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Acute Inhalation Toxicity Study of 2-Fluoroacetamide in Rats

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One of the most potent rodenticides is 2-fluoroacetamide (2-FA). Toxicity of this chemical is well documented. However, its inhalation toxicity data is not available in the literature. Hence, acute inhalation toxicity study was carried out by exposing male and female rats to aerosols of 2-FA at different concentrations for 4 h in a dynamically operated whole body inhalation exposure chamber. During and after the inhalation exposure the rats were less active, and showed mild tremors and convulsions. At higher concentrations the rats died after 2-3 days. The estimated 4-h LC_{50} for male and female rats was 136.6 and 144.5 $mg \cdot m^{-3}$ respectively. Exposure to 0.7 LC_{50} for 4 h duration showed an increase in the liver weight of male and female rats 7 days after exposure. Various haematological and biochemical variables determined were within the normal limits. However, histological findings showed injured lung as indicated by desquamation and necrosis of the epithelium of the respiratory tract. Marked hypertrophy of hepatocytes displaying strong acidophilic granulated cytoplasm was observed. Focal dilatation of renal proximal tubules in kidney with cytoplasmic vacuolation, and irregularly placed pyknotic nuclei were seen. The present study shows that 2-FA is a highly toxic chemical through the inhalation route based on the LC_{50} value. Consequently necessary precautions should be taken during its handling.

INTRODUCTION

One of the most potent rodenticides is 2-Fluoroacetamide (Klaassen, 1980). It is an "unclassified rodenticide" (status ISO 765) and listed under Schedule 1 of Poisons in USA. Since 2-fluoroacetamide (2-FA) and sodium fluoroacetate are highly toxic to other animals, their use is restricted to licensed pest control operators only, especially in poisoning large predatory mammals such as coyotes (Pradhan, Roger and Dutta, 1986). In handling such toxic substances, care must be taken to avoid accidental dermal contact, ingestion and inhalation of the powder.

2-FA and fluoroacetate are extremely toxic chemicals. The toxicity of fluoroacetate is due to the inhibition of citric acid cycle. Fluoroacetate is incorporated into fluoroacetyl coenzyme A and with oxaloacetate it forms fluorocitrate. Fluorocitrate inhibits the enzyme aconitase which converts citrate to isocitrate. As a result, large quantities of citrate accumulate and the citric acid cycle is blocked (Peters, 1963). The heart and CNS are the tissues most critically involved by a general inhibition of oxidative energy metabolism. The signs and symptoms of fatal poisoning with 2-FA and fluoroacetate in addition to non specific signs of nausea and vomiting, include cardiac irregularities, cyanosis, generalized convulsions, and death from ventricular fibrillation or respiratory failure (Brockmann, McDowell and Leeds,

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1955；Ecobichon，1991). Death in humans from fluoroacetate poisoning is usually due to cardiac failure (Pradhan，Roger and Dutta，1986).

As an effective rodenticide，2-FA can be used in the bait form. It has a very low vapor pressure. However，owing to its high toxicity，inhalation exposure of this chemical to humans at confined work places is possible. The purpose of the present study was to generate inhalation toxicity data pertaining to 2-FA exposed rats；such data is not available in the literature.

MATERIALS AND METHODS

Animals

Male and female Wistar rats weighing 120-190 g，bred and maintained in the animal house of our establishment were used for this study. The rats were housed in polypropylene cages with rice husk as bedding material and were provided with food (Amrut，India) and water *ad libitum*. The experimental protocol was approved by the ethical committee of the establishment.

Chemicals

2-FA is a colorless crystalline substance. This chemical was synthesized in the Chemistry Division of our establishment and the purity was checked by gas chromatographic method. Its purity was above 95%. All the other chemicals used were of analytical grade.

Inhalation Exposure

Acute inhalation toxicity study was carried out with vapors and aerosols of 2-FA. For studies with vapors，10 g of 2-FA was kept in an evaporator. Filtered air was passed through it at a rate of 25 L per minute (LPM) for generating vapors. Five male and five female rats restrained in individual wire mesh cages，were exposed to the vapors in a dynamically operated whole body inhalation exposure chamber (DRDE inhalation exposure chamber，Model 1B，50 L capacity). For studies with aerosols，2-FA was dissolved in distilled water and pumped into a glass airblast nebuliser using a low volume liquid delivery pump (Waters，USA). Two hundred millilitres of 2-FA solution was taken in a reservoir for aerosol generation. A modified nebuliser was used in which unaerosolized solution drained in the reservoir (Fig. 1). The 200 ml solution was used for the aerosol generation for 2 h. For a 4 h exposure the reservoir was replenished twice. By this method the quantity of 2-FA used was less and only 10% of the solution was aerosolized for period of 2 h. The aerosols generated were directed into the exposure chamber which was ventilated at a rate of 25 LPM. Air from the exposure chamber was passed through a sodium hydroxide wash bottle and a cotton-wool filter，before exhausting. For the determination of actual concentration，various percentages of 2-FA in distilled water (0.25%-8.0%) were taken，nebulized and passed inside the exposure chamber，without animals. Air from the exposure chamber was sampled from the breathing zone of the animals at a rate of 2 LPM for 2-5 min through a PTFE (0.2 μm) membrane filter and the concentration was determined gravimetrically. This procedure was repeated several times to arrive at a mean concentration for various percentages of 2-FA solution，taken in the reservoir. Nominal concentration was calculated by the rate of nebulization and the airflow inside the exposure chamber. The particle size was monitored using an optical

particle counter (Royco, USA) with five channels, counting particles less than 3.0 μm.

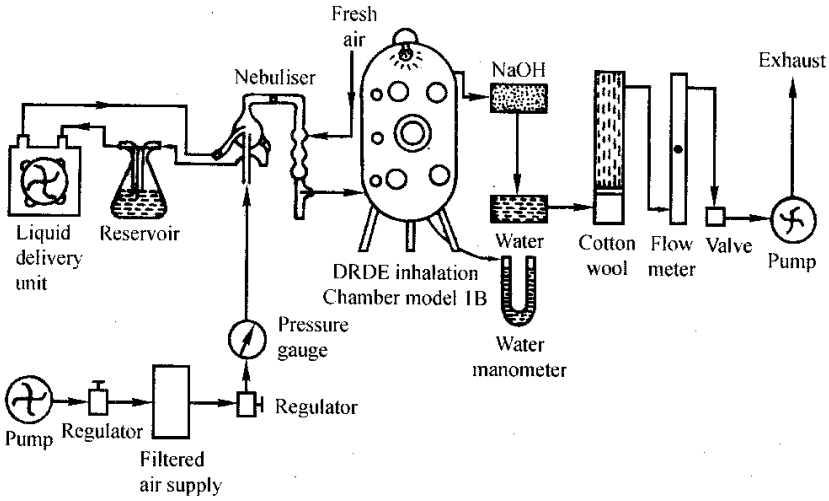

FIG. 1. Schematic diagram of 2-fluoroacetamide exposure assembly for whole body exposure of rats.

Acute Toxicity Study

The male and female rats were exposed in individual wire mesh cages (5 rats at a time) for 4 h to various concentrations of 2-FA aerosols. After exposure, the body weights of rats and mortality were observed for a period of 14 days. LC_{50} was calculated by the moving average method of Gad and Weil (1989). To study the acute toxic effects of 2-FA, 15 male and 15 female rats were exposed to 0.7 LC_{50} of 2-FA, for 4 h duration (5 rats at a time). Groups of five male and five female rats were sacrificed after 1 h, 24 h and 7 days post exposure. The rats were anesthetized with ether and blood was drawn from orbital sinus for analysis of hematological and biochemical parameters. Hemoglobin content (Hb), red blood corpuscle count (RBC), packed cell volume (PCV), white blood corpuscle (WBC) total and differential counts were estimated using standard procedures (Baker and Silverton, 1976). Total protein was estimated by the method of Lowry *et al*. (1951). Blood urea and blood glucose were determined using standard diagnostic kits (Ranbaxy, India). The rats were then sacrificed by cervical dislocation and the vital organs, viz., lung, liver and kidney, were dissected out for histology and weighed for calculation of organ to body weight ratios.

For histology, liver and kidneys were fixed in 10% buffered formalin. The middle portions of the kidneys were cut into 3 mm sections (2 per kidney), and two 3 mm sections of liver extending from the hilus to the margin of the left lateral lobe, were used for processing. The excised lungs with trachea were inflated with 10% buffered formalin solution through a tracheal cannula to an intra-pulmonary pressure of 10 cm water and the whole lung was then immersed in the fixative. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleaned with xylene and embedded in paraffin wax. Subsequently, 5-6 μm thick sections were deparaffinated and stained with hematoxylin and eosin (McManus and Mowry, 1965) for examination under the light microscope.

Statistical Analysis

The data were analysed by one way ANOVA and Dunnett's multiple comparisons procedure. SigmaStat (Jandel Corporation, USA) was used for the statistical analysis.

RESULTS AND DISCUSSION

The generated vapor concentration of 50 $mg \cdot m^{-3}$ did not show any toxic effect on the rats. Hence aerosols were generated and the rats were exposed to various concentrations. The aerosols generated were within the respirable range as monitored by the optical counter (more than 90% less than 3.0 μm). The theoretical and actual concentrations of the aerosols were very close to each other. During and after the inhalation exposure the rats were less active, and showed mild tremors and convulsions. At higher concentrations the rats died after 2-3 days. The estimated 4-h LC_{50} value was 136.6 (121.3-154.0) and 144.5 (121.5-171.7) $mg \cdot m^{-3}$ in male and female rats respectively (Table 1).

TABLE 1

Inhalation Exposure Concentrations of 2-FA and Percent Mortality of Exposed Rats

Concentration of 2-FA ($mg \cdot m^{-3}$)[a]	Percent Mortality	
	Male	Female
109.0	0	0
129.6	40	40
154.0	80	60
183.3	100	80
199.8	–	100

[a]Pre-calibrated actual concentration.

No mortality was observed following exposure to 0.7 LC_{50} of 2-FA. The effects of exposure to 0.7 LC_{50} of 2-FA on organ to body weight ratio, various hematological and biochemical parameters are shown in Tables 2—4. An increase in the liver weight was observed both in male and female rats 7 days post exposure (Table 2). Except for a decrease in Hb, PCV and RBC in female rats 24 h post exposure, there were no other significant changes in the hematological parameters of both male and female rats (Table 3). The various biochemical parameters were within the clinical limits (Table 4).

TABLE 2

Effect of Inhalation Exposure (0.7 LC_{50}) of 2-FA on Organ to Body Weight Ratio

Post Exposure Period	Lung	Liver	Kidney
Male			
Control	0.51 ± 0.03	3.51 ± 0.30	0.75 ± 0.03
1 h	0.57 ± 0.04	3.76 ± 0.26	0.82 ± 0.07
24 h	0.50 ± 0.03	3.98 ± 0.30	0.85 ± 0.04
7 days	0.59 ± 0.04	4.50 ± 0.21[a]	0.79 ± 0.01
Female			
Control	0.61 ± 0.11	4.06 ± 0.24	0.71 ± 0.03
1 h	0.55 ± 0.02	3.82 ± 0.42	0.76 ± 0.03
24 h	0.59 ± 0.06	4.14 ± 0.16	0.76 ± 0.08
7 days	0.66 ± 0.02	4.67 ± 0.05[a]	0.78 ± 0.01

Values are $\bar{x} \pm s$ of five rats.

[a]Significant difference from control by Dunnett's method, $P < 0.05$.

TABLE 3

Effect of Inhalation Exposure (0.7 LC_{50}) of 2-FA on Haematological Variables

Post Exposure Period	PCV (vol%)	Hb (g%)	RBC ($\times 10^6$/mm^3)	WBC (cell/mm^3)	Polymorphs (%)	Lymphocytes (%)	Eosinophils (%)
Male							
Control	39.3 ± 0.4	13.2 ± 0.3	4.30 ± 0.02	9283 ± 179	64.8 ± 2.2	34.5 ± 2.1	0.6 ± 0.3
1 h	38.8 ± 1.0	12.7 ± 0.4	4.30 ± 0.16	9450 ± 160	63.6 ± 2.3	35.5 ± 2.2	0.8 ± 0.4
24 h	38.5 ± 1.0	12.4 ± 0.4	4.20 ± 0.19	9550 ± 95	65.5 ± 2.1	34.0 ± 2.0	0.5 ± 0.3
7 days	39.5 ± 0.5	12.7 ± 0.2	4.10 ± 0.09	9150 ± 170	63.7 ± 4.5	35.5 ± 2.4	0.8 ± 0.3
Female							
Control	40.3 ± 0.8	11.9 ± 0.3	4.30 ± 0.15	9566 ± 186	63.1 ± 2.5	36.0 ± 2.4	0.8 ± 0.4
1 h	38.6 ± 0.8	12.5 ± 0.3	4.20 ± 0.16	9433 ± 207	62.1 ± 1.5	37.3 ± 1.4	0.5 ± 0.3
24 h	34.6 ± 1.2[a]	10.8 ± 0.4[a]	3.70 ± 0.06[a]	9316 ± 174	63.1 ± 2.1	36.0 ± 1.8	0.8 ± 0.4
7 days	39.2 ± 1.5	12.4 ± 0.4	4.20 ± 0.14	9100 ± 108	63.0 ± 1.5	36.5 ± 1.3	0.5 ± 0.3

Values are $\bar{x} \pm s$ of five rats.

[a]Significant difference from control by Dunnett's method, $P < 0.05$.

TABLE 4

Effect of Inhalation Exposure (0.7 LC_{50}) of 2-FA on Biochemical Variables in Blood

Post Exposure Period	Urea (mg/dl)	Glucose (mg/dl)	Protein (g/dl)
Male			
Control	39.6 ± 1.9	102.4 ± 2.6	8.30 ± 0.94
1 h	37.2 ± 2.8	113.1 ± 3.4	9.50 ± 0.30
24 h	39.2 ± 2.1	100.1 ± 0.6	7.60 ± 0.56
7 days	33.2 ± 0.9[a]	97.5 ± 0.6	8.70 ± 0.63
Female			
Control	36.1 ± 1.6	98.1 ± 2.1	8.10 ± 0.29
1 h	38.8 ± 1.3	90.7 ± 4.9	9.90 ± 0.39[a]
24 h	41.3 ± 1.2[a]	102.2 ± 1.4	7.60 ± 0.72
7 days	32.2 ± 0.9	100.3 ± 5.2	8.40 ± 0.29

Values are $\bar{x} \pm s$ of five rats.

[a]Significant difference from control by Dunnett's method, $P < 0.05$.

One hour after exposure, the lung histology showed mild inflammatory reaction Twenty four hour post exposure lung showed injured epithelium with focal necrosis and sloughing of surface cells. Many surface lining cells appeared enlarged with cytoplasmic vacuolation (Plate 1a). On 7th day post exposure, the injured epithelium was generally disrupted due to sloughing and necrosis of surface epithelial cells. The sloughed cells and cellular debris were admixed with polymorphonuclear cells and fibrinoid material, which appeared to be attached to the airway surface (Plate 1b). These lesions were common in both the sexes.

One hour post exposure to 2-FA, the rat liver did not show any significant change. Marked hypertrophy of hepatocytes and reduced staining of the nuclei were observed around the central vein 24 h after exposure. On 7th day post exposure, hepatocytes showed strong acidophilic granulated cytoplasm. Necrotic hepatocytes were observed randomly, throughout the liver lobules (Plate 1c). Liver histology also showed bile duct proliferation (Plate 1d).

Moderate histological changes occurred in the kidneys of exposed male and female rats.

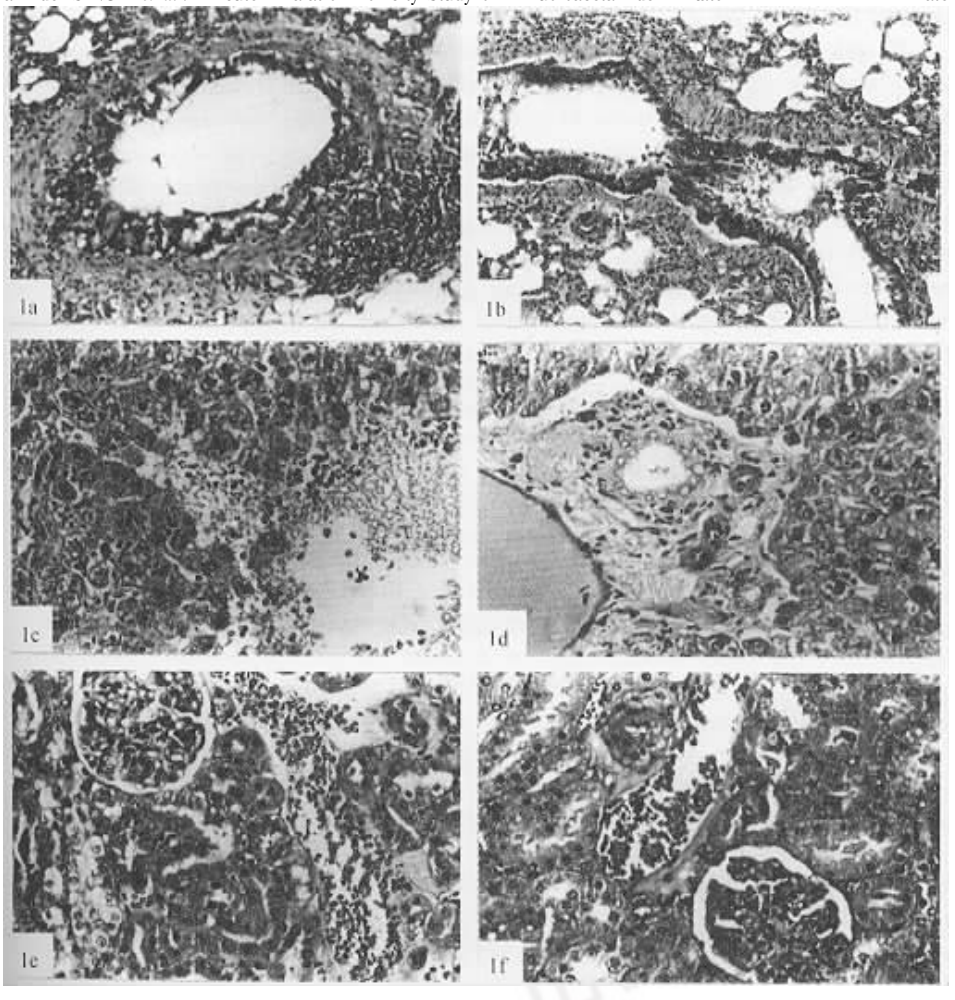

Plate 1a. 2-FA exposed rat lung, 24-h post exposure showing injured epithelial lining with sporadic necrosis; few cells were hypertrophic: ×65. Plate 1b. 2-FA exposed rat lung, seven days post exposure showing disrupted and sloughed epithelial lining of the bronchioles: ×32.5. Plate 1c. 2-FA exposed rat liver, seven days post exposure showing granulovacuolar degeneration of hepatocytes with pronounced acidophilia: ×65. Plate 1d. 2-FA exposed rat liver, seven days post exposure showing bile duct proliferation: ×65. Plate 1e. 2-FA exposed rat kidney showing congestion, mild hemorrhage and degeneration of renal parenchyma 24-h post exposure: ×65. Plate 1f. 2-FA exposed rat kidney showing normal renal parenchyma seven days post exposure: ×65.

Focal degeneration of renal proximal tubules and congestion in renal parenchyma were observed 24 h post exposure (Plate 1e). Tubular degeneration regressed and renal histology appeared normal on 7th day post exposure (Plate 1f).

Though the general toxicity and mechanism of action of 2-FA through oral route is well documented, the effect of 2-FA through inhalation route is not available in literature. The only document available was on its LC_{50} in mice as 550 $mg \cdot m^{-3}$ for which duration of exposure is not known. Since the vapors generated by passing air were very small, inhalation toxicity studies were conducted by generating aerosols of 2-FA. Chemicals whose LC_{50} lies between 100 to 1000 $mg \cdot m^{-3}$ are known as highly toxic. The acute inhalation toxicity studies of 2-FA revealed that it is a highly toxic chemical. Exposure to 0.7 LC_{50} of 2-FA revealed that this chemical may be hepatotoxic and nephrotoxic also. Exposure did not reveal any significant hematological and biochemical alteration. However, histological examination revealed that it can affect the liver and kidney similar to chlorinated hydrocarbons.

CONCLUSION

2-FA is extremely toxic through the oral route. Its oral LD_{50} in rats is 4-15 $mg \cdot kg^{-1}$ (Ecobichon, 1991). The present study shows that 2-FA is an highly toxic chemical through the inhalation route based on its LC_{50} value and that necessary precautions should be taken during its handling.

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