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Effect of Boron in Drinking Water on the Male Laboratory Rat

Bruce S. Seal and Howard J. Weeth
Division of Animal Science, College of Agriculture, University of Nevada, Reno, Nevada 89557

Boron is required by certain higher plants, but its role in animal systems has not been determined (NICHOLAS and EGAN 1975). A maximum safe level of 5 mg B/L in livestock drinking water has been proposed by the Environmental Protection Agency (1973). However, no maximum safe level has been set for public water supplies (EPA 1975). Boron can occur in surface waters due to industrial uses and negligible quantities are removed during sewage treatment (WAGGOT 1969). In a brief survey of waters in the Great Basin, GREEN and WEETH (1977) found concentrations of boron in a number of samples to exceed the safe maximum suggested for livestock by EPA (1973).

Recent work concerning the toxicology of boron in drinking water has been controversial. Workers in the USSR have suggested maximum safe concentrations as low as 0.5 mg B/L in drinking water (VERHITSKAYA 1975; BORISOV 1976; KRASOVSKII et al. 1976). MATTHEWS (1974) suggested a safe maximum of 20 mg B/L in drinking water for human consumption and GREEN and WEETH (1977) proposed a 40 mg/L maximum for livestock. DIXON et al. (1976) failed to show any biologically significant effects with 6.0 mg B/L in drinking water of rats, while much higher concentrations of boron in feed of laboratory rats have produced no toxic effects (WEIR and FISHER 1972).

Our purpose was to further evaluate boron toxicity in drinking water and investigate physiologic actions of boron at high concentrations. This study should further aid in establishing a defensible standard for boron in drinking water.

MATERIALS AND METHODS

Forty-five weanling (28 days old) Long-Evans hooded male rats were offered deionized drinking water, *ad libitum*, to which 0, 150, and 300 mg of boron per liter were added as $Na_2B_4O_7 \cdot 10H_2O$. Fifteen animals were placed randomly per treatment group. Experimental animals were housed in a controlled-environment animal room (12D:12L) for the 70-day treatment period. The rats were fed Purina Lab Chow *ad libitum* which contained approximately 54 micrograms of boron per gram of feed.

Animals were sacrificed before feeding at the end of the treatment period. Blood was drawn by cardiac puncture into heparinized

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syringes. A sample of blood was used to determine packed cell volume in microhematocrit tubes. Plasma was recovered from remaining blood and stored frozen for further analytical determinations. The following organs were removed, dissected free of extraneous tissue and weighed; adrenals (paired), spleen, kidneys (paired), seminal vessicles and coagulating glands (paired), testicles (paired), liver and right femur. The right femur was stored frozen for further analytical determinations. The testes and epididymus were examined for the presence of sperm, which was recorded as yes or no.

The following plasma analyses were made; triglycerides as described by FLETCHER (1968), urea nitrogen by the method of CROCKER (1967) and alkaline phosphatase as reported by SOMMER (1954). Plasma total protein concentrations were determined by refractometry (WEETH and SPETH 1968), while a vapor pressure osmometer was used to obtain osmotic pressures.

Wet weights of femurs were obtained at time of sacrifice. The femurs were then dried at 100°C for 24 hours, cooled to room temperature in a dessicator and weighed to obtain oven dry weight. Fat-free dry weight was obtained by extraction with petroleum ether for 72 hours in a soxhlet extractor. The femurs were again dried at 100°C and weighed.

For further chemical analyses, fat-free dry bones were ashed in an electric muffle furnace. The ash was weighed and suspended in five ml 6N HCL. This solution was brought to a 100 ml volume with distilled water. Following appropriate dilutions, phosphorus was determined by the method of FISKE and SUBBAROW (1925) and calcium by atomic absorption spectrophotometry.

Organ and body weight data were treated by analyses of covariance. This technique adjusts treatment means of the dependent variable for significant differences in the independent variable (organ weight to final body weight). Chi square was used to test for differences among treatments on the presence of spermatazoa. Plasma and bone data were treated by analyses of variance. Differences among treatment means were evaluated by use of Duncan's multiple range test. All statistical analyses were as described by STEEL and TORRIE (1960) and programmed by KOH (1973). Unless otherwise stated, all effects and differences noted in this study are significant at the 5% level of probability ($p<.05$).

RESULTS AND DISCUSSION

Laboratory rats drinking water containing boron at concentrations of 150 and 300 mg/L had body weights 7.8 and 19.8% less than the control group (Figure 1). This suggests a growth suppression of animals drinking water containing boron at concentrations of 150 mg/L or above. PFEIFFER et al. (1945) reported boron, as boric acid, in drinking water at 170 mg B/L did not affect rat growth. However, 440 mg B/L had an inhibitory effect after 20 to 30 days. WEIR and FISHER (1972) reported a 50% reduction in

growth and food utilization efficiency of rats when feed contained 1750 mg/kg of boron. No deleterious effects on growth or food utilization were detected at 520 mg B/kg of feed. Cattle were reported to have reduced feed consumption when drinking water contained 150 and 300 mg B/L (GREEN and WEETH 1977). In contrast, GREEN, et al. (1973) reported feed consumption of laboratory rats drinking water containing 300 mg B/L for 49 days was not significantly different from control animals while body weight gain was 21% less. Excessive boron ingestion reduces growth and is capable of causing a loss in body weight which may not be due entirely to reduced feed and water consumption.

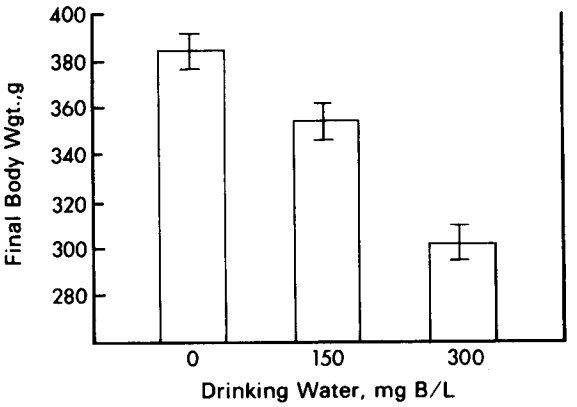

Figure 1. Effect of Boron in Drinking Water on Covariance Adjusted Final Body Weights of Rats. Fifteen Animals per Treatment.

When laboratory rats were offered drinking water containing 300 mg B/L they were overtly smaller in body size with long toenails, atrophic scrotal sacs and coarse pelages. WEIR and FISHER (1972) reported coarse hair coats, scaly tails, a hunched position, swelling and desquamation of the pads and paws, long toenails, small scrota, inflamed eyelids, and a bloody discharge around the eyelids of rats consuming boron at 1750 mg B/kg of feed for one year. GREEN et al. (1973) reported similar symptoms when rats consumed boron in drinking water at concentrations of 150 mg/L or greater. Cattle were reported to have erythematous skin around the dew claws and slight cases of diarrhea when drinking water contained 150 and 300 mg/L of boron (GREEN and WEETH 1977).

No differences among treatments were observed in the mean covariance weights of kidneys, liver or adrenals. The testes, seminal vesticles, spleen and right femur weights were significantly less with boron-drinking water treatments (Figures 2 and 3).

GREEN et al. (1973) reported no differences in adrenals, heart, and kidneys to body weight ratios in laboratory rats consuming boron in drinking water at 300 mg/L. However, they reported a reduction in testes and ovaries to body weight ratios of rats drinking boron in their water. WEIR and FISHER (1972) reported

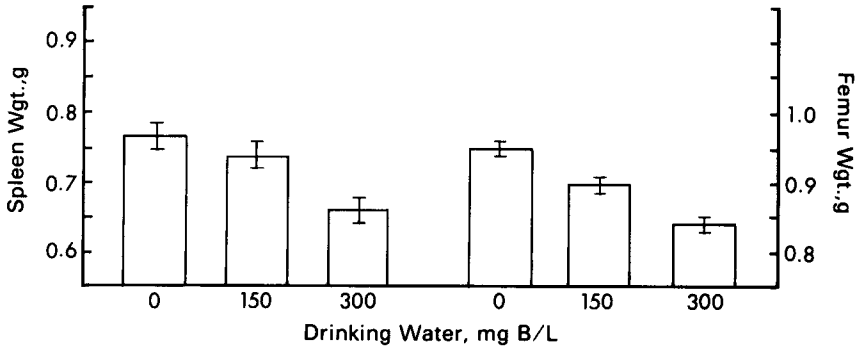

Figure 2 Effect of Boron in Drinking Water on Covariance Adjusted Spleen and Right Femur Weights. Fifteen Animals per Treatment.

that rats consuming feed with 1750 mg/kg of boron had larger organ to body weight ratios for brain, thyroids, adrenals and kidneys. Spleen and testes to body weight ratios were significantly less than controls. WEIR and FISHER (1972) saw no histopathology in any organs studied, except the testes.

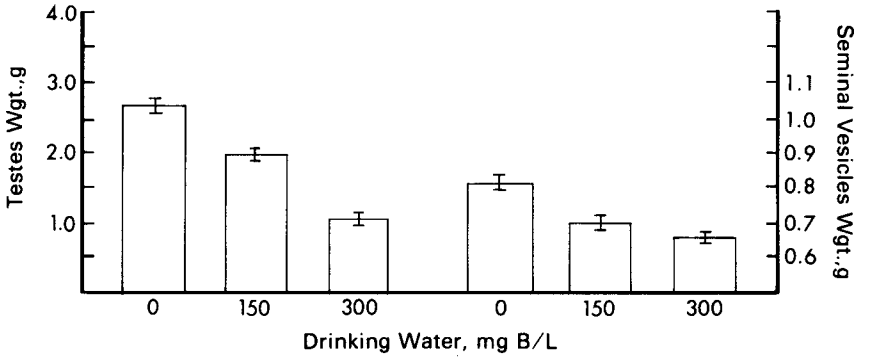

Figure 3. Effect of Boron in Drinking Water on Covariance Adjusted Testes and Seminal Vesicles Weights. Fifteen Animals per Treatment.

Boron at the highest concentration interferes with spermatogenesis. Only three out of fifteen animals had spermatazoa present in the group to which 300 mg B/L was added to the drinking water, a highly significant difference from the 0 mg/L and 150 mg/L treatment groups. GREEN et al. (1973) reported testicular atrophy with excessive boron intake with no recovery after 50 days of drinking water without boron. Electron microscopic studies by SILEVA et al. (1977) revealed destruction of the spermatogenic epithelium.

The seminal vesicles were also atrophic with boron-water treatments (Figure 3). Consequently, androgenic activity may have been reduced without subsequent maintenance of secondary sex character-

istics (TURNER and BARNARA 1976). However, GREEN et al. (1973) did not observe histologic change in interstitial tissue. They also saw no evidence of testicular effects in rats drinking 75 mg B/L.

Plasma parameters are presented in Table 1. Triglyceride concentrations were 14.0 and 31.2% lower than control animals when rats consumed 150 and 300 mg B/L, respectively. A low protein diet or an extended period of poor nutrition may cause low serum triglycerides (SEARCY 1969). Lipid transport in the blood is dependent upon lipo-protein complexes and as a consequence the concentration of circulating lipids is dependent in part upon plasma protein levels. Total protein concentration of plasma was 10.7% lower in rats drinking water with 300 mg B/L (Table 1). According to COLES (1974) hypoproteinemia is commonly associated with inadequate diet or poor absorption of materials from the intestinal tract. WEETH (1977) reported plasma total protein concentration of cattle was lower than that of controls when they consumed boron in drinking water at 120 mg/L. Inadequate protein intake, digestion or absorption may interfere with hemoglobin synthesis (COLES 1974). A slight anemia was indicated in animals drinking 300 mg B/L, whose hematocrit was 6.8% lower than controls (Table 1). In contrast, GREEN and WEETH (1977) reported no change in plasma protein concentration, but a lower hematocrit in cattle drinking water containing 150 and 300 mg B/L.

TABLE 1

Effects of Boron in Drinking Water on Plasma Composition of Male Laboratory Rats

	Drinking Water, mg B/L			
Item	0	150	300	SEM[a]
Triglycerides, mg/100 ml	95.7[b]	82.3[c]	65.3[d]	6.79
Urea Nitrogen, mg/100 ml	20.69[b]	19.40[b]	18.26[b]	1.178
Alkaline Phosphatase, IU/L	83.17[b]	82.13[b]	57.40[c]	5.042
Total Protein, g/100 ml	7.73[b]	7.57[b]	6.90[c]	0.106
Osmotic Pressure, mOsm/kg	333.44[b]	327.79[b]	322.03[c]	3.178
Hematocrit, %[e]	48.8[b]	47.8[b]	45.5[c]	0.61

[a]Standard error of the mean for all treatments, 15 per treatment.
[b,c,d]Means on same line followed by different superscripts differ at P<.05.
[e]Determined on whole blood by microhematocrit tube centrifugation.

Blood urea nitrogen (BUN) levels are dependent upon nitrogen metabolism and ability of the kidney to remove end products (MELBY and ALTMAN 1974). Urea nitrogen concentrations did not vary with any treatment (Table 1). This is in agreement with WEIR and FISHER (1972).

Plasma alkaline phosphatase activity was 31.0% lower in rats drinking water with 300 mg B/L (Table 1). Lower circulating levels may reflect a general arrest of growth, in particular some impairment of osteoblastic activity (SEARCY 1969). Plasma osmolality was also lower in rats consuming 300 mg/L of boron in their drinking water. Problems in the gastrointestinal tract or kidneys may cause changes in plasma osmolality (COLES 1974). GREEN and WEETH (1977) reported no changes of plasma osmotic pressure in cattle consuming water with 150 and 300 mg B/L.

Composition of the right femur was analyzed to determine any effects on calcified tissue. Percent fat was 53% lower in bones of rats drinking water containing 150 and 300 mg B/L (Table 2). A trend toward an increase in percent water as percent fat decreases in bone occurred, resulting in a trend toward ($r = -.2$) a negative correlation between the two. No change in the percent ash of bone (Table 2) occurred; however, calcium expressed as concentration in fat-free dry bone was 15.6% less than controls in animals consuming 300 mg B/L in their drinking water. The phosphorus concentrations were 10% ($P<.10$) less in the bones of rats consuming 300 mg B/L. The Ca:P ratio was unchanged (Table 2).

TABLE 2

Effects of Boron in Drinking Water on Composition of Right Femur of Male Laboratory Rats

	Drinking Water, mg B/L			
Item	0	150	300	SEM[a]
Percent Water	24.33[b]	26.08[b]	26.39[b]	0.871
Percent Fat[d]	1.56[b]	0.73[c]	0.72[c]	0.216
Percent Ash	68.94[b]	68.72[b]	68.48[b]	0.225
Calcium, mg/100 mg fat-free dry weight	22.48[b]	21.68[b]	18.96[c]	0.868
Phosphorus, mg/100 mg fat-free dry weight	10.76[b]	10.53[b]	9.69[b]	0.346
Calcium:Phosphorus	2.08[b]	2.06[b]	1.96[b]	0.045

[a]Standard error of the mean for all treatments, 15 per treatment.
[b,c]Means on same line followed by different superscripts differ at $P<.05$.
[d]Percent fat and ash are expressed as that of oven dry weight.

Intestinal malabsorption syndromes result in a loss of excessive foodstuffs in feces with a resultant systemic manifestation of nutritional defects which change the compositional nature of plasma (LATNER 1975). Plasma protein concentration was significantly lower along with triglyceride concentration and hematocrit, while the BUN was unchanged. This may indicate an interference with intestinal absorption. Intestinal malabsorption results primarily in decreased lipid absorption (LATNER 1975). Consequently, absorption of fat soluble vitamins may be decreased, specifically vitamin D. Percent fat and calcium concentration of bone were both lower in animals drinking boron in their water. The most apparent feature of intestinal malabsorption is reduced body weights seen in this study and others (PFEIFFER et al. 1945; WEIR and FISHER 1972; GREEN et al. 1973; GREEN and WEETH 1977).

Reported toxic concentrations vary considerably. A safe tolerance of boron has been suggested to be as low as 0.5 mg/L (VERHITSKAYA 1974; BORISOV 1976; KRASOVSKII et al. 1976), while much higher levels in feed and water have shown little or no effects (PFEIFFER et al. 1945; WEIR and FISHER 1972; GREEN et al. 1973; DIXON et al. 1976; GREEN and WEETH 1977; WEETH 1977). From this study it is apparent that 150 mg B/L in drinking water of rats is deleterious and the effects are more drastic but not lethal at 300 mg B/L. In general, the effects were reduced growth and suppressed germinal activity. Blood and plasma parameters studied suggest altered fat and protein metabolism with some hemodilution. A possible interference with bone calcium metabolism is indicated. The signs of boron toxicity appear to suggest the intestinal malabsorption syndrome described by LATNER (1975). The effect of boron on intestinal absorption has not been investigated. Further studies are clearly indicated.

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