

## Hepatotoxic Alterations Induced by Subchronic Exposure of Rats to Formulated Fenvalerate (20% EC) by Nose Only Inhalation

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**Objective** Fenvalerate (20% EC) is a synthetic pyrethroid, which is commonly used in India by farmers for the protection of many food and vegetable crops against a wide variety of insects. However, its inhalation toxicity data is very limited in the literature due to the fact that the exposure levels associated with these effects were usually not reported. Hence, inhalation exposure was carried out to investigate the hepatotoxic effects. **Method** Adult male rats were exposed to fen for 4 h/day, 5 days a week for 90 days by using Flow Past Nose Only Inhalation Chamber. Sham treated control rats were exposed to compressed air in the inhalation chamber for the same period. **Results** The results indicated hepatomegaly, increased activities of serum clinical enzymes (indicative of liver damage/dysfunction) along with pronounced histopathological damage of liver. **Conclusion** The hepatotoxic potential of formulated Fen (20% EC) in rats exposed by nose only inhalation is being reported for the first time and warrant adequate safety measures for human beings exposed to this insecticide, particularly by inhalation route.

**Key words:** Fenvalerate; Rat; Inhalation; Hepatotoxicity

### INTRODUCTION

Fenvalerate (an ester of 2-(4-chlorophenyl)-3-methyl butyric acid  $\alpha$ -cyano 3 phenoxybenzyl alcohol) is a synthetic pyrethroid insecticide active against a wide range of pests, particularly boring insects viz., lepidoptera, Diptera, Hemiptera etc., invading cotton, fruit and vegetable crop in India. It is also used in public health sectors and cattle sheds for controlling flies<sup>[1,2]</sup>.

Limited histopathological studies of liver have been reported in rodents fed with technical and/or formulated Fen<sup>[3,4]</sup>.

Fen has also been reported to increase the incidences of  $\gamma$ -glutamyltranspeptidase enriched hepatic foci in liver of rats fed with sublethal doses of Fen<sup>[5]</sup>. The detailed biochemical and histopathological alterations in liver of rat/mice exposed to formulated Fen by inhalation are not much known at present.

Hence, in the present study, histopathological investigation in liver along with the

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activities of serum clinical enzymes have been carried out in adult male rats exposed to different doses of formulated Fen (20% EC) by nose only inhalation for three months.

## MATERIAL AND METHODS

### *Animals and Chemicals*

Ten adult male albino Wistar rats ( $150 \pm 180$  g) obtained from Industrial Toxicology Research Centre, animal house colony that had been maintained under standard conditions of husbandry, were used. The rats were acclimatized for 7 days prior to exposure. All chemicals used were of analytical grade procured from SISCO Research Laboratories, India. The substrates for enzyme assay were purchased from Sigma chemical Co., USA. Formulated fenvalerate procured from M/s Motilal Pesticide Pvt. Ltd., Masani, Mathura, India and was stored in a sealed container under cold and dry conditions to avoid oxidation and/or hydrolysis.

### *Inhalation Exposure*

Animals were exposed in flow past nose only dynamic inhalation chamber (InTox Products, USA) to 1/5th, 1/10th, 1/15th  $LC_{50}$  i.e. ( $6500 \text{ mg/m}^3$ ,  $3500 \text{ mg/m}^3$  and  $2200 \text{ mg/m}^3$  respectively) (repeated exposure, 4 h/d, 5 days a week) for three months as reported by us earlier<sup>[6,7]</sup>. Age and sex matched control rats were exposed to a compressed air only under identical conditions. Fen samples were collected from inhalation chamber at regular intervals for analysis. The concentration of Fen in each sample collected at an interval of 30 min was analyzed by using Hewlett Packard (USA) Model 5890A Gas chromatograph. The amount of Fen (chamber concentration) found in the samples for different dosages are shown in Table 1.

TABLE 1  
Inhalation Exposure Doses and Chamber Concentration of Fen

Groups	Exposure Doses of Fen	Chamber Conc. of Fen
Control	Compressed Air	0
Fen 20% EC	1/5th $LC_{50}$	$6500 \pm 135 \text{ mg/m}^3$
Fen 20% EC	1/10th $LC_{50}$	$3500 \pm 70 \text{ mg/m}^3$
Fen 20% EC	1/15th $LC_{50}$	$2200 \pm 50 \text{ mg/m}^3$

### *Liver Body Weight Ratio*

Rats were sacrificed by cervical dislocation after three months (2 h after the last exposure). Livers were surgically removed and cleaned free from blood and other tissues. The relative weights of liver were calculated by dividing organ weight with body weight and quotient being multiplied by  $100^{[8]}$ .

### *Histopathological Studies*

Liver was fixed in 10% neutral buffered formalin. After routine processing it was embedded in paraffin and serial sections were cut at  $5 \mu\text{m}$  and stained with hematoxyline and eosin following standard methodology<sup>[9]</sup>.

### Enzyme Analysis

Five to seven mL of blood samples were collected from rats by retro orbital plexus puncture in a dry and clean 15 mL glass test tube. Sera samples were separated and acid phosphatase (ACP) and alkaline phosphatase (ALP) activity were estimated following the methodology of Wooton<sup>[10]</sup>, whereas the activity of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated following the Methodology of Reitman and Frankel<sup>[11]</sup>.

### Statistical Analysis

The data were analyzed by the Student's *t* test as described by Fischer<sup>[12]</sup> and  $P < 0.05$  was considered to be significant.

## RESULTS

The effect of formulated Fen (20% EC) inhalation on the weight of liver has been shown in Table 2. The results indicate significant increase in absolute as well as relative weights of liver in group of rats exposed to 1/5th LC<sub>50</sub> Fen for three months as compared to the control. The data shown in Table 3 indicates significant increase ( $P < 0.05$ ) in the activities of clinical enzymes viz., acid phosphatase (ACP), alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in sera of rats exposed to 1/5th LC<sub>50</sub> of formulated Fen (20% EC) by nose only inhalation for 3 months as compared to the control.

TABLE 2  
Effect of Fen (20% EC) Inhalation (4 h/day, 5 days a week) for Three Months on Liver Weight

Group	Absolute	Weight
Control	7.79 ± 0.26	3.25 ± 0.13
Exposed	9.64 ± 0.35 <sup>a</sup>	5.01 ± 0.01 <sup>a</sup>

Note. Data represent  $\bar{x} \pm s$  of 6 rats in each group. <sup>a</sup> $P < 0.05$ .

TABLE 3  
Effect of 90 Days Fen Exposure (1/5th LC<sub>50</sub>, 4 h/day) Through Nose Only Inhalation Chamber on Serum Clinical Enzymes

S. No.	Serum Enzymes	Control	Exposed
1	Acid Phosphatase	4.285 ± 0.180 <sup>a</sup>	12.0 ± 0.186 <sup>c</sup>
2	Alkaline Phosphatase	17.5 ± 0.150 <sup>a</sup>	45.0 ± 0.186 <sup>c</sup>
3	Serum Glutamate Oxaloacetate Transaminase (SGOT)	12.5 ± 0.150 <sup>b</sup>	35.0 ± 0.181 <sup>c</sup>
4	Serum Glutamate Pyruvate Transaminase (SGPT)	5.25 ± 0.178 <sup>b</sup>	18.5 ± 0.315 <sup>c</sup>

Note. Data represent mean  $\bar{x} \pm s$  of 6 rats in each group. <sup>a</sup>μ moles p nitro phenol formed/min/dL. sera. <sup>b</sup>μ moles pyruvate formed/min/dL. sera. <sup>c</sup> $P < 0.05$ .

The concomitant histopathological examination of liver from rats exposed to 1/5th LC<sub>50</sub> Fen (20% EC) showed fatty degenerative changes in centrolobular as well as mid zonal areas (Figs. 2 and 3). Hepatocytes showed marked cytoplasmic and nuclear damage along with the presence of large sized vacuoles in cytoplasm. Most of nuclei showed karyolysis.

Kuffper cells were prominent, bile ducts and blood vascular lesions were also seen. The enzymatic and histopathological changes in liver were not significant with lower doses of Fen treatment.

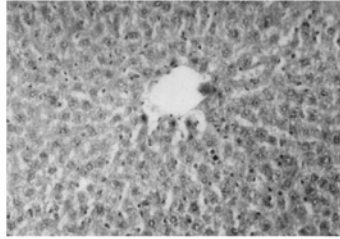


FIG. 1. Section of liver from control rat showing the normal hepatic architecture around the central vein. H and E  $\times$  200.

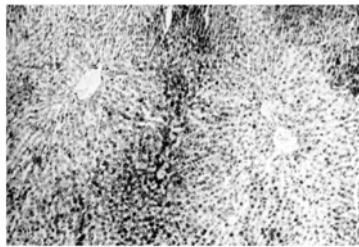


FIG. 2. Section of liver from rat three months post exposure to 1/5th  $LC_{50}$  of Fen showing uniform degenerative changes of hepatocytes around the centrolobular, midzonal and peripheral areas. H and E  $\times$  160.



FIG. 3. Section of liver from rat three months post exposure to 1/5th  $LC_{50}$  of Fen showing marked fatty degenerative changes in hepatocytes. H and E  $\times$  800.

## DISCUSSION

In the present study rats exposed to 1/5th, 1/10th and 1/15th  $LC_{50}$  of Fen by nose only inhalation for a duration of 3 months showed liver enlargement and significantly ( $P < 0.05$ ) increased its weights indicating the presence of hepatomegaly (Table 2). The increased weights were consistent with data from microscopic examination of the liver showing edema and compound related centrolobular hepatocyte hypertrophy in formulated Fen exposed rats. These observations are in agreement with the findings of significant increase in liver weights of rats fed with Fen<sup>[3,13-16]</sup>. Similar findings were also observed with another pyrethroid treated rats<sup>[17]</sup>.

The histopathological changes observed in livers of rats exposed to formulated Fen by

inhalation showed early degenerative changes of the hepatocytes in the centrilobular areas, less marked degenerative changes in the midzonal areas and least affected periportal areas. The enhanced activities of serum clinical enzymes may be due to lysis/damage of hepatocytes resulting in the permeation of these enzymes to serum. Clinical enzymes viz., ACP, ALP, SGOT, and SGPT have been commonly associated with liver dysfunction/damage. The histopathological changes observed in liver of Fen fed rats along with changes in serum clinical enzymes resembles the earlier findings<sup>[4,18]</sup>.

Mandal *et al.*<sup>[19]</sup> reported that single oral administration of Fen at 5 mg/kg produced marked degenerative changes in liver of goats after 60 days. Some workers reported that oral administration of Fen to animals may produce microgranulomatous changes in liver comprising of various mononuclear phagocytic cells (microgranuloma) and varying number of multinucleated giant cells<sup>[4,18]</sup>. However, the present study failed to show any such kind of effects in liver of rats exposed to formulated Fen by inhalation for a duration of 3 months.

The toxic effects of Fen in fish *Catla catla* showed decreased glycogen contents in liver, which indicated liver to be one of the vital organs of metabolism that could be adversely affected by Fen ingestion<sup>[20]</sup>.

Majumder *et al.*<sup>[21]</sup> reported focal areas of necrosis and proliferation of bile duct along with fibrosis after 28-day oral administration of Fen to broiler chicks. The present study did not show any kind of necrosis in the liver of rats exposed to Fen by inhalation for 3 months.

Dermal application of Fen (0.01%) produced fibrosis in the periportal areas of liver in broiler chicks<sup>[22]</sup>. However, the present study failed to show any such kind of effects in liver of rats exposed to formulate Fen by inhalation for 3 months.

These hepatic changes are consistent with the observed increase in the absolute and relative organ weights and identify liver as the principal target organ involved in metabolism. The observed changes suggest a continuum of hypertrophy with progressive evidence of cytotoxicity at the higher dose of Fen inhalation. Hepatomegaly and/or hypertrophy are often caused by the induction of the liver microsomal monooxygenase system in rats<sup>[18]</sup>.

Exposure to a wide range of foreign compounds leads to hyper functional liver enlargement<sup>[23-27]</sup>. The toxicological significance of this type of liver enlargement and its relevance in the term of "no-effect level" has been discussed by a number of workers<sup>[25, 28-30]</sup>. A widely held interpretation is that this phenomenon is a reversible physiologic adaptive response due to increased functional demand on the liver, resulting in the accelerated metabolism of the xenobiotics.

Thus, the results of the present study indicate hepatotoxic potential of a formulated Fen (20% EC) preparation (which is commonly used in India) in rat model exposed by nose only inhalation for three months and warrant adequate safety measures by human beings.

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