# Effects of Methylmercury on Approach and Avoidance Behavior of Mallard Ducklings

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Contamination of the environment with mercury has been shown to be a threat to the survival and reproduction of certain birds (BORG et al. 1969, LJUNGGREN 1968). Little is known, however, about the effects of mercury on the behavior of birds. The present experiment was designed to provide preliminary data to determine whether low dietary levels of methylmercury dicyandiamide fed to mallard ducks (*Anas platyrhynchos*) would affect approach and avoidance behavior of their offspring.

## Methods

Dosing of Adults: The breeders were 18-month-old game-farm mallard ducks purchased from Whistling Wings in Hanover, Illinois. Three males and 10 females were randomized to each of three 2.33 m x 3.56 m pens. Flowing water and commercial duck-breeder mash were always present. Each pen was equipped with a strawfilled nestbox, 0.61 x 1.6 x 0.61 m high. Morsodren (active ingredient: methylmercury dicyandiamide) was dissolved in propylene glycol in concentrations that would provide 0.5 ppm and 3 ppm mercury when mixed into the duck feed. The Morsodrencontaining propylene glycol was mixed into the feed in the ratio of 2 parts propylene glycol to 98 parts mash; controls received an equal amount of clean propylene glycol. Treatments consisting of the two concentrations of mercury and controls were randomized to the three breeding pens on January 27.

The dietary concentrations of 0.5 and 3 ppm mercury were mixed into a commercial duck breeder mash which contained only about 7% water. Had the mash contained as much water as do natural diets of wild mallard ducks, the concentrations of mercury would be lower. To convert the concentrations of mercury in the commercial mash to what the concentrations would be in a natural duck diet, one must divide the ppm mercury in the mash by some value greater than 1; the value for the divisor would depend upon how much water the natural diet contained.

MARTIN and UHLER (1939) analyzed the stomach contents of 7,998 game ducks in the United States and Canada and found that plant matter constituted an average of 73% by volume of the contents and animal matter 27%. RICKETT (1921) calculated that 20 aquatic plants used as duck foods contained an average of 88% water. MEEKS (1968) analyzed a small fish and a variety of invertebrates and found that these animals contained from 76% to 85% water. Based upon these values for the animal and plant composition of game-duck diets and the average water content of these two groups of food, one would divide the value for ppm mercury in the commercial duck mash by about 6.63 to convert it to ppm mercury in the average natural duck diet described above. This division would convert the concentrations of 0.5 ppm and 3 ppm mercury discussed in this paper to values of 0.08 ppm and 0.45 ppm respectively.

If the natural diet of a group of wild ducks contained large amounts of wild seeds or cultivated grains, the divisor used to convert ppm mercury in the mash to ppm in the natural diet would be a much smaller number. DILLON (1959) found that almost 90% of the diet of mallard ducks in certain Louisiana rice-growing areas was rice and the seeds of plants associated with rice growing; the rice contained about 12% water. McATEE (1939) reported the water content of corn and black bindweed (*Polygonum convolvulus*) seeds to be about 12% also. If the total diet of the mallards in the Louisiana rice-growing areas did not have a water content exceeding 20%, one would divide the mercury concentrations in the commercial mash by about 1.16 to convert the 0.5 ppm and 3 ppm mercury to 0.43 and 2.58 ppm respectively.

According to the MARTIN and UHLER (1939) data, one would generally use a divisor closer to the 6.63 value, which is based on a succulent natural diet. A useful rule of thumb might be to divide the ppm mercury in the commercial mash by 5 to arrive at the ppm mercury that would have been in the mash if the mash had about as much water content as does the average mallard duck diet in the wild. Dividing 0.5 ppm and 3 ppm mercury by 5 yields values of 0.1 ppm and 0.6 ppm as the concentrations converted from commercial mash to a natural mallard diet. To make a precise conversion for a particular group of wild ducks, one would have to know the amount of water in the average diet of those ducks.

Two samples of the control diet and 3 samples each of the 0.5 and 3 ppm mercury diets were saved for chemical analysis.

Collection and Incubation of Eggs: Eggs were collected each day and stored at  $13^{\circ}$  C. At 2-week intervals, eggs were set in a Petersime incubator at a temperature of  $37.5^{\circ}$  C and at a relative humidity of about 80%. For every 2-week collection period, 4 eggs were randomly selected from each mercury treatment; the contents of the 4 eggs were pooled, weighed, and saved in a frozen condition for mercury analysis. For the first and last 2-week collection periods, 4 control eggs were saved for mercury analysis. Data in this paper are for ducklings that hatched from eggs collected during the 2-week collection periods one through nine (February 3 through June 7, 1972). Mercury Analysis: Feed and egg samples were analyzed for mercury by WARF Institute, Inc. using cold vapor atomic absorption. A sample of feed or homogenized pool of eggs was digested by refluxing with sulfuric and nitric acids. Hydroxylamine hydrochloride and stannous chloride were added to the digest to reduce the mercury (II) ions to mercury metal. The sample was aerated and, using atomic absorption, the mercury was measured in the air stream passing through a gas cell. The lower limit of detection was about 0.05 ppm mercury. Mercury recoveries by this method were 93% and 94% with spiked feed samples and 96% and 97% with spiked egg samples.

Care of Young: Ducklings were kept in the hatching compartment of the incubator, without food, until their approach behavior was tested. Metal leg bands were placed on the ducklings shortly after hatching to identify parentage. After I had tested the approach behavior of the ducklings I placed them into heated Petersime brood-unit pens (temperature in the heated portion of the pen was about  $35^{\circ}$  C). A pen contained 16 birds from the same treatment. Ducklings were fed commercial duck-starter diets containing the same concentration of mercury that their parents had been fed. Water was available at all times. The room in which the ducklings were kept was artificially lighted from 7:00 am to 9:00 pm.

Behavior Test Apparatuses: The apparatus used to measure approach responses to maternal calls consisted of 10 identical runways that measured 25 cm high and 12 cm wide. A 12-cm-long area in the front was divided from the rest of the runway by a swinging translucent plexiglass gate; ducklings were held in this area at the start of a test. Beyond the holding area, the runway extended 50 cm; the floor for the last 20 cm was a treadle. The weight of a duckling on the treadle closed an electrical circuit, and the presence of the bird was recorded on an Esterline Angus event recorder. Each runway was covered by a translucent plexiglass lid. The floor was covered with a green paper mat that provided the duckling with a good footing. The mats were replaced periodically. The walls and floor of the runway were constructed of white acoustical tile.

The stimulus used to elicit approach was a tape-recorded maternal call of a mallard hen. The call was recorded on the tape at 1-second intervals and was played at  $70 \pm 3$  db (re. 0.0002 dynes/cm<sup>2</sup>), measured at the location of the bird in the holding area, from a Jenson FX-35 3.5-inch loudspeaker located in the end of the runway above the treadle. The speaker was centered 12 cm above the floor and was covered by white cloth. The ambient sound level within the holding area was 50-58 db.

The test apparatus used for measuring avoidance responses consisted of 16 identical runways that measured 15 cm high and 12 cm wide. The walls were gray and the floor was lined with green paper mats to provide for a good footing. The mats were replaced periodically. Transparent plexiglass lids covered each runway. A 17-cm-long area in the front was divided from the rest of the runway by a swinging transparent plexiglass gate; the front of this area was covered with 1/2-inch x 1-inch wire mesh. This area served as a place in which ducklings were held at the start of testing. Beyond the holding area the runway extended 200 cm. The frightening stimulus was a 5-cm<sup>2</sup> wooden axle whose sides were alternately colored black and white. Plastic blades, 5 cm long x 1 cm wide, extended at right angles from each surface of the axle. A falling weight mechanically turned the axle at an average speed of about 3 revolutions per second, causing a flashing black and white pattern and noise created by the raking of the plastic blades against the wire mesh front of the holding area. A Polaroid camera photographed the apparatus from the top to give a visual record of how far each bird had run.

Testing Procedures: In order to qualify for approach behavior testing, a duckling had to be at least 12 hours posthatch age (hours since hatching) and between 26 days, 0 hours, and 26 days, 20 hours, in developmental age (age from the onset of incubation). Ducklings were tested in groups of 10, proceeding from those that hatched first through those that hatched later. Birds were carried in a common container from the incubator into a different room for testing and were assigned 1 to a runway without conscious selection of any individual from the container. Sixty seconds after the last duckling was placed in its holding area, the test was begun by turning on the tape-recorded maternal call and simultaneously swinging open the plexiglass restraining gate. For the next 5 minutes each duckling could move about in its runway as it chose.

Ducklings were tested for avoidance behavior twice: first between the developmental ages of 27 days, 0 hours, and 27 days, 7 hours (about 2 days of age after hatching), and again between the developmental ages of 33 days, 0 hours, and 33 days, 7 hours (about 8 days of age after hatching). Pens of birds were tested in random order. A pen of ducklings was removed from the brooder and carried in a container into a separate room where testing was done. Sixty seconds after the last of the 16 birds was placed in its holding area, the test began by swinging open the holding gate and immediately presenting the frightening stimulus. Two seconds later the Polaroid camera photographed the test runways.

Scoring of Behavior and Statistical Design: The following 4 measurements of approach behavior were calculated from event recording charts: (1) the latency of approach to the maternal call (the time elapsed from the start of the test until the bird stepped onto the treadle near the loudspeaker), (2) the number of reversals of location within the runway (from being on to being off the treadle or vice versa), (3) the percentage of time after initial approach that the bird spent on the treadle, and (4) the number of times the bird jumped from the treadle toward the loudspeaker per 100 seconds the bird was on the treadle.

In the statistical analyses of the approach behavior data,

it was assumed that there were no pen effects on approach behavior (all adults of a dosage group were kept in the same pen) and, therefore, the behavior of each duckling could be treated as an individual datum. Consequently, a greater likelihood of finding statistically significant differences by chisquare and analysis of variance tests existed than if I had been able to use each hen as the sampling unit. For this reason, results of statistical tests that are barely significant should be accepted with caution.

An interaction chi-square test was computed for the number of ducklings in each of the 3 groups that approached the maternal call within 5 minutes contrasted to the number that failed to approach. An analysis of variance was computed for the log 10transformed latencies of approach for those ducklings in each group that approached within 5 minutes.

An interaction chi-square test was run on the proportion of ducklings in each group that made 1 reversal in runway location contrasted to the proportion that made more than 1 reversal. Data for ducklings in each group that made more than 1 reversal were analyzed by analysis of variance of the log  $_{10}$  transformations of the number of reversals. Ducklings that never approached the maternal call did not enter into these analyses.

The proportion of birds in each group that spent 100% of their time, after initial approach, on the treadle is the same as the proportion that made only 1 reversal; consequently no statistical analysis was required on these data. For those birds spending less than 100% of their time on the treadle, an analysis of variance was performed using the angular transformation of percentage of time on the treadle.

An interaction chi-square test was run on the proportion of ducklings in each group that made no jumps from the treadle toward the loudspeaker contrasted to the proportion that did jump. For ducklings that jumped, an analysis of variance was computed using the log  $_{10}$  transformation of the number of jumps/100 seconds on the treadle. Ducklings that never approached the maternal call did not enter into these analyses.

The avoidance response of each duckling was classified as (1) not leaving the holding area after 2 seconds, (2) running up to 10 cm, or (3) running beyond 10 cm. An interaction chi-square contingency test was computed for the proportions of birds in each category for the three groups of ducklings; each group was then compared to each of the other groups in 2 (groups) x 3 (categories) interaction chi-square tests. Analysis of variance was computed on the basis of log 10 transformations of the distance in centimeters run for those birds running more than 10 cm. In these two statistical tests of avoidance behavior, it is assumed that there are no pen effects on behavior; as with approach behavior tests, one should be cautious in accepting results that are just barely statistically significant.

## Results

Residues of Mercury in Feed and Eggs: Analysis of feed samples revealed that both samples of control feed contained less than 0.05 ppm mercury. Feed samples from the 0.5 ppm mercury treatment contained 0.49, 0.53, and 0.64 ppm mercury; and feed from the 3 ppm mercury treatment contained 3.32, 3.32, and 3.55 ppm mercury. These values seem to be within the range of accuracy of the analytical methods used. Pools of control eggs from the first and last 2-week collection periods contained less than 0.05 ppm mercury. Residues in eggs from the 0.5 ppm mercury dosage leveled off at about 1 ppm mercury after 4 to 5 weeks of dosage. Residues in eggs from hens receiving the 3 ppm mercury dosage rose gradually until weeks 8 and 9 after which residues ranged between 6.46 and 9.19 ppm mercury. These calculations were made on the basis of the wet weights of the feed (about 7% water) and eggs (about 70% water). If one expresses the concentration of mercury in both feed and eggs on a dry-weight basis, the concentration of mercury in eggs is about 6 times as great as that in the feed for ducks fed the 0.5 ppm mercury diet and about 6 to 9 times as great for ducks fed the 3 ppm mercury diet.

Approach Behavior: There were no significant differences among the 3 groups of ducklings in (1) the percentage of birds approaching the maternal call within 5 minutes  $[0.10 > P(\chi^2 \ 2df > 5.45) > 0.05]$ , (2) the percentage of approach-responding ducklings that made only 1 reversal in the runway (and, therefore, spent 100% of the time after this reversal on the treadle)  $[0.5 > P(\chi^2 \ 2df > 1.61) > 0.3]$ , and (3) the percentage of approach-responding ducklings that the loudspeaker  $[0.2 > P(\chi^2 \ 2df > 3.71) > 0.1]$ . Data are listed in Table 1.

|            | Percentage of ducklings that: |              |             |  |
|------------|-------------------------------|--------------|-------------|--|
|            | Approached                    | Made only    | Jumped at   |  |
| Group      | maternal call                 | one reversal | loudspeaker |  |
| Controls   | 87.06 a*                      | 33.73 a      | 81.53 a     |  |
|            | (286)†                        | (249)        | (249)       |  |
| 0.5 ppm Hg | 88.75 a                       | 38.04 a      | 76.45 a     |  |
|            | (311)                         | (276)        | (276)       |  |
| 3 ppm Hg   | 80.60 a                       | 39.81 a      | 84.26 a     |  |
|            | (134)                         | (108)        | (108)       |  |

#### TABLE 1

Behavioral responses of mallard ducklings to a maternal call

\* Groups with a common letter were not significantly different at  $\alpha = 0.05$  in chi-square tests.

+ Number of ducklings in sample.

Among ducklings that approached the maternal call within 5 minutes, there were no significant differences among the 3 groups in analysis of variance tests of latency of approach  $[0.25 > P (F_{2}, _{630} \ge 1.71) > 0.10]$  and number of jumps at the speaker per 100 seconds on the treadle  $[0.50 > P (F_{2}, _{502} \ge 0.90) > 0.25]$ .

Analysis of variance tests revealed that ducklings from parents fed control diet made more reversals than did ducklings from parents fed diets containing 0.5 or 3 ppm mercury [0.001 >P (F<sub>2,398</sub>  $\geq$  7.22)] and that ducklings in the control group also spent a greater percentage of their time on the treadle (in the time remaining in the test after initial approach) than did ducklings from parents fed 0.5 ppm mercury in the diet  $[0.05 > P (F_{2,398} \geq 3.16) > 0.025]$ . Controls did not differ in percentage time on the treadle from ducklings whose parents were fed 3 ppm mercury in their diets (Table 2).

#### TABLE 2

| Behavioral | responses | of m  | allard | i duckli | ings | that   | approached | а |
|------------|-----------|-------|--------|----------|------|--------|------------|---|
|            | mate      | ernal | call   | within   | 5 m  | inutes | 3          |   |

|            |                                | Mean            |                  |                        |
|------------|--------------------------------|-----------------|------------------|------------------------|
|            |                                |                 | Percentage time  | Number of<br>jumps per |
| _          | Latency of                     | Number of       | on treadle       | 100 seconds            |
| Group      | approach (sec)                 | reversals       | after approach   | on treadle             |
| Controls   | 55.35 a <sup>*</sup><br>(249)† | 9.35 a<br>(165) | 70.15 a<br>(165) | 4.76 a<br>(203)        |
| 0.5 ppm Hg | 50.24 a<br>(276)               | 7.58 b<br>(171) | 61.93 b<br>(171) | 5.47 a<br>(211)        |
| 3 ppm Hg   | 59.92 a<br>(108)               | 6.37 b<br>(65)  | 68.49 ab<br>(65) | 5.19 a<br>(91)         |

\* Groups that do not have a common letter were found significantly different a  $\alpha$  = 0.05 using Kramer's (1956) modification of Duncan's (1955) Multiple Range Test.

+ Number of ducklings in sample.

Avoidance Behavior: A greater proportion of ducklings from parents fed diets containing 0.5 ppm or 3 ppm mercury ran more than 10 cm from the frightening stimulus than did controls at 27 days developmental age [0.005 > P ( $\chi^2$  > 16.17) > 0.001]. Although about the same proportion of ducklings in each of the 3 groups remained within the starting area, a greater proportion of ducklings from the control group ran only 1-10 cm (Table 3).

|            |     | Percent | age of duckling | s that ran: |
|------------|-----|---------|-----------------|-------------|
| Group      | N   | 0 cm    | 1-10 cm         | > 10 cm     |
| Controls   | 319 | 32.92   | 31.97           | 35.11 a*    |
| 0.5 ppm Hg | 339 | 35.10   | 19.77           | 45.13 ь     |
| 3 ppm Hg   | 158 | 38.61   | 20.88           | 40.51 Ъ     |

Behavioral responses of mallard ducklings to a frightening stimulus at 27 days developmental age

\* Groups with a different letter were found significantly different at  $\alpha$  = 0.05 in chi-square tests.

Ducklings in all 3 groups ran farther at 33 days developmental age than at 27 days, but those from parents fed diets containing mercury were again more likely to run beyond 10 cm than were controls  $[0.01 > P (\chi^2_{4df} > 13.29) > 0.005]$  (Table 4).

#### TABLE 4

Behavioral responses of mallard ducklings to a frightening stimulus at 33 days developmental age

| Group      | <br>N | Percentage of duck1<br>0 cm 1-10 cm | ings that ran:<br>> 10 cm |
|------------|-------|-------------------------------------|---------------------------|
| GIOUP      |       |                                     |                           |
| Contro1s   | 319   | 17.55 19.44                         | 63.01 a*                  |
| 0.5 ppm Hg | 339   | 15.34 11.21                         | 73.45 b                   |
| 3 ppm Hg   | 158   | 15.19 10.76                         | 74.05 Ъ                   |

\* Groups with a different letter were found significantly different at  $\alpha = 0.05$  in chi-square tests.

Among ducklings that ran beyond 10 cm at 27 days developmental age, there were no significant differences by analysis of variance  $[0.75 > P (F_{2,326} \ge 0.63) > 0.5]$  in mean distance run by birds in the 3 groups. At 33 days, ducklings from parents fed a diet containing 3 ppm mercury ran significantly farther than ducklings from parents receiving 0.5 ppm mercury  $[0.05 > P (F_{2,564} \ge 3.50) > 0.025]$ . Control ducklings did not significantly differ from ducklings in either mercury-dosage group in distance run (Table 5).

|            | Mean distance run     | o (cm) at ages:       |
|------------|-----------------------|-----------------------|
| Group      | 27 days developmental | 33 days developmental |
| Controls   | 40.70 a*<br>(112)†    | 67.26 ab<br>(201)     |
| 0.5 ppm Hg | 41.51 a<br>(153)      | 57.86 a<br>(249)      |
| 3 ppm Hg   | 42.47 a<br>(64)       | 74.46 b<br>(117)      |

Distance run by mallard ducklings from a frightening stimulus

 $\sigma$  Based on ducklings that ran more than 10 cm.

- \* Groups that do not have one letter in common were found significantly different at  $\alpha = 0.05$  using Kramer's modification of Duncan's Multiple Range Test.
- + Number of ducklings in sample.

## Discussion

About the same percentages of ducklings in the control group and the 2 mercury-treatment groups responded to the tape-recorded maternal calls (by approach, reversals, and jumps toward the loudspeaker). However, the magnitude of certain approach behaviors differed among controls and ducklings from parents fed diets containing 0.5 or 3 ppm mercury. Based on those ducklings that made more than one reversal, control ducklings apparently made more, but shorter, trips off the treadle under the loudspeaker than ducklings whose parents were fed a diet containing 0.5 or 3 ppm mercury. It is possible that control ducklings were most strongly attracted to the maternal call and that they made more reversals because they were more actively searching for the calling hen. No data were collected on the activity levels of each group of ducklings; controls might simply have been the most active group at the time approach behavior was measured. Control ducklings also spent more time on the treadle than ducklings from parents fed a diet containing 0.5 ppm mercury, although the level of statistical significance was not strong.

Ducklings whose parents were fed a diet containing either 0.5 or 3 ppm mercury were hyper-responsive in avoidance behavior compared to controls. As with approach behavior, it would be helpful to know the activity levels of each group of ducklings at the time avoidance behavior was measured. LAHUE (1973) fed orb-weaving spiders (Araneus diadematus) 0, 1, 2, 5, or 50 pg of methylmercury chloride per day for 2 weeks. At 1 and 2 pg of methylmercury chloride the frequency of web construction increased over the control group, whereas the 2 higher levels of mercury treatment caused a decrease in the frequency of web construction.

There is additional evidence that methylmercury can affect the behavior of animals. ROSENTHAL and SPARBER (1972) found that detour learning behavior was impaired in domestic chicks (Gallus gallus) hatched from eggs injected with as little as 0.5 mg methylmercury dicyandiamide/kg of egg. SPYKER et al. (1972) intraperitoneally injected pregnant mice with 0.16 mg of methylmercury dicyandiamide per 20 g of body weight and observed open field and swimming behavior of their young; young from mothers treated with mercury were less active in open field tests and exhibited abnormal swimming behavior.

Some mallard duck eggs from the wild have been found to contain more mercury than eggs laid by hens I fed 0.5 ppm mercury; DUSTMAN et al. (1972) found up to 2.7 ppm mercury in mallard eggs in the Lake St. Clair area. Most mallard eggs sampled from the wild, however, have contained less than 1 ppm mercury (DUSTMAN et al. 1972, VERMEER 1971). Although there are no records of wild mallard eggs containing as much mercury as found in the eggs of parents fed a diet containing 3 ppm, eggs from other species of birds have contained such amounts of mercury. A common tern (*Sterna hirundo*) egg from Lake St. Clair contained 6.25 ppm mercury (DUSTMAN et al. 1972), and herring gul1 (*Larus argentatus*) eggs have been found to contain up to 15.8 ppm mercury (VERMEER et al. 1973).

Feeding parents a diet containing 3 ppm mercury, in addition to affecting the behavior of healthy hatchlings, also reduces hatching success and survival of young (HEINZ 1974). However, it is not known to what extent methylmercury has affected the behavior of the young of wild mallard ducks or the young of any other species of bird in the wild.

## Summary

Mallard ducks were fed a control diet or a diet containing 0.5 or 3 ppm mercury (as methylmercury dicyandiamide) based on the dry feed. These mercury diets are approximately equivalent to 0.1 and 0.6 ppm mercury in a natural succulent diet. I measured for the ducklings the approach behavior in response to a tape-recorded maternal call and the avoidance of a frightening stimulus.

There were no significant differences among controls and ducklings from mercury-treated parents in the percentage of ducklings that approached the tape-recorded call. Control ducklings, however, moved back and forth toward the call more than ducklings from mercury-treated parents and also spent more time in the end of the runway near the loudspeaker than ducklings whose parents were fed a diet containing 0.5 ppm mercury.

Compared to control ducklings, ducklings from parents fed a diet containing 0.5 or 3 ppm mercury were hyper-responsive in the test of avoidance of a frightening stimulus.

Mallard eggs collected in the wild have been found to contain levels of mercury exceeding the 1 ppm (wet-weight) found in the eggs of hens fed a diet containing 0.5 ppm, but there are no reports of mallard eggs collected in the wild that were found to contain as much mercury (6 to 9 ppm) as eggs from hens fed a diet containing 3 ppm mercury. On a dry-weight basis, the concentration of mercury in the eggs was about 6 times as great as that in the feed for ducks fed the 0.5 ppm mercury diet and about 6 to 9 times as great for ducks fed the 3 ppm mercury diet.

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