Investigation by the Ames test of urine samples from rats exposed to methyl isocyanate

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There is limited toxicological information on methyl isocyanate (MIC) which was the causal agent in the Bhopal disaster in December 1984 when several thousand people were killed or injured. Kimmerle and Eben established that MIC was highly irritant to skin and mucosae and caused pulmonary oedema.¹ Nemery *et al* suggested that at high concentrations MIC probably caused peracute death through reflex inhibition of breathing and rats surviving exposure exhibited narrowing of the airways and haemorrhagic pulmonary oedema.² The lesions were repaired rapidly but residual peribronchial fibrosis and signs of renewed inflammation were apparent. Nemery *et al* also showed that sodium thiosulphate does not protect rats from the acute and subacute effects of MIC and that cyanide intoxication is not concerned in the clinical syndrome seen after exposure to MIC.³

There is little information on the genotoxic potential of this compound but MIC has been reported to be non-mutagenic in the standard Ames assay.⁴ Compounds that are negative in the standard plate incorporation assay, however, may show positive results when urine from animals treated with the compound are tested.⁵

The objective of the present study was to assess the mutagenic potential of urine collected from rats exposed to MIC using the *Salmonella*/mammalian microsomal mutagenicity assay.^{6,7} The test was carried out on animals used in a separate study² and the present study was instigated as an additional assay. Hence the study size and exposure level were dictated by the prime experiment.

Chemicals may produce mutagens in rat urine and it is possible to disassociate various products detoxified by conjugation with a β -glucuronidase or a β -glucuronidase/sulphatase solution.⁸⁻¹⁰ Owing to the small volumes of urine produced in this study, conditions were optimised for the possible detection of mutagens. The test was carried out using a

β -glucuronidase/sulphatase incubation in the plate incorporation assay in the presence of S-9 with strains TA98 and TA100. These are the most sensitive strains and detect frame shift and base pair mutations respectively. These conditions for the urine assay were shown to be most sensitive in the in vivo international programme for chemical safety (IPCS).⁵

Materials and methods

Two groups of three LAC-P strain rats, body weight 140-160 g, were used. The first group were unexposed control animals. The second group were exposed to 12.5 μ l of MIC in the 50 l tank for 60 minutes giving a maximal exposure level of 0.25 mg/l (100 ppm) assuming total evaporation with no loss. The time weighted average concentration over the 60 minutes, however, was between 20 and 30 ppm. After exposure the animals were placed individually in metabowls and the urine collected daily, on ice, for three days. The urine was frozen at -20°C until required.

The urine samples were thawed at room temperature and centrifuged at 1000 g for 10 minutes to remove debris. The resultant supernatants were sterilised by filtration through a 0.45 μ m "Acrodisc" (Gelman Science Ltd, Northampton, UK). The samples were then incubated for 24 hours at 37°C, with 65 units/ml of β -glucuronidase/sulphatase solution (Sigma Chemical Co Ltd, Poole, Dorset) before use in the Ames test. Aliquots (0.5 ml) of the processed urines were incorporated, in triplicate, in the standard plate incorporation test as described by Maron and Ames⁷ in the presence of a 10% S-9 mix. The *Salmonella typhimurium* strains TA98 and TA100 were obtained from Professor B Ames, University of California, USA.

Unless otherwise indicated the media and chemical used were from standard sources as described elsewhere.

Results

All the animals in the exposed group showed obvious

Table 1 Effect of MIC on body weight and urine collected in LAC-P rats

Treatment	Rat No	Body weight (g)				Urine collected (ml)		
		0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	1	143	145	150	153	5.8	5.8	7.5
	2	148	140	129	149	7.0	8.0	9.7
	3	149	153	161	165	5.0	7.2	11.0
	Mean $\pm$ SD	147 $\pm$ 3	146 $\pm$ 7	147 $\pm$ 16	156 $\pm$ 8			
0.25 mg/l MIC	4	162	139	124	136	14.5*	15.5*	2.1
	5	148	126	120	129	7.6	4.4	5.7
	6	145	124	110	119	6.2	4.1	4.3
	Mean $\pm$ SD	152 $\pm$ 10	130 $\pm$ 8	118 $\pm$ 7	128 $\pm$ 8			

*Urine collected with ice water entering cracked tube.

Table 2 Examination of urine samples from rats treated with MIC on reversion in *Salmonella typhimurium* strains TA98 and TA100

Animal treatment	Rat No	Mean† revertants/plate								
		Test controls	TA98			Test controls	TA100			
			Day 1	Day 2	Day 3		Day 1	Day 2	Day 3	
		Solvent control	22				80			
		Positive control*	362				530			
Control	1			38	36	38		120	120	107
	2			44	42	27		115	98	96
	3			36	37	29		98	92	90
0.25 mg/l MIC	4			37	33	33		86	76	128
	5			47	41	36		122	97	94
	6			42	42	37		76	87	90

*Benzo(a)pyrene: 5 μ g/plate.

†Mean of triplicate plates.

signs of respiratory distress and lost weight over the first two days. By the third day the animals had recovered from the breathing problems and started to gain weight. The body weights and health of the control animals remained consistent through the entire period. Table 1 shows the body weights and urine volumes collected over the three day period.

Table 2 shows the effect of MIC on reversion in strains TA98 and TA100. No difference in revertant values was seen when comparing the non-exposed to MIC-exposed animals at one, two, or three days after treatment. Where sufficient urine was collected a repeat test using the same samples confirmed this result.

Discussion

The negative results from this study, where a sufficiently high dose of MIC was used to produce respiratory distress in the animals, would suggest that metabolites of MIC either do not reach the urine or that if they do they are not mutagenic.

The lack of genotoxicity of the urine samples is consistent with the lack of genotoxic effects in 500 babies examined from 1500 pregnant women who were exposed in the second and third trimesters to MIC in Bhopal.¹¹

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