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Toxic effects of sub lethal concentration of dioctyl phthalate on the histology of liver of *Clarias batrachus* (Linn.).

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Abstract: Dioctyl phthalate (DOP) commonly used as plasticizer enters into aquatic environment from the industries manufacturing plastic products, PVC resins, cosmetics and many other commercial products. Therefore, this study was designed to evaluate the acute toxicity effects of Dioctyl phthalate on fresh water fish *Clarias batrachus*. The 96 hour LC_{50} of Dioctyl phthalate in *Clarias batrachus* was estimated as 14.5ml/L. Histopathological changes in liver of *Clarias batrachus* were determined by exposing them to a fraction of $LC_{50}(1/5^{th})$ dose with every change of water for 30 days.The liver of *Clarias batrachus* was excised at every interval of 30 days and histological changes of liver were studied under light microscopy. Liver tissues showed abnormalities. Centrilobular vacuolation, necrosis, eccentric nuclei and enlarged nuclei, Centrilobular degeneration of hepatocytes were observed in liver tissue of fish.

Key word: Lethal concentration; Dioctyl phthalate; *Clarias batrachus*; Histology

Introduction

Phthalates are a class of high-production-volume synthetic chemicals that are ubiquitous in the environment. They are a group of similar diesters of phthalic acid which impart softening characteristic lending flexibility to the plastic. Phthalates are not covalently bound to the plastic matrix and leach out of PVC when they come in contact with lipophilic substances therefore absorbed by organic residues and solid surface in environmental water system. They can bio-accumulate in invertebrates, fish and plants. Phthalates have received considerable attention in recent years because of the potential hazards it causes to the environment. Globally more than 18billion pounds of phthalates are being used every year (Blount *et al.,* 2000a).

Dioctyl phthalate (DOP) is one of the most important phthalate used as plasticizers in poly vinyl chloride, Cosmetics, detergents, Soap, lubricating oils and in some medical devices. DOP is insoluble in water (23-340µg/l) but because of readiness with which it forms colloidal solution, its true solubility in water is to be 26-50µg/l and has a very low volatilization rate (WHO, 2003). In water, DOP predominantly attaches to suspended particles and sediments, but a small amount remains dissolved in water (EHHA, 2009). Since there is a growing concern that phthalate esters may be a menace to health and to our ecological system, there is a need to review the evidence on the toxicity and possible health threats that these esters present directly or indirectly to man. Thus, the objective of present study is to determine the median concentration of Dioctyl Phthalate and to investigate the histopathological changes in the liver of fish by exposing them to fraction of $LC_{50}(1/5th)$ dose.

Material and Methods

Collection and maintenance of fish

DOP toxicity was assessed using *C. batrachus* as an aquaculture model. In this experiment we collect 60 mature adult *C. batrachus* obtained from local fish farm, Chaibasa 22.57N: 85.82E., W. Singhbhum, Jharkhand, India and brought to avoid any injury and disinfected by giving a bath of 2 minute in 0.05% $KMnO_4$ solution thereafter, they were transferred to glass aquarium for two weeks for acclimatization in the laboratory condition before the commencement of the study. During the period of acclimation and the experiment, fish were fed two times daily with earthworms or tubifex or Blood worms. The size of the fish varied from 36.5±5.0cm in standard length and 85.3±2.0g in weight. They were reared in aquaria containing 50L dechlorinated tap water which was provided with continuous aeration. The water was renewed every other day. The mean temperatures, pH of the water used were 27.2^{0}C and 6.58 respectively.

Dioctyl phthalate (DOP) is a widely used plasticizer. It is slightly yellow transparent oily liquid soluble in ethanol, ether, mineral oil and majority of organic solvent.

All chemicals were analytical grade. Dioctyl phthalate ($C_{24}H_{38}O_4$, CAS registry number 117-81-7) was purchased from National Enterprises, Kolkata. DOP was dissolved in ethanol in ratio 1:1

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to prepare the desired degrees of concentration of chemical for toxicity test.

Lethality of *Clarias batrachus* in Dioctyl phthalate:

In this study, a static bioassay technique (ASTM 729-90) (ASTM, 1990) was adopted and preliminary screening was carried out to determine the appropriate lethal concentration of DOP (Solbe, 1995). Experiments were carried out in six groups. Five treatment group with DOP concentrations i.e. 1, 5, 10, 15 and 20mg/L and a control group with no DOP were conducted in glass aquariums. The cumulative mortality was recorded for 24 – 96 h and fish were examined to determine the cause of death. Immediate behavioral changes of the fish were recorded before death.

Median lethal toxicity of Dioctyl phthalate:

In this study, 60 adult *Clarias batrachus* were exposed to sub lethal concentrations (12,12.5,13,13.5,14 and14.5 mg/L) of DOP in water. The fish were randomly divided into six treatments groups (A-F) of 10 fish each. The temperature, pH and the dissolved oxygen of the tap water used in the study were 27.5°C, 7.2 and 6.7 mg/L respectively while the total hardness was 110 mg/L CaCO3.

Acute toxicity of *Clarias batrachus* in fraction of LC_{50} ($1/5^{th}$):

Ten fishes were exposed to 1/5th fraction dose of LC50. Experiments were run under static renewal bioassay for 30 days. After each of the exposure periods of 0, 15 and 30 days, respectively, fish from the experimental group as well as control aquaria were sacrificed and the liver collected. The collected tissues were fixed in bouin's solution, processed routinely, embedded in paraffin, sectioned at 6µm thickness, stained with heamatoxylin and eosin (H & E) and examined using trinocular light microscopy

Results

Toxicity of Dioctyl Phthalate:

The LC_{50} and LC_{100} were found to be 14.5 mg/L and 15 mg/L concentration for death and 96 h exposure respectively as shown in table 1. No adverse behavioral changes or mortality were recorded in the control fish throughout the period of the bioassay. The behavior of the control fish and their colour were normal. Symptomatic behavioral toxicities observed in DOP treated fishes were; agitated or erratic swimming, rapid air gulping, restlessness, sudden quick movement and rapid opercula movement. *C. batrachus* normal darkly pigmentation in the dorsal and lateral parts was changed to very light pigmentation in the dorsal and lateral parts.

Table 1: Morality of adult *Clarias batrachus on*24-96 hours exposure to varied concentration of Dioctyl phthalate.

Toxicant Concentration (Ml/L)	Exposure Time (H)				Total Mortality	Total No. Of Survived
	24	48	72	96		
	0	1	0	0	1	9
12ml/L	0	0	0	1	1	9
	0	0	0	1	1	9
	0	0	1	1	2	8
12.5ml/L	0	0	0	1	1	9
	0	1	0	1	2	8
	0	0	2	0	2	8
13ml/L	1	0	1	1	3	7
	0	1	1	1	3	7
	0	2	0	1	3	6
13.5ml/L	1	1	0	1	3	7
	0	2	1	2	5	5
	1	0	1	1	3	7
14ml/L	0	2	0	1	3	7
	1	0	1	2	4	6
	0	1	1	3	5	5
14.5ml/L	1	1	1	1	4	6
	1	0	2	2	5	5
	6	2	2	0	10	0
15ml/L	3	4	3	0	10	0
	4	3	2	1	10	0
	0	0	0	0	0	10
CONTROL	0	0	0	0	0	10
	0	0	0	0	0	10

Histopathological effects of Dioctyl phthalate on *Clarias batrachus*.

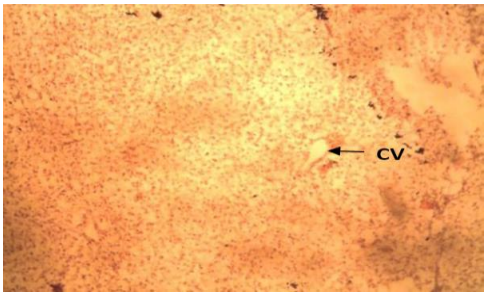

Figure 1: Centrilobular Vacuolation (CV) of hepatocytes of Liver section of treated group exposed to 2.9 ml/L for 30 days.

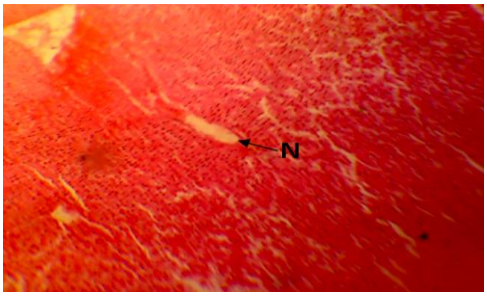

Figure 2: Necrosis (N) of Liver section of treated group exposed to 2.9 ml/L for 30 days.

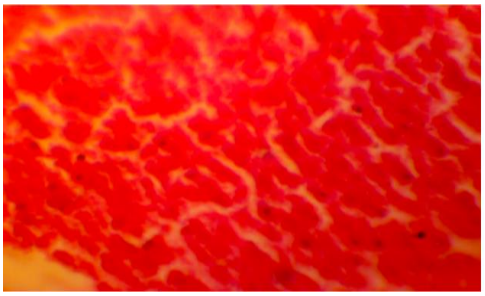

Figure 3: Centrilobular degeneration hepatocytes of Liver section of treated group exposed to 2.9 ml/L for 30 days.

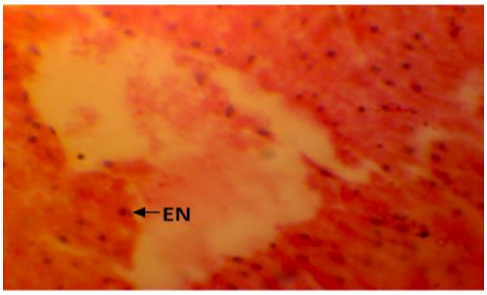

Figure 4: Eccentric nuclei (EN) and enlarged nuclei of hepatocytes of Liver section of treated group exposed to 2.9ml/L for 30 days.

Histopathological changes in liver:
In this group of *Clarias batrachus* which were treated with 1/5th of LC_{50} (2.9 ml/L) of Dioctyl phthalate; damage was observed in blood vessels of the liver. The histology of control fish liver revealed normal typical paranchymatous appearance. While Centrilobular Vacuolation, necrosis, Eccentric nuclei, enlarged nuclei of hepatocytes, Centrilobular degeneration of hepatocytes was observed in treated group.

Discussion

Some studies have been done on DOP toxicity in fish, but literature is scanty with regard to toxicity studies of this chemical using fraction of LC50. Moreover, 96 h LC_{50} in fish probably is not well established. The LC_{50} of DOP based on 96 h study in present experiment was 14.5 ml/l. The Present study showed that mortality in *Clarias batrachus* increased with increase concentration of DOP. The rapid opercular movement is due the solubility limit of DOP in water. Its solubility in water is to be 26-50µg/l and has a very low volatilization rate (WHO, 2003). Therefore, it could have separated and floated as a thin film on top of the aquarium water, thereby limiting the oxygen gas exchange at the surface.

Fish liver histopathology is an indicator of chemical toxicity and it is a useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment (Fernandes, *et al.,* 2008). Liver is a major site for biotransformation of toxic chemicals which usually makes them less toxic and more easily excreted. Any stress effect that occurs in fish may lead to change in physiology, haematology and histopathology of fish (Bulut *et al.,* 2012). The literature on histopathology effects of DOP on fish is still rare. The study revealed that DOP exposure induced histopathological alterations in liver of a freshwater catfish *Clarias batrachus*. Necrosis, Eccentric nuclei and enlarged nuclei of hepatocytes of the liver tissues in the study were observed, probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. The most frequent encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis. This also agrees with Babu *et al.,* (2007) in the exposure of fish to fenevalerate on the liver tissues of *C. mrigala*, when necrosis of tubular epithelium and pycnotic nuclei in the hematopoietic tissue occurred.

Conclusion

In present study, it has been observed that Exposure to sub lethal concentrations of DOP results into dose and duration-dependent histopathological alterations in the liver of *Clarias batrachus*. DOP showed degenerative changes in hepatic cells after 30 days exposure compared to control. Thus DOP is toxic and further research is required in this field.

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