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Embryotoxic Effects of Oral Ametryn Exposure in Pregnant Rats

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Ametryn (2-methylthio-4-ethylene-6-isopropylamino-5-triazine) is a s-triazine herbicide first synthesized in 1962 and approved for peas, cotton, wheat, and a variety of beans (Gysin 1962). It is a white crystalline substance, sparingly soluble in water (185 ppm) and having a melting point of 84-86°C. Like all s-triazine herbicides, ametryn brings about its herbicidal action by inhibiting photosynthesis (Kneulseli et al. 1969). Little information has been reported on toxicity of ametryn on laboratory animals as well as its occupational hazards (Loosli 1994). Oral LD_{50S} for rats range from 508-3000 mg/kg depending on the sex, age, and route of exposure (Gaines & Linder 1986). Residues of ametryn have been detected in forage and cattle milk (Viden et al. 1987) and minimal concentrations have also been found in root crops such as cassava and yams (Bardalaye & Wheeler 1984). Ametryn fed in the diet to two generations of male and female rats at 0, 20, 200, and 2000 ppm did not cause impairment in reproductive performance (Yau et al. 1988) , however, ametryn exposure to Wistar albino rats in utero was found to be teratogenic with skeletal abnormalities being the most common (Awad 1995). Following a single oral dose of ametryn to rats, only 2% remained in the body after 72h of exposure (Oliver et al. 1969).

As a result of the extensive use of ametryn in the northern region of Nigeria and the reported detection of the herbicide in some root crops, which form the stable food of the country, we decided to evaluate the effect of oral exposure to ametryn in its technical form on pregnant rats with particular interest on organogenesis of developing fetuses.

MATERIALS AND METHODS

Gesapex 500FW®, a technical grade ametryn (A3952G, G34163, P806054, June 1988) was supplied by Swiss -

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Nigeria Chemical Company, Lagos. A total of 300 4-month old female Sprague-Dawley rats was obtained from the Animal Center of the College of Medicine, University of Lagos. Rats were fed on commercial rat chow obtained from Pfizer Livestock PLC, Lagos. Five groups of 10 rats each weighing 212 ± 30 g (S.D) were used for acute toxicity studies. The median lethal dose (LD_{50}) and slope function were calculated using Litchfield & Wilcoxon (1949). The LD_{50} was 1616 mg/kg with a slope function of 2.27. The selected doses were 101, 202, 404, and 539 mg/kg and distilled water for the control. For five consecutive days, vaginal smears were taken every morning (0700h) to determine the stage of the estrus cycle (Ashling, 1968). At the estrus stage (i.e. nucleated cells), two females and a male were caged at 1800h and the next day, vaginal smears were examined microscopically to check for sperm plugs . Detection of sperm plugs was regarded as Day 0 of pregnancy. Female rats were assigned to treatment groups using a table of random numbers. Pregnant rats received appropriate doses of ametryn orally from day 6 to 15 of gestation.

Each rat was carefully examined for evidence of toxicity. Droplets of blood appearing at the vaginal opening between days 12 to 14 of gestation indicated resorption. Maternal weights were taken on days 6 to 15, and day 20 of gestation at which time they were sacrificed. Food consumption and water intake were measured on days 6-20. Maternal weight was determined as the overall weight on day 20 minus the weight of the uterus. At sacrifice, the maternal livers were removed, blotted, and weighed. Liver weight expressed over the maternal weight gave the relative liver weight. Ovaries and uteri were examined to determine the number of corpora lutes and live, dead and resorbed fetuses. The uterus of apparently non-pregnant rats was stained with a 10% solution of sodium sulphide and evaluated for evidence of early resorption or implantation sites. Placentae were weighed and the sex, tail length, and crown–rump length measured on all fetuses. Fetuses were examined for external morphological alterations and cleft palate. One–third of the fetuses in each litter, selected by a table of random numbers, were examined using a dissecting microscope for evidence of soft tissue alteration (Staples 1974). The rest of the fetuses were randomly divided into two groups, one group was stored in 95% ethanol, later cleared, and stained with alizarin red-S, for skeletal examination (True 1947). The second half was placed in Bouin’s solution for histopathological analysis. Thirteen treated and three control pregnant rats were allowed to deliver normally.

One-way analysis of variance was used for fetal weight, maternal weight gain, food consumption, water intake, liver weight, number of implantation sites, resorption,

live and dead fetuses, placental weight, fetal sex position in the horn, and when these were significant, least square difference was applied. Fetal sex ratio was analysed using a binomial distribution test, while the tail and crown-rump lengths were subjected to Student's t-test. Abnormalities were analysed using a Wilcoxon test.

RESULTS AND DISCUSSION

After the administration of the herbicide, we noticed a reduction in the activity of the rats, mainly at high doses. Maternal mortality increased with increased dosage; and a significant reduction was observed in food consumption, water intake, and maternal weight gain between days 6 and 15 of gestation for 404 and 539 mg/kg groups compared to control (table 1). Changes in absolute liver weight were inappreciable whereas relative weights were significantly increased at the two high doses, probably in response to body weight reductions. All maternal parameters recorded for rats dosed with 101 and 202 mg/kg were not significantly different compared to controls. Pregnant rats allowed to deliver, did so at 21 ± 1 (S.D) days for rats in 202, 101 mg\kg and control groups compared to 24 ± 2 (S.D) days for rats exposed to 404 and 539 m\kg. At the two high doses, ametryn was shown to significantly increase the number of resorption per litter (especially early resorption) and reduced termed fetuses per litter, fetal body weight, placental weight, tail length, and crown–rump length (Table 2). There was no effect on the number of implantations and fetuses per litter, fetal sex distribution and ratio, resorption site distribution in the uterus, or stillbirths. No cases of cleft palate or external abnormalities of the soft tissues were noticed for any of the fetuses. Stained skeletons did not reveal any axial or appendicular bone malformations. However, there was delayed ossification of some bones, including cranial bones, distal sternabrae, sacral and coccygeal bones, and hand and foot bones of fetuses exposed to 404 and 538 mg/kg (table 2). Histological analysis did not reveal any pathological changes.

This study has shown that technical ametryn at high doses affects both the mother and her fetuses. Maternal toxicity was indicated by hypoactivity which was subsequently followed by increased maternal mortality and decreased food consumption, water intake, and body weight gain. Toxicity was not evident for rats administered 202 or 101 mg/kg. The increase in relative liver weight at doses greater than 404 mg/kg was a result of decreased maternal weight, which was not accompanied by hepatomegaly. Decreases in fetal weight, tail length, and crown-rump length, increased early resorption, and

Table 1. Evaluation of toxicity in rats exposed to technical ametryn.

Parameter	Dose (mg/kg/day) 0	101	202	404	539
No. dams (deaths)	22(0)	24(0)	23(2)	32(10)	35(13)
% Pregnancy	91.0	87.5	91.0	68.9	62.8
Food intake (g/kg/d)	104.2± 10.2	99.8± 13.7	99.6± 11.3	74.3± 14.3*	43.9± 15.1**
Water intake (ml/kg/d)	174.6± 23.5	169.8± 18.7	164.7± 20.9	134.0± 09.2*	135.0± 08.4*
Weight gain (g):					
Day 6-15[a]	22±9	21±10	20±11	-14±18*	-17±20*
During gestation[b]	44±16	42±19	37±16	09±15**	06±11**
Liver Weight:					
Absolute (g)	6.6± 1.4	6.6± 1.6	6.9± 1.5	6.7± 1.4	6.9± 1.3
Relative ($x10^2$)	4.6± 0.7	4.8± 0.6	4.7± 0.7	8.6± 0.3**	8.1± 0.5**

Values are expressed as mean ± S.D

[a]) Values showing average change in total maternal body weight between day 6 to 15 of gestation.

[b]) Values showing difference in body weight on day between 20 of gestation.

* $p<0.05$, ** $p<0.01$ (Compared to control)

delayed ossification of some bones observed for fetuses at higher doses, characterized embryotoxicity (Aliverti et al. 1979) . The mechanism through which ametryn might have brought about its toxic effects are not well elucidated but are not unconnected to delayed implantation, alteration of intrauterine environment and reduction in the placental transfer of essential nutrients. Since all the rats exposed to 404 and 539 mg/kg of ametryn did not deliver after the normal time (day 21 or 22 of gestation), it implies the herbicide might have been administered prior to implantation thus possibly leading to delay and lengthening of gestation period. Delay in implantation could not have been the

Table 2. Reproductive and developmental performance of female rats administered technical ametryn during gestation (organogenesis period).

Parameter	Dose (mg/kg/day) 0	101	202	404	539
No. of litters	17	19	18	15	16
Implantations/ litter	11.0± 0.7	10.8± 0.8	10.6± 0.7	10.6± 0.5	10.2± 0.6
Corpora lutea/ ovaries	11.1± 0.6	11.4± 0.2	11.2± 0.5	11.6± 0.3	11.3± 0.7
Fetuses/litter	9.6± 2.4	9.4± 2.2	8.9± 2.0	8.8± 3.4	8.2± 2.7
Resorptions/ litter	1.6± 0.1	1.4± 0.1	1.5± 0.1	2.0± 0.2	2.1± 0.3
Termed fetuses/ litter	9.6± 2.4	9.4± 2.2	8.9± 2.0	5.2± 0.8**	0**
Fetal parameters: sex ratio (m:f)	1:0.8	0.9:1	1:1	1:0.9	1:0.9
Body weight (g)	4.2± 1.6	4.1± 1.8	3.9± 1.7	2.7± 0.9*	1.7± 0.8**
Placental weight (g)	0.47± 0.15	0.43± 0.14	0.41± 0.19	0.35± 0.17*	0.32± 0.15*
Tail length (cm)	1.42± 0.22	1.38± 0.21	1.39± 019	1.26± 0.06*	1.11± 0.02**
Crown-rump length (cm)	3.90± 0.44	3.82± 0.39	4.03± 0.31	3.26± 0.22*	2.29± 0.17**

Values are expressed as mean ±S.D * P<0.05, ** P<0.01

main factor of embryotoxicity because fetuses obtained from rats sacrificed on day 20, and those allowed to deliver, had low fetal body weights and other parameters compared to the control (table 2). No teratogenic effects were observed at any dosage levels, but the herbicide was embryotoxic at doses greater than 404 mg/kg.

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