Acute toxicity of Primextra Gold on freshwater fish, *Clarias gariepinus* (Burchell 1822)

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Abstract

The need to boost food productivity to meet the demands of the ever-increasing human population necessitates the frequent use of agrochemicals. Primextra Gold containing atrazine (329g/l) and S-metolachlor (400g/l) is commonly used in agriculture to control weeds, especially in rice paddies. Given that herbicides could harm non-target species like fish, this study examined the effects of Primextra Gold on the behaviour, haematology, blood biochemistry and kidney of *Clarias gariepinus*. Juvenile *C. gariepinus* mean weight (11.05±5.43g) and length (11.95±6.13cm) were exposed to lethal and sublethal concentrations of the herbicide. The toxicity assay showed that the median lethal concentration (LC_{50}) was 3.63µg/l. The fish exposed to sublethal concentrations exhibited signs of asphyxiation. The white blood cell (9.4-10.8×10^6), aspartate aminotransferase (40.01-60.01IU/l) and alanine aminotransferase (68.02-90.12IU/l) levels increased significantly ($p<0.05$) in the test group compared to the control. Contrarily, glucose (53.14-82.25mg/dl) and protein (3.12-9.38mg/dl) were lower in the herbicide exposed group compared to the control ($p <0.05$). Kidney histopathology revealed focal loss of the renal tissue, mild intra renal haemorrhage, moderate focal loss of renal tissue and severe focal loss of renal tissue in the herbicide exposed fish. The findings of this study suggest that this herbicide is hazardous to aquatic life and has negative consequences for non-target species when used indiscriminately. It is recommended that the herbicide should be used with caution, especially near aquatic habitat to maintain good water quality and ensure sustenance of aquatic biodiversity.

Keywords: *Clarias gariepinus,* Primextra, behavioural responses, haematology, blood biochemistry, histopathological alterations.

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Introduction

Food security in Africa, especially Sub-Saharan African Nations has become a global concern (Dodo 2020). Consequently, most farmers in Sub-Saharan Africa like Nigeria have turned to the use of herbicides to boost crop productivity and meet the demand of the ever-increasing human population. Primextra Gold, which contains atrazine (329g/l) and S-metolachlor (400g/l) is one of the widely used herbicides to control weeds in corn, sorghum, sugarcane, asparagus, bananas, citrus groves, coffee, conifer tree crop areas, forestry, fruit orchards, grasslands, grass crops, guavas, macadamia orchards, maize, oil palms, sorghum, sugar cane, pineapples, roses, vines, green vegetables and rice farms **(**Cui *et al* 2012; Blahova *et al* 2013; Akhtar 2021; Ahmad *et al* 2021; Puvvula *et al* 2021). Atrazine belongs to the group of triazine herbicides, which also include simazine and promazine (Olatoye *et al* 2021). It is a selective pre-and post-emergence herbicide for annual control of grass and broad-leaved weeds. It has also been used as a soil sterilant for airfields, parking lots and industrial sites and as an algicide in swimming pools (IARC 2019). Atrazine is banned in the European Union and some American countries, after reviewing the risks of the herbicide for more than 7 years but the US Environmental Protection Agency says the widely used herbicide can stay on the market with some new restrictions (Erickson 2020).

Atrazine rarely binds to foliage and may wash off from treated plants (Hanson *et al* 2020) into the environment. Unfortunately, the herbicide can reach aquatic ecosystems through run-offs and underground leaching (Todd and Leuwen 2002; Nwani *et al* 2014; Okogwu *et al* 2015; Ahmad *et al* 2021). It can accumulate in aquatic environment and organisms and thus, threaten water quality and non-target aquatic organisms such as fish (Shercan and Bacoon 2011; Gill and Garg 2014)**.** It can bioaccumulate in edible fish tissues, cause high fish mortality, decrease fish yield, impair healthy functioning of the aquatic ecosystem and can adversely affect human health (Adedeji *et al* 2009; Wells 2009). Fish exposed to herbicides and pesticides such as those containing atrazine exhibit poor growth (Opute *et al* 2021) and abnormal behavioural changes (Banaee *et al* 2011; Opute *et al* 2021), and suffer haematological, biochemical and histological damages (Van-derOost *et al* 2003; Okogwu *et al* 2015; Akhtar *et al* 2021).

Doherty *et al* (2019) showed that atrazine affected acetylcholinesterase activities, lipid peroxidation and testosterone levels in exposed fingerling and juvenile *Clarias gariepinus.* Atrazine based herbicides such as

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Primextra Gold is the most commonly patronized herbicide in Nigeria and it is environmental available (Olatoye *et al* 2021).

Environmental toxicity of the herbicide can be evaluated using well-known model bioassay organisms (Sani and Idris 2016). African mud catfish, *C. gariepinus* is among the most preferred species by fish farmers because of its acceptability and commercial value in Nigeria and several Africa countries (Adeshina *et al* 2017). It breeds in floodplain lakes that receive herbicides from nearby rice farms (Okogwu 2011) and it is likely that the adult and juveniles are exposed to the toxicity of herbicides such as atrazine. This study was undertaken to evaluate the toxicity of primextra on the behaviour, blood and kidney of juvenile *C. gariepinus*. The pool of knowledge on the toxicity of the herbicide is necessary in advocacy for policy change on healthy aquatic environment and protection of biodiversity.

Materials and methods

Collection and acclimatization of fish samples

A total of 370 juvenile *C. gariepinus* of mean weight (11.05±5.43g) and mean length (11.95±6.13cm) were obtained from a local fish farm and acclimated for two weeks at the Department of Applied Biology Laboratory, Ebonyi State University, Abakaliki, Nigeria. During acclimation, the fish were fed commercial diet at 3% of their body weight daily. The water was replaced daily to eliminate wastes (Okogwu *et al* 2015).

Acute toxicity test

Following a range finding test, ten acclimated fish each were randomly exposed to five different test concentrations; 0.00, 2.00, 4.00, 6.00, 8.00, and 10.00µl of the herbicide in static tanks containing 10 litres of water each and designated O (control), I, II, III, IV and V, respectively. All experiments were performed in triplicates. The LC_{50} was determined as described by Nwani *et al* (2014) with minor modifications.

Sub-lethal bioassay

Based on the 96-hour LC_{50}, three test concentrations; 1/10th of LC_{50} (0.36µg/l), 1/9th of LC_{50} (0.40µg/l) and 1/8th of LC_{50} (0.45µg/l) were selected for the sub-lethal acute test and designated A, B and C, respectively. The tests were set up in triplicates, with a control experiment running concurrently. At 24, 48, 76 and 96 hours intervals, water temperature, pH, dissolved oxygen (DO), conductivity and total dissolved solutes (TDS) were measured using established procedures (APHA 2012; OECD 2014).

Behavioural responses

Behavioural changes in the fish, such as hyperactivity, equilibrium status, swimming rate, convulsions, somersaulting activity, fin movement, and operculum movement, were observed and recorded in exposed and control groups at 24, 48, 76 and 96 hours after exposure, as recommended by OECD (2014).

Haematological analysis

At the end of the 96-hour bioassay, blood from six fish per group was collected through the caudal vein puncture using 20ml disposable heparinised syringe. The blood was transferred to tubes containing the anticoagulant, potassium salt of ethylene diamine tetraacetic acid (EDTA), sodium fluoride tubes, and plain tubes (Amegashie *et al* 2015). Haematological parameters, red blood cell (RBC), white blood cell (WBC), packed cell volume (PVC) and haemoglobin were analysed according to the methods of Ochei and Kolhatkar (2008). The mean cell corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean cell haemoglobin content (MCH) were computed according to the methods of Ayoola (2011).

Biochemical analysis

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the Randox kit by Reitman and Frankel (1957) following the manufacturer's guideline. Alkaline phosphatase (ALP) activity was determined following Kochmarand Moss (1976) methods. Total protein content was determined using the Tietz (1995) method. Glucose level in the plasma was measured using a commercial enzyme kit of tench (Glu L 1000, PLIVA-Lachema, Czech Republic). After 10 minutes of incubation in a solution containing glucose oxidase, peroxidase, and 4-aminoantipyrine, samples were transferred to a 96-well microtiter plate (200ml) and their absorbance (500nm) was measured by a plate reader (Tecan Sunrise, USA). This technique had a quantification limit of 0.021mmol/L, a working range of 0.065–45mmol/l, a repeatability of 1.05 percent, and a working volume of 10µl (Bartonkova *et al* 2017). Total bilirubin was determined by Jendrassik and Grof (1938) methods.

Histopathological analysis

Six fish from each group were dissected, the kidneys removed and processed for histopathological examinations, as described by Si-Tayeb *et al* (2010), with minor changes. The kidney tissues were preserved in 10% normal saline for one week and then dehydrated for 30 minutes in different grades of alcohol ranging from 50 percent to absolute alcohol. The dehydrated tissues were cleaned by immersing them in three (3) changes of xylene for 30 minutes each, impregnated in paraffin wax for 30 minutes in a hot oven at 60°C, blocks were made and sectioned into 5-micron thickness using rotatory microtome. The sectioned tissues were rehydrated in distilled water, stained with Hematoxylin-Eosin (H-E) and then viewed and micrographed using a microscope.

Statistical analysis

Based on the dose dependent mortality, probit technique was used to determine the median-lethal concentration (LC_{50}). The difference between the different groups was tested using one-way analysis of variance (ANOVA) and the means separated by Duncan range test. All statistical analyses were performed using Statistics Programme for Social Science (SPSS) software version 21.1.

Results

Mean lethal toxicity and water quality

The median lethal concentration (LC_{50}) of Primextra Gold was estimated as 3.63g/l (Figure 1). The

temperature (27.87-28.33ºC), pH (6.79-7.53), dissolved oxygen (DO; 3.07-5.07mg/l), conductivity (720.33-825.00µS/cm) and total dissolved solids (TDS; 333.33-409.00mg/l) of the test water varied significantly between the control and the herbicide exposed groups (Table 1).

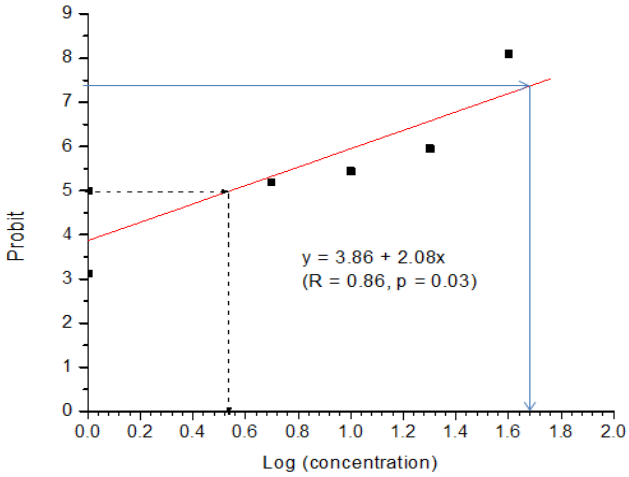

Figure 1. Probit method of estimating median lethal concentration (LC_{50}) of Primextra in *C. gariepinus.* (LC_{50} = 3.63 µg/l), Upper limit = 48.98 µg/l; Lower limit = 1.00 µg/l

Behavioural responses

The fish in the control group exhibited normal behaviour throughout the 96-hour sub-lethal bioassay while those subjected to different sub-lethal concentrations of Primextra, displayed various abnormal behaviours such as hypersensitivity, loss of equilibrium, erratic swimming, air gulping, and rapid operculum movement in time and concentration dependent manner (Table 2).

Haematological analyses

The red blood cell (RBC) varied between 8.28×10^6 and 10.42×10^6/µl while the WBC ranged between 9400×10^3 and 10800×10^3/µl. Haemoglobin concentration (Hb) varied between 8.50 and 10.70g/dl and the packed cell volume (PCV) ranged between 30.00 and 35.00%. The MCH varied from 10.07 to 10.27pg while mean corpuscular volume (MCV) varied between 33.59 and 36.89fl (Table 3). Only the WBC varied significantly ($p<0.05$) between the herbicide groups and the control.

Biochemical parameters after exposure to Primextra

The AST (40.01-60.01IU/l) and ALP (68.02 and 90.12IU/L) were significantly higher in the fish exposed to different concentrations of Primextra compared to the control ($p<0.05$), while glucose (53.14-82.25mg/dl) and protein (3.12-9.39mg/dl) were significantly lower in the test group than the control ($p<0.05$). However, ALT (64.04-69.13IU/l) and bilirubin (4.11-7.55mg/dl) varied insignificantly between groups ($p>0.05$) as shown in Table 4.

Effects of Primextra exposure on kidney histology

The kidney of the control group showed normal renal architecture, including normal glomeruli (NG) and renal tubules (RT). However, the kidneys of fish exposed to 0.36 and 0.40µg/l of atrazine showed moderate loss of focal renal tissue (FLRT) and mild intra renal haemorrhage (MIRH). The kidney of fish exposed to 0.45µg/l of atrazine showed severe loss of focal renal tissue, resulting in glomeruli loss (Plate 1).

Discussion

In the present study, dissolved oxygen (DO) showed an inverse proportional relationship with the concentration of the herbicide in that as the concentration of the herbicide increased, the DO decreased. Many authors have established such inverse relationship between oxygen and concentrations of toxicants (Barton 2002; Ayoola 2008a and 2008b; Dogan and Can 2011; Nwani *et al* 2013; Okoh 2015; Okogwu *et al* 2015). The decline in dissolved oxygen in the treated groups could lead to hypoxia (Mallya 2007), which may be responsible for abnormal behaviours observed in the pesticide treated fish. The test fish showed signs of asphyxiation such as frequent surfacing and gulping of air, somersaulting and erratic fin and opercula movement, which are attempts at oxygenation. These behaviours have been reported for fishes exposed to different types of toxicants (Nwani *et al* 2013; Nwani *et al* 2014; Okoh 2015; Okogwu *et al* 2015; Ašmonaitė *et al* 2016; Kalita and Coudhury 2018; Soni and Verma 2018; Sharma 2019; Salim *et al* 2021).

The white blood cell count (WBC) was significantly higher in primextra exposed fish ($p<0.05$) although other blood parameters varied insignificantly between test groups and control. White blood cells defend the system against assaults, elevation of WBC in the test fish could be attributed to an attempt to protect the fish from the toxicity of the pesticide as suggested by Okogwu *et al* (2022). Similar increase in WBC has been reported for different fish species exposed to pesticides (Ramesh *et al* 2009; Okogwu *et al* 2015; Popoola 2018; Khan *et al* 2016; Ghayyur *et al* 2021) and has been attributed to increased lucopoisis due to the presence of toxicants in the environment (Narra 2016). The decline in serum glucose in the current study could be linked to the stress generated by the herbicide on the fish species, which resulted in excessive serum glucose usage (Popoola 2018). This agrees with the work of Ramesh *et al* (2009), who also reported decrease in serum glucose in parameters of common carp, *Cyprinus carpio*.

Observed significant reduction in the protein level in the present study could be attributed to increased energy demand due to the toxicity of the herbicide, which might increase protein consumption, a process where protein is converted into energy, and therefore the serum protein will be reduced. Several researchers have reported similar reduction in serum protein in fish exposed to atrazine-herbicides and adduced that it could be linked to kidney damage and consequent declined in the fish's health condition (Akhtar *et al* 2021).

The significant elevation in the activities of liver enzyme, AST could be attributed to damage of hepatic cells as a result of necrosis as opined by Vitek and Ostrow (2009), while raised level of ALP may be due to cholestasis as suggested by Lala *et al* (2022). Kumar *et al* (2011) reported similar elevations in the activities of liver enzymes in fish exposed to cypermethrin and suggested that such increase could be a result of the transformation

Table 1: Physicochemical parameters of test water during 96-hour sub-lethal bioassay

Pesticide concentration (µg/l)	Temperature (^{O}C)	pH	DO (mg/l)	Conductivity (µS/cm)	TDS (mg/l)
Control	27.67±0.03^{a}	7.53±0.03^{a}	5.07±0.88^{a}	720.33±17.63^{a}	333.33±4.98^{a}
0.36	27.70±0.06^{b}	7.40±0.06^{b}	4.63±0.09^{b}	754.67±3.18^{b}	365.00±13.50^{b}
0.40	28.27±0.12^{b}	7.27±0.09^{b}	3.80±0.17^{c}	777.33±4.63^{b}	366.00±14.36^{b}
0.45	28.33±0.09^{b}	6.79 ±0.12^{b}	3.07±0.15^{c}	825.00±17.50^{b}	409.00±18.58^{b}

values along the same column with different alphabetic superscripts differ significantly ($p<0.05$).

Table 2: Behavioural responses of *C. gariepinus* to different sub-lethal concentrations of Primextra during the 96-hour bioassay

Pesticide Concentration (µg/l)	Control				0.36				0.4				0.45			
Behaviour/Time(h)	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
Hypersensitivity	-	-	-	-	+	++	+	+	++	++	+	+	+++	++	+	+
Loss of Equilibrium	++	++	++	++	++	+	++	++	+	+	+	+	+	+	+	+
Erratic Swimming	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Gulping of air	-	-	-	-	+	+	+	-	+	+	-	-	+	+	-	-
Convulsion	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-
Somersaulting	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-
Fin movement	++	++	++	++		++	++	++	++	++	++	++	+++	++	++	++
Opercular movement	++	++	++	++	++	++	++	++	++	++	++	++	+++	++	++	++

Keys: - =None, + = Mild; ++ = Moderate; +++ = Strong,

Table 3: Haematological parameters (Mean ± SE) of juveniles *C. gariepinus* at 96-hour exposure to sublethal concentrations Primextra Gold herbicide

Blood parameters	Pesticide concentration (µg/L)			
	Control	0.36	0.4	0.45
RBC (×10^{6}/µl)	10.42± 1.03^{a}	8.41 ± 1.02^{a}	8.28 ± 1.11^{a}	8.64 ± 1.03^{a}
WBC (×10^{3}µ/l)	9400 ± 10.50^{a}	10400 ±15.10^{b}	10800 ±20.01^{b}	10100 ±10.10^{b}
Hb (g /dl)	10.70 ±0.81^{a}	8.70 ± 0.80^{a}	8.50 ± 0.70^{a}	8.70 ± 0.65^{a}
PCV (%)	35.00 ± 2.80$_{a}$	31.00 ± 1.90^{a}	29.00 ± 1.55^{a}	30.00 ± 2.05^{a}
MCH (pg)	10.23 ± 0.86^{a}	10.35 ± 0.76^{a}	10.27 ± 0.76^{a}	10.07 ± 0.71^{a}
MCV (fl)	33.59 ±1.01^{a}	36.86 ±1.60^{a}	35.02 ±1.20^{a}	34.72 ±1.04^{a}
MCHC (g/l)	30.57 ± 1.11^{a}	28.07 ± 0.85^{a}	29.31 ± 1.14^{a}	29.00 ± 0.78^{a}

Table 4: Biochemical parameters (Mean±SE) of juveniles of *C. gariepinus* after 96-hour sub lethal exposure to Primextra Gold herbicide

Biochemical parameters	Pesticide concentration (µg/l)			
	Control	0.36	0.4	0.45
AST(IU/l)	40.01±0.78^{a}	58.02±0.49^{b}	60.01±0.73^{b}	56.03±0.89^{b}
ALP (IU/l)	68.02±0.79^{a}	94.05±0.86^{b}	89.13±0.39^{c}	90.12±0.78^{c}
ALT(IU/l)	64.04±0.58^{a}	70.07±1.13^{a}	69.13±0.19a	68.15±119^{a}
Glucose (mg/dl)	80.20±2.05*a*	61.30±1.77^{b}	50.12±1.05^{c}	54.11±0.97^{c}
Protein (mg/dl)	8.41±0.98^{a}	3.41±0.86^{b}	3.62±0.71^{b}	3.93±0.81^{b}
Bilirubin(mg/dl)	4.30±0.19^{a}	6.32±0.35^{a}	6.42±0.19^{a}	6.37± .18^{a}

Values with different alphabetic superscripts differ significantly ($p< 0.05$) between concentrations within the same column

action by the liver to cope with the assault of the xenobiotic.

The kidney cells were damaged in concentration dependent manner, the damages resulted in haemorrhage and focal loss of renal tissues, which could lead to erythropenia (Okogwu *et al* 2022), impaired distillation function of the kidney and kidney failure. The findings are consistent with those of Okogwu *et al* (2015) and Shahid *et al* (2021), that reported glomeruli loss, kidney vascular congestion, tissue loss and fragmentation, and intra-renal haemorrhages in fish exposed to pesticides.

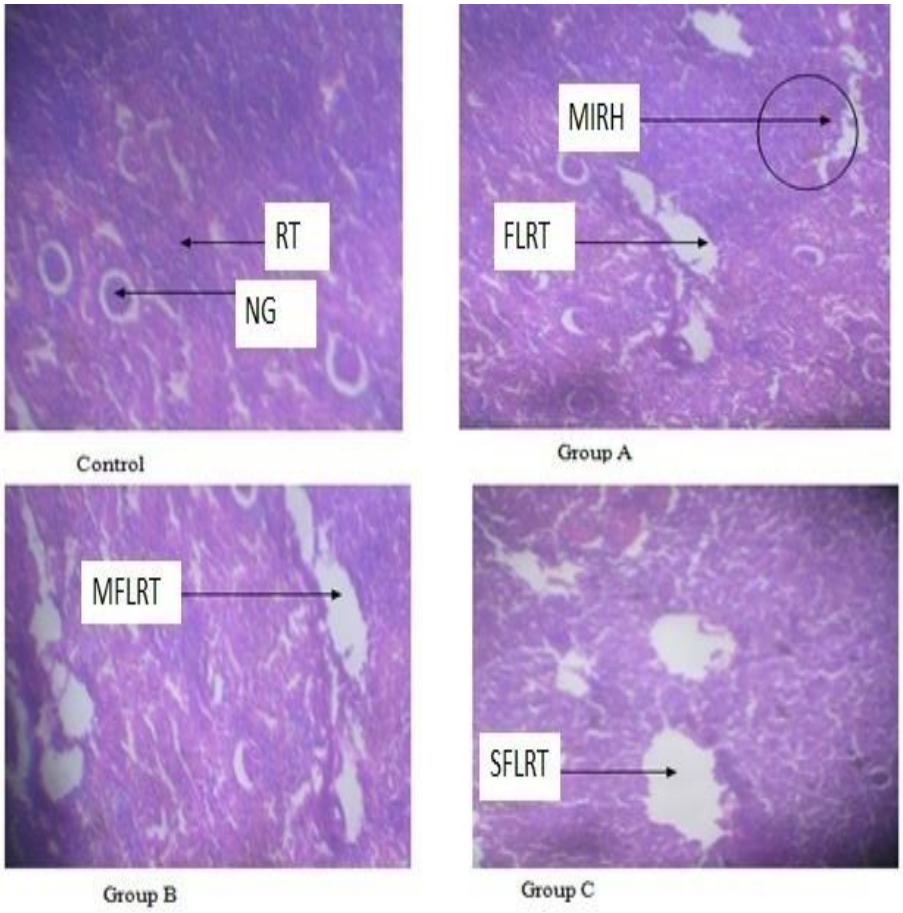

Plate 1. Histopathological effect of Primextra Gold herbicide on the kidney of *C. gariepinus* after 96-hour exposure. The control group showing normal glomeruli (NG) and renal tubules (RT), group A showed focal loss of renal tissue (FLRT) and mild intra renal haemorrhage (MIRH), group B showing moderate focal loss of renal tissue (MFLRT) and group C showing severe focal loss of renal tissue (SFLRT).

Conclusion

The results of the present study showed that Primextra Gold herbicide significantly lowered the dissolved oxygen level of the test water and induced detrimental behavioural changes in *C. gariepinus*. It also revealed that the WBC count, liver enzymes (AST and ALP), serum glucose and protein were significantly altered, while the kidney was severely damaged. The study contributes to knowledge on the toxicity of herbicides to non-target species and has shown that Primextra Gold contributes to environmental pollution and could decline biodiversity. Regular monitoring of the environment, herbicide use education and proper regulatory policies are advocated as panacea to the damaging effect of herbicides to the environment.

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