Environmental Toxicology and Chemistry, Vol. 10, pp. 911-916, 1991
Printed in the USA. Pergamon Press plc

0473

0730-7268/91 $3.00 + .00

Ref

EFFECTS OF DIETARY BORON AND ARSENIC ON THE BEHAVIOR OF MALLARD DUCKLINGS

MOLLY R. WHITWORTH,* GREY W. PENDLETON, DAVID J. HOFFMAN and MICHAEL B. CAMARDESE
U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20708

(*Received* 1 *June* 1990; *Accepted* 17 *October* 1990)

Abstract — High concentrations of boron and arsenic have been associated with irrigation drain water and aquatic plants consumed by waterfowl. Both compounds affect the central nervous system and cause generalized physiological distress in mammals and waterfowl. We examined sublethal effects of boron and arsenic on the behavior of developing mallard ducklings (*Anas platyrhynchos*). Day-old ducklings received an untreated diet (control) or a diet containing 100, 400, or 1,600 ppm boron, added as boric acid, or 30, 100, or 300 ppm arsenic, added as sodium arsenate. Activity schedules and behavior durations were analyzed for effects at the various treatment levels. Both boron and arsenic at the highest levels had significant effects on the activity schedules of developing ducklings, including increased time at rest and under the provided heat lamp. We also observed decreases in the amount of time treated ducklings spent in alert behaviors and in the water in comparison to control ducklings. High levels of boron (1,600 ppm) increased feeding time overall but did not increase the amount of food consumed. Arsenic had no effect on feeding behavior. There were no differences found in the durations of behaviors as a result of treatment. These findings, in combination with reported effects on the growth and physiology of ducklings under identical treatments, suggest that reported concentrations of these compounds in aquatic plants in the Central Valley of California could adversely affect normal duckling development and survival.

Keywords — Boron Arsenic *Anas platyrhynchos* Mallard Duckling Behavior

INTRODUCTION

Boron, arsenic and selenium have attracted attention in recent years due to potentially hazardous effects on wildlife exposed to them as constituents of irrigation drain water. Boron and arsenic become biologically available to waterfowl from soil leachate as a consequence of irrigation. Both boron and arsenic compounds have been used as growth additives in poultry feed, thus more data are available on their uptake, excretion and accumulation characteristics in birds than on their relative toxicity. Samples of aquatic plants in waste irrigation waters at several locations in the Central Valley of California have exhibited high concentrations of boron (wigeongrass [*Ruppia maritima*], mean of 371 ppm dry weight; grass seeds, mean of 1,860 ppm dry weight; algae, mean of 501 ppm dry weight) [1]. Boric acid, a common form of boron readily absorbed by plant tissues, has a relatively low toxicity; however, it has been found to accumulate in the brain, spinal cord and liver after ingestion [2] and has been associated with central nervous system excitation, ataxia, depression and convulsions. Boric acid in the diet has caused growth retardation in mammals [2]. The LD50 value for boric acid in rats has been reported from 3,160 to 5,140 mg/kg [3,4]. Doses of 1,000 and 2,000 ppm boron added to the diet of male rats resulted in germinal aplasia with associated reduction of enzymatic activities (hyaluronidase, sorbitol dehydrogenase and lactate dehydrogenase isozyme-x); 500 ppm had no effect [5]. Decreased hatching success and duckling survival have been demonstrated for adult female mallards given 1,000 ppm dietary boron [6]. Decreased growth and physiological effects, including altered concentrations of hepatic glutathione, plasma triglycerides and hematocrit values, have been found in mallard ducklings at 100 ppm [7].

Arsenic has been detected in wigeongrass from waste irrigation water at 430 ppm and is known to accumulate to high levels as sodium arsenate in plant roots and tubers [8]. Several comprehensive volumes on the action and toxicity of arsenic are available [8-11]. Sodium arsenate is the most prev-

*To whom correspondence may be addressed.
The current address of M.R. Whitworth is Water Policy Branch, PM-221, Office of Policy Analysis, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460.

9534
CTR POP HEALTH
PAGE 07

alent environmental form of arsenic but is not as toxic as the trivalent arsenite form. The LD50 of sodium arsenite in mallard hens is 323 mg/kg [12]. Symptoms of trivalent sodium arsenite poisoning in birds include muscular incoordination, slowness, fluffed feathers, huddled appearance, immobility and seizures. Chronic sublethal exposure is associated with weakness, paralysis and decreased growth, but rarely results in death because of rapid detoxification and excretion [9]. Sodium arsenate in the diet of ducklings decreased growth and affected blood, liver and brain biochemistry [13]. Teratological effects, including exencephaly, were caused by low levels of sodium arsenate in mice exposed in early gestation [14–16].

Our study was part of a larger effort to examine sublethal effects of boron and arsenic on mallard duckling growth and physiology. Dosages were selected to cover the range known to occur in irrigated drainwater environments [1] (David Gilmer, U.S. Fish and Wildlife Service, personal communication). In an effort to assess potential behavioral effects resulting from drainwater exposure, we examined time budgets and daily activity patterns of ducklings consuming a range of dietary levels of sodium arsenate or boric acid from age of hatching to nine weeks.

METHODS

Ducklings and treatments

One-day-old mallard ducklings obtained from Oak Ridge Game Farm, Gravette, Arkansas, were assigned randomly to seven treatment groups: 100, 400 and 1,600 ppm boron; 30, 100 and 300 ppm arsenic; and controls. Three replicates for each treatment group resulted in a total of 21 pens. Ten birds per pen were maintained for a nine-week period on treatment; for behavioral observation, four randomly selected birds per pen were color-marked with lightweight plastic tags adhered to feathers at the base of the neck. Boric acid (99% pure) or sodium arsenate (98% pure) (both from Fisher Scientific, Pittsburgh, PA) were mixed directly or in 2% water, respectively, into commercial duck starter mash (Beacon Milling Co., Cayuga, NY) to obtain the specified boron and arsenic equivalents. The duck starter mash contained 22% protein, 3% crude fat and 4% crude fiber. Moisture content ranged from 12.1 to 14.2%. Dietary exposure started as soon as the ducklings were placed into the pens; food was available on a continuous basis. Feed was mixed on a weekly basis, and samples of the diets were taken from each batch of feed to assure correct concentrations. Pen size was approximately 1.5 m × 3 m and contained a trough of flowing water (0.3 m × 1.5 m) at one end. These pens were in partially enclosed buildings and received the natural photoperiod during the spring and summer. Heat lamps were provided throughout the study period.

Behavioral observations and analyses

Quantitative data on seven behavioral activities (resting, feeding, bathing, preening, standing alert, moving and drinking) were recorded from day 1 of the experiment (duckling age, 2 d) on the four color-marked ducklings in each pen, using a portable microcomputer. The observation order of the pens was randomized. Observations were made through specially designed doors allowing full view of the pen but obscuring the observer. Observers were blind to dietary treatment throughout the study. Each of the four marked ducklings per pen was observed for five continuous minutes, resulting in one 20-min observation period per pen per day. During this time, the seven behaviors and corresponding pen locations of the focal animal were recorded. At the end of each 5-min interval, instantaneous data on activity and duckling location within the pen were collected for all four marked ducklings for each observation period. The computer was programmed to select the sequence of data collection randomly across all four ducklings. All data were collected between 0800 and 1600 h, and observation periods were evenly distributed across all weeks for a total of 187 h.

The seven behavioral activities were summarized in two ways. The average duration of each observed activity was determined by combining the continuously collected data from all sample birds within a pen and sampling period and dividing by the number of times the activity was observed. Similarly, the instantaneously collected data from each behavior were also combined within each pen and divided by 20, the number of instantaneous observations in a sample, to yield an estimate of the proportion of time spent in each activity. Location within the pen, including proximity to the heat lamp, was simultaneously recorded with each instantaneous activity record.

Preliminary analyses indicated that the durations and proportions of time in each activity changed during the study (Fig. 1). The study period was then divided into three equal time periods (period 1 = weeks 1–3; period 2 = weeks 4–6; period 3 = weeks 7–9). The time period was included in subsequent analyses. For each behavior, the duration and percentage of observations were compared with a two-way analysis of variance (ANOVA) having main effects of dietary treatment and time

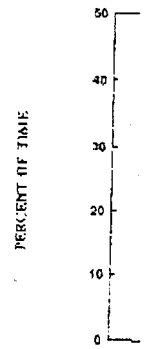

Fig. 1. Time bi
lings for period
1 to 3; period 2
9. Points are ba
resent means (/

period. Tuke
used to sepa
ANOVA indic

The results
neous behavio
collected only
yielded no sign
haviors, bathin
alert (both arse

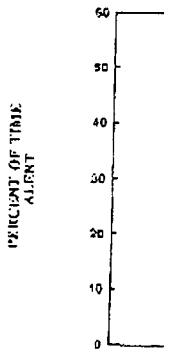

Fig. 2. The interac
in mallard ducklin

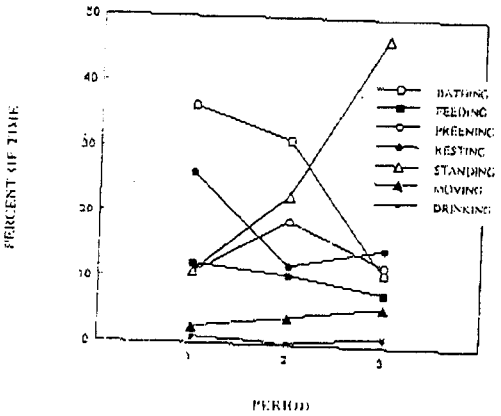

Fig. 1. Time budgets of untreated control mallard ducklings for periods 1 through 3. Period 1 consisted of weeks 1 to 3; period 2, weeks 4 to 6; and period 3, weeks 7 to 9. Points are based on instantaneous recordings and represent means ($n = 86$) for each behavior.

period. Tukey's studentized range test [17] was used to separate main effect means when the ANOVA indicated differences existed ($p < 0.05$).

RESULTS

The results presented are based on the instantaneous behavior data; continuous behavior data, collected only to examine durations of behaviors, yielded no significant treatment effects. Three behaviors, bathing (arsenic treatment only), standing alert (both arsenic and boron treatments) and resting (boron treatment only) showed significant period–treatment interactions ($p < 0.05$; Figs. 2a–3b).

Arsenic

Instantaneous behavior data yielded time percentages spent in each behavior type (Table 1). Dietary arsenic at 300 ppm had an effect ($p < 0.05$) on the amount of time spent resting and in alert posture. Ducklings on the 300-ppm diet spent more than 33% of their time resting, whereas controls rested 17.9% of the time. This effect directly influenced the amount of time available for alert behavior. Birds treated at 300 ppm arsenic spent only 14.9% of their time standing and alert, compared to 26.5% for controls.

There was an interaction of treatment and age on alert behavior in 300 ppm–dosed birds ($p = 0.0002$). Control ducklings increased the amount of time spent alert over time (see Fig. 1) from 10.9% (SE = 2.6) in period 1 to 46.8% (SE = 4.2) in period 3; the rate of increase (age effect) was higher than that in the 300-ppm treatment group (Fig. 2a).

There were significant arsenic treatment effects on time spent in various locations within the pen. Birds on 30- and 300-ppm diets spent less time in the water trough than did controls (26.6 and 24.9 vs. 43.7%; $p = 0.0001$). Thirty- and 300-ppm arsenic treatments resulted in decreases in bathing time (overall $p = 0.017$); however, separations of means were seen only in period 2 and for all periods combined (Fig. 2b). Ducklings on 300-ppm diets also spent significantly more time under or near the heat lamp than controls (10.9 vs. 4.6%; $p = 0.023$). This effect was evident, despite the signif-

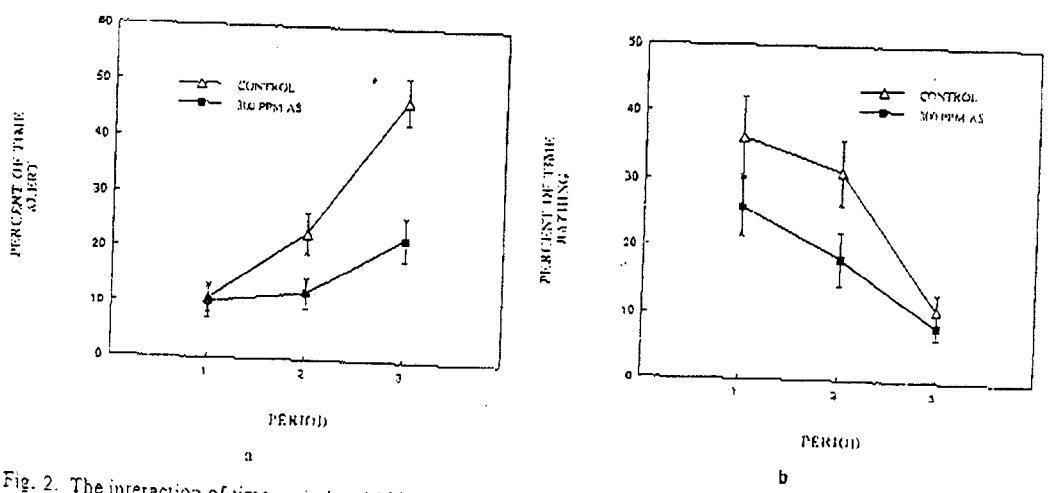

Fig. 2. The interaction of time period and 300-ppm arsenic-treated diet on (a) alert behavior and (b) bathing behavior in mallard ducklings. Points represent means ± 1 SE.

01/08/2001 14:03 8 CTR POP HEALTH PAGE 08

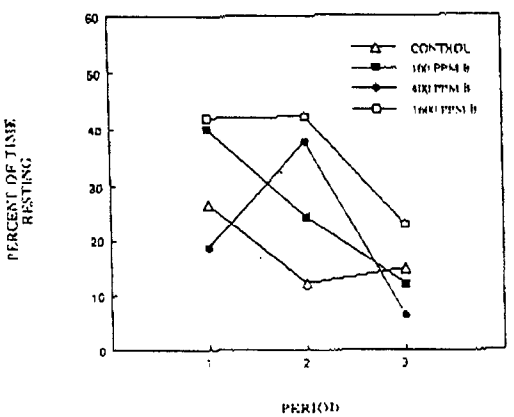

Fig. 3a. The interaction of time period and boron-treated diets on resting behavior in mallard ducklings. Points represent means; standard errors were omitted for clarity.

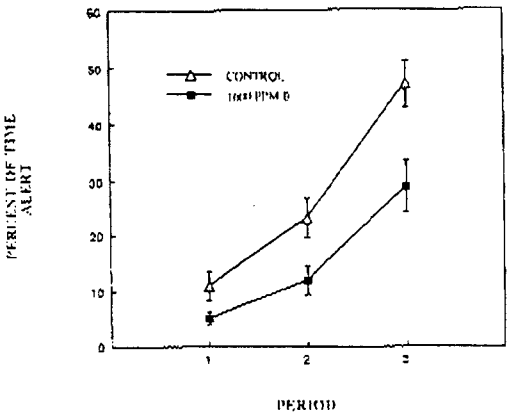

Fig. 3b. The interaction of time period and 1,600-ppm boron-treated diet on alert behavior in mallard ducklings. Points represent means ± 1 SE.

icant decreasing age effect on time spent under the lamp ($p = 0.001$); overall duckling time spent under the lamp decreased from 12.3% in period 1 to 3.1% in period 3.

Boron

Dietary boron in the form of boric acid had an effect on five of the seven observed behaviors (Table 2). Boron at 100 ppm and 1,600 ppm decreased ($p < 0.05$) bathing time from a control level of 26.4% to 12.8 and 14.4%, respectively. Both feeding and preening times were increased ($p < 0.05$) in treated ducklings (1,600 ppm and 100 ppm, respectively) over controls. Ducklings fed 1,600 ppm boron were affected ($p < 0.05$) in the amount of time spent resting and in alert posture. Treated birds rested 34.8% of the time, compared to controls at 17.9%. There were significant ($p <$

Table 1. Effects of dietary arsenic on the percent time spent in various behaviors by mallard ducklings (time periods combined unless indicated)

	Untreated controls ($n = 86$)	Arsenic in diet, ppm		
		30 ($n = 91$)	100 ($n = 97$)	300 ($n = 93$)
Bathing[a]	26.4 (2.9)[b]	17.6 (2.6)[c]	22.2 (2.7)	17.6 (2.2)[c]
Period 1	36.3 (5.9)	32.5 (5.3)	43.4 (5.4)	25.9 (4.3)
Period 2	31.2 (4.8)	11.5 (2.9)[d]	15.6 (2.7)[d]	18.4 (3.9)
Period 3	11.0 (2.3)	5.8 (2.3)	6.2 (1.9)	8.3 (1.9)
Feeding	10.3 (1.5)	13.5 (1.7)	11.6 (1.5)	11.7 (1.6)
Preening	13.9 (1.6)	12.8 (1.7)	16.0 (2.0)	17.6 (2.1)
Resting	17.9 (3.0)	17.7 (3.2)	12.8 (2.4)	33.7 (4.1)[c]
Alert[a]	26.5 (2.6)	33.1 (2.9)[c]	30.5 (2.4)	14.9 (1.9)[c]
Period 1	10.9 (5.9)	15.7 (3.5)	12.9 (2.5)	10.3 (2.9)
Period 2	22.9 (3.6)	45.6 (5.6)[d]	35.8 (4.1)	12.2 (2.7)
Period 3	46.8 (4.2)	42.8 (4.3)	44.0 (3.9)	22.3 (3.7)[d]
Moving	4.1 (0.8)	4.4 (0.7)	6.3 (0.8)	4.1 (0.7)
Drinking	0.9 (0.3)	0.9 (0.3)	0.7 (0.2)	0.3 (0.1)

[a]Period–treatment interaction was found with ANOVA ($p < 0.05$). Results of main effects tests and one-way analyses within periods are both presented.
[b]$\bar{x}$ (SE).
[c]Significantly different from controls in two-way analyses using Tukey's multiple comparison ($p < 0.05$).
[d]Significantly different from controls in one-way analyses within period using Tukey's multiple comparison ($p < 0.05$).

0.05) time per
spent resting,
treatment leve
nificantly ($p <$
26.5%). How
for the two l
reached contr
mained at a s

Boron trea
time duckling
heat lamp. B
spent a greate
heat lamp th
0.0001); this e
all decreasing
lamp ($p = 0.0$
was also affe
100 ppm, 31.
ppm), averagi
0.0001).

Time-activ
behavioral ec
in understanc
consumption.
ergy in develo

Table 2. Effect of dietary boron on the percent time spent in various behaviors by mallard ducklings (time periods combined unless indicated)

	Untreated controls (n = 86)	Boron in diet, ppm		
		100 (n = 108)	400 (n = 97)	1,600 (n = 97)
Bathing	26.4 (2.9)[a]	12.8 (2.3)[b]	19.5 (2.7)	14.4 (2.2)[b]
Feeding	10.3 (1.5)	10.9 (1.5)	13.4 (1.5)	17.2 (2.1)[b]
Preening	13.9 (1.6)	21.7 (2.5)[b]	15.0 (1.9)	11.8 (1.6)
Resting[c]	17.9 (3.0)	26.1 (3.5)	21.3 (3.5)	34.8 (3.8)[b]
Period 1[d]	26.3 (7.3)	39.6 (5.5)	18.6 (5.4)	41.6 (6.4)
Period 2	12.0 (2.9)	24.1 (5.7)	37.4 (7.3)[e]	41.9 (7.0)[e]
Period 3	14.7 (3.9)	12.1 (3.9)	6.1 (2.0)	22.7 (6.1)
Alert[c]	26.5 (2.6)	21.7 (2.1)	24.1 (2.6)	15.5 (2.1)[b]
Period 1	10.9 (4.6)	7.2 (1.8)	8.0 (1.8)	5.1 (1.1)
Period 2	22.9 (3.6)	21.8 (3.5)	16.5 (3.2)	22.9 (2.4)
Period 3	46.8 (4.2)	39.4 (3.7)	52.1 (4.6)	28.7 (4.6)[e]
Moving	4.1 (0.8)	6.1 (0.9)	6.2 (0.8)	5.3 (0.9)
Drinking	0.9 (0.3)	0.6 (0.2)	0.6 (0.2)	1.1 (0.4)

[a]$\bar{x}$ (SE).
[b]Significantly different from controls in two-way analyses using Tukey's multiple comparison ($p < 0.05$).
[c]Period–treatment interaction was found with ANOVA ($p < 0.05$). Results of main effects tests and one-way analyses within periods are both presented.
[d]Means are different based on ANOVA ($p < 0.05$), but no separation found with Tukey's multiple comparisons.
[e]Significantly different from controls in one-way analyses within period using Tukey's multiple comparison ($p < 0.05$).

0.05) time period effects on the proportion of time spent resting, but the effect was different at each treatment level (Fig. 3a). Time alert decreased significantly ($p < 0.05$) from that of controls (15.5 vs. 26.5%). However, by period 3, percent time alert for the two lowest treatment concentrations had reached control levels; only 1,600-ppm birds remained at a suppressed level (Fig. 3b).

Boron treatments had a significant effect on the time ducklings spent in the water and under the heat lamp. Birds treated with 1,600 ppm boron spent a greater amount of time under or near the heat lamp than controls (19.1 vs. 1.1%; p = 0.0001); this effect was significant despite the overall decreasing proportion of time spent near the lamp (p = 0.0004). Time spent in the water trough was also affected at all treatment levels (21.4% at 100 ppm, 31.2% at 400 ppm and 23.0% at 1,600 ppm), averaging 25.2 vs. 43.7% for controls (p = 0.0001).

DISCUSSION

Time–activity budgets are important aspects of behavioral ecology, and their documentation aids in understanding habitat utilization and energy consumption. Alterations in the allocation of energy in developing ducklings, as a result of toxicant exposure, could seriously jeopardize survival. In addition, behavioral adjustments made in response to environmental stressors may serve to mitigate potential effects, but such adjustments themselves may affect individual survival. Few detailed field studies are available on time budgets for developing ducklings, primarily because ducklings are so difficult to observe. This study utilized a controlled experiment to compare detailed effects of boron and arsenic on behavioral activities in an attempt to better understand the mechanisms by which environmental contaminants may be affecting waterfowl. Such a study could not practically be accomplished in a field setting. Although it can be argued that the absolute percentages spent in these measured behaviors are probably not identical to those in nature, these results suggest that both arsenic and boron may alter behavioral patterns and could affect survival of mallard ducklings.

Although none of the focal animals observed in the behavioral study died, results from a larger sample of birds in an associated study showed 10% mortality and decreased overall growth and rate of growth at 1,600 ppm boron [7]. In the same study, physiological measurements, including hematocrit, hemoglobin and plasma calcium concentrations, were lower in the 1,600 ppm treatment group.

01/08/2001 14:03 8 CTR POP HEALTH PAGE 09

Environmental To
Printed in the USA

Brain boron concentrations increased with dietary level, with brain ATP decreasing [7]. Arsenic at all treatment levels decreased growth and growth rates in females, and Arsenic in the diet at 300 ppm also altered brain biochemistry, reducing ATP concentration [13]. A significant negative correlation was found between brain boron concentration and decreased brain ATP concentration [7]. Treatments had no effect on the amount of time spent feeding or preening by arsenic-treated birds, and 1,600 ppm dietary boron actually increased feeding time over controls. These findings corroborate the observed behavioral effects; increased resting times were presumably linked to decreased brain energy levels.

It is noteworthy that, despite the obvious survival value of alert behavior, particularly to older fledged ducklings who no longer have constant adult protection, boron- and arsenic-treated ducks effectively sacrificed time spent standing alert, allowing for extra time in prolonged resting periods. They continued to devote normal or above-normal amounts of time in self-maintenance behaviors such as feeding. Whether wild ducklings would make similar tradeoffs remains unanswered. Because actual food consumption was not measured as part of the study and would be difficult to do with free-ranging ducklings, feeding efficiency and nutrient absorption cannot be estimated; both of these factors could influence general health and survivability.

In summary, developing mallard ducklings appeared to select increased rest and supplementary warmth in response to exposure to high levels of arsenic or boron. Their compensating shift in time budgets was only marginally successful in maintaining weight and survival in a small penned enclosure [7,13]; however, in the natural environment, decreased time available for normal alert behavior would have obvious drawbacks. Furthermore, energy required to secure food in a natural habitat would be far greater than walking less than 1 m to a full food trough in a confined pen. Therefore, both of these factors, predator avoidance and foraging strategies, may be affected negatively in wild birds exposed to similar levels of boron and arsenic.

Acknowledgement—Funding for this work was provided under the U.S. Bureau of Reclamation/U.S. Fish and Wildlife Service Intraagency Agreement No. 6-AA-20-04170. Bridgette Estel and Kelly Campbell assisted in data collection. We thank Marcia Holmes for typing portions of the manuscript and Thomas Custer and Michael Tome for manuscript reviews.

REFERENCES

1. **Ohlendorf, H.M., D.J. Hoffman, M.K. Saiki and T.W. Aldrich.** 1986. Embryonic mortality and abnormalities of aquatic birds: Apparent impacts by selenium from irrigation drainwater. *Sci. Total Environ.* 52:49–63.
2. **Beyer, K.H., W.F. Bergfield, W.O. Berndt, R.K. Bootwell, W.W. Carlton, D.K. Hoffman and A.L. Schroeter.** 1983. Final report on the safety assessment of sodium borate and boric acid. *J. Am. Coll. Toxicol.* 2:87–125.
3. **U.S. Environmental Protection Agency.** 1975. *Preliminary Investigation of Effects on the Environment of Boron, Indium, Nickel, Selenium, Tin, Vanadium and Their Compounds*, Vol. I—Boron. EPA 560/2-75-005a. Washington, DC.
4. **Weir, R.J. and R.S. Fisher.** 1972. Toxicologic studies on borax and boric acid. *Toxicol. Appl. Pharmacol.* 23:351–364.
5. **Lee, I.P., R.T. Sherins and R.L. Dixon.** 1978. Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. *Toxicol. Appl. Pharmacol.* 45:577–590.
6. **Smith, G.J. and V.P. Anders.** 1989. Toxic effects of boron on mallard reproduction. *Environ. Toxicol. Chem.* 8:943–950.
7. **Hoffman, D.J., M.B. Camardese, L.J. LeCaptain and G.W. Pendleton.** 1990. Effects of boron on growth and physiology in mallard ducklings. *Environ. Toxicol. Chem.* 9:335–346.
8. **National Academy of Science.** 1977. *Environmental Pollutants: Arsenic*. Committee on Medical and Biological Effects of Environmental Pollutants, Washington, DC.
9. **Woolson, E.A.** 1975. Arsenical pesticides. Symposium Series 7. American Chemical Society, Washington, DC.
10. **Pershager, G. and M. Vahter.** 1979. Arsenic—A toxicological and epidemiological appraisal. Naturvardsverket Rapp. SNV PM1128. Karolinska Institute, National Swedish Environmental Protection Board, Stockholm, Sweden.
11. **Eisler, R.** 1988. Arsenic hazards to fish, wildlife, and invertebrates: A synoptic review. *U.S. Fish Wildl. Serv. Biol. Rep.* 85(1.12).
12. **Tucker, R.K. and D.G. Crabtree.** 1970. Handbook of Toxicity of Pesticides to Wildlife. U.S. Fish and Wildlife Service Resource Publication 84.
13. **Camardese, M.B., D.J. Hoffman, L.J. LeCaptain and G.W. Pendleton.** 1990. Effects of arsenic on growth and physiology in mallard ducklings. *Environ. Toxicol. Chem.* 9:785–795.
14. **Willhite, C.C.** 1981. Arsenic-induced axial skeletal (dysraphic) disorders. *Exp. Mol. Pathol.* 34:145–158.
15. **Ferm, V.H. and S.J. Carpenter.** 1968. Malformations induced by sodium arsenate. *J. Reprod. Fertil.* 17:199–201.
16. **Morrissey, R.E. and N.K. Mottet.** 1983. Arsenic-induced exencephaly in the mouse and associated lesions occurring during neurulation. *Teratology* 28:399–411.
17. **Steel, R.G.D. and J.H. Torrie.** 1980. *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd ed. McGraw Hill, New York, NY.