TABLE 1

RESULTS OF FEEDING P^{32} TO *Aedes aegypti* DURING PERIOD OF EXTRINSIC INCUBATION OF *P. gallinaceum*

Mosquito lot no.	No. fed on gametocyte carrier (10-26)	Feeding during period of extrinsic incubation	Dissected for oöcysts (11-4)		Dissected for sporozoites (11-9)	
			No.	Positive	No.	Positive
544 A	50	Glucose solution only	5	3	4	2
691 A	50	Glucose solution only	5	5	11	9
688 A	50	P^{32} + glucose	5	5	36	none
692 A	50	P^{32} + glucose	5	5	25	none
692 B	50	P^{32} + glucose	none	..	32	none

estimate the total radiation delivered to the gland itself. The efficiency of our Geiger counter could be estimated as being approximately 20%, and this would mean that there were in the vicinity of 12,000 disintegrations per minute in the average glands during the early part of the feeding process. Owing to the extremely small amount of tissue involved, this would suggest a very high equivalent roentgen dosage. On the other hand, in spite of such a relatively high degree of radioactivity, it was surprising that there were no apparent deleterious effects on the mosquitoes themselves during the period studied. No gut examinations were carried out on the 14th day, and therefore there are no data as to presence or absence of oöcysts at this time in the 93 mosquitoes that showed no sporozoites in the salivary glands.

It appears that the amount of radiation in the salivary glands and/or the mosquito as a whole was sufficient to arrest development of the parasites during the oöcyst stage.

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Toxicity to Mice of Chlordane Vapor and Solutions Administered Cutaneously[1]

Hubert Frings and Joseph E. O'Tousa

Pennsylvania State College, State College, Pennsylvania

The toxicity of DDT to mammals has been rather thoroughly investigated (*2, 4, 7–9*), and it is now known that acute intoxication causes nervous disorders, and chronic intoxication results in fatty degeneration and necrosis of the liver. The newer insecticide, Chlordane,[2] however, has received much less attention. In general, its action is similar to that of DDT, but it is more readily absorbed through the skin (*1, 2, 6, 8*). Like DDT, it causes hepatic necrosis in chronic intoxication (*4, 6–10*). The present investigation was instituted to determine the effects of long-continued application of small quantities of Chlordane to the skin, and also to discover the possible toxicity of its vapor.

Five groups of virgin female Swiss albino mice were used in the experiments: Ten mice received no treatment and served as a control group; 16 mice received cutaneously 0.01 ml of odorless kerosene (Deobase) daily for 5 days per week; 16 mice received cutaneously 0.01 ml of 2% Chlordane solution in odorless kerosene (7.4 mg of Chlordane per kg of body wt per day) daily for 5 days per week; 16 mice received cutaneously 0.04 ml of 2% wettable powder suspension of Chlordane daily for 5 days per week; 60 mice in four subgroups were exposed to Chlordane vapor for different periods of time.

The amounts selected for application to the mice corresponded to doses which a man might receive were he to have his hands and arms wetted to the elbows. Because of the higher surface tension of the wettable powder suspension, more of this than of the kerosene solution is required to effect the same wetting. The solutions were applied from a micropipette to the intrascapular region of the mice. When applied at that spot, the solutions are difficult or impossible for a mouse to remove by itself. Extended observations showed that the mice made no effort to lick each other after one or two tastes of the kerosene or Chlordane solutions. Thus the materials entered the body through the skin and were not ingested. Since some of the Chlordane evaporated from the skin, there must have been some inhalation of the vapor, but probably not in appreciable amounts.

The cutaneous treatments were continued for a total of 20 weeks, 5 days per week. The total amount of kerosene or Chlordane-kerosene solution applied to each mouse was 1 ml and it contained 20 mg of Chlordane (740 mg/kg body weight). The total amount of Chlordane applied to each mouse in the group receiving applications of the wettable powder was four times this. Every month one mouse from each group, selected at random, was sacrificed for autopsy, and the tissues were fixed for histological study. Only the livers have been studied histologically so far.

For tests of vapor toxicity, two types of setup were used. In the first, 16 mice were housed in a wire-mesh cage supported in a box (12 in. × 20 in. × 36 in.) without touching the sides. This box was covered with three pieces of glass with 4-in. spaces between for ventilation. Once every 3 weeks, a strip about 2 in. wide around the inside of the box was painted with about 5 g of Chlordane. The concentration of Chlordane vapor was estimated at about 25% saturation at the start of the experiment, and as a result of accumulation of material on the strip, at about 50% saturation at the conclusion. Since quantitative tests for Chlordane vapor in air do not exist, these are only approximate estimates. The amount applied to the box was considerably higher than that which would be applied in pest control practice, and

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this was only partially offset by the fact that ventilation of the box was greater than that of an ordinary room. In the second setup, mice were exposed in a treatment chamber to air which had been bubbled through Chlordane in a saturation chain. Twenty mice were exposed to this continuously, 12 were exposed for 15 hr every day, and 12 were exposed for 9 hr every day.

A refined grade of Chlordane, containing 60%–75% Chlordane and 25%–40% related compounds also insecticidal in nature, was used in the experiments. This and the kerosene used as the solvent are standard products present in household insecticidal sprays. The carrier in the Chlordane wettable powder was Attaclay.

In view of the report by Ingle (*6*) that rats treated with Chlordane convulsed when subjected to intense auditory stimulation, the animals were tested every 20 days for susceptibility to "audiogenic" seizures. The technique was like that used by Hall (*5*) in his work on mice. A mouse was placed in a metal washtub to which an electric doorbell was fastened. After a period of 1 min, during which the mouse usually explored the tub, the bell was sounded for 75 sec or until the animal convulsed. The intensity of sound produced in the tub was 102 ± 2 db (relative intensity 10^{-16} watts/sq cm) at the periphery and 95 ± 2 db at the center, with the major frequency components between 0.7 and 1.2 kc.

The seizure pattern is striking. When the bell is turned on, the susceptible mouse cringes at the side of the tub. After a variable period of time, the mouse starts a series of short jerking runs. Finally it breaks into a frenzied run around the tub and after a few circuits of the tub falls over in a coma which usually culminates in death if the sound is not turned off. In our experiments, an effort was made to stop the bell just as soon as it was obvious that the wild running fit had started, so that the mice could be saved for further testing.

Although the physiological and psychological mechanisms involved in the seizure pattern are still obscure, it seems certain that susceptibility to seizure is a rough estimation of nervousness in the animals (*3, 11*). This gives us a method for detecting possible toxic effects on the nervous system before these become incapacitating.

The mice in the control group and in the group receiving applications of kerosene gained weight normally and appeared at all times in good health. The kerosene caused a loss of fur at the site of application, but there was no dermatitis. There were no deaths other than routine sacrifices. The livers of mice in both groups were normal both macroscopically and microscopically. In seizure tests, two mice in each group convulsed at the first two tests, but only one mouse, this in the kerosene group, continued to have seizures after the second test. Mice of this strain lose the tendency to seizure with increasing age; therefore, these results may be considered as representative of normal mice. The seizure results correspond well with those obtained in our laboratory with mice of the same strain being tested in a totally different experiment.

Mice in the groups receiving applications of Chlordane, either in the kerosene solution or the wettable powder suspension, reacted quite differently. After about 3 weeks of application, the mice became distinctly excitable, running to and fro in the cage without seeming purpose. They would jump from almost any height and became difficult to handle. Weight gains were normal, and appetites were unimpaired, however. Seven of the Chlordane-kerosene group and nine of the wettable powder group died before the end of the experiment. On autopsy, these were found to exhibit a rather typical liver damage. The kerosene solution brought about loss of fur, probably the result of the kerosene, and a dermatitis of variable severity characterized by roughening of the skin with local areas of bleeding and ulceration. The wettable powder suspension, on the other hand, caused only slight loss of fur and little or no dermatitis. The kidneys and liver of mice treated for 10 weeks or more appeared lighter than normal with white blotches, the lungs were occasionally hemorrhagic, and other organs appeared normal. Histologically the livers of mice from both groups showed a typical centrolobular necrosis after about 10 weeks of treatment, with the left lateral lobe most severely affected.

The mice in the treated groups exhibited a much higher percentage of seizures than normal mice. In the kerosene-Chlordane group, the number of seizures rose steadily from the start of the experiment; after 6 weeks of treatment the level of incidence was greater than 30%, and it remained over that for the rest of the experiment, the highest percentage being 45%. Three mice died in seizures in this group, the only mice which died during testing. Since in this group every mouse which convulsed continued to convulse in all subsequent trials, these three, which died early in the experiment, would probably have been among the individuals which had seizures in later tests, and thus the percentage incidence would undoubtedly have been about 45%–60%. The seizures of treated mice also were preceded by a shorter latent period than those of normal mice, and they were more severe. In the wettable powder group, the incidence of seizures remained about normal until after 9 weeks of treatment. Then this climbed to about 50%, where it remained until the end of the experiment. As a critical test of the effects of Chlordane wettable powder suspension on seizure incidence, mice about 40 days old were tested, and 7 of these which did not exhibit seizures were selected for treatment with the suspension. These young mice, all of which were negative at the start, showed 70% seizures following 3 weeks of cutaneous applications. There was no apparent correlation between the incidence of seizures and liver damage.

The mice in the group breathing a relatively low concentration of Chlordane vapor showed, after a few weeks, a characteristic loss of activity and muscular coordination. Weight gains and appetite, however, were normal. All the mice were dead after 16 weeks of exposure, half the deaths occurring one by one before the fifteenth week, and the remaining half immediately following renewal of the coating of the strip in the box at that time. There was evidence of liver damage after 6 weeks of exposure. Histologically, the liver presented the same picture of necrosis as was found in the other treated groups.

In sound tests, the group exhibited the normal low percentage of seizures up to 9 weeks of treatment. Following that, the percentage rose sharply to 50% just 2 weeks before all the mice died.

The mice in the groups breathing air saturated with Chlordane reacted quite differently. They immediately ceased feeding and drinking and huddled together, seeming loath to move at all. They apparently became blind and within relatively few hours lost all power of coordination. All the mice in the group exposed continuously died within 4 days, four of the 20 dying during the first day. The mice in the groups exposed for 15 hr/day and 9 hr/day were exposed for only 4 days. Half of the 15-hr group were dead at that time, and the others died within a few days. Two of the mice in the 9-hr group were dead at the end of 4 days (36 hr of exposure), and all but four died within the next 10 days. This confirms the statement of Radeleff (*10*) that animals "poisoned by Chlordane do not seem to recover, once they have manifest toxic symptoms." There were no observable anatomical differences between these mice and normal mice, except for extreme emaciation in mice which lived for some days. No sound tests could be given because the mice survived too short a time and seemed incapable of moving about with rapidity after 6 hr–8 hr of exposure to the vapor.

In general, results of this study indicate that Chlordane is very similar to DDT in its toxic action in mammals. The first system affected is the nervous system, and nervous symptoms predominate in acute toxicity. In chronic intoxication, however, the liver seems to be most affected. We were unable to determine to our satisfaction whether Chlordane accumulated in the fat of the mice or not. Possibly the cumulative toxicity is owing merely to destruction of the liver cells, along with effects on other organs of the body such as the kidneys.

Because of the widespread use of Chlordane in structural pest control, the rather striking toxicity of the vapor is significant. In this particular, Chlordane differs from DDT, just as it does in the apparent relative ease with which it is absorbed through the unbroken skin. A surprising feature is the relatively high toxicity of wettable powder preparations, since it is usually assumed that this type of preparation is less readily absorbed than kerosene solutions. All these results indicate that repeated exposure to Chlordane preparations on the skin or continued inhalation of the vapor may be deleterious.

Another striking point is that the treated animals gained weight normally, and except for greater irritability than the controls, seemed in good health. If transposition of results on mice to man is permissible, this indicates that the general level of health, appetite, weight maintenance, and the like would be a poor index of early chronic intoxication by Chlordane. The established fact that the chlorinated hydrocarbon insecticides have a similar cumulative action makes it imperative that clinical tests for detection of early intoxication in man be developed.

In experimental animals, the method of "audiogenic" seizures seems promising as a test for early intoxication. From our results with Chlordane, it is seen that the incidence of "audiogenic" seizures in a population of treated mice rises rather sharply a few weeks before clinical symptoms and deaths appear. With proper standardization of the test situation and with selected mice, this test may provide a good indication of early intoxication.

Finally, the results suggest that Chlordane may have value as a rodenticide. Since it is toxic to animals when absorbed through the skin and lungs, as well as when ingested, it might be used under favorable conditions for control of mice and possibly rats.

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Stability of the Adenosinetriphosphatase System in Animal Tissues[1]

Mona Marquette and B. S. Schweigert

Division of Biochemistry and Nutrition, American Meat Institute Foundation, and Department of Biochemistry, University of Chicago, Chicago

In the course of experiments on the stability of respiratory enzyme systems in beef tissues obtained from carcasses of different grades and aged for varying periods, the stability of the adenosinetriphosphatase (ATP-ase) system in intact tissues was investigated. Data on the nature and occurrence, metabolic function, and specificity of this system have been reviewed (*2, 3*); however, little attention has been devoted to studies on the stability of the system in intact tissues stored for varying periods of time after the animals are killed. In the present study the ATP-ase activity of animal tissues stored at $-2°$ C and at $+5°$ C for periods up to 15 days was investigated.

Preliminary experiments were conducted with rat tissues and the study was then extended to beef muscle tissue. The procedure used for determining the ATP-ase activity of the tissues was essentially that described by Umbreit, Burris, and Stauffer (*5*). Young adult rats that were fed stock ration were killed by decapitation. Approximately 1 g of muscle tissue, and in certain experiments liver and kidney, were removed and prepared

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