hoped to realize a monetary return from the application. This certainly limits the use of these materials to their proper places.

Many states as well as the federal government are now seeking appropriations for further research on the relationship of agricultural chemicals to wildlife populations. This, we feel, is a good thing. The use of agricultural chemicals is becoming quite widespread and all the implications should be studied.

Most certainly this is an area where industry and the conservation groups can work together. As I have already indicated, our files contain volumes of data on the toxicity of our products toward various types of plant and animal life. We have data on the rate of disappearance of these chemicals. We have years of experience in working with these materials and in explaining their use to the public. This, certainly, is one of the greatest areas where industry and conservation agencies can and should work together on a point of mutual interest.

TECHNICAL GAME SESSION

A LABORATORY STUDY OF AN ARKANSAS DUCK DIE-OFF [1]

By CALVIN A. PAGE and JOHN J. LYNCH [2]

In January, 1956, a "die-off" of Mallard Ducks (*Anas platyrhynchos*) was reported in the Jonesboro, Arkansas area. Estimations of the number of birds involved have ranged between 15,000 and 20,000 from a flock concentration that varied between 250,000-500,000 birds localized over an area of approximately 700-1,000 acres. Field studies of this "die-off" indicated that the major cause of death was acute lead poisoning as based upon acid scarring of the gizzard, bile excretion and the presence of lead pellets in the gizzard. Just preceding the "die-off," a period of drought coupled with snow coverage forced the birds to dry-feed on soybeans from areas that had been hunted with the ground, therefore, heavily contaminated with lead pellets. In addition to lead poisoning, a great many of the birds showed a condition resembling crop-impaction.[3] A total of thirty specimens showing mixed symptoms of lead poisoning, "crop-impaction," and tissue necrosis were brought into the laboratory for examination.

Preliminary autopsy findings, Table I, indicated the probable causes of death to be lead poisoning and "crop-impaction" as a result of soybean engorgement. Similar impaction "die-offs" in Canadian Geese (*Branta canadensis*) have previously been reported by Hanson and Smith (1950),[2] and it was found by these investigators that dried soybeans would undergo an 85% increase in bulk within three after immersion into water. Durant (1956),[1] studying crop impactions in geese, reported that death would occur between 4-16 hours after drinking water following feedings on dry, hulled soybeans. An extreme shortage of water and other weather conditions in Arkansas just preceding this "die-off" were highly inducive to "crop impaction" and lead poisoning.

TABLE I

AUTOPSY SUMMARY

Findings	*Male**	*Female†*	*Total*
Gross Appearance			
Heavy	5	3	8
Normal	4	9	13
Emaciated	5	4	9
Rectal Staining	12	16	28

1 Financed by the Department of Bacteriology, Southwestern Louisiana Institute, Lafayette and the United States Fish and Wildlife Service, Lafayette.

2 Present address: United States Fish and Wildlife Service, Lafayette, Louisiana.

TABLE I—*Continued*

AUTOPSY SUMMARY

Findings	*Male**	*Female†*	*Total*
Crop Appearance			
Normal	6	6	12
Engorged	6	8	14
Proventriculus Engorged	5	2	7
Ruptured Crop	3	6	9
Gizzard Appearance			
Normal	9	5	14
Flaccid	5	11	16
Lead Pellets	7	7	14
Liver Appearance			
Normal	4	3	7
Reduced	9	11	20
Bile Sac Enlarged	10	10	20
Bile Staining	1	3	4
Intestine Appearance			
Normal	4	3	7
Flaccid	10	13	23
Lung Appearance			
Normal	12	10	22
Inflamed	2	6	8
Brain Appearance			
Normal	3	2	5
Inflamed	12	14	26
Hemorrhage	6	12	18

* Total males 14. † Total females 16.

In view of the autopsy findings of lead pellets in 14 duck gizzards and other evidences of lead poisoning, all gizzards were analyzed for lead in the tissue with negative results in those specimens not showing lead on autopsy. Hence, the presumptive diagnosis could not be substantiated.

EXPERIMENTAL PROCEDURES AND RESULTS

Necrosis of crop tissue in severe impactions led us to suspect the possible presence of a proteolytic microorganism. Therefore, crop contents were collected and pulverized portions were cultured for the presence of known aerobic or anaerobic pathogenic bacteria. No such organism could be demonstrated. To further determine the presence of a biologically active agent, feeding experiments were conducted using three groups of adult white mice, Manor Farm Strain, with eight mice per group. The first group was fed Purina Small Animal Chow. The second group was fed dried, hulled soybeans from the epizootic area, and the third group was fed crop contents taken from impacted specimens. This feeding regime was continued for two weeks. Three days after the start of the program, two mice from the third group died. Extreme distention of the intestine and inflammation of the lung was noted in both animals on autopsy. Intestinal contents from these animals were cultured for aerobic and anaerobic bacteria. These results were negative in so far as the presence of pathogenic organisms is concerned. Autopsy of all surviving animals at the end of the feeding regime did not reveal the presence of any morphological abnormalities.

Lung, brain, heart, and crop tissues from each specimen were removed at the time of duck autopsy. Each tissue was ground in a sterile mortar with pestle, using sterile sand as an abrasive. One ml of nutrient broth was added to the minced tissue and grinding was continued until a homogeneous paste was obtained. To this paste, 4.0 ml of nutrient broth was added and the material was transferred to a sterile centrifuge tube and centrifuged at 3,500 r.p.m. for 15 minutes at 4° C. The supernatant fluid was transferred to a sterile vial.

10,000 units of penicillin and 0.05 gram of streptomycin in suspension were added for each milliliter of fluid. The mixture was gently agitated for 15 minutes and aerobic and anaerobic sterility controls were made and found to be negative. The fluids were frozen and stored at —5° C. until used.

Virus survey was made using embryonated chicken eggs and white mice. Ten-day white leghorn embryos were employed with inoculation of 0.1 cc per embryo via the allantoic cavity route. Five embryos were used per tissue fluid. Death of the embryo during the first 24-hour post-inoculation period was considered to be trauma induced and was therefore not considered in the final results. Death following this period was considered to be evidence of virus activity. Embryos killed by the inoculum were chilled and the allantoic fluid was harvested, tested for bacterial sterility and used for a second embryo passage, Table II.

TABLE II

SUMMARY OF BIOLOGICAL SURVEY, EMBRYO PASSAGE

	First Passage		*Second Passage*	
Tissue Extract	*A*	*M*	*A*	*M*
Crop	1/30	0.7	0/1	0.0
Lung	5/30	4.2	1/5	4.0
Brain	0/30	0.0	...	..
Heart	3/30	3.4	1/3	6.6

A—Number of active tissues as evidenced by embryo mortality.
M—Per cent mortality for all embryos inoculated for each tissue type. (150 embryos.)

A very low incidence of biological activity was detected as a result of embryo inoculation of the tissue extract. Lung tissue had the highest activity both in the number of active tissues found and in the percent mortality of the embryo. This biological activity could be carried through a second embryo passage, but was lost on the third passage.

Mouse inoculations was made using two adult white mice per tissue. The route of inoculation was determined by the type of tissue extract to be used. Brain tissue extract was inoculated via the intracerebral route with an inoculum size of 0.1 cc, and lung tissue extract was inoculated via the intra-nasal route with an inoculum of 0.03 cc. All mice not killed by the inoculum were autopsied at the end of ten days and evidence of virus activity was taken to be distention of the intestine, inflammation of the lung or inflammation of the brain. All suspicious tissues were harvested and processed for embryo inoculation and a second mouse passage (Table III). Biological activity in mice was also very slight; however, two animals died as the result of tissue extract inoculation.

TABLE III

SUMMARY OF BIOLOGICAL SURVEY, MOUSE PASSAGE

Tissue Extract	*Dead*	*GDI*	*IB*	*IL*	*No Effect*
Crop	0	2	2	2	25
Lung	1	0	0	1	28
Brain	1	5	3	1	25
Heart	0	3	0	0	27

GDI—Gas distention of intestine.
IB—Inflammation of brain. IL—Inflammation of lung.

Autopsy of the survivors revealed a rather high number of intestinal distentions. All distended intestines were harvested and prepared for embryo inoculation. Bacterial sterility controls on these specimens were negative. Tissue extracts from mouse tissues were inoculated into ten-day embryonating chicken eggs via the allantoic cavity route. A high degree of activity was found on the first passage embryos, but it was not demonstrable for more than two serial passages (Table IV). Normal mouse tissue showed no significant activity.

TABLE IV

EMBRYO PASSAGE OF MOUSE TISSUE

Egg Passage	*Crop #4**	*Crop #30**	*Brain #14**	*Brain #18**
First	7/9†	7/9	7/10	2/10
Second	0/10	0/10	3/9	0/10
Third	0/10	0/10	0/10	0/10

* Number refers to original autopsy number given to specimen.
† Ten embryos were inoculated per tissue.

The specifically active tissues from mice were traced back to duck origin and a striking similiarity was found for each tissue. On initial duck autopsy, specimens numbered 4 and 14, arbitrarily, showed engorged and necrotic crops, brain inflammation and no lead in the gizzard. Specimens numbered 18 and 30 showed all but necrosis of the crop. All were impacted. Tissue extracts from these specimens produced gas distention of the intestine and brain inflammation on mouse passage.

DISCUSSION

The laboratory findings indicate that the Mallard "die-off" in Arkansas was due primarily to three causes: namely, lead poisoning, crop engorgment suggesting "impaction," and a micro-biological agent of low virulence to chicken embryos and white mice. It is postulated by the investigators that this low-virulent agent could be the biological factor responsible for a condition conducive to "crop impaction" since it is the opinion of these investigators that "impaction" would not occur or cause death unless there was present some other disturbing factor.

REFERENCES

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THE OCCURRENCE OF TUMORS IN WILD ANIMALS

By LAWRENCE KILHAM

Hunters have noticed "warts" or "horns" on cottontail rabbits for many years and have usually thrown snch animals away as unfit to bring home. In 1931, however, one hunter, who happened to be a member of the Rockefeller Institute for Medical Research, brought his cottontail, warts and all, back to the laboratory. This episode was the start of a chain reaction of investigations on virus tumors. The particular cottontail shot near princeton, New Jersey, had fleshy growths on its skin known as fibromas and in efforts to transmit the virus which causes them, Dr. Richard E. Shope obtained shipments of live cottontails from Kansas. Chance favors in the prepared mind in science. These western cottontails had a second type of tumor,—hard and shaped like a horn—of a type known as a papilloma. A proporation of rabbits afflicted with papillomas eventually die of cancer. While the present report consideres tumors as infectious diseases of wildlife, the principle investigations regarding them have been motivated by an interest in medical research.

The two tumors of cottontails, fibromas and papillomas, are distinct in their geographical distributions. The papillomas, or "horns," occur in states such as Minnesota, Iowa and Kansas west of the Mississippi and are found mostly on the heads and necks of afflicted animals. Fibromas or "warts," however, are limited to eastern cottontails. They have been encountered from Michigan to New York and Maryland and are located mostly on the feet and lower legs. A Surprising thing is that thousands of western cottontails have been liberated