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IMPACT OF ORGANOPHOSPHATE PESTICIDES ON THE LIVER OF *Etroplus maculatus*, A FRESHWATER FISH OF KERALA, INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with both the authors. Author BTS conducted the experiment, managed the analyses of the study, wrote the protocol and the manuscript. Author TVAM designed the study and managed the literature searches. Both the authors read and approved the final manuscript.

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ABSTRACT

Water pollution induces histological changes in organisms like fishes and it leads to different changes ranging from biochemical alterations in single cell up to changes in fish population. The present sublethal toxicity study reveals the potential adverse effects of monocrotophos and phosphamidon, two widely used organophosphate pesticides in the paddy fields of Kuttanad, on a freshwater fish, *Etroplus maculatus*, a true denizen in the paddy fields of Kuttanad. Histopathology was used to assess the nature and extent of pesticide induced pathogenesis in *E. maculatus.* Liver plays a key role in metabolism and subsequent excretion of xenobiotics. The study reveals histological changes in liver included fatty-vacuolation and the displacement of nuclei to the periphery of the hepatocytes, coalescence of vacuoles, coagulated blood, necrosis, neoplasm, etc. Effects at the histological level are usually considered to be an early warning indicator of potential health impacts.

Keywords: Histopathology; monocrotophos; phosphamidon; liver; *Etroplus maculates.*

1. INTRODUCTION

Pesticides are applied to enhance the agricultural production, but the deleterious effects of them are often noticed in non-target organisms like fishes. Assessment of environmental hazards due to pesticides is an important challenge to toxicologists. In the acute toxicity test of chemicals to fish, death still represents an unequivocal end point in toxicology. Because of the deleterious impact of

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pollutants in aquatic ecosystems, the histocytological responses of fishes to various classes of xenobiotic compounds need to be determined and characterised. Fish liver structure and ultra structure proved to be valuable, sensitive indicators of toxicant-induced injury [1].

Physiological stress in fishes due to the pesticides can cause certain changes in metabolic functions. Liver, the centre for metabolism and the detoxifying locus for many poisons that enter the animal body appear to be variously affected when exposed to pesticides [2,3]. This pollution leads to morphological and cytological changes in the liver [4]. Liver of teleost fish does not show the diversity of pathology as in higher animals, probably due to the lack of Kupffer cells in the liver sinusoids. However, it is susceptible to a number of toxic and metabolic disturbances [5]. The analysis of liver responses in experimentally exposed fish can provide a basis for recognizing the toxicity syndromes and should, therefore, be of use in the early prognosis of the effects of environmental aquatic pollutants. A number of studies revealing the changes in functions are initiated by changes at cellular level [3,6,7,8].

The present sublethal study was carried out to assess the nature and extent of pesticides, Monocrotophos and Phosphamidon, two widely used pesticides in the paddy fields of Kuttanad, Kerala, India, induced histological changes in the hepatocytes of *Etroplus maculatus* (Bloch), an inhabitant of the paddy fields of Kuttanad. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

2. MATERIALS AND METHODS

The study on the sublethal toxicity of monocrotophos and phosphamidon on the juveniles of *Etroplus maculatus* were conducted for a period of 30 days. The experiments were conducted in the wet lab which has concrete floor with gentle slope, having proper drainage to remove pesticide contaminated water to minimize the risk of hazards. There were provisions for water supply, lighting and adequate ventilation in the shed. The sublethal bioassay was done in cement cisterns of 35 litres capacity. Clear filtered fresh water drawn from an open well subjected to a fine filtration using nylon bolting cloth, was used for the experiment and the tanks were filled with 32 litres of water.

Juveniles of *Etroplus maculatus* were collected from pollution free ponds from the natural habitat. The average size of *E. maculatus* was 4.75 ± 0.90cm in total length and 3.30 ± 0.80gm in weight were used for monocrotophos exposure while 6.70 ± 0.30cm in total length and 5.00 ± 1.00gm in weight were used for phosphamidon exposure. During these periods, they were fed *ad libitum* once a day on fresh clam meat. Monocrotophos and Phosphamidon are water soluble organophosphate and is a broad -spectrum systemic and contact insecticide-cum-acaricide with long term residual action. This is effective against sucking, chewing and mining insects on paddy, maize, barley, etc. Based on the LC_{50} values (3.36ppm for monocrotophos and 2.97ppm for phosphamidon) obtained [9] five nominal concentrations of the pesticides were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on [10,11]. The concentrations of pesticides used for each sublethal exposure were 0.1ppm, 0.3ppm, 0.6ppm, 1.0ppm and 1.5ppm of monocrotophos and 0.06ppm, 0.1ppm, 0.3ppm, 0.5ppm and 1.0ppm of phosphamidon.

Sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 hr to maintain the desired pesticide concentration. A control free of pesticide was also maintained in each experiment. All the treatments and the controls were made in triplicates. Ten healthy fishes, chosen at random from the acclimated stock were reared in 32 litres of water in seasoned cement cisterns. Fresh well water was used for the experiment and it was filtered using nylon bolting cloth and aerated to saturation prior to use.

Water quality parameters in the experimental tanks were measured by the following methods. Modified standard Wrinkler's method [12] was used for measuring the dissolved oxygen. pH was tested using universal pH indicator solution method. Temperature tested using thermometer with an accuracy of 0.1^{o}C.

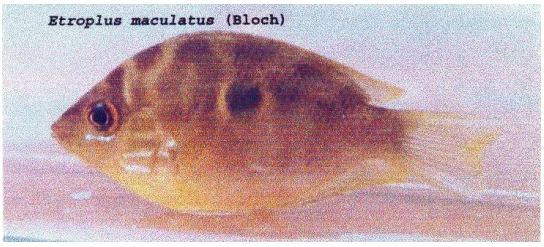

Fig. 1. *Etroplus maculatus*

After 30 days of the experiments five specimens from each of the treated as well as the control group were sacrificed and the target organ was dissected out and fixed immediately in Bouin's fluid. The organs fixed in Bouin's fluid were washed, dehydrated, cleared

and embedded in paraffin wax. Serial sections of each organ were taken at 3 to 5 m thickness and stained with Hematoxylin-abo staining procedures [13]. Detailed histological observations were carried out with the help of a binocular microscope.

3. RESULTS

3.1 *Etroplus maculatus* exposed to monocrotophos

3.1.1 Physico-chemical parameters

Weekly mean temperature, pH and DO values ranged from 28.0 to 28.4^{o}C, 6.8 to 7.51 and 6.2 to 7.1 mg.l^{-1}, respectively.

3.2 Morphology of the Liver of *Etroplus maculatus*

The liver is bilobed, consisting of two subequal lateral lobes, disposed longitudinally and confluent anteriorily for a portion of their extent. Anteriorly it is moulded to the posterior face of the transverse septum between the pericardial and abdominal portions of the coelom, and from thence extends backwards into the abdominal cavity.

3.3 Histopathological Observations

Control: The liver of fishes maintained in control consisted of the parenchymatous, homogenous, polygonal cells formed from double layers of liver cells separated from each other by capillary blood spaces called liver sinusoids. These hepatocytes were compact and carried centrally placed nucleus with distinct nucleolus. These hepatocytes and liver sinusoids were observed as intact (Fig. 2 A).

0.1 ppm: The hepatocytes of fishes in this exposure were normal. All the nuclei could be seen as round, regular in outline and located approximately in the centre of the cells. Liver sinusoids also were normal in this concentration. (Fig. 2 B)

0.3 ppm: A few hepatocytes, at this concentration, have denoted round and prominent vacuolation. The vacuoles varied in size but had similar shape (Fig. 2 C).

0.6 ppm: The hepatic parenchyma exhibited cytologically detectable perturbations in this exposure. Most of the hepatocytes varied in size and shape. They possessed large, inhomogeneous round vacuoles. The large vacuoles pushed the nuclei towards the peripheral side of the hepatocytes and these nuclei could be observed as darkly stained. Slightly condensed hepatocytes were also observed in this exposure (Fig. 2D).

1.0 ppm: The hepatocytes in this treatment showed detectable pathological alterations. Most of the hepatocytes of almost all the fishes depicted round vacuoles. The size of these vacuoles varied from cell to cell and these vacuoles forced the nuclei towards the margin of the cells. The condensed hepatocytes were seen in between the severely vacuolated hepatocytes (Fig. 2 E, F).

1.5 ppm: Almost all the hepatocytes consisted of round vacuoles. The extensive intracellular vacuolation resulted in the displacement of the nuclei towards the cell margin. As the intensity of vacuolation increased the vacuoles were coalesced (Fig. 2 G, H). Coagulated blood cells inside the liver sinusoids and constricted sinusoids could also be noticed in this exposure (Fig. 2 G).

***Etroplus maculatus* exposed to phosphamidon**
Physico-chemical parameters:

Weekly mean temperature, pH and DO values ranged from 27.8 to 28.35^{o}C, 7.26 to 7.4 and 6.2 to 7.01 mg.l$^{-1,}$ respectively.

3.4 Histopathological Observations

Control: These hepatocytes were compact and carried centrally placed nucleus with distinct nucleolus. These hepatocytes and liver sinusoids were observed as intact (Fig. 3 A).

0.06 ppm: No pathological conditions could be observed in this exposure (Fig. 3 B).

0.1 ppm: Most of the hepatocytes of fishes in this exposure were also similar to that of the hepatocytes of control fish. But one fish exhibited slight vacuolation and damaged hepatocytes at certain regions near the liver sinusoids (Fig. 3 C).

0.3 ppm: Some hepatocytes of fishes in this concentration exhibited slight vacuolation and the corresponding displacement of nuclei towards the periphery (Fig. 3 D). All other hepatocytes and liver sinusoids were normal in structure.

0.5 ppm: All the fishes that were exposed in this concentration were noted for the complete vacuolation of hepatocytes. The vacuoles varied in

size but most of them were round in shape. The vacuoles forced the nuclei towards the periphery and the blood cells inside the liver sinusoids appeared as coagulated (Fig. 3E).

1.0 ppm: The liver cells were characterised by clear round vacuoles. The vacuoles appeared in various sizes and the vacuolation forced the nuclei towards the periphery of the hepatocytes as in the previous case. Severe vacuolation merged the vacuoles together (Fig. 3F). In two fishes, the hepatocytes have undergone necrosis (Fig. 3G). Certain regions in one of the fish marked with a special structure i.e. distinctively separated vacuolated area from a non-vacuolated area. The vacuolated area appeared with peripherally situated nucleus, but at the immediate vicinity of this vacuolation there were non-vacuolated cells consisting of regularly arranged cells with centrally placed nuclei having nucleolus similar to normal hepatocytes. This may be due to the growth of neoplasm (Fig. 3H).

4. DISCUSSION

In the present study, the histopathological effects exhibited in the liver of *E. maculatus* document a dose-dependent reaction of liver histology. The pathological changes included fatty-vacuolation and the displacement of nuclei to the periphery of the hepatocytes, congested and constricted liver sinusoids, condensed hepatocytes, destructed cellmembrane, necrosis and pyknotic nuclei and neoplasm.

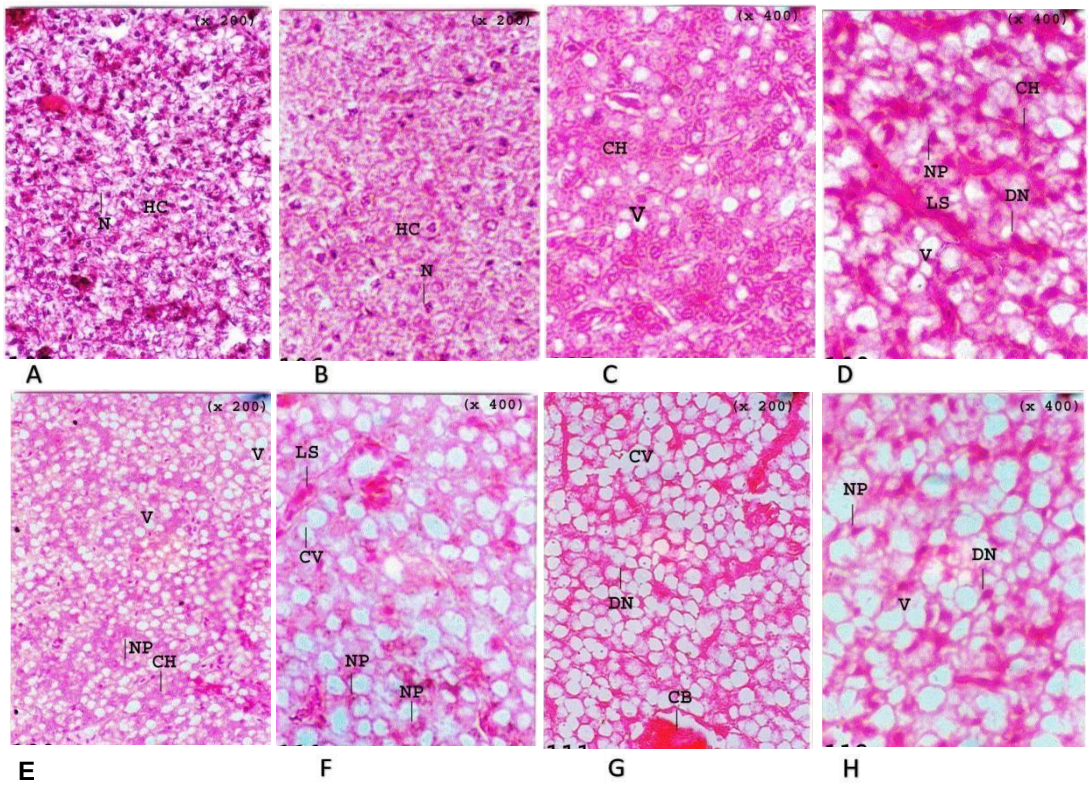

Fig. 2. Liver of *E. maculatus.* (A) Control - hepatocytes (HC) and nucleus (N). H + E x 200. (B) Treated with 0.1ppm monocrotophos - hepatocytes (HC) and nucleus (N). H + E x 200. (C). Treated with 0.3 ppm monocrotophos - vacuoles (V) and condensed hepatocytes (CH). H + E x 400. (D) Treated with 0.6ppm monocrotophos - vacuoles (V), liver sinusoid (LS), peripherally located nucleus (NP), condensed hepatocytes (CH) and darkly stained nucleus (DN). H + E x 400. (E). Treated with the 1.0 ppm monocrotophos - vacuoles (V), peripheraly located nuclei (NP) and condensed hepatocytes (CH). H + E x 200. (F). Treated with the 1.0 ppm monocrotophos - liver sinusoids (LS), coalescence of vacuoles (V) (CV) and peripherally located nucleus (NP). H + E x 400. (G). Treated with the 1.5 ppm monocrotophos - coalescence of vacuoles (V) (CV), darkly stained nuclei (DN) and coagulated blood (CB). H + E x 200. (H) Treated with the 1.5 ppm monocrotophos - vacuoles (V), peripheraly located nuclei (NP) and darkly stained nucleus (DN). H + E x 400

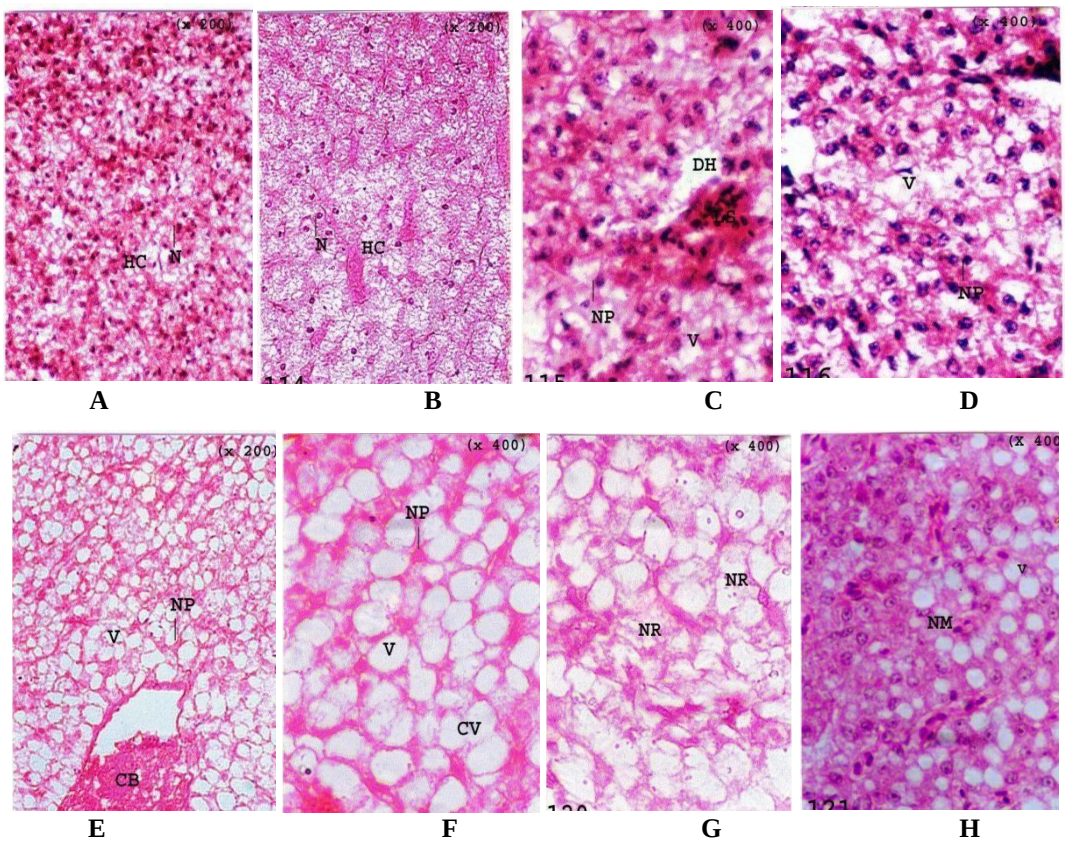

Fig. 3. Liver tissue of *E. maculatus.* (A) *C*ontrol - hepatocytes (HC) and nucleus (N). H + E x 200. (B) Treated with 0.06 ppm phosphamidon - Hepatocytes (HC) and nucleus (N). H+E x 200. (C) Treated with 0.1 ppm phosphamidon - destructed hepatocytes (DH), vacuolation (V) and peripherally located nuclei (NP). H + E x 400. (D)Treated with 0.3 ppm phosphamidon - vacuoles (V) and peripherally located nuclei (NP). H + E x 400. (E) Treated with 0.5ppm phosphamidon - vacuoles (V), peripherally located nuclei (NP) and coagulated blood (CB). H + E x 200. (F) Treated with 0.1 ppm phosphamidon - vacuoles (V), coalescence of vacuoles (CV) and peripherally located nuclei (NP). H + E x 400. (G) Treated with 1.0 ppm phosphamidon - Necrosis (NR). H + E x 400. (H) Treated with 1.0 ppm phosphamidon - vacuoles (V) and neoplasm (NM).H+Ex400

The liver of *E.maculatus* treated with monocrotophos and phosphamidon, (except 0.1 ppm monocrotophos and 0.06 and 0.1 ppm phosphamidon treated *E. maculatus*), showed hepatocellular vacuolation. As the concentration increased, the size of the vacuoles become increased due to coalescence of small vacuoles. This extensive intra- cellular vacuolization resulted in the displacement of nucleus to the cell margin. Most of the scientists considered this vacuolation as the accumulation of fat in the hepatocytes [2,14,15,16]. During the tissue processing the fat is extracted from the cells, leaving empty vacuoles.

The phenomenon of fatty liver or better defined, the storage of large quantities of fats in the fish liver, is quite normal at certain times, including periods of sexual maturation, when both flounders and ruffe store considerable amount of fat in the hepatocytes [17] and dietary imbalance in cultured fishes. However, in the present study, the diet was normal, the fishes were juveniles and therefore, the observed fatty livers must undoubtedly be considered as pathological condition due to pesticides. According to [16], accumulation of fat gave the fish modicum of protection from its toxic effects. Accumulation and sequestration of contaminants can only be effective as long as the capacities of the organ involved are not overloaded [18]. [16] found that, after 21 days of exposure to atrazine, liver lipoid degeneration was revealed. Such pathology indicated the transition to the third step of the stress process (exhaustion) as revealed by the increase in mortality rates. Similarly, a transition stage to third step of the stress was noted

in higher concentrations of the present sublethal toxicity studies in *E. maculatus*. In the present study the vacuolation is due to the storage of fat in the hepatocytes in *E. maculatus* as they are clear, round and sharply lined as suggested by [19].

In the present study, all the fishes in the highest concentration (1.5 ppm monocrotophos treated and 1.0 ppm phosphamidon treated *E. maculatus*) exhibited the coalascence of vacuoles. The size of the vacuoles also varied between cells. [17] found similar observations, that all hepatocytes contain fat vacuoles, the size of which varied between cells. As fat deposition increases the vacuoles become merged.

The hepatocytes of almost all of the fishes treated with the pesticides showed vacuolation and the resulting displacement of nuclei towards the periphery. Several scientists have reported this type of displacement of nuclei [3,20]. According to [16], the association of small lipid droplets may have led to the formation of larger inclusions, as their diameter increased up to 20µm. This extensive intracellular lipid vacuolisation resulted in the displacement of the nucleus to the cell margin. In the present study also the vacuolation resulted in the displacement of the nuclei to the cell margin as suggested by [16].

According to [21], coagulative necrosis is a sudden cessation of blood flow to an organ. With coagulative necrosis, shape of cells and their tissue arrangement are maintained, facilitating recognition of the organ and tissue. Necrotic changes occur after cell death and represent the sum of degradative process [20]. In the present study, the coagulative necrosis could be observed in 1.0 ppm phosphamidon treated *E. maculatus*. According to [5], acute and extensive necrosis of liver cells occur in toxic conditions but focal necrosis is more common. In the present study, the condensed or shrunken hepatocytes were noted in 0.6 and 1.0 ppm monocrotophos treated *E. macualtus*. This might be due to the fact that in higher concentrations, during the experiment, we could see the food wastage and thereby the food intake was less and this starving condition might have resulted in the use of stored glycogen in the liver, which might have led to the shrinkage of liver cells. Such a condition was reported by [22] who could induce shrinkage of the liver cells in post larval, juvenile milk fish *Chanos chanos* bycomplete starvation for 9 days. [23] found that the hepatocytes had been reduced in size chiefly due to the loss of stored glycogen and fat.

In the present study, *E. maculatus* treated with 1.5 ppm monocrotophos exhibited the constricted liver sinusoids. This constriction of liver sinusoids might be due to the pesticide reaction in the wall of the blood vessel during the detoxification process of the pesticides.While studying the pathological conditions in the liver of ruffe, *Gymnocephalus cernua* from the highly polluted Elbe estuary [17] also observed similar constriction of sinusoids in fish.

Blood coagulation inside the liver sinusoids could be noticed in 1.5 ppm monocrotophos treated and 0.5ppm phosphamidon treated *E.maculatus*. It might be due to the pesticide reaction during the detoxification process of liver. In the pollutant - induced hepatopathology, a gradual increase in damages is noticed with larger duration of test, showing significant damages in hepatocytes and coagulation of blood in sinusoids [24,25] also noted the coagulation of blood in the sinusoids of *Channa punctatus* treated with $HgCl_2$. In the present study, 1.0ppm phosphamidon treated *E. maculatus* have exhibited an early neoplastic condition. This exposed an appearance of clear separation of vacuolated and non-vacuolated area. All the fishes remained healthy during the study period even after being affected with neoplasm. Previously [26] reported a similar condition in channel cat fish treated with aflatoxin B_1. In the present study this might be due to a carcinogenic action of the pesticide, which might be attributed to the cumulative toxicity as reported by Ram and Singh, 1988.

5. CONCLUSION

Liver exhibited a dose-dependent degeneration in histology when it was exposed to various sublethal concentrations of pesticides. Since the liver has a regenerating capacity, it is not much affected by the exposure to toxicant medium. Sublethal exposure to the toxicant medium will definitely damage the organ. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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