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TOXIC EFFECTS OF BORON ON MALLARD REPRODUCTION

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Abstract—Boron, a naturally occurring trace element generally considered environmentally innocuous, was documented to severely impair mallard reproduction. Boron is leached from irrigated agricultural soils and transported in drainage water that contaminates wetlands. Until now, only the selenium accumulated in aquatic food chains has been documented to pose a toxic hazard to wildlife in drainage water wetlands. Adult mallard (*Anas platyrhynchos*) ducks were fed diets supplemented with 0, 30, 300 or 1,000 ppm boron (fresh weight; diets contained about 10% moisture). The hatching success of fertile eggs was significantly reduced by 1,000 ppm boron, less than one-third the highest boron concentrations found in plants in California's San Joaquin Valley. Hatching weights, duckling survival and duckling weight gain were also reduced by 1,000 ppm boron. Boron concentrations in mallard egg, liver and brain tissues were dose-related. Boron did not affect adult survival or egg fertility. Management of drainage water-contaminated environments must now also consider the adverse effects of boron, as well as the possible interactions of drainage water contaminants.

Keywords—Boron Mallard Reproduction Boric acid Agricultural drainage water Embryo survival

INTRODUCTION

Millions of hectares of arid and semi-arid lands are irrigated each year for agriculture. Naturally high concentrations of salts and trace elements, combined with poor drainage conditions, have necessitated the construction of elaborate drainage systems such as those in the San Joaquin Valley of California and other areas of the western United States [1]. These agricultural drainage waters may transport salts and trace elements to wetlands used by fish and wildlife species [1,2].

The Kesterson Reservoir, located in the Kesterson National Wildlife Refuge in Merced County, California, received irrigation drainage water containing high concentrations of selenium [2,3]. Selenium contamination of the aquatic food chains of fish and wildlife was found at Kesterson, and mortality and reproductive failure was observed in fish and wildlife inhabiting the reservoir [2,4]. Concentrations of selenium in food organisms at Kesterson were sufficiently high to produce death and developmental malformation of avian embryos, as confirmed by field sampling [2,5] and laboratory experimentation [6].

Aquatic food organisms sampled at Kesterson also contained very high concentrations of boron [2,7], another naturally occurring trace element found in soils of the western United States [8]. Boron leached from agricultural soils and transported to Kesterson Reservoir in drainage water had accumulated in aquatic plants used by waterfowl as food [2,7]. Vegetative material including seeds, rhizomes and whole plants collected at Kesterson contained boron residues [7]. The lowest mean value for any plant group analyzed was 38.9 ppm boron in cattail seeds. The highest mean value was 1,860 ppm boron in submerged aquatic vegetation seeds [7]. Eighteen different plant groups and matrices were analyzed and the minimum and maximum values were 21 and 3,500 ppm boron, respectively [7]. High levels of boron in irrigation water in the San Joaquin Valley were first reported in 1928 [9], and boron concentrations exceeding 2,000 ppm (dry weight) were quantified in plants more than 50 years ago [10].

Toxicological interpretation of the high environmental levels of boron found in aquatic food chains at the Kesterson Reservoir was difficult because of the paucity of information about the potential effects of boron on birds [2]. Studies of the effects of boron on mammals have shown reduced fertility in male rats [11]. Injection of boron compounds into chicken eggs produced teratogenic effects and embryo death [12,13]. Laying hens on a

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diet containing 0.5% boric acid stopped laying eggs; however, the hatching success of the eggs produced while on the boron-treated diet was not determined [14].

The high concentrations of boron in aquatic plants at Kesterson Reservoir, and possibly in other wetlands receiving agricultural drainage water, suggested the need for studies to assess the potential effects of dietary boron on avian reproduction. This article reports the results of a study designed to determine the effects of boron on mallard reproduction and to identify a dietary concentration that will produce an adverse effect in that species.

METHODS

Mallards obtained from a commercial game farm were randomly assigned to groups to receive one of four different diets. Each group was initially housed, separately by sex, in 3.5 × 4.5 m buildings. Females were kept on an 8-h light:16-h dark photoperiod to prevent egg laying. After a 2-week acclimation period, both sexes were switched from untreated diets to experimental diets. These diets were prepared with duck developer mash supplemented with powdered boric acid to achieve added boron concentrations of 0 (controls), 30, 300 and 1,000 ppm. Fifteen birds of each sex received either the 30, 300 or 1,000 ppm boron diet, and 25 pairs received an unsupplemented control diet. More pairs were allocated to the control group to optimize comparisons between controls and each treatment group using Dunnett's procedure [15].

After receiving the experimental diets for three weeks, males and females were paired, by treatment group, and each pair was randomly assigned to a 4.6 × 4.6 m outdoor breeding pen. Each pen had a continuous water supply, natural cover and a nest box. After birds were paired and moved to the breeding pens, the diets were mixed using duck breeder mash.

Mallard diets were mixed at approximately two-week intervals in 20-kg batches. The moisture content of the duck mash ranged from 9.7 to 11.8%. Therefore, fresh-weight concentrations of boron in the diet were nearly equal to the dry-weight concentrations. Samples of the diets were taken from each batch of feed mixed, and 24 of these samples were randomly selected for boron residue analysis. The geometric mean boron concentrations determined for the control (unsupplemented), 30, 300 and 1,000 ppm diets were 8, 35, 288 and 1,000 ppm on a dry-weight basis, respectively.

Female mallards incubated their eggs and each clutch contained 20 eggs or fewer. Each egg was numbered sequentially in the nest until incubation began, and in nests where the clutch size exceeded 20 eggs, the lowest numbered eggs were removed. By regulating the clutch size using this method, hens did not incubate the atypically large clutches that are sometimes laid by commercial-stock mallards. The eighth egg laid by each female was collected for boron analysis. Body weights of mallards were measured when treatment began, when males and females were paired and when they were killed.

Eggs were candled at 14 d of incubation to determine fertility and embryo viability. Males were removed from the pens at the time of candling unless the initial clutch of eggs was infertile. In that case, the male remained with the hen and a second nesting attempt was permitted after the removal of the infertile eggs. Ducklings were weighed at hatch, 7, 14 and 21 d and received the same experimental diet as the adults from that pen. Adults and young from the same pen were killed using carbon dioxide when the young reached 21 d of age.

All adults and one randomly selected duckling from each brood were necropsied and examined for macroscopic pathological changes in organs. Samples of brain, liver, kidney and heart were preserved in neutralized buffered formalin and later examined for histopathological lesions. Egg measurements were made prior to opening, eggshell thicknesses were measured with a micrometer and eggshell weights were taken after drying the shells for at least one month. The index of eggshell quality was determined according to the methods of Ratcliffe [16].

In each experimental group, 10 adult pairs and one of each pair's ducklings were randomly selected for boron analysis of tissues. Five randomly selected adult pairs and one of each pair's ducklings were chosen from the control group for liver and brain boron residue analysis.

Samples of feed, liver tissue and eggs were stored frozen until the end of the study and then sent to the Environmental Trace Substances Research Center in Columbia, Missouri, for boron analysis. Samples were homogenized, lyophilized and percent moistures determined. Moisture was determined by placing a weighed aliquot of the sample in a Fisher Isotemp oven and drying at 103 to 105°C. Approximately 0.5 g of sample was weighed into a freshly cleaned 100-ml quartz Kjeldahl flask. Slowly, 15 ml of concentrated sub-boiled nitric acid and 2.5 ml of concentrated sub-boiled perchloric acid were added. After the initial reaction had subsided, the sample was placed on low heat until the evolution of dark red fumes had ceased. Gradually,

the heat was increased until the nitric acid began refluxing, and the samples were allowed to reflux overnight. After the refluxing, the heat was gradually increased until the nitric acid was driven off, and the reaction with perchloric acid had occurred. Two milliliters of concentrated sub-boiled hydrochloric acid were added, heated to boil, removed from the heat and 5 to 10 ml of deionized water added. Samples were allowed to cool, diluted using deionized water in a 50-ml volumetric flask and transferred to a clean polyethylene bottle.

Boron concentrations were determined by inductively coupled plasma emission spectroscopy using a Jarrell-Ash Model 1100 Mark III with 40 analytical channels, controlled by a Digital 11/23+ computer. The instrument was standardized with a series of seven standards containing 36 elements. After the standardization, the detection limit was determined by taking 10 integrations of the zero standard; three times the standard deviation of the mean was then used as the detection limit. Instrumental quality control samples were then analyzed to check the operation. Standards were run every 10 to 15 samples to check for drift. If the drift was more than 5%, the instrument was restandardized. After the analysis was completed, the data were transferred to the Perkin-Elmer LIMS 2000 computer for calculation. Boron tissue concentrations are expressed as micrograms boron per gram of sample on a dry-weight basis.

Boron concentrations reported by the analytical laboratory as below the limit of quantification were assigned the value of one-half of the lower limit of detection for the particular matrix analyzed. All boron residues were subjected to a log transformation prior to analysis of variance and Tukey's multiple comparison procedure, if the F value was significant at $p < 0.05$ [17]. Percentage data were subjected to an angular transformation prior to statistical analyses [17].

RESULTS

The hatching success of fertile eggs laid by mallards fed 1,000 ppm boron was reduced to about 52% of the control value (Table 1). The hatching success of fertile eggs for mallards fed diets supplemented with 30 or 300 ppm boron did not differ from that of the control group (Table 1). Fertility of eggs, eggshell thicknesses and the Ratcliffe index of eggshell quality were not affected by any of the dietary boron treatments. Boron supplementation did not delay the time when hens began laying after receiving treated diets, nor did it affect the length of the incubation period. Embryo mortality during the first 14 d of incubation, approximately the first half of the incubation period, did not differ between controls and any of the boron treatment groups; however, embryo mortality in the 1,000 ppm treatment group during the entire incubation period was significantly higher than in the other groups (Table 1). Mortality of embryos produced by mallards fed the 1,000 ppm boron diet was higher during the second half of incubation, compared with controls (Table 1). Of the embryos that failed to hatch and of those that did hatch, only two had developmental malformations and both died prior to hatching. These two embryos included one from the 1,000 ppm group and one from the control group.

Body weights of hatchlings were lower in both the high-boron treatment group (1,000 ppm) and the 300 ppm group, compared with controls (Table 2). The mean weight gain over 21 d for all ducklings fed boron diets was lower than the weight gain for controls (Table 2).

Weekly mortality rates were measured for ducklings from the time of hatch until three weeks of age. Ducklings fed the 1,000 ppm boron diet had higher mortality rates during their first week of life than did controls (Table 3). The mortality rate of this cohort declined during the next two weeks and did not differ from controls after the second week (Table 3). The total number of ducklings produced per female (incubating a fertile clutch) was also significantly reduced in the 1,000 ppm boron treatment group (Table 3).

Boron did not produce any overt signs of toxicosis in either adult or young mallards, and none of the adult mallards on treatment died of boron toxicosis. Measurements of adult body weights dur-

Table 1. Reproductive parameters (mean ± SD) for mallards fed a control (0 ppm) or boron-supplemented diet

Boron added to diet (ppm)	N	Hatching success in fertile eggs (%)	Embryo mortality (%) Day 1 to day 14	Embryo mortality (%) Day 15 to hatch
0	23	88.5 ± 17.4	5.2 ± 12.8	6.7 ± 11.0
30	14	89.9 ± 18.7	5.3 ± 17.8	4.8 ± 8.9
300	11	93.2 ± 7.1	0.6 ± 2.0	6.2 ± 6.8
1,000	15	46.3 ± 36.7[a]	17.4 ± 27.0	50.2 ± 34.7[a]

Statistical analyses were performed on data subjected to an angular transformation. Sample sizes (N) are the number of breeding pairs incubating fertile clutches.
[a]Significantly different from controls (0 ppm) at $p \leq 0.05$ using Dunnett's procedure [15].

ing three time periods allowed for comparisons of weight change among experimental groups. Weight changes among males were not significant (Table 4). Body weight changes among female treatment groups were significantly different only between two of the three times examined, the onset of treatment and the time when males and females were paired (Table 4). Females fed the 1,000 ppm boron diet lost more weight during this one-month period before reproduction began. However, by the end of the reproductive cycle, body weights of female mallards fed the high-boron diet did not differ from controls. In a 5-d feeding trial prior to this study, one pair of mallards was each fed a diet supplemented with 0, 300 or 1,000 ppm boron. Each pair fed these respective diets consumed an average of 222, 184 or 209 g food/bird per day.

Adults and young mallards were necropsied and examined for gross pathological changes; tissues were taken for histopathology. Of the four organs sampled for histopathology, none had histopathological lesions or abnormalities that were related to boron treatment. Mallard duckling body weights at 21 d of age were significantly lower in both the 30 and 1,000 ppm treatment groups, compared with controls (Table 5). Liver and brain weights were also significantly lower for 21-d-old ducklings fed 1,000 ppm boron (Table 5); however, the mean brain-to-body-weight ratio for this group was significantly higher, compared with controls, suggesting that the relative size of the body was smaller for ducklings in this cohort (Table 5).

The mean percent moistures for brain tissues of adult and young mallards were 79.2 and 80.3%, respectively. Liver moisture content averaged 72.0 and 73.8% for adults and young, respectively. Differences among mean boron concentrations in eggs produced from the different groups were determined using analysis of variance and increased from below the lower analytical detection limit to 49 μg/g on a dry-weight basis (Table 6). The maximum boron concentration quantified in an egg was 81 μg/g (dry weight). Boron concentrations in liver and brain tissue did not differ between males and females, and sexes were combined in the statistical analyses. Boron levels in livers of both adult

Table 2. Hatching weights and weight gain (mean ± SD) for mallard ducklings fed a control (0 ppm) or boron-supplemented diet

Boron added to diet (ppm)	Hatching weight (g)	Weight gain (g)		
		Hatch to day 7	Day 8 to day 14	Day 15 to day 21
0	36.2 ± 0.7 (23)	52.8 ± 2.4 (22)	125.4 ± 5.7 (22)	163.0 ± 8.8 (23)
30	37.3 ± 1.1 (13)	37.6 ± 2.4 (11)[a]	114.5 ± 5.6 (11)[a]	149.9 ± 8.9 (12)[a]
300	34.9 ± 0.9 (11)[a]	44.0 ± 2.4 (10)[a]	105.5 ± 3.3 (10)[a]	140.9 ± 4.0 (11)[a]
1,000	31.3 ± 1.3 (12)[a]	26.1 ± 2.9 (10)[a]	76.4 ± 5.8 (10)[a]	122.6 ± 8.2 (10)[a]

Sample sizes are given in parentheses and changes in sample sizes represent mortality or missing data.
[a]Significantly different from controls (0 ppm) at $p \leq 0.05$ using Dunnett's procedure [15].

Table 3. Weekly duckling mortality rates (%) and mean numbers of ducklings produced per female for mallards fed a control (0 ppm) or boron-supplemented diet

Boron added to diet (ppm)	Duckling mortality (%)				Productivity per female	
	N	Hatch to day 7	Day 8 to day 14	Day 15 to day 21	N	Mean no. of 21-d-old ducklings
0	23	11.6 ± 22.1	8.1 ± 18.8	3.3 ± 11.0	23	8.61 ± 5.06
30	13	8.3 ± 13.1[a]	4.9 ± 8.9	7.0 ± 25.2	14	10.00 ± 4.66
300	11	9.6 ± 16.2	5.0 ± 7.9	5.2 ± 15.0	11	10.09 ± 3.67
1,000	10	21.0 ± 21.5[b]	12.3 ± 18.1	5.7 ± 15.7	15	3.47 ± 3.78[b]

Ducklings were from clutches produced by pairs fed the same respective diets. Mean values (±SD) with sample sizes (N) represent the numbers of breeding pairs that produced ducklings or incubated fertile clutches. Females laying infertile clutches were excluded from the analysis.
[a]$N = 14$, all ducklings from one pair did not survive beyond day 7.
[b]Significantly different from controls (0 ppm) at $p \leq 0.05$ using Dunnett's procedure [15].

and 21-d-old mallards were greater for the 300 and 1,000 ppm treatment groups than for mallards fed 30 ppm boron or controls (Table 6). Boron residues in brain tissue of young and adult mallards increased with increasing dietary concentrations and were statistically higher with each dietary concentration administered (Table 6). The highest concentration determined for any of the tissues analyzed was 100 μg/g (dry weight) for one duckling brain sample (Table 6). Concentration ranges for brain and liver tissues from mallards fed the 1,000 ppm boron diet overlapped with those resulting from the 300 ppm dietary exposure; however, mean values for all tissues analyzed from the 30, 300 and 1,000 ppm groups were significantly different (Table 6).

DISCUSSION

Boron is widespread in nature and the natural boron content of soils is usually between 10 and 300 ppm, depending on a soil's origin [18]. Borate mineral deposits that are economically feasible to mine are generally derived from lacustrine sediments or rocks in arid regions [8]. Borate deposits of economic interest have been identified in Asia, Europe, South America and the arid and semi-arid regions of the western United States [8]. Moreover, like selenium, boron has also been identified as an important constituent of coal ash, occurring at concentrations of up to 1,900 ppm [19]. The geologic setting associated with high soil boron levels in the United States is one that is also commonly associated with agricultural conditions that require irrigation [18], and boron in irrigation water has been a long-standing problem for agriculture [10,18]. Moreover, the uptake and accumulation of boron by plants can result in extremely high boron levels [10,20,21], including those plants used by waterfowl for food [1,7,20].

Boron has long been recognized as a micronutrient essential for normal plant growth [18,22].

Table 4. Weight changes in adult mallards fed a control diet (0 ppm) or a boron-supplemented diet

Boron added to diet (ppm)	N	Mean weight (g) ± 1 SD					
		Males			Females		
		Onset of treatment[a]	Time at pairing[b]	Time at sacrifice[c]	Onset of treatment	Time at pairing	Time at sacrifice
0	25	1227 ± 122	1180 ± 115	1161 ± 124	1076 ± 118	1010 ± 104[d]	1016 ± 131
30	15	1230 ± 126	1170 ± 127	1139 ± 94	1086 ± 98	1060 ± 82	1036 ± 138
300	15	1275 ± 140	1223 ± 139	1165 ± 117	1010 ± 114	1023 ± 104	1021 ± 149
1,000	15	1282 ± 132	1191 ± 121	1092 ± 104	1075 ± 105	991 ± 101	1020 ± 140

[a]4 March 1986, placed on treated diets 2 d later.
[b]4 April 1986, males and females paired and transferred to outdoor breeding pens.
[c]Both males and females were killed when duckling broods were 21 d old.
[d]Adult female weight changes between onset of treatment and time of pairing were significantly different ($p = 0.0001$) among groups. Females on 300 ppm diet gained more weight than did controls or those on 1,000 ppm diet (both lost weight). Females on 1,000 ppm lost significantly more weight than did those on 30 ppm (one-way analysis of variance, Tukey's range test).

Table 5. Body and organ weights (mean ± SD) of 21-d-old mallard ducklings fed a control (0 ppm) or boron-supplemented diet

Boron added to diet (ppm)	N	Body weight (g)	Liver weight (g)	Brain weight (g)	Liver/body weight ratio	Brain/body weight ratio
0	23	386.3 ± 46.8	20.50 ± 3.56	2.98 ± 0.20	0.053 ± 0.007	0.008 ± 0.001
30	13	340.7 ± 52.1[a]	18.95 ± 3.58	2.89 ± 0.27	0.056 ± 0.005	0.009 ± 0.001
300	11	347.4 ± 22.8	18.61 ± 4.11	3.14 ± 0.58	0.054 ± 0.012	0.009 ± 0.001
1,000	10	255.3 ± 80.5[a]	13.25 ± 4.18[a]	2.43 ± 0.47[a]	0.052 ± 0.005	0.010 ± 0.003[a]

Each duckling was randomly selected from a brood produced by a female fed the same respective diet. Sample sizes (N) are the numbers of breeding pairs incubating fertile clutches.
[a]Significantly different from controls (0 ppm) at $p \leq 0.05$ using Dunnett's procedure [15].

Table 6. Geometric mean concentrations (μg/g, dry weight) of boron in eggs and tissues of mallards fed a control or boron-supplemented diet

Boron added to diet (ppm)	Boron concentration (μg/g, dry weight)					
	Egg	Liver			Brain	
		Adult	Duckling	Adult	Duckling
0	NQ[a] (NQ; $n = 5$) A	NQ (NQ; $n = 10$) A	NQ (NQ; $n = 5$) A	NQ (NQ; $n = 10$) A	NQ (NQ; 4; $n = 5$) A
30	3 (NQ–5; $n = 15$) B	NQ (NQ–4; $n = 20$) A	3 (NQ–5; $n = 13$) A	NQ (NQ–6; $n = 20$) B	4 (NQ–7; $n = 13$) B
300	13 (5–21; $n = 14$) C	15 (7–24; $n = 20$) B	17 (5–36; $n = 11$) B	14 (5–24; $n = 20$) C	19 (6–44; $n = 11$) C
1,000	49 (26–81; $n = 14$) D	33 (6–74; $n = 20$) C	51 (23–89; $n = 10$) C	41 (9–89; $n = 20$) D	66 (27–110; $n = 10$) D

Concentration ranges and sample sizes (n) are given in parentheses. Means sharing the same letter within each column are not statistically different at $p \leq 0.05$ using a one-way analysis of variance (on log-transformed data) with Tukey's multiple comparison procedure ($p \leq 0.05$). Boron concentrations in livers and brains of adult ducks were not significantly different ($p > 0.05$) between males and females.

[a]Concentrations reported as not quantifiable (NQ) were assigned a value equal to one-half the lower limit of quantification (3–4 μg/g dry weight, depending on sample matrix) for statistical analyses.

However, its role in animal nutrition, if any, remains unknown [22]. Boron is usually considered relatively innocuous from a toxicological perspective, and both boric acid and borax have very high LD50 values in laboratory rats, suggesting low acute mammalian toxicity [18]. In addition to acute toxicity studies, some data are available for chronic effects in rats and domestic birds. Male rats fed diets containing 1,000 ppm boron or more had reduced fertility [11] and egg injection studies done with chickens produced teratogenic effects and embryo death. The LC50 values for borax and boric acid injected directly into eggs were 0.5 and 1.0 ppm, respectively [13]. Injection of boric acid into chicken eggs has caused facial malformations and deformed extremities of embryos [12]. Laying hens placed on a diet supplemented with 0.5% boric acid produced eggs that showed a marked increase in boron by the second day of treatment; however, all birds stopped laying after 6 d [14]. Although these studies showed that development and survival of chicken embryos are affected when boron compounds are injected into the egg, the potential embryotoxic effects resulting from a dietary exposure to boron were not known.

During the course of this study, adult body weights did not change significantly for males; only female weights measured between the time treatment started and the time sexes were paired (1 month) changed significantly. Females fed the 1,000 ppm boron diet did enter the reproductive season at a mean body weight lower than other groups; however, this weight was regained during the reproductive season (Table 4). Moreover, preliminary feeding trials over a 5-d period did not suggest strong aversion to boron-treated diets. Although female mallards fed 1,000 ppm boron did enter the reproductive season at a lower body weight than did other experimental groups, the number of eggs laid, the fertility of eggs and the size of individual eggs were not affected by boron treatment. These data indicate that the adverse effects of boron were at the level of the egg, and that boron present in high concentrations in the egg increased embryo mortality.

The results of the present study indicate that boron levels in aquatic plants and plant seeds at the Kesterson Reservoir were greater than the dietary concentration required to significantly impair reproduction in mallards. Hatching success of fertile eggs, body weights of ducklings at hatch and survival of ducklings from hatching to day 7 were all significantly reduced when breeding adults and their offspring were maintained on a diet supple-

mented with 1,000 ppm boron. Moreover, the weight gain of ducklings from the time of hatch through 21 d of age was reduced by all dietary concentrations of boron when compared with controls. Mean brain-to-body-weight ratios were higher for ducklings exposed to 1,000 ppm boron, compared with controls, also suggesting that growth and development were adversely affected by boron treatment. However, histological evaluations of liver, brain, kidney and spleen showed no pathologies that were related to boron exposure. Mallards fed concentrations of up to 2,000 ppm boron did not exhibit any histological pathologies. These data suggest that histology may not prove to be an adequate means of assessing boron exposure or toxicosis in mallard ducks. Moreover, because no adults died as the result of dietary boron treatment, it appears that embryos and hatchlings are the most sensitive mallard life stages to boron toxicosis.

Boron levels in egg, liver and brain tissue increased as dietary concentrations of boron increased; however, these tissues contained residues that were at least one order of magnitude lower than the dietary concentration administered. Boron in brain tissue increased significantly with each greater dietary level; however, the 30 ppm dietary boron treatment did not result in liver concentrations that were different from controls.

Mallards fed diets supplemented with 1,000 ppm boron had markedly impaired embryo survival, although the teratogenic effects previously described in boron egg-injection studies were not observed in the present study. Boron is known to affect many biologically important reactions [12] and can competitively inhibit several dehydrogenases [23]. Moreover, boron has an affinity for binding to polyhydroxy compounds, such as riboflavin, and developmental malformations produced by egg injection resemble those associated with riboflavin deficiency [12]. Riboflavin supplementation was effective in reducing boric-acid-induced malformations in chick embryos and the mechanism of teratogenicity was believed to be boron complexation with riboflavin-containing enzymes [12]. Studies done in vitro have confirmed the boron-riboflavin complex formation [24], and chicks given riboflavin in excess of their dietary requirement were shown to have a lower incidence of boric acid toxicity [25].

Results of biochemical studies of the effects of boron suggest that the embryo mortality observed in mallards fed boron-supplemented diets may have been caused by the propensity for boron to complex with riboflavin or other coenzymes critical for normal cellular respiration. Mallard embryo mortality was greatest during the second half of incubation when energy demands for embryonic growth were great. Further studies of the biochemical changes associated with boron-induced embryo toxicity are needed to elucidate the specific toxic mechanisms.

CONCLUSIONS

In the past, high concentrations of boron in agricultural irrigation and drainage water have created concern because of the known phytotoxic effects of boron on crops. Recent studies that have sampled wildlife food chain organisms in wetlands supplied with agricultural drainage water have found extremely high concentrations of boron in certain plants. These concentrations exceeded the 1,000 ppm dietary level found in this study to significantly impair mallard reproduction. Although earlier studies of the effects of boron on avian embryos using egg injection techniques showed teratogenic effects, mallard embryo deformities caused by boron were not observed during this study. Unlike selenium, which causes a variety of embryo malformations that have been observed in both field and laboratory studies, boron appears to have the potential to kill embryos during development without causing deformities. This suggests that boron-induced reproductive failure in wild populations may be more difficult to detect than problems caused by selenium. Cleanup or treatment of wetlands supplied with agricultural drainage water has been focused primarily on reducing the environmental hazard posed by selenium. The results of this study indicate that strategies to solve problems caused by contamination of wetlands with drainage water constituents should also consider boron contamination of wildlife food chains.

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