Arch. Environ. Contam. Toxicol. 26, 444–451 (1994)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Main and Interactive Effects of Arsenic and Selenium on Mallard Reproduction and Duckling Growth and Survival

T. R. Stanley Jr.*, J. W. Spann, G. J. Smith**, R. Rosscoe***

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20708, USA

Received: 13 September 1993/Revised: 24 November 1993

Abstract. Arsenic (As) and selenium (Se) occur together in high concentrations in the environment and can accumulate in aquatic plants and invertebrates consumed by waterfowl. Ninety-nine pairs of breeding mallards (*Anas platyrhynchos*) were fed diets supplemented with As (sodium arsenate) at 0, 25, 100, or 400 μg/g, in combination with Se (seleno-DL-methionine) at 0 or 10 μg/g, in a replicated factorial experiment. Ducklings produced were placed on the same treatment combination as their parents. Arsenic accumulated in adult liver and egg, reduced adult weight gain and liver weight, delayed the onset of egg laying, decreased whole egg weight, and caused eggshell thinning. Arsenic did not affect hatching success and was not teratogenic. In ducklings, As accumulated in the liver and reduced body weight, growth, and liver weight. Arsenic did not increase duckling mortality, but it did decrease overall duckling production. Selenium accumulated in adult liver and egg, was teratogenic, and decreased hatching success. Selenium did not affect adult weight, liver weight, survival, onset of egg laying, egg fertility, egg weight, or eggshell thickness. In ducklings, Se accumulated in the liver and reduced body weight and growth, and increased liver weight. Selenium increased duckling mortality and decreased overall duckling production. Antagonistic interactions between As and Se occurred whereby As reduced Se accumulation in liver and egg, and alleviated the effects of Se on hatching success and embryo deformities. It was demonstrated that As and Se, in the chemical forms and at the dietary levels administered in this study, can adversely affect mallard reproduction and duckling growth and survival, and that As can alleviate toxic effects of Se.

Present address: National Biological Survey, National Ecology Research Center, Fort Collins, CO 80525

**Present address:* Wildlife International Ltd., 305 Commerce Drive, Easton, MD 21601

***Present address:* National Zoological Park, Smithsonian Institution, Washington, DC 20008

Correspondence to: T. R. Stanley Jr.

Arsenic in U.S. surface waters commonly ranges from < 0.01 to 1.1 mg/L (Durum *et al.* 1971), but can reach 14 mg/L in agricultural wastewater (Moore *et al.* 1989). Arsenic accumulates in aquatic plants to levels as high as 1,450 μg/g (dry wt) (Lancaster *et al.* 1971), and in aquatic invertebrates concentrations can be 131 times greater than the water concentration (Spehar *et al.* 1980). Waterfowl consuming aquatic plants and invertebrates from As-contaminated sites are exposed to As and may be adversely affected. In two recent studies (Camardese *et al.* 1990; Hoffman *et al.* 1992), arsenate in the diet of mallard ducklings (*Anas platyrhynchos*) decreased duckling weight and overall growth, and caused physiological abnormalities. The effects of As consumption on adult waterfowl and waterfowl reproduction has not been investigated. However, in chickens fed diets supplemented with an organoarsenical, As accumulated in the eggs (Daghir and Hariri 1977), and As injected into eggs was teratogenic and embryotoxic (Peterkova and Puzanova 1976). These studies suggest that reproduction in waterfowl exposed to As might be impaired.

Selenium occurs naturally in soil and can leach into surface waters and accumulate in aquatic plants and invertebrates (Hothem and Ohlendorf 1989). At Kesterson Reservoir, CA, average Se concentrations as high as 73 μg/g and 100 μg/g have been found in aquatic plants and insects (Ohlendorf 1989). In 1983, poor reproductive success and malformed embryos and chicks were reported for ducks and other aquatic birds breeding at Kesterson (Ohlendorf *et al.* 1986). Subsequent field and laboratory investigations provided convincing evidence that high levels of Se in agricultural wastewater and food chain organisms was responsible (Ohlendorf *et al.* 1989; Heinz *et al.* 1989; Hoffman and Heinz 1988; Hothem and Ohlendorf 1989).

Arsenic and Se occur together in high concentrations in the environment (Moore *et al.* 1989), and may interact in organisms exposed to these elements. Arsenic is known to interact antagonistically with Se in mammals (Rhian and Moxon 1943; Palmer *et al.* 1983), and Hoffman *et al.* (1992) found As prevented Se-induced mortality and impaired growth in mallard ducklings. In poultry, dietary As counteracts decreases in adult weight, egg production, egg weight, hatching success, and juvenile weight caused by excess Se in the diet (Thapar *et al.* 1969). Currently there is little information on the interactive effects of As and Se on waterfowl reproduction. The objective

of this study was to determine the main and interactive effects of As and Se on mallard reproduction and duckling growth and survival.

Materials and Methods

Adults and Dietary Treatments

Ninety-nine pairs of 1-year-old mallard drakes and hens were purchased from a game farm (Frost Waterfowl Trust, Coloma, WI) in March 1988. All birds were banded, wing-clipped, segregated by sex, and then randomly assigned to indoor holding pens for a 10-day conditioning period. During this period, both sexes received an untreated diet of commercially available developer mash (14.5% crude protein) (all feed was supplied by Chesapeake Feed Company, Beltsville, MD), and females were kept under controlled lighting (8 h/day) to delay the onset of egg laying and to synchronize their cycles. Following conditioning, birds were weighed and randomly assigned to one of eight treated diets in a 4 × 2 replicated factorial arrangement (N = 12 pairs per treatment combination). Diets consisted of feed supplemented with As at 0 (As control), 25, 100, or 400 μg/g, in combination with Se at 0 (Se control) or 10 μg/g. Drakes were then placed in 1-m square outdoor pens, one bird per pen, while females remained in indoor pens under controlled lighting. After 4 weeks on treated diets, females were randomly paired with males on the same treatment combination in the outdoor pens. The three remaining pairs of mallards were placed on the 400 μg/g As and 10 μg/g Se treatment combination. All pens contained a water pan, feeder, and nest box. At pairing, birds were weighed and switched to treated diets prepared with breeder mash (17% crude protein).

Sodium arsenate (99.2% pure; Mallinckrodt Inc, Paris, KY) was used to prepare diets and was mixed into the feed as a dry powder. Sodium arsenate was used because it is the most common form of As in the environment (Braman and Foreback 1973). Selenium, in the form of seleno-DL-methionine (98–99% pure; Bachem Inc, Torrance, CA), was dissolved in distilled, deionized water (1% by weight) before addition to the feed. Selenomethionine was used because it is the most likely form of selenium in food chain organisms (Ohlendorf 1989). One percent water, by weight, was added to all Se control diets to ensure moisture content was consistent across treatments. A 20 g sample of feed was collected from each batch of feed mixed. Five feed samples per treatment combination were randomly selected for As and Se analysis.

Nesting, Eggs, and Incubation

Nest boxes were checked daily, and new eggs numbered sequentially. Cracked or broken eggs were removed from the nest, and eggs laid outside the nest were placed in the nest. From each nest the eighth egg was removed and the following measurements were taken: whole egg weight, egg length, egg width, shell weight, shell thickness, and egg contents weight. The contents of each egg were frozen and retained for chemical analysis. Eggshells were air-dried for 30 d before weighing and measuring shell thickness. Ratcliffe Indices (Ratcliffe 1967) were computed. From the nests of the three extra pairs of mallards, every third egg laid was removed (beginning with the second egg), measured as above, and the contents frozen for chemical analysis. This was done to detect trends in egg As and Se concentrations relative to laying order. From the eggs that remained for the three extra pairs of mallards, a random sample of six eggs was removed from each nest and measured as above, and the yolk and albumen were separated and frozen for chemical analysis. This was done to determine the distribution of As and Se in the egg.

Hens incubated their own clutches, but were not allowed to incubate more than 20 eggs. When a clutch exceeded 20 eggs, successive eggs, beginning with the first, were removed up until incubation began.

On day 14 of incubation, all eggs in the nest were candled and dead or infertile eggs were removed. When egg removal exceeded 50% of the clutch, extra eggs, removed from nests prior to incubation, were marked and added to the nest to bring clutch size up to 50%. This was done in an attempt to reduce nest desertion. Males were removed from the pen on day 14 of incubation and placed in holding pens where they were maintained on treated diets. In nests where the entire clutch of eggs was infertile the male was left in the pen and the nest was allowed to recycle. In cases where a nest recycled, the eighth egg of that nest was removed, measured, and submitted for chemical analysis.

Ducklings and Necropsies

On the day of hatching ducklings were weighed, marked with a web tag, and placed on the same diet as their parents. Eggs that failed to hatch and ducklings that failed to survive the first 12 h were removed from the pen and the embryos and ducklings examined for deformities. In pens where an incubating hen deserted the nest, eggs were removed, and the embryos examined for deformities. Treated diets supplied for adults and ducklings beginning with the day of hatching, or beginning with the day the nest was deserted, were prepared using starter mash (22.0% crude protein).

Ducklings were checked daily for survival, were weighed again at 7 d, and at 14 d were euthanized in a CO_2 chamber along with their parents. In pens where the nest was deserted, the adults were euthanized 14 d after nest desertion.

At the time of sacrifice, adults were weighed and the liver removed, weighed, and frozen for chemical analysis. Ducklings were also weighed at the time of sacrifice, and one was randomly selected for removal of the liver for weighing and chemical analysis. Samples of liver, kidney, and spleen from adults and a second randomly selected duckling from the following treatment combinations: As control and Se control, 400 μg/g As and Se control, As control and 10 μg/g Se, and 400 μg/g As and 10 μg/g Se, were saved in 10% buffered formalin for histopathological analysis (hematoxylin and eosin staining with examination by light microscope by Donald A. Willigan Inc, Germantown, MD).

Chemical Analysis

All samples were analyzed for As and Se according to the method described by Krynitsky (1987). Percent recovery from spiked adult liver, juvenile liver, whole egg, yolk, and albumen, was 93%, 95%, 88%, 97%, and 82%, respectively, for As, and 99%, 113%, 98%, 121%, and 94%, respectively, for Se. The limit of detection for As and Se in adult liver, juvenile liver, whole egg, yolk, and albumen, on a dry-weight basis, was 0.34, 0.38, 0.32, 0.16, and 0.67 μg/g, respectively. Arsenic and Se concentrations reported by the analytical laboratory (Patuxent Analytical Control Facility, Patuxent Wildlife Research Center, Laurel, MD) as below the limit of detection were assigned a value of one-half the limit of detection. Average recovery from National Bureau of Standards reference material (NRCC DOLT-1) was 91% for As and 93% for Se. Results of all residue analyses are reported on a μg/g dry-weight basis.

Statistical Methods and Data Analysis

Histopathological data for adults and ducklings were analyzed by logistic regression using a model with parameters for main effects and

Table 1. As concentration (μg/g, dry wt) in adult liver, egg, and duckling liver for mallards fed diets supplemented with As (sodium arsenate)[a]

	As added to the diet (μg/g)			
	0	25	100	400
Days on treated diet (adults only)[b]	115 (92–144)	117 (91–160)	117 (92–160)	128 (81–173)
Adult liver (sexes combined)	0.23 (0.057) A [34][c]	0.49 (0.066) B [47][d]	2.4 (0.28) C [48]	6.6 (0.63) D [48]
Whole egg	0.23 (0.031) A [17]	0.46 (0.049) B [24]	1.8 (0.14) C [24]	3.6 (0.44) D [22]
Duckling liver	0.20 (0.004) A [5]	0.65 (0.069) B [11]	4.5 (0.87) C [10]	33 (5.7) D [9]

[a] Values are arithmetic means (SE) and were computed by pooling data over the two levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

[b] Range of values is in parentheses

[c] Only 10 livers from adults receiving the As control and Se control treatment combination were submitted for chemical analysis (five males and five females)

[d] Excludes a male placed on the wrong diet 2 weeks prior to necropsy

interactions between As and Se. Parameters were tested for significance using *z*-tests.

All remaining response variables were analyzed by analysis of variance (ANOVA) under a model appropriate for the main effects and interactions being tested, and an error term suitable for unbalanced data. Tests of hypotheses which included more than one bird per pen were made with F-tests under a mixed effects model using an appropriate error term. All other F-tests assumed a fixed effects model and used the residual mean square as the error term. Means used to test for differences in duckling weight and growth were weighted by the number of ducklings alive in the pen at the time of sampling. Means used to test for differences in duckling mortality were weighted by the number of ducklings alive at the beginning of the time interval. When interactions between As and Se were detected, a one-factor ANOVA was used to test for differences among As treatments within levels of Se. Normality of residuals was evaluated using the Shapiro-Wilk statistic (Shapiro and Wilk 1965) and normal probability plots. Studentized residuals were plotted to assess homoscedasticity. Data that did not meet the normality or homoscedasticity assumptions necessary for ANOVA were transformed so as to meet the assumptions. Consequently, all percentage data were arc-sine-transformed and all residue data were $\log_e$-transformed before analysis. Multiple comparisons were made using Tukey's multiple comparison procedure (MCP) at $\alpha = 0.05$.

Results

Residue Analyses

The mean concentration (SE) of As in the As control, 25 μg/g As, 100 μg/g As, and 400 μg/g As treated diets (data pooled over the two levels of Se), was 0.26 (0.119), 22 (0.9), 93 (2.4), and 403 (17.6) μg/g, respectively (n = 10 for each level of As). The mean concentration (SE) of Se in the Se control and 10 μg/g Se treated diets (data pooled over the four levels of As) was 0.37 (0.035) and 6.5 (0.18) μg/g (n = 20 for each level of Se).

Arsenic accumulated in adult mallard liver (sexes combined), whole egg, and duckling liver (Table 1). No interactions occurred between As and Se, and laying order did not affect As accumulation in whole egg. The concentration (SE) of As in albumen and yolk, for eggs from the three extra pairs of mallards receiving 400 μg/g As and 10 μg/g Se, was 7.2 (0.35) μg/g and 3.5 (0.18) μg/g. The difference was significant ($P < 0.0001$, n = 18).

Table 2. Se Concentration (μg/g, dry wt) in adult liver, egg, and duckling liver for mallards fed diets supplemented with Se (seleno-DL-methionine)[a]

	Se added to the diet (μg/g)	
	0	10
Days on treated diet (adults only)[b]	115 (81–160)	124 (93–173)
Adult female liver[c]	2.4 (0.15) A [41][d]	31 (1.5) B [48]
Adult male liver[c]	3.9 (0.90) A [40][e]	34 (1.9) B [48]
Whole egg	1.4 (0.06) A [40]	37 (2.1) B [47]
Duckling liver	0.92 (0.113) A [28]	20 (4.1) B [7]

[a] Values are arithmetic means (SE) and were computed by pooling data over the four levels of As; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

[b] Range of values is in parentheses

[c] The average concentration of Se in the liver (data pooled over the two levels of Se) differed by sex (P = 0.0032)

[d] Only five livers from adult females receiving the As control and Se control treatment combination were submitted for chemical analysis

[e] Only five livers from adult males receiving the As control and Se control treatment combination were submitted for chemical analysis. Sample excludes a male placed on the wrong diet 2 weeks prior to necropsy

Selenium accumulated in female and male liver, whole egg, and duckling liver (Table 2). The average concentration of Se in adult liver (data pooled over the two levels of Se) differed by sex ($P = 0.0032$), and for sexes combined there was an antagonistic interaction between As and Se ($P = 0.0336$). The concentration of Se in adult liver was lower in birds receiving the 400 μg/g As and 10 μg/g Se treatment combination than in birds receiving As control, 25 μg/g As, or 100 μg/g As, in combination with 10 μg/g Se ($P < 0.0001$) (Table 3). In whole egg As and Se interacted antagonistically ($P = 0.0004$). The

Table 3. Interactive effects of As and Se on adult mallards and ducklings fed diets supplemented with As (sodium arsenate) and Se (seleno-DL-methionine)[a]

As in diet (μg/g)	0	25	100	400	0	25	100	400
Se in diet (μg/g)	0	0	0	0	10	10	10	10
Se in adult liver (sexes combined) (μg/g, dry wt)	2.8 (0.25) [10]	3.0 (0.39) [23]	3.9 (1.49) [24]	2.7 (0.18) [24]	35 (2.7) [24]	37 (2.7) [24]	33 (2.2) [24]	23 (1.4) [24]
Se in whole egg (μg/g, dry wt)	1.6 (0.22) [5]	1.6 (0.15) [12]	1.2 (0.06) [12]	1.4 (0.08) [11]	42 (3.3) [12]	40 (2.3) [12]	42 (2.5) [12]	24 (6.2) [11]
Hatching success (%)	91.4 (4.43) [11]	91.7 (7.54) [11]	90.8 (5.07) [8]	74.5 (8.64) [7]	8.5 (4.14) [10]	9.8 (8.66) [8]	11.9 (4.99) [10]	59.7 (17.16) [7]
Embryo deformities (%)	0 [11]	0 [11]	1.9 (1.33) [8]	2.9 (2.86) [7]	57.5 (12.17) [10]	23.4 (12.31) [8]	2.4 (1.60) [10]	0 [7]
Duckling weight at hatching (g)	36.0 (3.58) [11]	34.7 (3.28) [11]	33.7 (3.56) [8]	30.9 (1.91) [7]	28.2 (3.39) [3]	27.8 (0.19) [2]	28.0 (1.28) [4]	33.6 (4.45) [5]
Duckling mortality (%)	17.5 (11.45) [11]	20.3 (19.71) [11]	17.6 (11.59) [8]	56.7 (22.05) [7]	90.0 (0.00) [3]	51.4 (29.46) [2]	68.1 (23.05) [4]	63.8 (12.83) [5]
Duckling production	10.3 (1.50) [11]	10.1 (1.23) [11]	11.0 (1.25) [8]	1.6 (0.61) [7]	0 [4]	1.5 (1.50) [2]	0.8 (0.48) [4]	1.2 (0.49) [5]

[a] Values are arithmetic means (SE); bracketed numbers are sample sizes. For all As and Se interactions $P \leq 0.0336$

Table 4. Main effects of As on weight gain, liver weight, and reproduction in adult mallards fed diets supplemented with As (sodium arsenate)[a]

	As added to the diet (μg/g)			
	0	25	100	400
Weight gain, treatment onset to necropsy (sexes combined) (g)[b]	52.8 (14.73) A [48]	78.1 (16.63) A [47][c]	22.0 (13.82) A [48]	26.9 (14.90) A [48]
Ratio of liver weight to body weight (sexes combined)	0.024 (0.0008) A [48]	0.025 (0.0011) A [47][c]	0.024 (0.0008) A [48]	0.021 (0.0005) B [48]
Pairs of breeders	24	24	24	24
Hens laying eggs	24	24	24	24
Days between pairing and first egg	16 (1.2) A [24]	16 (2.0) A [24]	16 (1.1) A [24]	25 (1.6) B [24]
Whole egg weight (g)	57.5 (0.90) A [24]	57.3 (0.75) A [24]	55.1 (1.04) AB [24]	51.6 (1.85) B [22]
Eggshell thickness (mm)	0.38 (0.005) AB [24]	0.39 (0.004) AB [24]	0.40 (0.007) A [24]	0.37 (0.009) B [22]
Ratcliffe Index	2.16 (0.026) AB [24]	2.21 (0.023) AB [24]	2.28 (0.048) A [24]	2.06 (0.058) B [22]

[a] Values are arithmetic means (SE) and were computed by pooling data over the two levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

[b] Means in this row differed by ANOVA ($P = 0.0468$) but could not be separated by Tukey's MCP at $\alpha = 0.05$

[c] Excludes a male placed on the wrong diet 2 weeks prior to necropsy

concentration of Se in whole egg was lower in birds receiving the 400 μg/g As and 10 μg/g Se treatment combination than in birds receiving As control, 25 μg/g As, or 100 μg/g As, in combination with 10 μg/g Se ($P < 0.0001$) (Table 3). Laying order did not affect Se accumulation in whole egg. The concentration (SE) of Se in albumen and yolk, for eggs from the three extra pairs of mallards receiving 400 μg/g As and 10 μg/g Se, was 59 (2.1) μg/g and 10 (0.7) μg/g. The difference was significant ($P < 0.0001$, n = 18).

Treatment Effects on Adults

Two adult males receiving the 400 μg/g As and Se control treatment combination died 31 d and 84 d after treatment onset. Liver concentrations of As for these birds were 6.2 and 0.69 μg/g. Post mortem examinations revealed severe emaciation, but nothing else unusual.

For all levels of As and Se there were no differences in adult body weight at treatment onset, pairing, or necropsy. Weight gain in adult mallards (sexes combined) between treatment onset and necropsy differed among levels of As ($P = 0.0468$) (Table 4), but means could not be separated using Tukey's MCP. There was weak evidence that weight gain in adults (sexes combined) between pairing and necropsy was lower in birds receiving 10 μg/g Se than in birds receiving Se control diets ($P = 0.0531$).

Liver weight (mean ratio of liver weight to body weight) in adults (sexes combined) was lower for birds receiving 400 μg/g As than for the other levels of As ($P = 0.0071$) (Table 4), but was not affected by Se.

Glycogen depletion in the hepatic cell parenchyma was

Table 5. Main effects of Se on reproduction in adult mallards fed diets supplemented with Se (seleno-DL-methionine)[a]

	Se added to the diet (μg/g)	
	0	10
Pairs of breeders	48	48
Hens laying eggs	48	48
Hatching success (%)	88.2 (3.31) A [37]	20.0 (5.36) B [35]
Embryo deformities (%)	0.9 (0.61) A [37]	22.4 (5.93) B [35]

[a]Values are arithmetic means (SE) and were computed by pooling data over the four levels of As; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

scored as minimal, slight, moderate, or marked. The frequency of occurrence of moderate or marked glycogen depletion in adult mallards (sexes combined) receiving the As and Se control, 400 μg/g As and Se control, As control and 10 μg/g Se, or 400 μg/g As and 10 μg/g Se treatment combinations (n = 24 birds per treatment combination), was 6, 12, 2, and 14, respectively. Logistic regression indicated that glycogen depletion occurred more frequently in birds receiving 400 μg/g As than in birds receiving As control diets ($P = 0.0003$). Selenium did not affect glycogen depletion, and there was no interaction between As and Se. No other histopathological effects were detected in adults.

Treatment Effects on Reproduction

Arsenic at 400 μg/g increased the number of days between pairing and laying of the first egg ($P < 0.0001$) and decreased whole egg weight ($P = 0.0023$) (Table 4). Shell thickness and the Ratcliffe Index for eggs from hens receiving 400 μg/g As were lower than for 100 μg/g As ($P = 0.0094$ and $P = 0.0032$), but did not differ from hens receiving As control or 25 μg/g As diets (Table 4). Egg fertility (percentage of fertile eggs per clutch) was not affected by As. Selenium had no affect on egg laying, egg weight, eggshell thickness, Ratcliffe index, or egg fertility.

Arsenic in the diet did not affect hatching success (percentage of fertile eggs that hatched) or embryo deformities (percentage of fertile eggs having deformed embryos or deformed ducklings failing to survive the first 12 h post-hatching). Selenium decreased hatching success ($P < 0.0001$) (Table 5), and there was an antagonistic interaction between As and Se ($P = 0.0003$). Hatching success was higher in birds receiving the 400 μg/g As and 10 μg/g Se treatment combination than in birds receiving As control, 25 μg/g As, or 100 μg/g As, in combination with 10 μg/g Se ($P = 0.0026$) (Table 3). Selenium increased embryo deformities ($P < 0.0001$) (Table 5) and there was an antagonistic interaction between As and Se ($P < 0.0001$). There were fewer embryo deformities in birds receiving 100 or 400 μg/g As, in combination with 10 μg/g Se, than in birds receiving the As control and 10 μg/g Se treatment combination ($P = 0.0002$) (Table 3).

Treatment Effects on Ducklings

Ducklings fed diets supplemented with 400 μg/g As had lower body weights at 7 d ($P < 0.0001$) and 14 d ($P < 0.0001$) post-hatching, decreased growth (measured as weight gain) between hatching and 7 d ($P < 0.0001$) and 7 d and 14 d ($P < 0.0001$), and decreased liver weight (mean ratio of liver weight to body weight) ($P = 0.0006$) when compared to ducklings receiving As control or 25 μg/g As diets (Table 6). Duckling weight at hatching was not affected by As. Ducklings receiving 10 μg/g Se had lower body weights at hatching ($P = 0.0035$), and 7 d ($P = 0.0344$) and 14 d post-hatching ($P = 0.0231$); decreased growth between hatching and 14 d ($P = 0.0424$); and increased liver weight ($P = 0.0131$) when compared to ducklings receiving Se control diets (Table 7). For duckling weight at hatching, there was a significant interaction between As and Se ($P = 0.0317$) (Table 3). However, because the one-factor tests for differences among As treatments within levels of Se were not significant, it is not clear whether the interaction was antagonistic. Arsenic and Se had no detectable histopathological effects in ducklings.

Duckling mortality (percentage of ducklings dying between hatching and 14 d) was not affected by As, but was higher in ducklings fed 10 μg/g Se when compared to ducklings fed Se control diets ($P < 0.0001$) (Table 7) and there was a significant interaction between As and Se ($P = 0.0140$) (Table 3). Once again, the one-factor tests for As within levels of Se were not significant so it is not clear whether the interaction was antagonistic. Duckling production (number of ducklings alive at 14 d for nests producing ⩾ 1 duckling) was lower in birds receiving 400 μg/g As ($P < 0.0001$) (Table 6), was lower in birds receiving 10 μg/g Se ($P < 0.0001$) (Table 7), and there was a significant interaction between As and Se ($P = 0.0004$) (Table 3). Duckling production was lower in birds receiving the 400 μg/g As and Se control treatment combination than in birds receiving As control, 25 μg/g As, or 100 μg/g As, in combination with Se control ($P < 0.0001$) (Table 3). In contrast, duckling production was higher in birds receiving the 400 μg/g As and 10 μg/g Se treatment combination when compared with birds receiving As control, 25 μg/g As, or 100 μg/g As in combination with 10 μg/g Se (Table 3).

Discussion

Main Effects of Arsenic

Arsenic in the diet of adult mallards reduced weight gain, reduced liver weight, delayed egg laying, reduced egg weight, and caused eggshell thinning. It is suspected that these effects were due to insufficient food intake. Adults receiving 400 μg/g As wasted more feed than birds on the other treatments, and there was evidence of glycogen depletion in their livers. Furthermore, the cause of death of two adult males receiving the 400 μg/g As and Se control treatment combination, appeared to be starvation. Thus, it is likely that adults detected the arsenate in the feed and consumed less. Despite the negative effects of As on egg laying, egg weight, and eggshell thickness, As did not reduce overall hatching success. It is possible, however, that under natural conditions these effects would contribute to some decrease in hatching success.

Arsenic accumulated in mallard eggs but there was no apparent trend in whole egg As relative to laying order. Thus, use of the eighth egg for As analysis seems sufficient.

The As concentration observed in duckling liver was higher than has previously been reported. In 10-week-old mallard

Table 6. Main effects of As on mallard ducklings fed diets supplemented with As (sodium arsenate)[a]

	As added to the diet (μg/g)			
	0	25	100	400
Duckling weight at 7 d (g)	78.1 (7.63) A [10]	78.8 (9.51) A [11]	64.4 (8.09) AB [10]	38.7 (1.66) B [10]
Duckling weight at 14 d (g)	185.5 (21.89) A [10]	190.5 (23.63) A [11]	146.1 (24.29) A [10]	67.7 (6.51) B [10]
Duckling growth between hatching and 7 d (g)	41.8 (6.02) A [10]	43.9 (7.77) A [11]	30.7 (8.42) AB [10]	6.2 (2.63) B [10]
Duckling growth between 7 and 14 d (g)	106.7 (17.85) A [10]	111.0 (16.15) A [11]	81.0 (18.30) AB [10]	27.3 (5.23) B [10]
Ratio of duckling liver weight to body weight	0.057 (0.0026) A [10]	0.057 (0.0019) A [11]	0.052 (0.0031) AB [10]	0.045 (0.0021) B [10]
Duckling production	7.5 (1.63) A [15]	8.8 (1.38) A [13]	7.6 (1.68) A [12]	1.4 (0.40) B [12]

[a]Values are arithmetic means (SE) and were computed by pooling data over the two levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

Table 7. Main effects of Se on mallard ducklings fed diets supplemented with Se (seleno-DL-methionine)[a]

	Se added to the diet (μg/g)	
	0	10
Duckling weight at hatching (g)	34.6 (1.79) A [37]	30.7 (2.27) B [14]
Duckling weight at 7 d (g)	73.6 (6.46) A [34]	42.9 (4.66) B [7]
Duckling weight at 14 d (g)	173.7 (18.11) A [34]	85.9 (15.02) B [7]
Duckling growth between hatching and 14 d (g)	138.6 (17.50) A [34]	55.8 (17.21) B [7]
Ratio of duckling liver weight to body weight	0.052 (0.0015) A [34]	0.055 (0.0038) B [7]
Duckling mortality between hatching and 14 d (%)	21.8 (9.92) A [37]	67.4 (9.99) B [14]
Duckling production	8.7 (0.85) A [37]	0.8 (0.28) B [15]

[a]Values are arithmetic means (SE) and were computed by pooling data over the four levels of As; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$

ducklings receiving 100 μg/g As, Camardese *et al.* (1990) found liver residues of 0.3 μg/g As (dry wt.). This is much lower than the 4.5 μg/g As observed for 2-week-old ducklings receiving 100 μg/g As in this study. It is possible that as ducklings age they are able to eliminate As more effectively. If this were true, then adult liver residues should be less than or equal to those observed for 10-week-old ducklings. This was not the case, however. The mean liver concentration of As for adults receiving 100 μg/g As in the present study was 2.4 μg/g As.

Arsenic (400 μg/g) decreased duckling growth and body and liver weight. Hoffman *et al.* (1992) observed lower body and liver weights in ducklings receiving 200 μg/g As, and Camardese *et al.* (1990) found duckling growth to be lower for birds fed 300 μg/g As. As in this study, Camardese *et al.* (1990) did not observe reduced growth at 100 μg/g As. It is suspected that the effects of As on duckling growth and weight were due to insufficient food intake. Ducklings receiving 400 μg/g As wasted more feed than birds on the other treatments, and it has been documented that ducklings fed 300 μg/g As consume less over the first 3 weeks post-hatching than controls (Camardese *et al.* 1990).

In this study, and in others (Camardese *et al.* 1990; Hoffman *et al.* 1992), As did not increase duckling mortality. Arsenic (400 μg/g) did, however, lower overall duckling production by 81%. Thus, at high levels, continuous exposure to As can adversely affect mallard reproductive success.

No residue data could be found for adult or duckling livers collected from As-contaminated sites. Thus, it is unknown how representative these data are for birds under natural conditions. Arsenic concentrations in eggs from birds receiving 400 μg/g As were considerably higher than concentrations in eggs collected from As-contaminated sites in the Tulare Basin, CA (J.P. Skorupa, U.S. Fish and Wildlife Service, pers. comm.). At one such site, a single killdeer (*Charadrius vociferus*) egg had 1.8 μg/g As (dry wt), which is comparable to concentrations found in eggs from birds receiving 100 μg/g As; however, such high concentrations are considered unusual. Thus, wild birds in the Tulare Basin are probably not being exposed continuously to As levels in excess of 100 μg/g, or the predominant form of As differs. Indeed, data for other areas indicate that organic rather than inorganic forms of As predominate in food-chain organisms (Phillips 1990), and it is known that some organic forms of As do not accumulate in eggs (Daghir and Hariri 1977). Consequently, diets supplemented with As, as sodium arsenate, may not be the best model for As toxicity under natural conditions.

Main Effects of Selenium

Selenium as selenomethionine did not have adverse effects on adult mallards in this study or a similar study by Hoffman *et al.* (1991). Selenium concentrations in adult liver were within ranges reported for ducks collected from Se-contaminated sites (Ohlendorf *et al.* 1986). The sex difference in liver Se observed was probably due to the female eliminating Se through egg laying (Heinz *et al.* 1987). Selenium did not affect the onset of

egg laying, eggshell thickness, egg weight, or egg fertility. Similar findings were reported by Heinz *et al.* (1987, 1989).

In the present study, whole egg Se averaged 37 μg/g in birds receiving 10 μg/g Se. This was well above concentrations known to have reproductive affects but within ranges found under field conditions (Ohlendorf *et al.* 1986). There was no apparent trend in whole egg Se relative to laying order. Thus, use of the eighth egg for Se analysis seems sufficient. The Se concentration in egg albumen was greater than in the yolk and has previously been reported in the literature (Heinz *et al.* 1987).

Several laboratory and field studies have documented the adverse effects of Se on hatching success and embryos (Ohlendorf *et al.* 1986, 1989; Heinz *et al.* 1987; Hoffman and Heinz 1988). In the present study, 10 μg/g Se reduced hatching success, and caused embryo deformities including hydrocephaly, improperly developed bills, and missing toes and feet. Similar deformities have been reported by other investigators (Heinz *et al.* 1987, 1989; Hoffman and Heinz 1988).

Duckling growth was lower for birds receiving 10 μg/g Se than for birds receiving Se control diets. Heinz *et al.* (1989) also observed reduced growth in ducklings but, in another study in which there was no exposure through the egg (Heinz *et al.* 1988), found 10 μg/g Se as selenomethionine did not affect duckling growth. Thus, reduced growth in ducklings appears to be a consequence of embryonic exposure to Se.

Ten μg/g Se in the diet increased duckling mortality and reduced duckling production. In a study in which there was no embryonic exposure to Se, Heinz *et al.* (1988) observed no increase in duckling mortality at 10 μg/g Se but did observe mortality at higher levels. Heinz *et al.* (1987) reported a decline in duckling production for adults fed diets supplemented with 10 μg/g Se.

This study demonstrated that 10 μg/g dietary Se can have a significant impact on mallard reproduction and duckling growth and survival. Levels of Se in aquatic plants and invertebrates from Se-contaminated sites are often in excess of the levels administered in this study (Ohlendorf 1989; Hothem and Ohlendorf 1989), and the effects observed are known to occur under field conditions (Ohlendorf *et al.* 1986, 1989). Available laboratory and field data would suggest that selenomethionine, at the levels administered in this study, is a good model for Se toxicity under natural conditions (Ohlendorf *et al.* 1989; Heinz *et al.* 1989; Hoffman and Heinz 1988; Hothem and Ohlendorf 1989).

Interactive Effects of Arsenic and Selenium

In the present study, antagonistic interactions occurred between As and Se whereby As reversed the negative effects of Se. Antagonistic interactions between As and Se have been reported for a number of species (Hill 1975; Levander 1977). Arsenic decreases the retention time and toxicity of Se (Dubois *et al.* 1940) and increases the excretion of Se from the liver into the bile (Hill 1975). Sodium arsenite and sodium arsenate have been found to be equally effective in preventing Se toxicity, whereas the As sulfides were found to be ineffective (Dubois *et al.* 1940). Several organic arsenicals have shown partial protective action against Se toxicity (Levander 1977).

Accumulation of Se in adult liver was reduced by the presence of As in the diet. This effect has also been reported for chickens (Thapar *et al.* 1969). Arsenic in the diet reduced deposition of Se in whole egg, reversed Se-induced decreases in hatching success, and decreased embryo deformities. Antagonistic interactions between As and Se have been observed for hatching success in chickens (Thapar *et al.* 1969). It was notable that at 100 μg/g As in the diet, there was no statistical difference in the percentage of embryo deformities between birds receiving Se control diets and those receiving 10 μg/g Se in the diet.

Interactions occur when the difference in mean responses for two levels of one factor (As) is not constant across levels of the second factor (Se). For duckling production, the significant interaction observed occurred because 400 μg/g As decreased production (relative to lower levels of As) when in combination with Se control, but not when in combination with 10 μg/g Se. Thus, despite the significant interaction, there was no evidence that As counteracted Se-induced decreases in duckling production.

The results of this study demonstrate that As, as sodium arsenate, can reverse some of the adverse effects of Se on mallards. The importance of this interaction in the environment is unknown. Exposure to As and Se at contaminated sites may not be in the chemical forms administered in this study, and exposure levels, especially for As, may be lower than those administered. The latter, in particular, is an important consideration. For many of the response variables where there were antagonistic interactions, there appeared to be a threshold between 100 and 400 μg/g As where the protective effect of As was manifested. Arsenic levels in food chain organisms from As-contaminated sites in CA would suggest that such high levels are rare (Hothem and Ohlendorf 1989; Moore et al. 1989). Thus, the interactions observed may not occur under natural conditions and, therefore, may not be an important consideration in the management of contaminated sites.

Acknowledgments. We sincerely thank Grey Pendleton for advice concerning statistical analyses, and Peggy Fitzgerald and Carol Sanderson for providing technical assistance. We also thank Gary H. Heinz, Elwood F. Hill, and David J. Hoffman for helpful comments on an earlier draft of this manuscript. This study was done in cooperation with the San Joaquin Valley Drainage Program under Intraagency Agreement No. 6-AA-20-04170.

References

Braman RS, Foreback CC (1973) Methylated forms of arsenic in the environment. Science 182:1247–1249

Camardese MB, Hoffman DJ, LeCaptain LJ, Pendleton GW (1990) Effects of arsenate on growth and physiology in mallard ducklings. Environ Toxicol Chem 9:785–795

Daghir NJ, Hariri NN (1977) Determination of total arsenic residues in chicken eggs. J Agric Food Chem 25:1009–1010

Dubois KP, Moxon AL, Olson OE (1940) Further studies on the effectiveness of arsenic in preventing selenium poisoning. J Nutr 19:477–482

Durum WH, Hem JD, Heidel SG (1971) Reconnaissance of selected minor elements in surface waters of the United States, October 1970, Geol. Survey Circ. 643. U.S. Department of the Interior, Washington, DC, 1971, 49 pp

Heinz GH, Hoffman DJ, Krynitsky AJ, Weller DMG (1987) Reproduction in mallards fed selenium. Environ Toxicol Chem 6:423–433

Heinz GH, Hoffman DJ, Gold LG (1988) Toxicity of organic and

inorganic selenium to mallard ducklings. Arch Environ Contam Toxicol 17:561–568

———, ———, ———(1989) Impaired reproduction of mallards fed an organic form of selenium. J Wildl Manage 53:418–428

Hill CH (1975) Interrelationships of selenium with other trace elements. Fed Proc 34:2096–2100

Hoffman DJ, Heinz GH (1988) Embryotoxic and teratogenic effects of selenium in the diet of mallards. J Toxicol Environ Health 24:477–490

Hoffman DJ, Heinz GH, LeCaptain LJ, Bunck CM, Green DE (1991) Subchronic hepatotoxicity of selenomethionine in mallard ducks. J Toxicol Environ Health 32:449–464

Hoffman DJ, Sanderson CJ, LeCaptain LJ, Cromartie E, Pendleton GW (1992) Interactive effects of arsenate, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. Arch Environ Contam Toxicol 22:55–62

Hothem RL, Ohlendorf HM (1989) Contaminants in foods of aquatic birds at Kesterson Reservoir, California, 1985. Arch Environ Contam Toxicol 18:773–786

Krynitsky AJ (1987) Preparation of biological tissue for determination of arsenic and selenium by graphite furnace atomic absorption spectrometry. Anal Chem 59:1884–1886

Lancaster RJ, Coup MR, Hughes JW (1971) Toxicity of arsenic present in lakeweed. New Zealand Vet J 19:141–145

Levander OA (1977) Metabolic interrelationships between arsenic and selenium. Environ Health Perspect 19:159–164

Moore SB, Detwiler SJ, Winckel J, Weegar MD (1989) Biological residue data for evaporation ponds in the San Joaquin Valley, CA. San Joaquin Valley Drainage Program, Sacramento, CA

Ohlendorf HM (1989) Bioaccumulation and effects of selenium in wildlife. In: Selenium in agriculture and the environment, SSSA Special Publication No. 23. Soil Sciences Society of America, Madison, WI

Ohlendorf HM, Hoffman DJ, Saidi MK, Aldrich TW (1986) Embryonic mortality and abnormalities of aquatic birds: Apparent impacts by selenium from irrigation drainwater. Sci Total Environ 52:49–63

Ohlendorf HM, Hothem RL, Welsh D (1989) Nest success, cause-specific nest failure, and hatchability of aquatic birds at selenium-contaminated Kesterson Reservoir and a reference site. Condor 91:787–796

Palmer IS, Thiex N, Olson OE (1983) Dietary selenium and arsenic effects in rats. Nutr Rep Int 27:249–258

Peterkova R, Puzanova L (1976) Effect of trivalent and pentavalent arsenic on early developmental stages of the chick embryo. Folio Morphologica 24:5–13

Phillips DJH (1990) Arsenic in aquatic organisms; a review emphasizing chemical speciation. Aquat Toxicol 16:151–186

Ratcliffe DA (1967) Decrease in eggshell weight in certain birds of prey. Nature 215:208–210

Rhian M, Moxon AL (1943) Chronic selenium poisoning in dogs and its prevention by arsenic. J Pharmacol Exp Ther 78:249

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

Spehar RL, Fiandt JT, Anderson RL, DeFoe DL (1980) Comparative toxicity of arsenic compounds and their accumulation in invertebrates and fish. Arch Environ Contam Toxicol 9:53–63

Thapar NT, Guenthner E, Carlson CW, Olson CW (1969) Dietary selenium and arsenic additions to diets for chickens over a life cycle. Poult Sci 48:1988