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UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

WILDLIFE RESEARCH LABORATORY
BUILDING 45, FEDERAL CENTER
DENVER 2, COLORADO

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APPENDIX E 2 F R 10

June 4, 1952

Col. Kellogg
Commanding Officer
Rocky Mountain Arsenal
Derby, Colorado

Dear Col. Kellogg:

I am transmitting herewith a copy of a report prepared by Mr. George W. Sciple of this Laboratory with respect to his findings in connection with waterfowl mortality at the Arsenal Lakes. Whereas, we do not have the chemical facilities to perform all the analysis necessary in determining the identity of modern insecticides, it would appear quite evident from the work done by Mr. Sciple that toxic material of some kind has been present in these bodies of water.

Very truly yours,

/s/E. R. Kalmbach

E. R. Kalmbach
Director

Attachment

- cc: Director, Fish & Wildlife Service
- Dept. of Game Management
- Food & Drug Administration, Denver
- Mr. Dale Prentice, Rocky Mountain Arsenal
- Dr. Charles Davis, Bureau of Animal Industry
- Mr. Gurney Crawford, State Game Department
- Mr. George Sciple

STUDY OF AVIAN MORTALITY
ON THREE LAKES AT THE ROCKY MOUNTAIN ARSENAL
DERBY, COLORADO

by
George W. Sciple, Biologist
Wildlife Research Laboratory
Denver, Colorado

The program of investigation by the Wildlife Research Laboratory of duck mortality on lakes at the Rocky Mountain Arsenal was carried out in four phases. These were:

1. A field epidemiological survey.
2. Field toxicity experiments.
3. Chemical, gross pathological, and histopathological studies.^{1/}
4. Feeding and skin absorption tests.

The purpose of the first phase was to assess the mortality and to determine from field evidence the most likely source of the malady. The findings here guided operations in the other phases.

The purpose of the second phase was to determine whether healthy penned ducks could be intoxicated and killed by exposure on the waters of the Arsenal lakes.

Gross pathological and histopathological studies were undertaken to determine what lesions occurred, if any, in the dead birds, and whether pathognomonic lesions might be present.

Feeding and skin absorption tests were undertaken when strong indications of the source of the difficulty were found.

1. Field Epidemiological Survey

The field survey initially revealed many dead and dying birds on the water and along the shorelines of Upper Derby, Lower Derby, and Ladora Lakes. Many more carcasses were found far out in the surrounding grasslands and fields. Sick birds were seen to go into convulsions and fall while in flight. The many undisturbed carcasses found in the fields surrounding the lakes appeared from their distribution pattern to have come to their final resting places in this manner. A very rough estimate of total losses on the Arsenal lakes and on areas peripheral to them (during the period of investigation) was in the neighborhood of 1,200 ducks. In addition, several species of birds other than ducks were found dead on the shores of the lake. Carcasses of six species of mammals were reported in similar locations.

^{1/} The latter carried out by Dr. Charles L. Davis, Pathological Laboratory, U.S. Bureau of Animal Industry, Denver, Colorado

A characteristic syndrome was observed in the sickened free birds. The individual duck would sit quietly on the bank when approached closely by the observer. Its only movements would be an unusual repetitive "feeding" tremor of the mandibles and a coarse tremor of the head and neck. Depending upon the severity of the symptoms, the bird would either allow itself to be picked up without a struggle or would suddenly become alert as the observer came within a few feet of it. In the first situation, the duck seemed unable to coordinate muscular activity and could not escape. In the second situation, the bird apparently had a greatly reduced sensorium and was unable to respond except to the strongest stimuli. These latter birds, once they became alerted, would fly up strongly as the observer approached within 4 - 5 feet of them. They would rise sharply, but as an altitude of perhaps 50-75 feet was gained, the ducks would go into violent convulsions and fall back into the water. The convulsions were of a mixed sort with some clonic features, but also with a constant final opisthotonic component. Several birds were observed which were less heavily affected. They rose from the water and got into flight fairly well when flushed. After flight was underway, however, they were unable to maintain direction, level flight, equilibrium, or to judge distance. Several instances occurred where birds flew at full speed into the sides of buildings or into the ground, or folded their wings and "landed" while still 25 or 30 feet above the surface of the water.

These bizarre findings indicated strongly the action of an agent or agents affecting the central nervous system of the birds.

It was found that resident individuals (banding data) were the hardest hit by the mortality. While migrant species were also found to be susceptible to the agent or agents, they were found not to sicken immediately after their arrival in the area, but only after a time lag of about two weeks or slightly more. A similar time-lag was observed in experimental birds, as will be described later.

In every one of more than 30 daily periods of observation, resident species were found to be present on Upper Derby, Middle Derby, and Ladora Lakes. Only twice were birds seen on the toxic lake north of the plant area. On both of these occasions, the birds were found to be migrant species and not the resident birds.

Degree of mortality on the three industrial lakes appeared to be closely correlated with the population of birds resident on the lakes for a considerable period. Although "waves" of heavy mortality appeared to occur, it is believed that these were in reality reflections of heavy population use of the lakes through an extended time period precedent to the high mortality. In addition, as a fairly steady day-to-day mortality went on carcasses piled up to the point where they were noticed by the casual observer, who then tended to conclude that all the dead seen were representative of a single heavy mortality episode. The preponderance of field evidence was contrary to this point of view. Mortality actually seemed to be proportional to degree and intensity of population usage of the area, once an initial time-lag of two to three weeks had passed.

2. Field Toxicity Experiments

Field toxicity experiments were carried out by penning wild mallard drakes in various types of locations on all three of the industrial lakes. The experimental birds were obtained from a distant area free of any possible arsenal influences. This area was the Two Buttes Reservoir near Springfield, Colorado. (The full day to day record of the experiments is on file at the Laboratory. Because of its length an abbreviated summary will follow.)

A total of 12 mallards was used. Two of these were wing clipped, held on a small pool at the Federal Center, and were used as controls. Their diet was identical with that offered the experimental birds at the Arsenal. Both these birds remained well throughout the period of the experiment. They finally escaped after a storm wrecked a barrier enclosing them.

The experimental birds were at first exposed in one large cage on Upper Derby Lake for a one week adjustment and initial exposure period. All 10 birds were then moved to smaller cages, and were placed two to a cage in 5 locations on the three lakes. Two birds in one cage escaped due to the tampering with the cage by uninformed laborers. They were ruled out of the experiment. All of the 8 experimental birds died and both controls remained healthy.

The conditions preceding the deaths of these birds are of interest. Some of the caged birds died soon after the "lag period" mentioned above, while the others did not. It was at first thought that the locations were the differential factor. This view had to be modified, however, when it was found that the differential factor was surface exposure and exposure to the oily foam on the water surface. Ducks did not die when the base-boards of the cage projected above the water and closed off the cage from the general lake water surface. When water-surface and foam exposure were constantly present, the birds died without exception. Birds were taken from non-surface exposed cages where they had lived for extended periods and placed in cages offering surface and foam exposure. These birds then died after passage of the lag period. This evidence links toxicity directly to surface and foam exposure.

3. Chemical, Gross Pathological and Histopathological Studies

Sixteen necropsies were performed on ducks dying on the lakes. 1/ * The following were the findings in the 12 carried out by the author. With exception of brain, intestine, and lungs no pathology was noted grossly. A rather constant finding of unknown significance was a heart with ventricle in systole, auricles in diastole. The auricles were massively engorged with blood. Liver appeared normal, grossly, in all cases and no evidence of phosphorus poisoning could be detected. Gizzards were all normal and no

1/ Dr. Charles L. Davis, Pathological Laboratory, U.S. Bureau of Animal Industry, did three of these and Mr. Casimir Kuzniar, Rocky Mountain Arsenal, did one.

traces of lead shot could be detected. (One test for lead by the acetate method was negative also.) The intestine in several cases showed marked vascular congestion. In several cases the intestinal lumen, though clear of food material, was filled with an opaque, viscid, custard-yellow mucoid material. The lungs in 7 cases were markedly edematous, congested, and filled with a thin, blood-tinged effusion. The thin bloody fluid was also found in some quantity within the coelomic cavity in these cases. The brain showed considerable vascular congestion and pinkish coloration throughout. The meninges likewise showed vascular engorgement. A strong "chemical" odor, similar to that smelled on the lakes, was noted in the flesh and in the plumage of the birds.

Dr. Davis reports, in the histopathological examination, that no apparent histological changes were observed in the visceral organs. The appearance of possible tissue alterations in the brain has necessitated further examination of the material in hand. This examination has not yet been completed.

Gutzeit tests for the occurrence of arsenic were run on the organs of six birds. 1/ All were negative for the presence of arsenic.

Duck fat from birds dying on the lakes was supplied to Dr. Y.P. Sun, of Julius Hyman & Company, for bioassay and spectrophotometric analysis. His results were incorporated in a report to the Division Engineer dated 14 May, 1952.

Benzene extracts were carried out on several crude foam deposits found on Upper Derby Lake. One of these products was supplied to Dr. Sun for analysis.

Duck fat similar to that given Dr. Sun was turned over to Mr. Macdonald of Food and Drug Administration for bioassay and analysis.

4. Laboratory Experiments

Since a detailed account of the protocols of these experiments would be too voluminous for incorporation in this report, a condensation will be made.

The experiments involved feeding and skin absorption tests in mice and ducks. The material tested was an oily liquid, lighter than water, and with a viscosity and color roughly comparable to that of molasses. The odor was very strong and similar to that noted in the plant area of the Arsenal. This material was obtained by a simple benzene extraction of foam collected on the inlet stream (effluent from the plant) of Upper Derby Lake.

Practical obstacles hindered this work considerably and precluded the running of any but acute toxicity tests. Chief among these practical obstacles were limited time and the non-availability of wild male mallard ducks. In addition, the spring filling of the Arsenal lakes flooded out

* 1/ One of these was run by Mr. Casimir Kuznair of the Rocky Mountain Arsenal. Five were run by Messrs. Crabtree and Robison of the Wildlife Research Laboratory.

scum board and collection area. A single sample was available for tests on ducks. Three samples had previously been available for tests on mice.

Twenty-three to twenty-eight gram white mice were stomach tubed with as little as 0.05 cc. of the brown oily extract. Convulsions and death occurred in some of these animals within a minimum of an hour and fifteen minutes. This amount was uniformly fatal to all mice so dosed. Controls having "Mazola" corn oil in like amount remained unaffected.

Crystals were obtained from the brown oily extract. These were cleaned with benzene, air dried, ground to dust in a mortar, and the finely divided resulting material suspended in corn oil. Varying doses of this suspension stomach tubed into mice resulted in no deaths. The only observable symptom was a slight reflex hypersensitivity to sudden aural stimuli. The brown oil from which the crystals were isolated was then re-tested in mice and was found to retain its toxicity.

In a test of skin absorption, mice had an area of bare skin exposed by shaving the hair on the back above the pectoral girdle. Three drops of the brown oil were applied to this area. All mice so treated died within 24 to 28 hours. Terminal convulsions were observed in one case. Controls treated similarly with 3 drops of a bland corn oil ("Mazola") remained well.

Only 6 ducks and a single sample of foam extract were available for testing. An attempt was made to stomach tube the brown oil, undiluted, into a male mallard. It was found that the material was not retained. Vomiting occurred. It was found necessary to mix the brown oil with corn oil in order to avoid loss of the material through vomiting. This mixture was stomach tubed for three successive days. Neither death nor marked debility occurred. Some rather slight repetitive tremors of mandibles, head and neck, and wings were found to occur.

Control dosed only with corn oil appeared normal during the course of the experiment.

A bird was prepared for a skin absorption test by removing the plumage from an area on the back between the wings. Bare skin was exposed. The brown oil (0.2 cc.) was skin-applied at approximate 2 hour intervals. Neither death nor debility resulted. Some slight tremors of mandibles, head and neck, and wings were observed.

A control handled in a similar manner with corn oil applied to the skin surface appeared normal.

The only conclusion which can be drawn from the above is that, in the amounts administered, this sample of air-dried benzene extract of foam was not acutely toxic to male mallard ducks.

The need for more extensive studies is evident. Chronic toxicity studies should be run.

Conclusions

On the basis of the information gathered, it seems indicated that the avian mortality occurring on Upper Derby, Lower Derby, and Ladora Lakes is related etiologically to a surface-borne agent, or agents, toxic to the central nervous system. Though not yet proven, it is believed probable that this surface agent is contained in an oily scum which entered the lakes through a process-water drain from the plant area. The drain comprised one of the two sources of the "plant effluent" channel. This channel drains directly into Upper Derby Lake.

28 May 1952