Bull. Environ. Contam. Toxicol. (1993) 51:211-218

Environmental Contamination and Toxicology

Bile Duct Hyperplasia and Transient Liver Parenchymal Cell Alteration by Methylisocyanate

Usha Arora,[1]* R. Vijayaraghavan,[1] K. Sugendran,[1] and R. Mathur[2]

[1]Division of Pharmacology and Toxicology, Defence Research & Development Establishment, Gwalior 474 002, India and [2]School of Studies in Zoology, Jiwaji University, Gwalior, India

Several reports have appeared recently indicating adverse pharmacological, toxicological and physiological effectes of methylisocyanate (MIC) leading to severe and irreversible pulmonary damage both in human as well as in experimental animals (Bucher et al 1987, Fowler and Dodd 1986). A fraction of MIC can escape localized hydrolysis and can reach other organs through blood in its active form (Varma et al 1987, Bhattacharya et al 1988, Jeevarathinam et al 1988, Arora and Vijayaraghavan 1989,Slatter et al 1991).The present study demonstrates dose dependent effects of inhaled MIC on liver histopathology and on hepatic biochemical variables, which have not been reported so far.

MATERIALS AND METHODS

Ninety-six male Swiss albino mice(23± 3g) maintained on standard pellet diet (Lipton's India) and water ad libitum were divided into three groups. Group I,II & III were exposed to three concentrations of MIC(Synthesized in chemistry division of our establishment, purity 99%), through inhalation route as follows:

Group I 1 LC50 (0.268mg/L) MIC aerosols (no.=30)
Group II 1/2 LC50 (0.134mg/L) MIC aerosols (no.=30)
Group III 1/4 LC50 (0.067mg/L) MIC aerosols (no.=18)

Eighteen animals (no.=18) were exposed to fresh, filtered and non toxic air which served as control.

Animals were exposed in an all glass static inhalation chamber of 20 L capacity for 30 min (Vijayaraghavan and Kaushik 1987).Animals were observed for mortality in all the groups.The three doses were chosen to study the dose dependent effcts.LC50 was calculated as described by Vijayaraghavan and Kaushik (1987).

*send reprint requests to Usha Arora at above address

Blood was collected,in heparinized and non heparinized vials, from the optic plexus at 1 hr,24 hr,72hr, d 8 and d 15 in Group II and 1 hr, 24 hr and 72 hr in Group III along with control for biochemical variables. The animals were sacrificed at the following intervals for histological studies:

Group I 1hr, 24hr & 72hr (no.=4 at each time intervals)
Group II 1hr, 24hr,72hr,d8 & d15 (no.=6 at each time intervals)
Group III 1hr, 24hr & 72hr (no.=6 at each time intervals)

Whole blood glucose and plasma protein were determined by the method of Nelson (1944) and Lowry et al (1951) respectively. Activities of serum glutamate pyruvate transminase (S-GPT) (Reitman and Frankel 1954) and serum Alkaline phosphatase(S-ALP) (Wooton et al 1982) were measured following standard procedures. S-GPT activity is expressed as n mols pyruvate formed/min/mg protein and S-ALP activity as n mols phenol liberated/min/mg protein.

Two portions selected at random, from each liver of 4-6 animals, were fixed in aqueous Bouin's fluid. Tissues were processed by conventional method for routine paraffin sections. Three to four um thick sections were stained with hematoxylin and eosin (Culling 1974). The liver lesions and morphological changes were examined under light microscope by two authors for confirmation.

Mean values for each group of animals were tested for significance by Student's 't' test. A level of significnce of $p < 0.001$ was chosen.

RESULTS AND DISCUSSION

With 1 LC50 MIC, mortality was higher in the first two days (15/30) and three more animals died at 72hr (18/30). There was no mortality in Groups II & III. Liver to body wt ratio did not show significant change in any of the groups.

Blood glucose level increased significantly at 1hr and 24hr following 1\2 LC50 MIC exposure and returned to normal level at 72hr (Tabel-1). No significant change in plasma protein was observed. A marginal increase, in the activity of S-GPT though, statistically not significant, was indicative of mild injury to liver parenchyma. Significant increase in S-ALP at 1hr could be due to damage to bile duct epithelial lining (Hyde and Draisy 1974) and injury to hepatic parenchyma (Crofton et al

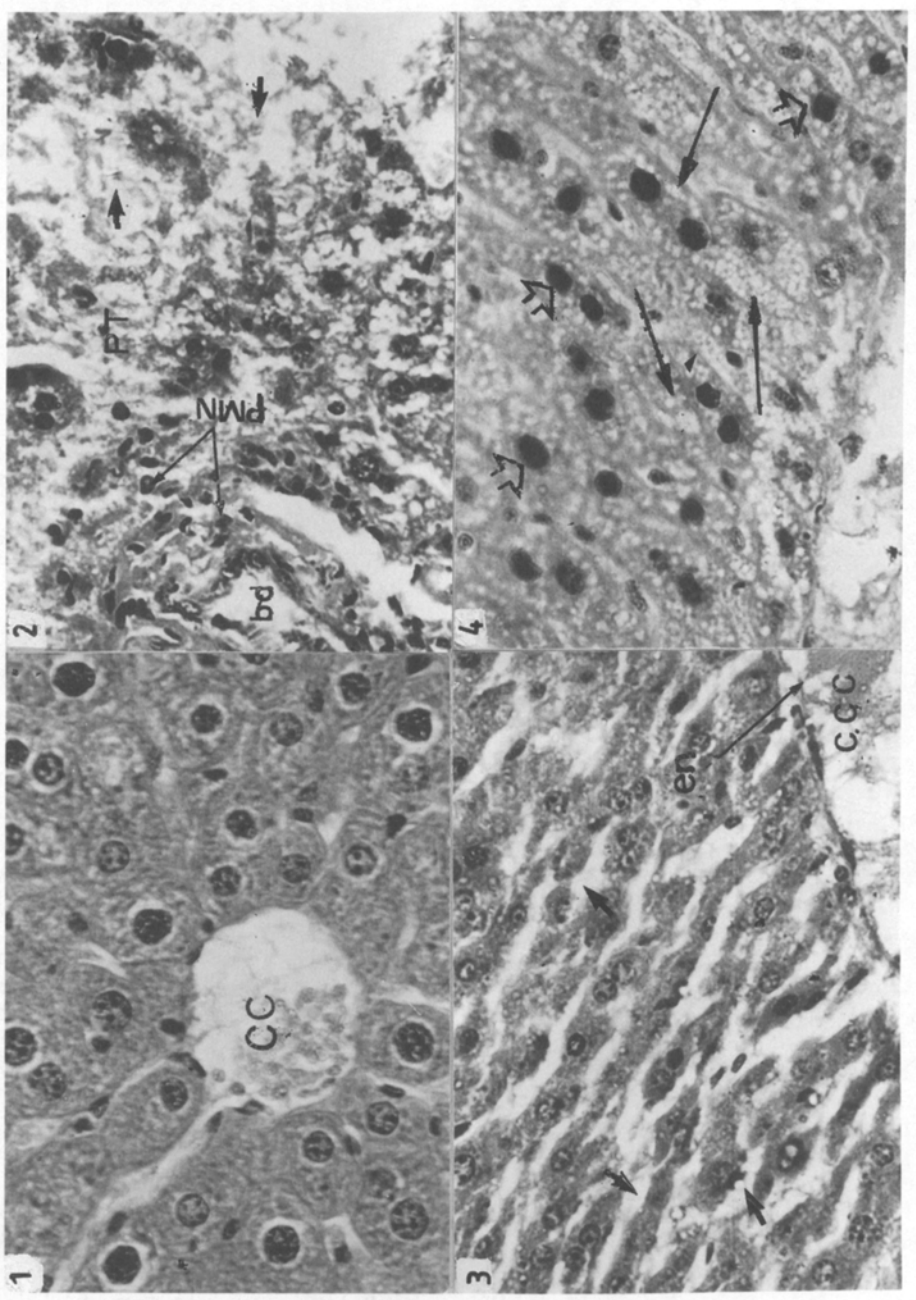
1979).Increase in corticosteroid level (Varma et al 1987) and stress (Chowdhury et al 1985) may lead to increase in blood glucose level (Husain and Matin 1987).Animals receiving 1\4 LC50 MIC exhibited no significant change in biochemical variables at any time intervals.

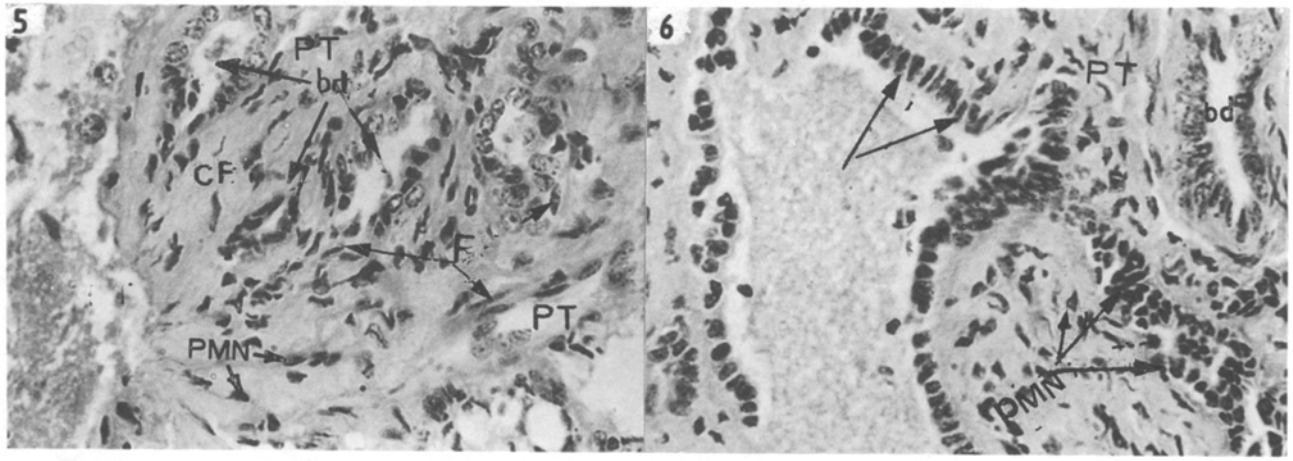
Table 1. Effect of methylisocyanate (1\2LC50) on some biochemical variables in blood plasma and serum with time.

Time	Blood glucose mg $100ml^{-1}$	Plasma protein mg ml^{-1}	S-GPT	S-ALP
Control	111.7±2.1	77.3±0.8	125.o±1.2	57.7±0.8
Treated				
1hr	238.8±16.3*	76.4±0.1	137.2±1.7	67.4±0.7*
24hr	146.9± 6.7*	77.3±0.8	126.1±1.1	57.2±0.6
72hr	94.3± 8.8	76.2±0.8	126.4±1.0	57.0±0.9
d 8	119.4± 7.4	76.9±o.9	128.3±1.2	58.6±0.7
d 15	117.6± 4.1	77.0±0.0	127.0±1.7	57.0±0.6

(Values are mean ±SE,no.=6,*p<0.001 compared to control)

The control liver did not show overt pathology (Fig.1).Histopathological observation in Group I mice (1 LC50 MIC) showed that at 1 hr central and periportal region (zone-1 of Rappaport 1969) has become eosinophilic. The sinusoids were dilated. At 24 hr sporadic karyolysis was observed in periportal and central regions. There was complete loss of hepatocytes and stroma (necrosis) at some foci, the polymorphoneutrophils (PMN) infiltrated the portal triads (PT). A few cuboidal cells in the bile duct (bd) epithelium were lost (Fig. 2). There was more marked necrosis of hepatocytes at 72 hr. In Group II mice (1/2 LC50 MIC), the sinusoids were dilated at 1 hr and central canal (CC) was congested. Damaged endothelial cells were observed (Fig. 3). Bile thrombi were present in sinusoids. At 24 hr cellular debris and proteinaceous fluid infiltrated into the CC. Moreover the nuclei of hepatocytes were pycnotic, hyperchromatic and of various sizes. The cytoplasm of hepatocytes was vacuolated (Fig. 4). Bile duct was proliferated but few cuboidal cells of bd were lost. At 72 hr after 1/2 LC50 MIC inhalation the Kupffer cells became hyperactive. Portal triad, particularly hyperplastic bd were surrounded by fibroblasts (F), collagen fibres (CF)and PMN (Fig. 5). On d 8, numerical nuclear density was found to be increased in the survived stroma, due to presence of large number of binucleated hepatocytes, showing rapid regenration of hepatocytes in an orderly fashion. At d


1
cc
2
PMN
bd
3
en
ccc
4



Photomicrographs of (1) control liver of mouse (2) after 1 LC50 and (3-6) 1/2 LC50 MIC treatment; H&E x 400 show 1. prominent nuclei, sinusoids and central canal (CC) of normal liver 2. complete loss of hepatocytes and stroma (arrows), bile duct (bd) and polymorphoneutrophils (PMN) in portal (PT) (24 hr) 3. damage to endothelium (en), dilated sinusoids (arrows) and congested CC (ccc) (1hr) 4. cytoplasmic vacuolations (arrows), hyperchromatic nuclei (open arrows) (24 hr) 5. bd surrounded by PMN, fibroblasts (F) and collagen fibres (CF) in PT (72hr) 6. metaplasia of cuboidal cells (arrows) of bd, PMN infiltration in PT (day 15).

15 liver histopathology showed loss of hepatic architecture at few foci due to metaplastic changes in epithelial cells of bd and PMN infiltration (Fig. 6). Proliferation of bd was persistent feature from 24 hr to d 15. In Group III mice (1/4 LC50 MIC), only mild inflammatory response was seen at 1 hr and 24 hr. MIC did not produce any marked change at 72 hr.

Alpha naphthylisothiocyanate (ANIT) has been shown to cause biliary hyperplasia following subacute exposure (Goldfarb et al 1963) and cessation of bile flow within 24 hr (Goldfarb et al 1962). We observed biliary hyperplasia and cytoplasmic vacuolations at 24 hr following 1/2 LC50 MIC exposure. MIC may act through the same mechanism as of ANIT, as protein binding properties of MIC are similar to that of ANIT. Isocyanate and isothiocyanates can act on several functional groups of protein (Cohen and Oppenheimer 1977; Bhattacharya et al 1988).

In the pathogenesis mechanism, anoxia resulting from lowered oxygen carrying capacity of blood, following MIC inhalation may cause periacinar necrosis (Fig. 2) (Jubb and Kennedy 1970). Significant number of fibroblast and collagen fibres appeared in Zone-1 at 72 hr, indicative of scarring. Biliary duct hyperplasia was the consistent feature at all time intervals in animals receiving subacute dose. With 1/2 LC50, regeneration occurred in survived recticulum, in the damaged area more rapidly to establish original pattern. Renewal of hepatocytes from the stem cell takes place around Zone-I, consistent with the finding of Zajicek et al (1985). MIC induced hyperplasia of bile duct without change in liver to body wt ratio supports the view of Lopez and Mazzanti (1955).

Bucher et al (1987) have reported mild cytoplasmic vacuolation in liver in mice exposed to 20-30 ppm MIC through inhalation for 2 hr. While Boorman et al (1987) did not find any changes in liver morphology exposed to various concentration (3 to 30 ppm) of MIC. Congestion, inflammation, degeneration and necrosis have been reported by Jeevarathinam et al (1988) following s.c. administration of 1 LD50 (126 mg/kg) and 0.5 LD50 to rabbits. Liver histopathology following MIC inhalation has not yet been reported.

The significant finding of our study is the proliferation of bile duct metaplastic changes and transient cytoplasmic vacuolation on following subacute inhalation of MIC. Formation of new bile ducts may be an adaptive mechanism to maintain bile flow through collateral channels. The lower dose (1/4 LC50) MIC did not produce any overt pathology. The study suggests that the degenerative changes induced in liver parenchyma by

inhaled MIC are transient (1/2 LC50) and dose dependent.

Acknowledgments. The authors thank Dr. R.V. Swamy Director and Dr. S. Das Gupta, Head, Pharamcology and Toxicology Division, DRDE, Gwalior for their encouragement and valuable suggestions. Authors thank Dr. S.J.S. Flora for the review of the manuscript.

REFERENCES

Arora Usha, Vijayaraghavan R (1989) Effect of sublethal exposure to methylisocyanate on testicular histomorphology in mice. Ind J Expt Biol 27:247-249

Bhattacharya BK, Sharma SK, Jaiswal DK (1988) In Vivo binding of ($1\text{-}c^{14}$) methylisocyanate to various tissue proteins. Biochem Pharmacol 37:2489-2490

Boorman GA, Brown R, Gupta BN,Uraih LC, Bucher JR (1987) Pathological changes following acute methylisocyanate inhalation and recovery in B6C3F1 mice. Toxicol Appl Pharmacol 87:446-456

Bucher JR, Gupta BN, Adkins Jr B, Thompson M, Jameson CW, Thigpen JE, Schwetz BA (1987) Toxicity of inhaled methylisocyanate in F344/N rats and B6C3F1 mice. 1 Acute exposure and recovery studies. Environ Hlth Perspect 72:53-61

Chowdhury AR, Rastogy VK, Arora Usha, Saxena Chhaya (1985) Biochemical and morphological alteration of adrenal under the exposure of orthochlorobenzylidene malononitrile. Mikroskopie 42:151-156

Cohen S, Oppenheimer E (1977) Biological formation and reactions of cyanates. In:The chemistry of cyanates and their thioderivatives.Patai S.(ed). John Wiley and Sons. New York

Crofton PM, Elton RA, Smith AF (1979) High molecular weight alkaline phosphatase in clnical study. Clin Chim Acta 98:263-275

Culling CFA (1974) Handbook of histopathological and histochemical technique 3rd ed. Butterworth & Co Ltd, London

Fowler EH, Dodd DE (1986) Acute inhalation studies with methylisocyanate vapour. Part *II* Respiratory tract changes in guinea pigs, rats and mice. Fund Appl Toxicol 6:756-771

Goldfarb S, Singer EJ, Popper H (1962) Experimental cholangitis due to alpha naphthyl isothiocyanate (ANIT). Amer J Pathol 40:658-698

Goldfarb S, Singer EJ, Popper H (1963) Biliary ductules and bile secretion. J Lab Clin Med 62:608-615

Husain K, Matin MA(1987) Role of adrenals in the production of hyperglycaemia and changes in hepatic carbohydrate metabolism in diazinon treated rats. Adv Biosci 61:101-105

Hyde TA, Draisy TF (1974) Principals of chemical pathology. Butterworths and Co Ltd, London

Jeevarathinam K, Selvamurthy W, Ray US, Mukhopadhyay S, Thakur L (1988) Acute toxicity of methylisocyanate, administered subcutaneously in rabbits, changes in physiological,clino chemical parameters. Toxicology 51:223-240

Jubb KVF, Kennedy PC, (1970) Pathology of domestic animals. Second ed. Academic Press,New York, San Francisco, London

Lopez M, Mazzanti L (1955) Experimental investigation on alpha- naphthylisocyanate as a hyperplastic agentes of the biliary ducts in rats. J Pathol Bacteriol 69:243-250

Lowry OH, Rosebrough NJ, Farr AL, Randall (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275

Nelson N (1944) A photometric adaptation of Somogyi method for the determination of glucose. J Biol Chem 153:375-380

Rappaport AM (1969) Anatomical consideration in Leon Schiff ed."Disease of the liver" 3rd ed. J Lippincott Co Philadelphia 1-49

Reitman S, Frankel S (1954) A colorimetric method for determination of serum oxaloacetic and glutamate.Amer J Clin Pathol 28:56-63

Slatter JG, Rashed MS, Pearson PG, Han DH, Baillie TA (1991) Biotransformation of methylisocyanate in the rat. Evidence for glutathion conjugation as a major pathway of metabolism and implication for isocyanate mediated toxicities. Chem Res Toxicol 4:157-161

Varma DR, Ferguson JS, Alarie Y (1987) Reproductive toxicity of methylisocyanate in mice. J Toxicol Environ Hlth 21:265-275

Vijayaraghavan R, Kaushik MP (1987) Acute toxicity of methylisocyanate and ineffectiveness of the sodium-thiosulphate preventing its toxicity. Ind J Exp Biol 25:531-534

Wooton IDP, Freeman H (1982) Microanalysis in medical biochemistry, 6th ed. Churchill Ltd, London

Zajicek G, Oren R, Weinreb M Jr (1985) The streaming liver. Liver 5:293-300

Received January 5, 1992; accepted February 20, 1993.