ppm⁹ was performed for four hours with 10 subjects. The concentration profile was stable and the presence of additional pollutant could be exclu-

Table 1 Common mean values for toluene in blood and urinary metabolites from two exposure experiments

		Before exposure	After 4 hours' exp	4 hours after exp	20 hours after exp	44 hours after exp
Toluene in blood	x	10	755.3	58.4	21.9	—‡
	s	—	159.6	27.7	8.8	—‡
µg/l	n	20	20	20	20	—‡
Hippuric acid in urine	x	0.823	4.208	1.663	1.074	0.680
	s	0.518	2.589	0.977	0.519	0.688
g/l	n	16	19	19	18	5
o-Cresol in urine	x	0.159	1.603	1.400	0.495	0.130
	s	0.110	0.796	0.688	0.236	0.048
mg/l	n	18	19	19	18	5
m-Cresol in urine	x	<0.2*	0.570	0.599	0.527	<0.2*
	s	—†	0.255	0.316	0.324	—†
mg/l	n	18	19	18	18	5
p-Cresol in urine	x	31.206	39.590	40.968	39.965	40.275
	s	17.780	24.673	26.578	23.540	30.376
mg/l	n	16	19	19	17	4
Phenol in urine	x	4.869	3.483	4.017	6.900	5.033
	s	2.282	2.453	4.169	4.076	1.966
mg/l	n	16	18	18	17	3
Density of urine	x	1010.8	1007.7	1011.4	1011.8	1014.8
	s	6.3	5.4	6.1	4.4	7.6
g/l	n	18	19	18	17	5

*Below detection limit. †Not computable. ‡Missing data.

Table 2 Results of linear regression $Y = A + B \times X$

Parameter combination X Y	Correlation coefficient	N	A	B	Significance < 2 p
Toluene/hippuric acid	0.6984	70	1.070	4.094 10 ⁻³	0.001
Toluene/o-cresol	0.5531	72	0.634	1.337 10 ⁻³	0.001
Toluene/m-cresol	0.2296	71	0.430	2.049 10 ⁻⁴	0.05
Hippuric acid/o-cresol	0.7036	74	0.334	0.293	0.001
Hippuric acid/m-cresol	0.2284	74	0.398	0.035	0.05
Hippuric acid/p-cresol	0.3292	72	30.958	3.920	0.01
o-Cresol/m-cresol	0.5067	76	0.294	0.191	0.001
Phenol/urine density	0.4510	69	1006.756	0.757	0.001

N = Number of single measuring values. A = Intercept. B = Slope.

ded. There was no statistical difference between the results obtained from the two experiments, so they are combined in table 1.

The correlation between the excretion of the various metabolites was shown by linear regression. Table 2 shows the results of this analysis; no correction for urine density is given.

The mean values for all metabolites in the urine increased after four hours' exposure except phenol and urine density. The subjects were allowed to drink during exposure, which accounts for the decrease in urine density. As phenol is not involved in the detoxication of toluene but only to urine density, it is to be expected that phenol excretion would also decrease.

Hippuric acid as a metabolite of toluene¹⁰⁻¹⁴ is a highly suitable parameter for the biological monitoring of toluene. From the present results it can be

estimated that a concentration range from 2 g/l to 6 g/l (mean value 4 g/l) at a urine density of about 1012 g/l indicates exposure near the MAK-value of 200 ppm.

In addition to hippuric acid, o-, m-, and p-cresol are also metabolites of toluene, the formation taking place concomitantly. Participation of p-cresol in toluene metabolism could not be shown clearly, as the increase during exposure is small compared with the normal range. In fig 2 the excretion of metabolites after correction for urine density of 1012 g/l¹⁵ is shown. The increase of all urinary metabolites becomes more obvious after correction. The formation of o-cresol is due entirely to toluene metabolism; values > 1 mg/l at a urine density of about 1012 indicate exposure near the MAK-value.

Analysis of hippuric acid and o-cresol by capillary gas chromatography is advantageous for the bio-

Fig 2 Excretion of urinary metabolites of toluene, corrected for mean density of 1012 g/l. Exposure interval four hours.

logical monitoring of even large numbers of samples: the method described is fast, simple, and accurate, requiring spot samples of urine only, and moreover it can be automated. If the results of urine analysis indicate exposure near the MAK-value, this may be determined by a personal air sampling technique developed previously.¹⁶

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