TIH

Toxicology and Industrial Health
27(6) 483–488

DOI: 10.1177/0748233710387011
tih.sagepub.com
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# Endosulfan-induced hepatotoxicity is route of exposure independent in rats

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## Abstract

Endosulfan is an important hepatotoxic agent that generates free oxygen radicals in liver. With the widespread use of endosulfan in agriculture, human beings are most likely to be exposed to it by eating food contaminated with endosulfan, exposure to its low levels by skin contact with contaminated soil, smoking cigarettes made from tobacco that has endosulfan residues on it, or by nose and whole body inhalation exposure in the farms during its application. Since endosulfan is a frequently used pesticide, and the incidence of toxic injury to the liver tissue in relation to its widespread use reported in the literature, we considered it necessary to investigate whether endosulfan-induced liver injury could be route of exposure dependent. Eighteen mature male albino Wistar rats, weighing between 180 and 220 g, were used in this study. The hepatotoxic effects of oral administration of endosulfan (5 mg/kg body weight) daily for 30 days, and 30 days whole body inhalation exposure to ungraded concentration of endosulfan were investigated in rats using serum liver enzymes and histopathological assay. At the end of the experimental period, serum alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, and creatine kinase activities obtained for the group of rats exposed orally to endosulfan were not significantly different ($p \geq 0.05$) from the activities obtained for rats exposed by whole body inhalation. However, the activity of these enzymes obtained for the rats exposed to endosulfan by both oral and inhalation routes were significantly increased ($p \leq 0.05$) compared, respectively, to the control. Also, on microscopic examination, the liver tissues of experimental groups exhibited severe damage histopathologically. The results of the enzyme and histological analyses showed that both oral and whole body inhalation exposure to endosulfan may cause liver tissue damage in rats. The exposure to endosulfan in rats caused liver tissue damage independent of the route of exposure.

## Keywords

Endosulfan, hepatotoxicity, liver enzymes, histopathology, route of exposure

## Introduction

Most of the pest control chemicals (pesticides) are known to be poisonous, presenting immediate or delayed adverse effects to the users if not improperly handled. Among the important pesticides that have been widely used over the years are the organochloride products (Lodha and Saxena, 1991). According to Hargrave et al (1992), Fossi et al (1995), and Nichols et al (1995), extensive use and limited biodegradation of these chemical agents are among the major contributing factors to their worldwide contamination and biomagnifications. Endosulfan is an example of a widely used insecticide belonging to the cyclodiene group of organochlorine pesticides, constituting important pollutant in the environment. Literature report indicates that endosulfan is highly toxic, regardless of the route of its exposure (Smith, 1991), causing in-coordination, imbalance, difficulty in breathing, gagging, vomiting, diarrhea, agitation, convulsions, loss of consciousness, and central nervous system disorders (Dutta and Arends, 2003). It

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is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenase systems which may cause oxidative stress (Liang et al., 1992). It has been reported that the metabolites of endosulfan have strong tendencies to get accumulated in different organs and tissues of the body, including the adipose tissue, liver, and food items (Thao et al., 1993; Winter and Street, 1992).

A positive correlation between changes in liver structure and biochemical constituents of the liver and serum has been shown in a number of studies on different mammals exposed to various pesticides (Ali and Shakoori, 1990, 1996, 1999; Boulecache and Spiess, 1974; Gertig and Nowaczyk, 1975). Hence, chemical pesticides like endosulfan and their metabolites may induce metabolic changes, which are indicators of toxicity, in liver and other tissues. Several underlying mechanisms have been highlighted to explain the nature of changes in liver under given conditions of pesticidal exposure and dosage (Kimbrough et al., 1971; Meany and Pocker, 1979; Yavuz et al., 2007). Among the suggested mechanisms include the generation of reactive oxygen species (ROS) and other free radicals into the system during the process of detoxification of pesticides. Free radicals generated during oxidative stress cause lipid peroxidation of cell membranes, which is in turn prevented by antioxidant enzymes (Kurutas et al., 2001; Kurutas and Tuncer, 2000). The generated ROS then provoke certain unwanted reactions in the cell and lead to membrane damage, alterations in metabolic activity, necrosis, and cell death. The action of these reactive species on the cell membranes alters the permeability of the membrane, impairs the functionality of the plasma membrane, causing the intracellular constituents to leak out into the extracellular compartment. The leakage of the intracellular constituents, including enzymes, is obviously due to impaired functions of plasma membrane and it has been reported that administration of lindane significantly decreases the brush border sialic acid content of the membrane, which alters membrane permeability (Labana et al., 1997).

With the widespread use of endosulfan in agriculture, human beings are most likely to be exposed to it by eating food contaminated with endosulfan. However, humans may also be exposed to low levels of endosulfan by skin contact with contaminated soil, by smoking cigarettes made from tobacco that has endosulfan residues on it, or by nose and whole body inhalation exposure in the farms during its application (Lonsway et al., 1997). Since endosulfan is a frequently used pesticide, and the incidence of toxic injury to the liver in relation to its widespread use reported in the literature, we considered it necessary to investigate whether endosulfan-induced liver injury could be route of exposure dependent.

## Materials and methods

### *Animals experimental design*

Eighteen mature albino Wistar rats, weighing between 180 and 2000 g were obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria. They were fed with a standard laboratory diet and tap water. Illumination was 12 hours light/dark cycle and room temperature was 25 $\pm$ 2°C. The animals were divded into experimental and control groups, which consisted of apparently normal albino Wistar rats. The experimental group was further divided into two groups (groups A and B) of six rats each. Rats in group A were exposed to endosulfan by oral administration (5 mg per kg body weight) daily for 30 days, while the rats in group B were exposed to ungraded concentrations of endosulfan by 4 hours daily nose and whole body exposure method for a period of 30 days. The endosulfan fraction administered orally was solubilized in Goya Olive oil as a vehicle. In this study, all animal experiment followed the guide for the care and use of laboratory animals obtained from the Institutional Animal Ethics Committee.

## Nose and whole body inhalation exposure to endosulfan

The liquid endosulfan (Thionex 35EC) used in this study was obtained from ABC Agrochemical shop in Watt Market, Calabar, Nigeria. The animals in the test group B were exposed to ungraded concentrations of endosulfan vapors in an exposure chamber. A modified nose and whole body inhalation exposure method previously described for gasoline vapors (Uboh et al., 2005, 2008, 2010a) was used to expose the animals in this group to ungraded concentrations of endosulfan vapors. In this method, endosulfan vapors were generated from 100 mL of liquid endosulfan pumped daily by a manual spraying machine into the exposure chamber (1.5 m $\times$ 0.9 m $\times$ 2.1 m), simulating an endosulfan-polluted agricultural environment. The chamber compartment was fully

**Table 1.** Effect of differences in the route of exposure to endosulfan on some serum liver enzymes in rats[a]

| Group | Treatment | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | CK (IU/L) |
|---|---|---|---|---|---|
| I | Control | 8.72 ± 0.54 | 8.80 ± 0.66 | 18.10 ± 0.29 | 21.60 ± 0.40 |
| II | Endosulfan by inhalation | 15.84 ± 0.28[b] | 13.50 ± 0.32[b] | 22.10 ± 0.56[b] | 26.40 ± 0.51[b] |
| III | Endosulfan orally | 15.80 ± 0.56[b] | 13.96 ± 0.16[b] | 22.70 ± 0.34[b] | 25.60 ± 0.51[b] |

Abbreviations: AST: aspartate amino transferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, CK: creatine kinase.
[a]Values are presented as mean ± SEM, $n = 6$.
[b]$p \leq 0.05$ compared respectively with group I.

saturated with the endosulfan vapors before the animals were transferred into it. These animals were then allowed to freely inhale the vapors in the chamber during the exposure period through nose and whole body route of exposure. At the end of each day's exposure period, the animals were transferred to a non-endosulfan-contaminated section of the animal house. An exposure period of 4 hours (9 a.m. to 1 p.m.) daily, 6 days per week, was adopted for 30 days.

### *Collection of blood and liver tissues for analyses*

At the end of the experimental period, the animals were sedated with chloroform and dissected for collection of liver tissue and blood specimens. The liver tissues were quickly removed and cleansed for histopathological investigations. The blood samples were collected by cardiac puncture using syringe and needle into non-heparinized sample tubes, centrifuged at 2000 rpm for 20 minutes to obtain clear supernatant sera for enzyme analyses. Serum alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and creatine kinase (CK) were assayed by spectrophotometric determination of their absorbances, using analytical grade laboratory reagent kits obtained from Biosystems Laboratories (S. A. Costa Brava, Barcelonia, Spain). The liver tissues were fixed in 10% buffered formaldehyde and processed routinely. They were sliced and embedded in paraffin; 5-µm sections were obtained, stained with Harris hematoxylene-eosin, and examined under light microscope (Bancroft and Stevens, 1977).

### *Statistical analysis*

Results were presented as mean ± SEM (standard error of mean). The data were statistically analyzed using the one-way ANOVA, followed by Student's *t*-test for pairwise comparison of the means. Results were considered to be statistically significant when $p$ values are less than 0.05 ($p \leq 0.05$).

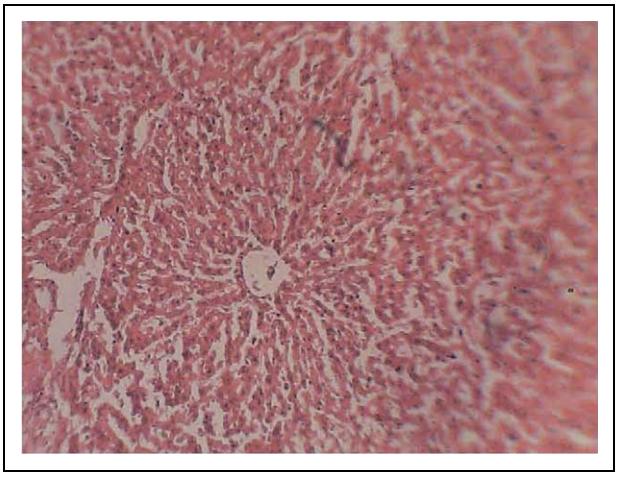

**Figure 1.** Liver tissue of rats in the control group showing relatively normal cellular architecture.

## Results

The effect of oral and whole body inhalation exposure to endosufan on the activities of some serum liver enzymes; AST, ALT, ALP, and CK was studied in rats. The result, presented in Table 1 and Figures 1−3, showed that the activities of serum AST, ALT, ALP, and CK obtained for rats exposed to endosulfan by inhalation and oral routes were significantly ($p \leq 0.05$) higher, compared, respectively, to the activities obtained for the control. It was also observed that the activities of these enzymes in rats exposed orally to endosulfan were not significantly ($p \geq 0.05$) different from the activities obtained for rats exposed by whole body inhalation (Table 1). Microscopic examinations of liver tissues of rats exposed to endosulfan by both oral and inhalation routes demonstrated some histopathological degenerations and liver cell necrosis, compared to the liver tissues of rats in the control group (Figures 1−3). The liver section of rats in the control group showed normal hepatocytes with well-preserved cytoplasm, prominent nucleus, and central vein (Figure 1). Whereas,

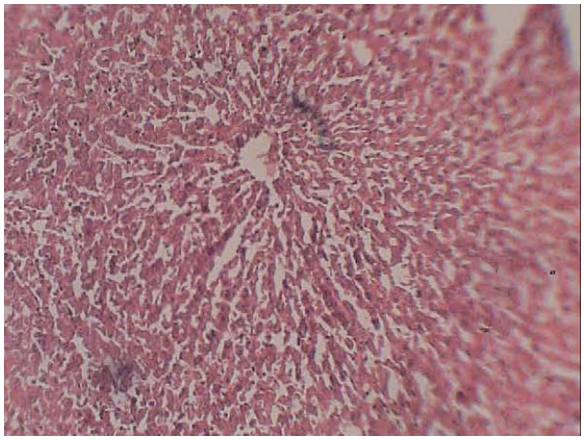

**Figure 2.** Liver tissue of rats exposed to endosulfan by inhalation. The plate indicates a distorted architectural structure and necrosis of the liver cells, with widespread distribution of sinusoids and reduced hepatic plates with less-prominent nuclei.

the liver sections of the test rats showed advanced changes in the liver architecture along with disarrangement of the hepatic strands (Figures 2 and 3). Also, hepatocytes around the central vein indicated a relatively high number of apoptotic and necrotic cells. Moreover, some inflammatory cells were also observed around the necrotic cells, as well as the enlargement of the sinusoids and vacuole formations in the hepatocytes. With the corroboration of the histopathological observations, this result gave a strong indication that exposure to endosulfan may cause hepatotoxicity in rats, independent of the route of exposure.

## Discussion

Pesticides play an important role in modern agriculture by providing dependable, persistent, and relatively complete control against harmful pests with less expense and effort. They have, no doubt, increased crop yields by killing different types of pests, which are known to cause substantial or total crop damage. At the same time, these chemicals are considered as potent pollutants of the environment with undesirable effects on non-target organisms. Among pesticides, the chlorinated hydrocarbon insecticides are generally much more persistent and have long residual properties that have placed them in excessive usage in agriculture and forestry throughout the world. Endosulfan is one of the important environmental pollutants of organochloride pesticide origin. It is widely used in insect control and is absorbed by both humans and animals through the intestinal tract, the lungs, and the skin. Organochlorine insecticides are highly toxic compounds that are responsible for a number of severe intoxications worldwide, with several deaths (Yavuz et al., 2007). Endosulfan causes many intentional and unintentional toxicities in many developing countries. The main site of organochloride storage in the body is the adipose tissue. It is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by cytochrome P450 oxygenase system, producing highly reactive free radicals that cause oxidative stress. Free radicals generated during oxidative stress cause lipid peroxidation of cell membranes which is in turn prevented by antioxidant enzymes (Omurtag et al., 2008).

The functional integrity of the liver tissues is commonly assessed from the activities of such enzymes as ALT, AST, ALP, γ-glutamyl transferase, and CK in the serum and the histopathological findings (Uboh et al. 2007, 2008, 2009, 2010b). In the present study, it has been revealed that low-level sublethal oral and whole body inhalation exposure to endosulfan affects the activities of serum liver enzymes (AST, ALT, ALP, and CK) in rat model. There was increase in the activity of the enzymes in rats that were exposed to endosulfan and the histology of the liver showed some distortions in the hepatic plates and distribution, and the stress on the sinusoid varies due to changes in the activity of the liver enzyme, an indication of liver injury. The result obtained in this study agrees with the available literature reports for oral exposure (Boereboom et al., 1998; Blanco-Coronado et al., 1992; Choudhary et al., 2003; Junqueira et al., 1994; Kurutaş et al., 2006; Nazir et al., 2000; Omurtag et al., 2008). In one of these reports, extremely high serum liver enzyme levels was observed to be due to ingestion of extremely high amount endosulfan with or without prolonged anoxia and that congestion of liver with perivenular steatosis presumptively attributed to endosulfan intoxication at forensic autopsy (Boereboom et al., 1998; Blanco-Coronado et al., 1992). It was therefore interesting to observe in this study that both oral and whole body inhalation exposure have almost similar adverse effect on the assayed serum indices of liver function and its histopathological findings in rats.

The organochlorine lindane causes an increase in activity of the pro-oxidant enzyme NADPH oxidase in isolated neutrophils (Khuns et al., 1986) and in liver microsomal preparations (Junqueira et al., 1994). In the liver, this pro-oxidant activity is accompanied by lipid

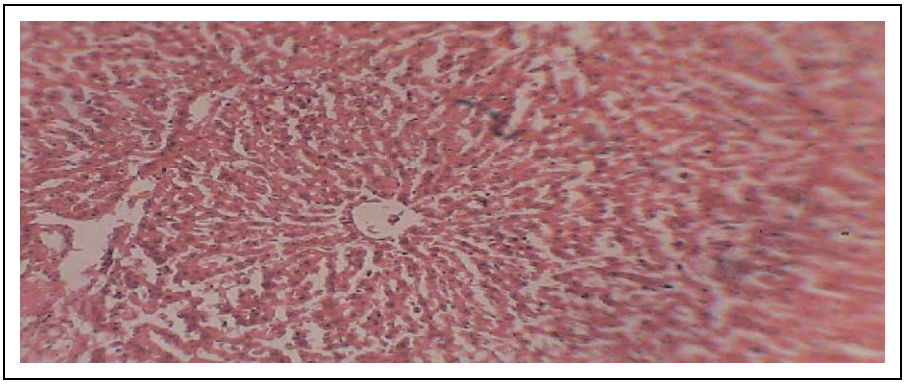

**Figure 3.** Liver tissue of rats orally exposed to endosulfan. The hepatic plates are hypertrophied with distorted architectural structure, indication of cellular necrosis. Also, the sinusoids appear smaller in size.

peroxidation and hepatocellular injury (Junqueira et al., 1986, 1994). Similarly, the other organochlorine, dieldrin, induces a state of oxidative stress in liver by causing an increase in the production of ROS with a concomitant decrease in antioxidant concentrations and an increase in hepatic DNA synthesis. These reports revealed that oral exposure to endosulfan is highly toxic for rat livers, with the most prominent gross findings at the necropsy showing swollen and pale state of the liver tissues. Liver tissue degenerations and necrosis were among the marked histopathological findings observed to be associated with exposure to endosulfan. The results of our present study showed that both oral and inhalation exposure of rats to endosulfan caused liver tissue damage revealed by increased serum levels of such liver enzymes ALT, AST, ALP, and CK in rats. Since rats have relative biochemical and physiological similarities with many other mammals including human beings, it is likely that the toxicity effects observed to be associated with endosulfan in rats may also be applicable to human tissues. In conclusion, this study also revealed that the hepatotoxic effects observed to be associated with endosulfan in rats is not route of exposure dependent.

## Funding

This research received no specific grant from any funding agency in the public, commercial, or not for-profit sectors.

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