EFFECTS OF AN ORGANOPHOSPHORUS PESTICIDE ON REPRODUCTION IN THE RAT

Leonard C. Ryan, M.S.
Boyd R. Endecott, B.S.
Gerald D. Hanneman, D.V.M.
Paul W. Smith, Ph.D.

Approved by

J. Robert Dille, M.D.
Chief, Civil Aeromedical Institute

Released by

P. V. Siegel, M.D.
Federal Air Surgeon

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The animals used for this experiment were lawfully acquired and treated in accordance with the "Principles of Laboratory Animal Care" issued by the Animal Facilities Standards Committee on the Animal Care Panel, U.S. Department of Health, Education, and Welfare, Public Health Service, March 1963.

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I. Introduction.

The toxic effects of organophosphorus insecticides are commonly ascribed to cholinesterase (CHE) inhibiton. However, recent reports in the literature indicate that a number of other enzyme systems may be affected. Freedland and McFarland reported that the pesticides Co-Ral, Ruelene, and malathion inhibited the glutamate deyhdrogenase reaction.[1] Williams reported up to a 400-fold increase in serum β-glucuronidase activity following administration of sublethal doses of paraoxon to rats.[2] Physostigmine significantly decreased the concentration of glycogen in rat brain.[3] In view of these observations it is reasonable to expect a broader spectrum of physiological disturbances than those resulting from CHE inhibition alone.

A search of the scientific literature revealed the well-established fact that a number of chlorinated pesticides adversely affect reproduction in mice and rats.[4 5 6 7 8 9] However, little is known about the effects of organophosphorus pesticides on reproduction. Kimbrough and Gaines reported that a single dose of parathion caused a high incidence of resorptions and/or weight reduction of the fetus in rats.[10] In their study only the females received the insecticide.

These and other considerations prompted us to conduct this preliminary study of the effects of oral exposure to O,O-diethyl S-[2-(ethylthio) ethyl] phosphorodithioate (disulfoton, Di-Syston) on reproduction in rats.

Disulfoton was selected for this study because it is a widely used organophosphorus insecticide for which the acutely toxic, nonlethal dose in rats has been established.[11 12 13]

II. Materials and Methods.

Albino rats obtained from Cheek-Jones (Houston, Texas) were held in our animal quarters for 2 weeks after arrival.

The disulfoton was technical grade, 97%, and was obtained from Chemagro Corporation, Kansas City, Missouri. Cholinesterase measurements were made using the automated method of Levine *et al.*,[14] as modified by Fowler and McKenzie.[15]

The test diet was prepared by grinding pellets of Purina Micro-Mixed Laboratory Chow and by mixing with the appropriate amount of disulfoton dissolved in 95% ethanol. After mixing, the material was placed in a flat pan and the ethanol was allowed to evaporate. The control feed was prepared in the same way except that no disulfoton was added.

Virgin male and female rats, 12 weeks of age, were housed five per cage. Each cage contained animals of a single sex. The test animals were placed on the diet containing 10 ppm of disulfoton. The control animals were maintained on the control diet. All animals were allowed to eat *ad libitum*.

After the animals had been on the experimental diet for 60 days, they were arranged into five groups; each group contained five males and five females. The grouping was done in the following manner:

Group 1: both the males and the females had been on the test diet and remained on the test diet during the mating period.

Group 2: males that had been on the test diet were placed with females that had been on the control diet. They were given the control diet during the mating period.

Group 3: both the males and the females had been on the control diet and they remained on the control diet during the mating period.

Group 4: females that had been on the control diet were placed with males that had been on the test diet, they were given the test diet during the mating period.

Group 5: males that had been on the control diet were placed with females that had been on the test diet; they were given the test diet during the mating period.

The mating was accomplished by placing one male and one female together in an individual cage.

Since the length of the normal estrus cycle for rats is reported to be 4 days, the males and females were left together for 8 days to insure inclusion of at least one complete cycle.[16] The rats were then re-segregated into their original groups and maintained on their original diet. Each female was placed in an individual cage a few days prior to parturition.

Ample time was allowed for all pregnant animals to deliver, then brain cholinesterase activity was measured in the adult males and females. Brain cholinesterase also was measured in the offspring of mothers which had been fed either the test or control diet throughout the experimental period.

III. Results and Discussion.

The female rats on the test diet exhibited more severe signs of poisoning than did the males. This was attributed to the difference in oral toxicity for male and female rats.[12] That the female rats are more susceptible than the males to the toxic effects of the organophosphorus insecticides is also apparent from Table 1, which shows that the brain cholinesterase in the females was depressed to about 20% of control levels, whereas in the males it was depressed only about 50%.

Due to our inability to exercise 'round-the-clock surveillance during parturition it was not possible to make an accurate count of litter size. It is our impression that it was not markedly different in the various pairings studied. The primary effect of disulfoton appears to have been on the number of pregnancies that occurred (Table 2). When both the male and female rats received control feed all of the females became pregnant. In group 2, where the males were taken off the test diet and placed with control females, four of the females became pregnant. In group 1, where both the males and females were on the test diet, three of the females became pregnant. In group 4, where the female controls were placed with males that were on the disulfoton, three of the females became pregnant. In group 5, where control males were placed with females that were on the test diet, again, only three females became pregnant.

Table 3 indicates that the offspring of female rats receiving disulfoton during the entire experimental period have only about 68% of the brain cholinesterase activity exhibited by the offspring of mothers not receiving disulfoton.

These results indicate that the pesticide disulfoton, under the conditions of these experiments, does have effects on reproduction. The number of pregnancies was decreased in the animals receiving disulfoton. This decrease in the number of pregnancies in poisoned females could be attributed to such factors as alteration in the estrus cycle or the receptivity of the female animal. The decrease in pregnancies in females mated to poisoned males, on the other

TABLE I.—Cholinesterase Activity of Rat Brain in μmoles of Acetylthiocholine Hydrolyzed Per Minute Per Gram of Wet Tissue

No. of animals	Diet			Activity ±S.D.	Percent of control
	First period 60 days	Mating period 8 days	Final period 27 days		
Males					
5	Control	Control	Control	9.41±1.11	(100)
5	Control	Disulfoton	Control	8.69±0.31	92.3
10	Disulfoton	Disulfoton	Disulfoton	4.56±0.58	48.4
5	Disulfoton	Control	Disulfoton	4.83±0.55	51.3
Females					
10	Control	Control	Control	8.79±0.82	(100)
5	Control	Disulfoton	Control	6.79±0.58	77.2
10	Disulfoton	Disulfoton	Disulfoton	1.65±0.16	18.7

hand, could be attributed to decrease in sperm concentration or viability. Further studies will be necessary to determine the cause of the decrease in pregnancies; and also the possible effects of the insecticide on the reproductive efficiency of the offspring.

TABLE II.—Effect of Disulfoton on the Number of Pregnancies

Group	No. of animals	No. of pregnancies
1. Males and females on disulfoton	5	3
2. Males on disulfoton put on control while mating with control females	5	4
3. Males and females on control	5	5
4. Female controls put on disulfoton while mating with males on disulfoton	5	3
5. Control males put on disulfoton while mating with females on disulfoton	5	3

TABLE III.—Brain Cholinesterase Levels in 10-Day-Old Rats in μmoles of Acetylthiocholine Hydrolyzed Per Minute Per Gram of Wet Tissue

No. of animals	Treatment	Cholinesterase activity ± S.D.	Percent of control
10	Control mothers	5.23±0.75	(100)
10	Mothers on disulfoton for 95 days	3.55±0.66	67.9

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