Acute toxicity of methyl isocyanate: a preliminary study of the dose response for eye and other effects

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ABSTRACT Acute toxic effects of methyl isocyanate in the rat were determined for two hour exposures to concentrations in the range 11 ppm (very slight effect) to 65 ppm (lethality: pulmonary oedema). Changes in the eye, lungs, and behaviour were noted. Eye changes were confined to erosions of the corneal epithelium and were most severe at intermediate levels of exposure. A comparison was made of the effects noted in rats with reported effects on survivors of the Bhopal disaster. Urinary thiocyanate concentrations in exposed rats were found to be reduced relative to control values.

The catastrophic consequences of the release of methyl isocyanate (MIC) from the Union Carbide plant at Bhopal in India have naturally focused attention on the toxicity of this material. It was already known to be a highly toxic and irritant material: the LD_{50} for four hours inhalation by rats has been reported to be 5 ppm in one study,[1] and between 30 and 60 ppm in another.[2] These numerical results by themselves, however, make only a limited contribution to the understanding of possible effects on man,[3] and there is relatively little published information on the effects at sublethal concentrations, particularly as regards effects on the eye, which was found to be an important target organ in survivors of the Bhopal incident. In view of this lack of information, and in support of epidemiological studies at Bhopal,[4 5] an investigation of these phenomena in rats after inhalation of sublethal concentrations of MIC was undertaken.

In addition to the lack of information on the dose response, time dependence, and outcome of the sublethal effects a further problem has been the uncertainty about the metabolic or chemical breakdown products of MIC. We have previously reported that the main hydrolysis product of MIC is n,n'-dimethyl urea.[5] We also suggested that formation of some toxic materials by reaction of MIC with endogenous substances could occur.

On the other hand, some reports of studies on exposed survivors in India have suggested that metabolism to cyanide is important, that urinary

thiocyanates are raised, and that thiosulphate is a useful treatment.[6] We therefore decided to examine the urinary thiocyanate concentrations of control and exposed rats. The following report describes the initial results of this study.

Methods

Male Lister hooded rats (weight 170–200 g) were exposed by inhalation to air containing various concentrations of MIC. This strain was chosen in preference to the more usual albino strains since effects on the eye and related neural pathways were of particular interest. Albino mammals are generally found to have abnormalities of those parts of the central nervous system concerned with vision and were therefore considered unsuitable subjects for these experiments. A two hour single acute exposure was chosen as the most appropriate model for the type of exposure experienced in India, and also of most direct relevance to other possible accidental exposures. No attempt was made to determine an accurate LD_{50} value, since this is known. Instead, for this initial study, animals were exposed in pairs to different concentrations, starting at a level expected to have minimal effects during the two hour exposure period, and stopping once the LD_{50} range had been reached. In this way it was hoped to obtain the maximum information with exposure of the minimum number of animals to this highly noxious material.

Exposures were performed in a glass chamber (in two cases with urine and faeces collectors, see below) through which an airflow of 10 l min was maintained. This air was previously filtered through a charcoal

Table 1 *Effect of exposure to MIC on breathing and behaviour*

Dose (ppm MIC) Animal Nos		Observations: breathing	Behaviour
Air control	11, 12	Breathing normal, ~2 Hz	Normal
11	1, 2	Slight slowing of breathing rate	Sedative effect, reversed immediately at end of exposure
21	3, 4	Sensory response: breathing slow and irregular. Reversed immediately at end of exposure	Prounounced sedative effect: animals asleep or unconscious (difficult to arouse by tapping chamber), recovered immediately at end of exposure. Animal 3 suffered from diarrhoea
31	5, 6	Breathing very slow (0·3–0·5 Hz) and irregular. Breathing still laboured 20 h after exposure but normal after 48 h	Sedative effect, but effort in breathing leading to greater arousal. Animals appeared asleep, but restless. Considerably more sensitive to ether anaesthesia 20 h after exposure
65	7, 8	Breathing very slow and irregular: some reversal of effect after exposure, but severe respiratory distress noted 20 h later. No 8 died 45 h after exposure. No 7 still distressed at 50 h when killed	Sedative effect, but also arousal due to irritation during exposure. Extremely sensitive to anaesthesia 20 h after exposure
200 (10 min)	9, 10	Severe irritation with coughing and slow breathing rate: slightly laboured breathing at 20 h after exposure, but normal at 48 h	Severe irritation and coughing noted, but sedation pronounced. More sensitive than control to ether anaesthesia 20 h after exposure

filter to remove organic contaminants but was not dried. Airflow was maintained by a stainless steel bellows pump. MIC was introduced from a microsyringe into the airstream at a steady rate by infusion pump and the atmospheric concentration monitored at the chamber outlet by Miran infrared spectrometer. A continuous chart record of concentration was obtained. Effluent airflow from the system was directed through a scrubber system with a continuous flow of water to remove MIC from the airstream before discharge to the atmosphere. In addition to the two hour exposure series a single experiment was performed with a ten minute exposure to a much higher concentration, which was below lethal for this duration.

The highest concentration two hour exposure and the air control exposure were performed in a glass

Table 2 *Effect of exposure to MIC on the cornea and other eye structures*

Dose (ppm MIC)	Animal		Observations: pre-dose	20 h after start of exposure
	No	Eye		
Air control	11	L	Normal	Normal
		R	Discrete anterior Stromal opacities	No change
	12	L	Normal	Small traumatic abrasion
		R	Normal	Normal
11	1	L	Normal	Faint interpalpebral epithelial haze
		R	Normal	Normal
	2	L	Normal	Normal
		R	Normal	Normal
21	3	L	Normal	Band shaped area of epithelial change: 6–8 vertically orientated folds. Epithelium grey, oedemtous: ulceration at base of folds
		R	Normal	Central area of grey epithelium within which fluorescein staining was widespread
	4	L	Normal	Central band of punctuate epithelil erosions
		R	Normal	Fine crisscross pattern of epithelial change in band, with clear cornea above and below
31	5	L	Normal	Normal
		R	Normal	Normal
	6	L	Scratch scar	Punctate interpalpebral epithelial erosions
		R	Normal	Punctate interpalpebral epithelial erosions
65	7	L	Normal	Normal
		R	Normal	Fine confluent epithelial erosions
	8	L	Normal	Punctate epithelial erosions
		R	Normal	Punctate epithelial erosions
200	9	L	Facet + old stromal foreign body scar	Diffuse epithelial erosions
		R	Normal	Central area of fine erosions
	10	L	Scattered punctate epithelial opacities	Fine epithelial microerosions
		R	Peripheral epithelium pigmented	Fine epithelial microerosions

chamber with urine and faeces collectors to provide uncontaminated urine samples for thiocyanate analysis. The animals remained in the chamber with clean air at a reduced flow rate for the remainder of a 24 hour period. Measurement of urinary thiocyanate was by the method of Aubry *et al*.[7]

The eyes of the animals were examined before and 20 hours after exposure, under light anaesthesia with ether. Biomicroscopic examination was performed with a Haag-Streit slit lamp microscope, and 1% fluorescein drops were applied to stain damaged corneal epithelium. The pupils were dilated with 10% phenylephrine drops to facilitate examination of the lens.

Results

Tables 1 and 2 record the actual mean concentrations of MIC produced in the chamber during exposures, and the observations of effects on breathing, behaviour, and eyes. After exposure, abnormalities of the corneal epithelium were noted, particularly at intermediate exposure levels, but no animal showed abnormalities of the lens or other internal structures of the eye. At the highest concentration (65 ppm) to which rats were exposed for two hours, one of the two animals died 45 hours after exposure: the other showed severe and persistent respiratory distress and was therefore killed at 50 hours. Postmortem examination of both animals showed haemorrhagic patches on the lungs, particularly the upper lobes, and the presence of fluid in the lungs and bronchi. Other organs appeared normal. The lungs of both animals were fixed for histology and examination of sections confirmed the diagnosis of pulmonary oedema and petechial haemorrhages. The remaining animals all appeared normal after 48 hours apart from eye changes, and were retained for observation of the long term outcome of these effects.

Urinary thiocyanate concentrations and 24 hour output are reported in table 3. The urinary concentration and 24 hour output of thiocyanate in the exposed animals were substanitially lower than the control results (urines from two animals were collected over 24 hours and pooled).

Discussion

MIC at low concentrations caused severe sensory irri-

tation, leading to slowing of the breathing rate from about 2 Hz to about 0·5 Hz: breathing also became irregular and laboured. This is similar to the effect of other respiratory irritants on the rat.[8] Additionally, however, there appeared to be a pronounced narcotic or sedative effect that produced unconsciousness at low concentrations such as 11 ppm, which caused only minimal changes in the eye or breathing rate. This was masked at higher concentrations of MIC by the arousal resulting from respiratory distress. At these higher concentrations the sensory response of the respiratory system was accompanied by damage shown by persistent respiratory distress and pulmonary oedema, which was fatal in one case. After sublethal doses, the respiratory problems resolved spontaneously and apparently completely; the narcotic effect was reversed on cessation of exposure, but a residual neurological or pulmonary change may be indicated by the hypersensitivity to ether anaesthesia 20 hours later, which is noted in table 1. Allowing for the interaction of these effects, there appears to be a progressive trend in the relation between dose and degree of response for the respiratory and central nervous system effects, so far as may be determined from qualitative observations. By contrast, the severity of effect on the eye was considerably greater at intermediate levels of exposure, suggesting that at high doses some protective response was evoked which, though effective in reducing damage to the corneal epithelium, was insufficiently evoked by lower levels that none the less caused superficial eye damage. In all cases the eye damage observed was confined to the epithelial layer.

When these observations are compared with those recorded in a survey of effects on the survivors of the Bhopal incident[45] it is apparent that there is a clear relation between the animal and human data. In both cases the fatal effects related to damage to the lungs, and there appears to be a simple relation between the severity of this effect and the degree of exposure.[45] On the other hand, the effects on the eye in the rat appeared most severe at intermediate exposure levels, again mirroring the observations in Bhopal. The damage caused to the eye in both rats and man was superficial in character, as expected from the highly reactive nature of MIC and the short lifetime therefore expected in vivo.[5] Findings such as dizzyness or unconsciousness, nausea, and other central nervous

Table 3   *Urinary thiocyanate after exposure to 65 ppm methyl isocyanate for two hours*

Sample	Creatine (nmol/l)	Thiocyanate (ug/ml)		Volume (ml) (2 rats)	24 h output (µg per rat)
		Measured	Corrected		
Control	6·0	6·6	1·7	9	7·7
Exposed	2·0	0·5	0·3	8	1·2

system related phenomena, and diarrhoea, are seen principally among survivors at Bhopal who suffered intermediate exposures. In the rat similar responses were apparent at intermediate dose levels but were masked or reversed at the highest concentrations. From this correlation of effects and dose dependence two initial conclusions may be drawn. Firstly, the use of death rates and incidence of pulmonary damage as a crude index of exposure in the Bhopal epidemiological study (in the absence of more definite measurements) is supported. Secondly, it is apparent that the sublethal acute effects in the rat offer a useful model of the effects on man, which will be exploited in further work to clarify epidemiological findings and to investigate possible long term outcomes of exposure to MIC. In view of the evident correspondence between acute toxic effects in man and rat, it does not appear unreasonable to use the rat as a model for investigations of other aspects of MIC toxicology, including metabolism.

On the basis of the thiocyanate data presented here there are no grounds for asserting a role for conversion to cyanide, or for the usefulness of thiosulphate treatment and the measurement of urinary thiocyanate concentrations. There is an important control level of thiocyanate in the urine of both rat and man resulting from endogenous metabolism and the conversion of dietary and other sources of cyanides. The reduction of thiocyanate concentrations below normal in the exposed rats probably relates to the inhibition of general intermediary metabolism and food intake by the sedative and toxic effects of MIC or its products. This finding is consistent with the absence of a chemically plausible route for the conversion of MIC to cyanide, either in vivo or in vitro. There appear to be three possible explanations if urinary thiocyanate concentrations are indeed raised in Bhopal survivors and visitors. Firstly, there could be a "rebound" effect after inhibition of endogenous thiocyanate metabolism by exposure to MIC.

(This could explain the effect in survivors but not in subsequent visitors to the area.) Secondly, the raised thiocyanate excretion may be related to some other environmental contaminant or dietary factor affecting the population. Thirdly, some additional material which can act as a source of cyanides may have been released during the course of the accident. We are not a present able to distinguish between these possibilities, although work is in progress to evaluate the time course of the change in thiocyanate excretion after MIC exposure of rats.

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