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ISSN 0971–765X

Assessment of the sub-lethal effects of Sarosate® on the liver and gills of *Clarias gariepinus* juveniles (Burchell, 1822)

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(Received 2 March, 2019; accepted 3 April, 2019)

ABSTRACT

One hundred and twenty (120) *Clarias gariepinus* juveniles of average weight 24.8 ± 15.6 g were exposed to sub-lethal doses of Sarosate® (0 mg, 0.10 mg, 0.15 mg and 0.20 mg) per liter of clean borehole water for 14 days after acclimatization to assess the toxicity on some internal organs. Exposed fishes were selected at day 1, 7 and 14, sacrificed and the liver and gills removed, fixed immediately in Bouin's fixative and thereafter processed for photomicrograph. Normal liver and gill architecture were observed in the control. However, effects ranging from mild infiltration of inflammatory cells to portal aggregate of inflammatory cells were observed in exposed fish liver samples. Also, mild detachment of gills from the epithelium, distortion of gill filament and some ghost like appearance of the gill (necrosis) were observed. The observed features indicated toxicity effects of the herbicide on *Clarias gariepinus* hence could be useful biomarkers of toxicant exposure.

Key words : *Clarias gariepinus, Histopathology, Liver dysfunction, Sarosate®, Sublethal toxicity*

Introduction

The consistent and increasing use of agrochemicals (fertilizers, herbicides and pesticides) in agricultural sites to improve soil fertility, control weeds and pests to improve crop yield have consequently been reported to exert degrading effects on freshwaters and its biodiversity (Anna-lissa and Galina, 1999). Agrochemicals can enter into aquatic ecosystem either by direct spray or run-off. The effects on the ecosystem might be on change in water chemistry, nutrient load (Olson *et al.,* 2005) with a resultant modification of food web (Pretty *et al.,* 2005; Moss, 2004) and increase in nutrient (eutrophication) of such ecosystem. Hence, agrochemicals (herbicides) have been reported to cause deleterious effects to non-target organisms including fishes, even at sub lethal concentrations (Jiraungkoorskul *et al.,* 2003).

Glyphosate based herbicide formulations in different brands such as Sarosate®, Round up®, Tackle®, Uproot®, General® are some of the most commonly used herbicides today (Eva and Mae-Wan, 2012). In Nigeria, these herbicides are applied mainly on rice paddies to get rid of weeds during land preparation before planting. From the point of application, the herbicides could be washed by run-

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ning water or rain into nearby water bodies. With a half-life range of 7 to 14 days in aquatic environment (Giesy *et al.,* 2000), glyphosate have been detected in many rivers around agricultural sites and residential areas (Pesce *et al.,* 2008). This might lead to the loss of biodiversity (fish) of unprotected permanent and temporal natural water bodies (ponds, lakes and rivers) within agrarian communities.

Some researchers have reported disruption of the synthesis of some essential aromatic amino acid that promote growth in plants, algae, bacteria and fungi by glyphosate (Della-Cioppa *et al.,* 1986; Franz *et al.,* 1997), and its effects on the morphology and physiology of functional organs in most animals (Modesto and Martinez, 2010). Although animals lack the shikimate pathway which is the major site of toxicity of glyphosate herbicide to plants, production of reactive oxygen species (ROS) due to the presence of the pollutants as well as development of oxidative stress could be possible indications of toxicity on exposed organisms (Oropesa *et al.,* 2008). A study conducted by Jiraungkoorskul *et al.,* 2002, showed histological changes in the liver, gills and kidney of young and adult Nile tilapia, *Oreochromis niloticus* exposed to acute doses of glyphosate based herbicide at mean concentrations of 17.15 ppm and 41.85 ppm, respectively. In the report of (Olurin *et al.,* 2006), hepatocytes enlargement with large vacuoles, sinusoid congestion, pyknosis and karyolysis were the most frequently observed degenerative changes on fish exposed to 0.05 % and 0.1 % v/v glyphosate based herbicide. Also, (Ayoola, 2008) studied the histopathological effects of glyphosate herbicide on juvenile African catfish (*Clarias gariepinus*) at the concentrations (1.9, 4.1, 9.0, 21.0, 45.0) mg/L and observed infiltration of leukocytes, increasing hepatocyte size with pyknotic nuclei, and vacuolation in the liver. Therefore, this study is aimed at assessing the sublethal effects of Sarosate® on the liver and gills of *Clarias gariepinus.* The will provide farmers and the general public with additional requisite information on the toxicity of the herbicides for regulators to properly assess the risk of the formulations and set specific requirements related to the over-water application.

Materials and Methods

Collection and Acclimatization of Fish Samples

Two hundred and fifty (250) *Clarias gariepinus* juveniles were purchased from Fishery and Aquaculture Department's farm in Ebonyi State University, Abakaliki at the early hours of the day and were transported to the Faculty of Science laboratory in 30 litres capacity plastic aquaria for acclimatization. Ethical clearance for animal health was secured from the committee on ethical clearance in Faculty of Science of Ebonyi State University, Abakaliki.

The juveniles were acclimatized to laboratory condition at variable temperature on static renewal method for two weeks in ten 30 litres capacity plastic aquaria each containing 20 liters of clean borehole water of pH 6.90 and dissolved oxygen of 4.30 mgL^{-1}. The juveniles were fed on a commercial pellet diet at 5 percent of their average body weight twice per day. The water was renewed every day in order to remove fecal matter and unconsumed feeds.

Procurement of the Test Herbicide

The test herbicide "Sarosate®", a glyphosate herbicide formulation was procured from Agro-chemical shop in Abakpa main market, Abakaliki. The herbicide according to the manufacturer is composed of 360 gL^{-1} of glyphosate in the form of 480 gL^{-1}isopropylamine salt. It is of World Health Organization Class III analytical grade.

Range Finding Test

Screening test was conducted with the herbicide to determine the appropriate concentration range for testing chemical as described by (Solbe, 1995). The doses of the test herbicide used for the range finding test after the screening test to establish the LC50 were 1.0, 1.5, 2.0, 2.5 and 3.0 mg per liter of water. The concentrations in milligram acid equivalent per liter as calculated from the administered doses were 35.0 mg a.e/L, 53.0 mg a.e/L, 71.0 mg a.e/L, 88.4 mg a.e/L and 106.1 mg a.e/L, respectively.

Definitive Test

After the range finding test, ten per cent (10 %) of the first three concentrations 0.10, 0.15, 0.20 mg L^{-1} in weight per volume (3.50 mg a.e/L, 5.30 mg a.e/L, 7.10 mg a.e/L) and a control were used to assay the sub lethal effects of the test herbicide formulation on the histopathology (liver and gills) of the juvenile. Ten (10) *Clarias gariepinus* juveniles of average weight 24.8 ± 15.8 g were randomly selected and stocked in each of twelve 30 liters capacity plastic aquaria containing 20 liters of water each. The treatments were in triplicates including the control.

The treatments were studied for fourteen days on 24 hours static renewal method. After day 1, 7 and 14, fish samples were randomly selected from different treatments and sacrificed. The liver and gills were removed and fixed immediately in Bouin's fixative for one week to prevent autolysis and improve staining quality. Thereafter, the tissues were dehydrated in different grades of alcohol ranging from 50 % to absolute alcohol for 3 minutes each to remove water which is not miscible with wax. The dehydrated tissues were cleared by immersing it through three changes of xylene for 3 minutes each to remove alcohol. They were thereafter impregnated in the hot oven by passing it through three changes of molten paraffin to remove the clearing agent and embedded with molten paraffin wax. The solidified tissues were mounted on wooden block and sectioned to 5 microns using rotary microtome. Each section was stained using haematoxylin/eosin stain and the photomicrograph taken at x150 magnification.

Results

Effects of Sarosate® on the Liver of the exposed Juveniles

Effects of Sarosate®on the Liver of the exposed Juveniles: Plate 1a-4c showed the liver micrograph of *Clarias gariepinus* juveniles exposed to Sarosate® at different concentrations and time. Plate 1a, 1b and 1c were the control (0 mg L^{-1}) for day 1, 7 and 14 and showed normal hepatic architecture with normal hepatocytes (NH). Plate 2a, 2b and 2c were the liver micrographs of juveniles exposed to 0.10 mg of the herbicide per litre of water for day 1, 7 and 14. The observed effects were mild infiltration of inflammatory cells, mild fatty change (Plate 2a); mild congestion of blood vessels, mild distortion of hepatic architecture (Plate 2b); extensive fatty change and necrosis of hepatic cells (Plate 2c). Plate 3a, 3b and 3c showed the liver micrographs for juveniles exposed to 0.15 mg of herbicide per litre of water for day 1, 7 and 14 and perivascular aggregate of inflammatory cells, moderate fatty change (Plate 3a); focal aggregate of inflammatory cells (Plate 3b); cytoplasmic ground glass appearance and moderate portal aggregate of inflammatory cells (Plate 3c) were observed. Lastly, plate 4a, 4b and 4c showed the liver micrographs of juvenile exposed to 0.20 mg of herbicide per litre of water for day 1, 7 and 14. There are indications of extensive fatty change, focal aggregate of inflammatory cells (Plate 4a); dilation of central vein with inflammatory cell, moderate inflammatory cells (Plate 4b); focal loss of tissue, ground glass appearance of the cytoplasm and portal aggregate of inflammatory cells (Plate 4c) in the liver.

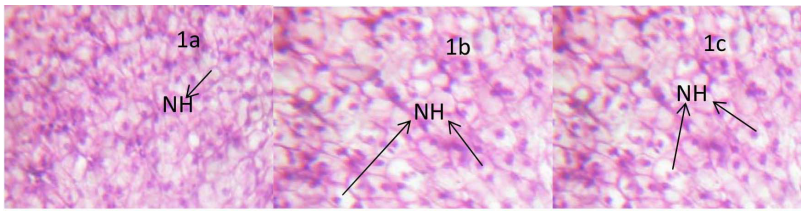

Plate 1a, b and c: Liver micrograph *C. gariepinus* juvenile exposed to 0 mg a.e/L (control) of Sarosate ® for day 1, 7 and 14, respectively (x150) (H&E) showing normal hepatic architecture with normal hepatocyte (NH).

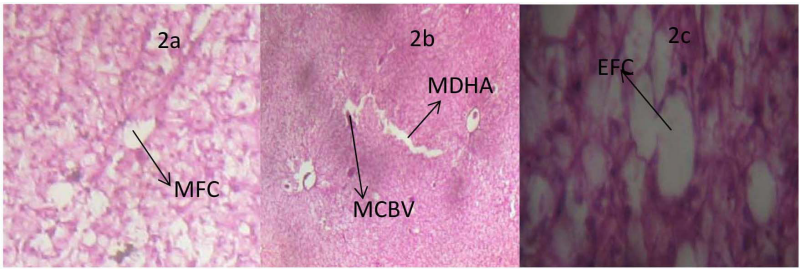

Plate 2a, b and c: Liver Micrograph of *C. gariepinus* juvenile exposed to 0.10 mgL^{-1} of Sarosate® for day 1, 7 and 14 (x150) on (H&E) showing mild fatty change (MFC), mild congestion of blood vessels (MCBV), mild distortion of hepatic arctitecture (MDHA) and extensive fatty change(EFC).

Effects of Sarosate®on the Gills of the exposed Juveniles

The effects of the test herbicide on the gills of the exposed fish juveniles at day 1, 7 and 14 are showed in Plates 5a – 8c). Normal gill architecture with well projected gill filament (WPGF), gill cartilage (GC) and gill epithelium (GE) were observed in the gills of the juveniles in the control (Plates 5a, b and c). In the juveniles exposed to 0.10 mgL^{-1} of the test herbicide, mild detachment of the gills from the epithelium (MDG), mild dilation of the gill filaments (MDGF), focal area of haemorrhage (FAH), ghost – like appearance (GA) or necrosis, dilation and distortion of the of gill filament were observed (Plates 6a, b and c). At 0.15 mgL^{-1}, moderate distortion of

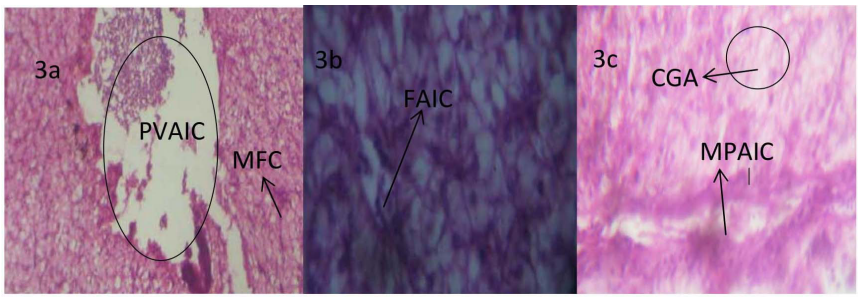

Plate 3a, b and c: Liver micrograph of *C. gariepinus* juvenile exposed to 0.15 mgL^{-1} of Sarosate® for day 1, 7 and 14 (x150) (H&E) showing perivascular aggregate of inflammatory cells (PVAIC), moderate fatty change (MFC), focal aggregation of inflammatory cells (FAIC), cytoplasmic ground glass appearance (CGA) and moderate portal aggregate of inflammatory cells (MPAIC).

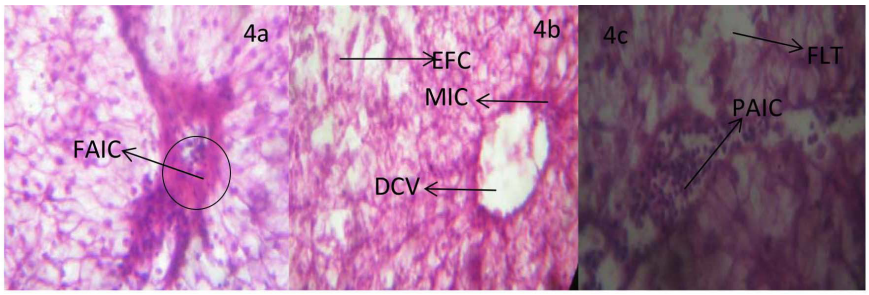

Plate 4a, b and c: Liver micrograph of *C. gariepinus* juvenile exposed to 0.20 mgL^{-1} of Sarosate ® for day 1, 7 and 14 (x150) (H&E) showing focal aggregate of inflammatory cells (FAIC), extensive fatty change (EFC), dilation of central vein (DCV) with inflammatory cells around it, moderate inflammatory cells (MIC), focal loss of tissues (FLT) and portal aggrrgate of inflammatory cells (PAIC) also known as portal hepatitis

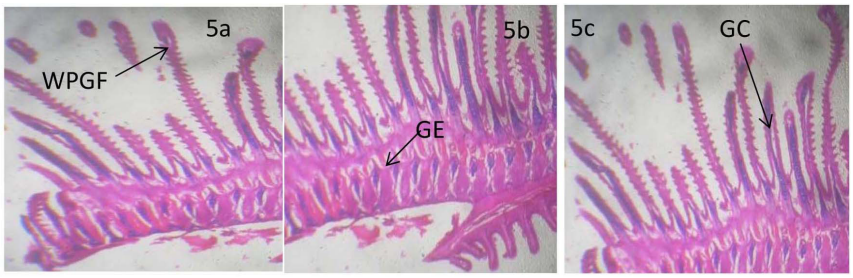

Plate 5a, b and c: Photomicrograph of gills (X150(H&E) of *Clarias gariepinus* juvenile exposed to 0 mg a.e/L (control) of Sarosate ® for day 1, 7 and 14, respectively showing normal gill architecture with well projected gill filament (WPGF)gill cartilages (GC) and the gill epithelium (GE).

the gills (MDG), moderate dilation of the gill filaments (MDGF), thinning and dilation of the gill filaments (T/DGF), clumping of gills (CG) in some areas, and focal areas of haemorrhage were observed (Plates 7a, b and c). At 0.20 mgL^{-1}, moderate clumping of the gill filaments (MCGF) was observed at day 1 and more severe effects such as loss of gill filament (LGF), focal areas of haemorrhage (FAH), and necrosis at day 7 and 14.

Discussion

Abnormal changes in the tissues of organisms could be in response to environmental stress. The stress

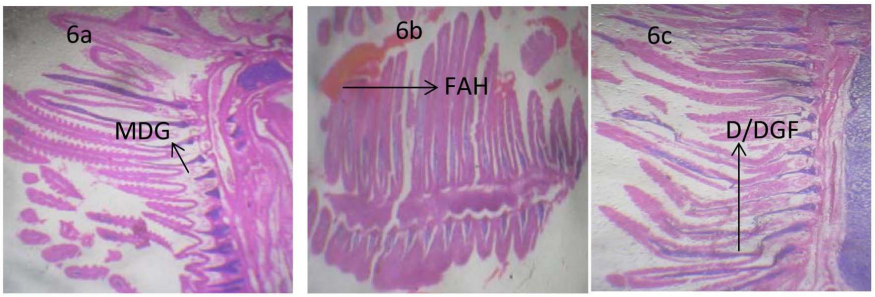

Plate 6a, b and c: Photomicrograph of gills (X150(H&E) of *Clarias gariepinus* juvenile exposed to 0.10 mgL^{-1} of Sarosate® for day 1, 7 and 14, respectively showing mild detachment of gill from the epithelium (MDG), focal area of hemorrhage (FAH), and dilation and distortion of gill filament (D/DGF).

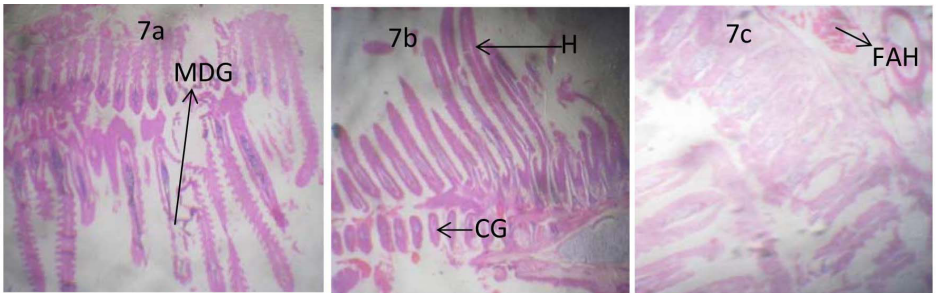

Plate 7a, b and c: Photomicrograph of gills (X150(H&E) of *Clarias gariepinus* juvenile exposed to 0.15 mgL^{-1} of Sarosate® for day 1, 7 and 14, respectively showing MDGF moderate distortion of gill (MDG), clumping of gills (CG), hypertrophy (H), focal area of hemorrhage (FAH)

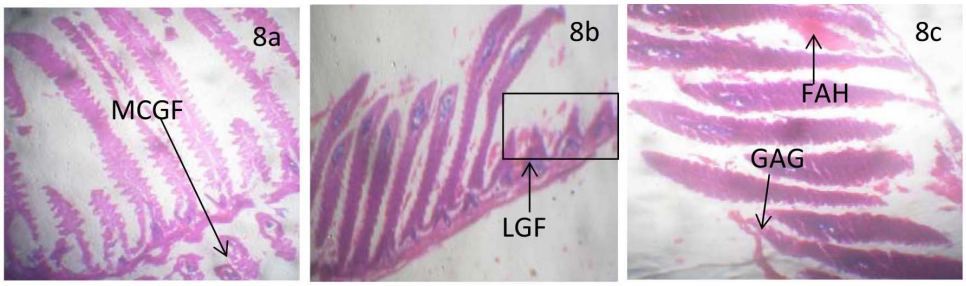

Plate 8a, b and c: Photomicrograph of gills (X150(H&E) of *Clarias gariepinus* juvenile exposed to 0.20 mgL^{-1} of Sarosate® for day 1, 7 and 14, respectively showing moderate clumping of gill filament (MCGF), loss of gill filament (LGF), hypertrophy (H), focal area of hemorrhage (FAH) and ghost-like appearance of the gills (necrosis) (GAGs).

may arise from variability in some climatic factors such as change in environmental temperature, salinity of an aquatic ecosystem or change in water chemistry due to the influence of human activities like use of agrochemicals (herbicides, fertilizer, pesticides). These can affect the vital organs that play important roles in the sequestration, biotransformation and elimination of chemicals (toxicants) in the body of organisms. Among vertebrates, fish have been used widely as bioindicators of aquatic pollution (Evans *et al.,* 1993). This might be due to the immune response system of fish which is made of connection of cells that proliferate and differentiate rapidly and are regulated by different soluble factors that act directly on its health.

According to Jiraungkrooskul *et al.* (2002), the recommended dose for field application of Roundup, a glyphosate herbicide used in their study range from 1500 to 2000 ppm. Although the brand of glyphosate formulation used in this study differs, the concentrations used for the definitive test were close to the range that other researchers adopted in their test. So, toxicants may exert varied effects on organisms at different concentrations and time.

The liver of *C. gariepinus* juvenile in the control showed normal hepatic architecture with normal hepatocyte (NH) throughout the study. This agreed with the report of other researcher who made similar observations in their studies. The result implied that the juvenile in the control treatment were in good state of health through out the experiment. In this study, the herbicide exerted various degree of damage on the liver of exposed fish at different concentrations and time. These ranged from mild fatty change, mild congestion of blood vessels, mild distortion of hepatic architecture, perivascular aggregate of inflammatory cells, cytoplasmic ground glass appearance to dilation of central vein and extensive fatty change. This is similar to the report of Opeyemi and Egbamuno (2012) who exposed *Clarias gariepinus* of average weight (40 – 50 g) to sub lethal concentrations of 0.02 %, 0.04 %, 0.08 % v/v of glyphosate herbicide and observed extensive vacuolation of hepatocytes after five days of exposure which progressed to mild portal and central venous congestion on the 10^{th} and 15^{th} day of the experiment. Olurin *et al.* 2006, diferred in their report which they observed that liver damage was not diverse in *Clarias gariepinus* exposed to sublethal concentrations of glyphosate herbicide. This they attributed to lack of Kuppfer cells in the liver sinusoid according to (Ellis, 1976). Risbourg and Bastide, (1995) reported increase in the size of lipid droplets and vacuolation in the liver of fish exposed to atrazine which are indications of the toxic effects of the herbicide on the test organism. Ayoola (2008) observed increased hepatocyte size with pykrotic nuclei, cloudy swelling, vacuolization and focal necrosis in the liver of catfish exposed to1.9, 4.1, 9.0, 21.0 and 45.0 mg/L of glyphosate herbicide which, was attributed to the inability of the liver to regenerate damaged cells. Similarly, portal hepatitis observed in this study could also be due to the inability of the fish to detoxify the toxicant and regenerate damaged liver as the time of exposure increased at the highest concentration. Since the liver is the major defense organ in fish, inflammation of liver cells could be due to the infiltration of the peripheral tissue with leucocytes which, according to House and Thomas, (2002) enhances the release of some factors that elicit non – specific physiological defense mechanisms. The histological alterations observed in this study such as mild distortion of hepatic architecture, dilation of central vein, portal hepatitis may be due to the accumulation of lipids and glycogen as a consequence of liver dysfunction arising from exposure to glyphosate herbicide as suggested by Opeyemi and Egbamuno, (2012).

The gills of *Clarias gariepinus* juvenile in the control experiment had normal gill architecture which, are indications of no degenerative effects on the test organisms. However, in fish exposed to different concentrations of glyphosate, degenerative features including mild detachment of gills from the epithelium, focal area of hemorrhage, moderate distortion of gills, clumping, necrosis, loss of gill filament, hypertrophy and ghost-like appearance were observed. This is in line with the reports of other researchers including Ayoola (2008) that observed swollen tip of the gill filament, congestion, and heterophilic infiltration at different glyphosate concentrations. Braz-Mota *et al*. 2015, observed hyperplasia and hypertrophy in the epithelial filaments, epithelium lifting and proliferation of mitochondria – rich cells in the gills of fish exposed to sub lethal concentrations (10 mg/L and 15 mg/L) of Roundup, a glyphosate herbicide which is similar to some of the observation made in this study.

The study showed that the test herbicide exerted significant alterations in the liver and gills of the exposed fish at different concentrations. The ob-

served effects increased significantly with concentrations and time. Therefore, regulators should properly assess the risk of the formulations and set specific requirements as regards its application over or around water bodies so as to protect the integrity of the ecosystem and its biota.

Acknowledgement

I wish to acknowledge Tertiary Education Trust Fund, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria for the grant given to Ecology, Hydrobiology and Fishery Research Group on a project titled "Assessment of the Impacts of Agricultural activities on some Aquatic ecosystems in Ebonyi State, Nigeria and the Health implications".

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