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<tr>
<td>Publisher</td>
<td>Journal of Tropic Agriculture, Food, Environment and Extension</td>
</tr>
<tr>
<td>Publication Date</td>
<td>May, 2008</td>
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CONTAMINATION OF DRINKING WATER WITH INORGANIC FERTILIZER: EFFECTS ON REPRODUCTIVE PERFORMANCE.

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Summary

Inorganic fertilizers used to boost crop production can contaminate surface and groundwater when improperly or excessively applied, and grazing/extensively raised animals commonly drink from these water sources. The effects of drinking water contaminated with variable low percentages of NKPC (10:15:15) fertilizer on reproductive performance were investigated using the albino rat as a model. Five groups (A-E) of 15 rats (10 females and 5 males) each were given drinking water contaminated with fertilizer at 0% (control), 0.1%, 0.5%, 1% and 2% respectively for a 12-week period during which the females and males were brought together for breeding for 6 weeks. Group E females (2% contamination) had a significantly lower (p<0.01) number of females that conceived, longer (p<0.01) time before conception, higher (p<0.05) number of mates before pregnancy resulted, higher (p<0.05) number of abortus recorded, and lower (p<0.05) litter size and weight, area of foetal attachment in utero, post-maternal survival index and live birth index. Group E females (1% contamination) had a significantly longer (p<0.05) time before conception, lower (p<0.05) litter size and weight, and higher (p<0.05) number of recorded foetuses. There were no significant variations (p>0.05) between the gestation length of the female, epididymal sperm reserve of the males, body weight and feed consumption of all the rats (males & females) in the different groups. It was concluded that contamination of drinking water with fertilizer at 1% & 2% adversely affected the reproductive performance of the females rats.

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Introduction

Inorganic fertilizers are raw materials containing either one or two or all the three basic nutrients (nitrogen, phosphorus and potassium) needed for plant growth and cost-effective replacement of crop-land nutrients that have been consumed by previous plant growth (EFNA, 1997; USEPA, 2001; ASF, 2006). Farmers (especially those in developing countries) rarely make any distinction between fertilizer types and do not usually recognize the need to adapt their application rates and timing to different soils and plant development, with a resultant excessive and/or improper application of inorganic fertilizers (USEPA, 2001; Toner & Burkart, 2004; Field, 2004). In addition, government subsidies on agricultural inputs especially fertilizers and the current tendency towards intensive and monocrop production further lead to very high levels of application of inorganic fertilizers (FAO, 1999; Horigan et al., 2002; USDA, 2003; Fields, 2004).

Studies have however shown that crops actually absorb only 10% of the minerals applied as fertilizers (Toner & Burkart, 2003; Fields, 2004). Excessive and improperly applied inorganic fertilizer not only become sources of non-point pollution of surface and groundwater (Schilling & Linski, 2000; USEPA, 2001; Toner & Burkart, 2003). Fertilizer-derived component chemicals of concern to source water quality (nitrates and phosphates mainly) have been associated with environmental degradation and a variety of adverse health outcomes in humans and animals (Cervey & Pretty, 1998; Flynn et al., 2000; WHO, 2003; Criss & Davison, 2004; Fewtrell, 2004; Werd et al., 2005).

Some reproductive disorders associated with fertilizer-derived nitrates and phosphates include spontaneous abortions, embryonic and foetal deaths, intrauterine growth retardation, congenital malformations and neonatal deaths (Schmitz, 1991; Bergin et al., 1982; Dorsch et al., 1981; Ahrnborg et al., 1988; Aashtaghau et al., 1993; Grant et al., 1996, Dukowski et al., 2001; Brender et al., 2004; Ward et al., 2003). To date, there had been no comprehensive report on the effects of subchronic fertilizer contamination of drinking water on reproductive performance. This present study investigated the effects of contamination of drinking water with low sub-lethal levels of fertilizer on reproductive performance, using the albino rat as a model. Fertilizer contamination of both surface and ground water is considered to be of great significance for animals, especially livestock that graze and/or are raised extensively which move about from one point to the other drinking from shallow water pools.

Materials and Methods

Seventy-five mature, maiden Sprague-Dawley albino rats made up of 30 females and 25 males were used for the study. The rats were aged 11-12 weeks and weighed between 111-142 g for the females and 130-175 g for the males at the commencement of the study. They were procured from the Faculty of Veterinary Medicine, Laboratory Animal Unit, University of Nigeria Nsukka. They were fed ad libitum on standard pelleted feed (Grand Cerезes & Oil Mills Ltd, Jos, Nigeria), and freely provided drinking water all through the study. At the commencement of the study proper ("day 0"), the rats were randomly divided into five groups (groups A - E), each comprising 10 females and 5 males, and identified using an indelible marker. Body weights and daily feed consumption (DFC) and urinary water consumption
(DWC) were determined for the rats in each group. Group A rats served as the control which was given clean uncontaminated drinking water. Groups B, C, D and E were given drinking water contaminated with 0.1%, 0.5%, 1% and 2% of NP/PC15-15-15 Etizeril (AFFCOTT Pte, Lagos, Nigeria) respectively all through the 14 weeks of the study. NPK:15-15-15 was chosen for the study because it is the major type of fertilizer available to farmers, being considered broad and equinominate in composition, and is the one most used by farmers (ASPF, 2006). Each rat was given their group specific drinking water as the only source of water throughout the 14-week study period.

The females and males in each group were kept in separate cages for the first 8 weeks of the study period during which period body weights, daily food and water consumption were determined at 4-week intervals. Afterwards, the corresponding males and females were brought together in the same cage (breeding cages) for the remaining part of the study period (6 weeks). During this period the males and females were kept in the same cage for breeding. The females were observed twice daily (morning and evening) for evidence of mating using the modified vaginal plug method (Kennon & Vickery, 1970; Ojehode et al, 2006). The number of males that mated before pregnancy was recorded for each rat in the different groups. Pregnant females in each group were separated from their group mates during the last trimester of pregnancy and kept in individual nursing cages where they littered. At delivery, gestation length, litter size and litter weight were noted for each rat. Three (3) days after delivery, the females were humanely sacrificed; the reproductive tracts were removed and opened to count the number of places of implantation in the uterus. The number of fetuses resorbed was determined by subtracting the litter size at birth from the number of sites of fetal attachment. The live-born index (LBI) and placental implantation survival index (PISS) were calculated using the standard formulae. Also, the males were humanely sacrificed and the epididymal tail was dissected out, weighed, homogenized and the sperm reserve was quantified haematometrically (Eghbokile et al, 1970).

Results generated from the study were subjected to Chi-square and one-way analysis of variance as appropriate using SPSS version as well as other statistical packages and variant means were separated by LSD (method).

Results and Discussion

Among the 10 female rats in each group, only 2 (20%) conceived in group E (2% contamination) as against 10 (100%) in all other rat groups. And the significantly lower (p<0.01) number of rats that conceived in group E was strongly associated (p<0.01) with fertilizer contamination of the drinking water. The mean ± SEM length of time before conception ranged from 1.49 ± 0.27 days in the group A rats (Control) to 6.00 ± 3.00 days in the group E rats (Table 1). The length of time before conception was found to be fertilizer concentration-dependent and that of group D and E rats (1% & 2% contamination) were found to be significantly higher (p<0.05) than that of group A rats. Also, the mean number of males before pregnancy resulted was lower in groups A rats (1.0 ± 0.0) and highest in the group E rats (2.79 ± 0.15); the value for the group E rats was significantly higher than that of the group A and other rat groups (B & D) (Table 1). There was however no significant differences (p>0.05) between the mean gestation lengths of the rats that conceived in all the groups (Table 1). The significantly lower conception rate in groups D and E were the significantly longer length of time before conception in groups D and E rats, and also the significantly higher number of males before pregnancy resulted in group E rats suggest that the fertilizer contamination may have led to either anovulation in the rats that did not conceive or interference with normal oestrous cycle, or may have caused early embryonic death with consequent return to oestrus (Capo, 1994; Wulreich, 1995). Such lower conception rates and higher number of services per conception had earlier been reported in rats and sheep given drinking water contaminated with a combination of fertilizers and pesticides at levels reported in ground water at certain locations (NDECC, 2002; WO01, 2003).

The mean litter size and litter weight of the groups D and E rats were significantly lower (p<0.05) than that of the group A rats; and the trend across the groups showed a fertilizer concentration-dependency (Table 1). The mean litter size decreased from 7.700 ± 4.00 reported for group A to 1.00 ± 0.74 reported for group E, while the litter weight decreased from 43.62 ± 5.35 , in group A, to 6.40 ± 0.28 in group E (Table 1). On examination of the reproductive tract, the number of fetuses reached was significant in group E rats was found to be significantly lower (p<0.05) than that of the group A rats and all the other groups (Table 1). The mean number of fetuses reached was found to be significantly higher (p<0.05) in the group D and E rats when compared to the group A rats (Table 1). The mean PISS and mean LBI of the group E rats were found to be significantly lower (p<0.05) than that of group A and other groups (Table 1). The significantly lower litter size and litter weight at birth in groups D and E rats showed that beyond affecting conception and embryonic survival, fertilizer contamination at 2% and 1% also affected intrauterine growth and fetal survival. This is further confirmed by the significantly higher mean number of fetuses resorbed in the groups D and E rats and the significantly lower mean PISS in these groups (Table 1). Earlier studies using nitrate and nitrates had reported their teratogenic effects (Spriggs et al., 1997; WO01, 2003). Also, high nitrate levels in water of humans have been associated with intra-uterine growth retardation in humans (Bikawasari et al., 2001). The significantly lower mean LBI reported in group E rats is in agreement with earlier reports in humans of a positive association, 64
between high drinking water nitrate and neonatal deaths (Aschengrau et al, 1993). The low LBI recorded in group E rats is thought to be a result of congenital malformations that do not permit postnatal survival; congenital malformations especially central nervous system malformations had earlier been reported to occur in humans in association with high levels of nitrate in drinking water (Scruggs et al, 1982; Dorsh et al, 1984; Aschengrau et al, 1988; Cohen et al, 2001; Want et al, 2003).

There were no significant variations (p=0.05) between the groups (A-E) in the epididymal sperm reserve; the sperm counts of the groups given contaminated water did not significantly differ (p=0.05) from the control group (Table 1). The lack of significant variations in the epididymal sperm reserve of the different groups is an indication that fertilizer contamination of drinking water did not significantly affect spermogenetic (Witnach, 1993).

Results of the body weight, DFC and DWR are not presented in table form because of space constraints in manuscript preparation. The body weight and DFC measurements during the first 8 weeks of the study before the females and males were brought together for breeding showed no significant variations (p=0.05); but for DFC, groups B and C females (0.1% and 0.5% contamination) drank a significantly higher (p=0.05) volume of water all though the 8 weeks of measurements when compared to the group A females (control), but the group B females drank a significantly lower (p=0.05) volume of water at week 8 when compared to the group A females. The group C males on the other hand drank significantly (p=0.05) higher volume of water when compared to the controls while groups D & E males drank a significantly lower (p=0.05) volume of water. The lack of significant variations in the body weight and feed consumption of the different rat groups indicates that fertilizer contamination of the drinking water in this study did not lead to any adverse effects on feed consumption and weight gain. These findings agree with the reports of Steigled & Atallah (1968) in guinea pigs given nitrates and nitrites in water and that of Heidel et al (1996) in rats and mice given combined fertilizer-pesticide contaminated water. The significantly lower water consumption of the groups D & E rats and significantly higher water consumption in groups B & C imply that contamination at 2% & 1% may have made the water unpalatable, while contamination with 0.1% & 0.5% may have made the water more palatable.

Based on the findings in this study, it was concluded that fertilizer contamination of drinking water at the levels of 0.1% and 1% as used in this study had a significant adverse effect on the reproductive performance of female albino rats. It is expected that such adverse reproductive effects will be more severe in ruminants as earlier studies had shown that ruminants are more sensitive to the effects of nitrates as a result of high nitrate reduction in the rumen (Speijers et al, 1987; WHO, 2003); and in addition, they are the animals that usually graze freely and commonly drink from shallow water pools.

References


Environmental Health Perspectives, 112-A:557-563.

Table 1. The reproductive indices of nine given drinking water contaminated with varying low percentage of NPS-15:15:15 fertilizer: the controls given uncontaminated water are also presented.

<table>
<thead>
<tr>
<th>Reproductive indices</th>
<th>Group A (Control)</th>
<th>Group B (5%)</th>
<th>Group C (10%)</th>
<th>Group D (15%)</th>
<th>Group E (20%)</th>
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<tr>
<td>Leech (female before</td>
<td>1.68m ± 0.27</td>
<td>1.70m ± 0.21</td>
<td>1.22m ± 0.86</td>
<td>4.01 ± 0.09*</td>
<td>6.90 ± 2.00*</td>
</tr>
<tr>
<td>% of females observed</td>
<td>1.10m ± 0.10</td>
<td>1.50m ± 0.22</td>
<td>1.56m ± 0.29</td>
<td>2.90m ± 0.22</td>
<td>2.70m ± 0.15</td>
</tr>
<tr>
<td>Genetation length (days)</td>
<td>21.00 ± 0.22</td>
<td>21.50 ± 0.10</td>
<td>23.50 ± 0.20</td>
<td>15.00 ± 0.00</td>
<td>17.00 ± 0.20</td>
</tr>
<tr>
<td>Gestation time (days)</td>
<td>19.00 ± 0.22</td>
<td>19.50 ± 0.10</td>
<td>20.50 ± 0.20</td>
<td>22.00 ± 0.20</td>
<td>23.50 ± 0.20</td>
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<tr>
<td>Incubation period (days)</td>
<td>23.50 ± 0.27</td>
<td>23.75 ± 0.10</td>
<td>24.50 ± 0.20</td>
<td>23.00 ± 0.20</td>
<td>24.50 ± 0.20</td>
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<tr>
<td>Leech (female before</td>
<td>1.40m ± 0.27</td>
<td>1.50m ± 0.22</td>
<td>1.56m ± 0.29</td>
<td>2.90m ± 0.22</td>
<td>2.70m ± 0.15</td>
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* Asterisk indicates a means significantly different from that obtained for Group A (Control): p < 0.05