# REPRODUCTIVE TOXIC EFFECTS OF INGESTION OF SODIUM FLUORIDE IN FEMALE RATS

Ahmad S Al-Hiyasat,[a] Ahmed M Elbetieha,[b] Homa Darmani[b]
Irbid, Jordan

**SUMMARY: The objective of this study was to investigate the toxic effects of different concentrations of sodium fluoride (200, 400 and 600 ppm NaF), administered in drinking water for 30 days, on the reproductive system of adult female Sprague-Dawley rats. The rats in the two higher-dosed groups showed clinical signs of toxicity unlike those exposed to NaF at a concentration of 200 ppm. Ingestion of 200 ppm NaF had no effect on the pregnancy rate of the rats nor on the number of implantations. However, the number of viable fetuses was significantly lower than in the control group. Furthermore, the pregnant rats with resorptions and the total number of resorptions increased in the NaF-treated group. There was also a significant increase in maternal organ weights. Rats which had ingested NaF showed increases in both the absolute and relative weights of the ovaries and in the relative weights of the uterus and kidney. The maternal body weights and water consumption were significantly reduced in the treated rats. The results indicate that exposure of female rats to NaF in drinking water has adverse fetotoxic effects.**

Keywords: Female rats, Fetotoxicity, Fluoride toxicity, Reproduction, Sodium fluoride.

## INTRODUCTION

Although fluoridation of water supplies is practiced in many places in the hope of reducing the incidence of dental caries, there is continuing concern that exposure to fluoride could cause toxic effects. An epidemiological study to assess whether fluoride could affect human birth rates using a U.S. database of drinking water systems showed an association of decreasing total fertility rate with increasing fluoride levels.[1] Decreased testosterone concentrations have also been reported in skeletal fluorosis patients and in males drinking the same water as the patients but with no clinical manifestations of the disease compared with those of normal, healthy males living in areas non-endemic for fluorosis.[2] These studies have suggested that fluoride toxicity may cause adverse effects in the reproductive system of males living in fluorosis endemic areas.[2] Additionally, it was found that *in vitro* exposure of human sperm to fluoride (250 mM) results in altered lysosomal activity, altered glutathione levels and morphological anomalies producing a significant decline in sperm motility.[3]

Multiple animal models have also shown that fluoride toxicity decreases fertility in most species studied, but so far these studies have concentrated mainly on the reproductive toxic effects of fluoride on male animals. Recently, Heindel *et al*[4] investigated the developmental toxicity of fluoride and reported no adverse effect of sodium fluoride on the embryonic and fetal developments in rats or rabbits at doses of 27 mg/kg/day in the rat and 29 mg/kg/day in rabbits.

[a]For Correspondence: Dr Ahmad S. Al-Hiyasat, [a]Department of Restorative Dentistry, Faculty of Dentistry, Jordan University of Science & Technology, PO Box 3030, Irbid, 22110, Jordan. Telephone: 02-7095111 (Ext. 23595). Fax: 02-7095123. Email: hiyasat@just.edu.jo. [b]Department of Biology, Faculty of Science.

Thus, at present, the toxicity of fluoride is well established, but animal studies addressing the effects on reproduction and development are limited in number. Furthermore, the mechanisms responsible for the effect of fluoride on reproduction and development are not well understood. In this study on female rats, we hope to clarify the outcome of sodium fluoride exposure on fertility in order to gain further insight into the mechanisms of the reproductive toxic effects of NaF.

## MATERIALS AND METHODS

*Animals and husbandry:* 40 Adult female Sprague - Dawley rats that weighed between 300 and 350 g were used in this study. The rats were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology. They were maintained in stainless steel cages on a standard laboratory feed diet of undetermined F content and were provided 0.5-ppm fluoride tap water *ad libitum*. The animals were kept at a controlled temperature of 21 ± 1.0°C on a 12-h light/dark cycle.

*Administration of sodium fluoride:* Sodium fluoride (NaF) (Sigma Chemical Company, St Louis, MO, USA) was dissolved in tap water at a concentration of 200, 400 and 600 ppm The rats were randomly divided into four groups of 10. The first group served as the control and the animals were allowed *ad libitum* access to the 0.5 ppm fluoride tap water without any added NaF for 30 days. The other three groups were allowed *ad libitum* access to tap water containing either 200, 400 or 600 ppm NaF for 30 days.

*Evaluation of reproductive toxic effects of NaF ingestion:* Animals were observed for clinical signs of toxicity daily from the first day of exposure to NaF. Their water consumption was also measured daily and body weights were checked weekly.

The effect of NaF ingestion on the occurrence of implantation was estimated in the adult rat and in their control counterparts after the 30-day exposure period. Treated rats and their control counterparts were divided randomly into groups of two animals each and housed for ten days with a sexually mature untreated male of proven fertility. During this period, at least two estrous cycles should have elapsed.[5] One week after removal of the untreated males, the treated females and their control counterparts were killed by cervical dislocation under light ether anesthesia. During autopsy, the following measurements were recorded: number of pregnant rats, number of implantations, number of viable fetuses, and number of resorptions. Furthermore, the maternal body, kidney, uterus, and ovary weights were measured, and the embryo weights were also recorded.

*Statistical Analysis:* The data were analyzed using the Student's t test and Chi-squared test using Minitab statistical package (Minitab Release 9, Minitab Inc., State College, PA, USA).

## RESULTS

*Exposure levels and toxicity of NaF:* the daily water consumption for the four groups of animals was as follows: control: 14.5 - 42.5 mL; 200 ppm NaF: 14.0 - 34.0 mL; 400 ppm NaF: 6.11 - 14.0 mL; 600 ppm NaF: 5.0 - 15.0 mL. The actual doses the animals received based on the water consumption per kg body weight per day were 22.6, 18.4, and 28.0 mg NaF for the 200, 400 and 600 ppm groups, respectively. The $LD_{50}$ for NaF is reported to be at least 52 mg/kg/day, equivalent to 350 ppm if administered in drinking water.[6] In the present study, we found none of the controls nor the 200 ppm group showed any clinical signs of toxicity. However, of the 10 rats exposed to 600 ppm NaF, none survived the duration of the experiment. Of the group exposed to 400 ppm NaF, only three survived the 30-day exposure period. The rats in the higher dose groups (400 and 600 ppm NaF) showed clinical signs of toxicity — dehydration, lethargy and hunched posture.

*Effect of NaF on body weight:* In the present study, rats exposed to 200 and 400 ppm NaF showed a statistically significant decrease in body weight and water consumption in comparison to the controls ($p<0.00001$) (Table 1). There was also a statistically significant reduction in the body weight of animals exposed to 600 ppm NaF for 21 days, in comparison to the control group ($p<0.0001$) (Table 1). The animals in the latter group did not survive the 30-day period of exposure to NaF.

**Table 1.** Effect of Sodium Fluoride on maternal body weights and water consumption

| Treatment | No. of dead females[a] | No. of pregnant females[a] | Body weight (g)[b] | Water consumption (mL/day)[b] | Dose of NaF from water (mg/kg/day)[b] |
|---|---|---|---|---|---|
| Control | 0 | 10/10 | 361.2 ± 1.6 | 37.6 ± 1.4 | 0 |
| NaF (200 ppm) | 0 | 10/10 | 249.8 ± 0.71* | 28.2 ± 0.91* | 22.58 ± 0.79 |
| NaF (400 ppm) | 7 | 1/3 | 221.7 ± 2.0* | 10.17 ± 1.2* | 18.35 ± 2.16 |
| NaF (600 ppm) | 10 | 0 | 195.0 ± 38*Δ | 9.11 ± 0.4* | 28.03 ± 1.2 |

[a]On date of sacrifice. [b]Results are expressed as means ± SEM.

*$p<0.0001$ significantly different from the control group (Student's t-test).

Δ Weight after 21 days of exposure.

*Effect of NaF on fertility:* As previously stated, only 3 animals survived in the 400-ppm NaF group. None survived from the group exposed to 600 ppm. Thus, for the rest of the study we only took into consideration the effects of 200 ppm NaF on fertility of treated rats in comparison to their control counterparts.

Table 2 shows the effect of 200 ppm NaF ingestion for 30 days on the fertility of female rats. There were no differences between the control and the NaF-treated group in the number of rats becoming pregnant nor in the number of implantations. However, the number of viable fetuses was significantly lower in the NaF-treated group ($p<0.005$). In the control group, only 2 pregnant rats

from the group of 10 showed resorptions compared to the NaF-treated group in which all the pregnant rats had resorptions ($p<0.001$). Furthermore, the total number of resorptions was much greater in the NaF-treated group than in the control group ($p<0.001$). If we look at the number of resorptions out of the total number of implantations in the control rats, we can see that the percentage of resorption was 14% (Table 2). In the rats exposed to 200 ppm NaF, the percentage of resorption was 71%, showing a statistically significant increase in resorptions ($p<0.0005$) (Table 2).

**Table 2.** Effect of long-term exposure to sodium fluoride via drinking water on fertility of female rats

| Treatment | Pregnant females | No. of implantations[a] | No of viable fetuses[a] | Rats with resorptions | Resorptions/total No. of implantations (%)[a] |
|---|---|---|---|---|---|
| Control | 10/10 | 7.00 ± 0.45 | 6.00 ± 0.56 | 2/10 | 10/70 (14 ± 8.4%) |
| NaF (200 ppm) | 10/10 | 8.20 ± 0.37 | 2.40 ± 0.78* | 10/10 | 58/82[†] (71 ± 9.8%)[‡] |

[a]Results are expressed as means ± SEM.

*p<0.005. [†]p<0.001 significantly different from the control group (Chi-squared).
[‡]p<0.0005 significantly different from the control group (Student's t-test).

*Effect of NaF on maternal organ and embryo weights:* Table 3 shows that NaF ingestion also resulted in a significant increase in both the absolute and relative weights of the ovaries ($p<0.005$ and $p<0.0005$, respectively). There was also a statistically significant increase in the relative uterine weights ($p<0.01$) and kidney weights ($p<0.0001$) compared to the control counterparts and a statistically significant increase in relative embryo weights ($p<0.01$).

**Table 3.** Effect of sodium fluoride on maternal organ and embryo weights

| Treatment | No. of females | Ovary weight (g) (mg/100g Bwt)[a] | Uterus weight (g) (mg/100g Bwt)[a] | Kidney weight (g) (mg/100g Bwt)[a] | Embryo weight (g) (mg/100g Bwt)[a] |
|---|---|---|---|---|---|
| Control | 10 | 0.18 ± 0.005 (50.94 ± 1.3) | 0.48 ± 0.01 (132 ± 3.7) | 1.44 ± 0.09 (398.7 ± 24) | 0.31 ± 0.03 (81.7 ± 8.3) |
| NaF (200 ppm) | 10 | 0.38 ± 0.042[†] (151.30 ± 17)[‡] | 0.52 ± 0.07 (225 ± 27)* | 1.63 ± 0.05 (651.7 ± 21)[§] | 0.29 ± 0.009 (113.4 ± 5.1)* |

[a]Results are expressed as means ± SEM of absolute and relative weights; relative weights expressed as mg/100g body weight

*p<0.01, [†]p<0.005. [‡]p<0.0005. [§]p<0.0001 significantly different from the control group (Student's t-test).

## DISCUSSION

The animal model in this study has been used previously in other investigations to assess the adverse effects of different compounds on reproduction in laboratory animals.[7,8] The concentrations employed in our study were chosen

after consideration of the data from the National Toxicology Program (NTP)[9] 14-day study wherein female rats given 400 ppm NaF in drinking water showed decreased body weight and water consumption and clinical signs of toxicity, while 800 ppm killed all the animals.[4,9] In our 30-day study, we found that 600 ppm killed all the animals, 400 ppm killed 70% of the animals and 200 ppm did not result in any deaths but there were reductions in water consumption and maternal body weight.

Our work has shown that long-term exposure of adult female rats to NaF at a concentration of 200 ppm administered in drinking water had no effect on the occurrence of pregnancy. However, there was a statistically significant reduction in the number of viable fetuses in the group ingesting NaF compared to the control group. The percentage of viable fetuses in NaF-treated rats was reduced to 40% that of the controls. Furthermore, there was a statistically significant increase in the number of resorbed fetuses, with the NaF-treated group showing 500% increase in the number of resorptions out of the total number of implantations in comparison to the control group.

In line with these results, a study on screech owls in fluoride-polluted areas showed a significant impairment of overall reproduction and suggested that NaF could cause slight to moderate reproductive disorders.[10] Chronic dietary NaF administration at 200 ppm resulted in lower egg weights and lengths and in a 10% reduction of the weights of day-one hatchlings in comparison to their control counterparts. We also found a slight but not statistically significant reduction (6%) in absolute embryo weights of rats exposed to 200 ppm NaF compared to the controls.

Other studies have shown that a reduction in food-borne fluoride in a skulk of silver foxes resulted in decreased neonatal mortality and increased kit production during a two breeding and whelping season period.[11] High-fluoride concentrations in drinking water are also reported to be associated with decreased human birth rates.[1]

Heindel *et al*[4] reported no adverse effects of NaF administered to Sprague-Dawley rats on gestation days 6 through 15 at levels of 0, 50, 150 or 300 ppm and at levels of 0, 100, 200, or 400 ppm to New Zealand White rabbits. They reported that doses up to 27 mg/kg/day in the rat or 29 mg/kg/day in the rabbit throughout major organogenesis caused no definitive developmental toxicity. They found no significant differences between the NaF groups and the control group in the average number of corpora lutea, implantations, live fetuses, or in the percentage of early deaths (resorptions), or late fetal deaths per litter. The percentages of pre- and post-implantation losses per litter and mean pup weights were not significantly different from control values. Another study by Collins *et al*[13] reported no developmental toxicity in rats exposed to NaF in drinking water throughout pregnancy (GD 0-20) at doses of 175 and 250 ppm with reported intakes of 24.7 and 25.1 mg/kg/day. These reports are in contrast to our study wherein we provide conclusive evidence that chronic exposure of female rats to 200 ppm NaF results in adverse effects on fertility.

In summary, we found that sodium fluoride administered in drinking water to rats for 30 days at doses averaging 22.6 mg/kg/day caused definite fetotoxic effects. There was a reduction in the number of viable fetuses and an increase in the number of pregnant rats with resorptions as well as an increase in the total number of resorptions.

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