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Multigeneration Reproductive Effects of Three Pesticides in Rats

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Multigeneration Reproductive Effects of Three Pesticides in Rats. KENNEDY, G. L. JR., FRAWLEY, J. P. AND CALANDRA, J. C. (1973) *Toxicol. Appl. Pharmacol.* **25**, 589–596. Administration of Delnav to 3 successive generations of albino rats at dietary levels of 3 and 10 ppm caused no measurable abnormalities among either parental animals or their progeny. Toxaphene at dietary levels of 25 and 100 ppm exerted no effects on litter size, pup survival, or weanling body weights but parental animals at 100 ppm showed liver changes consisting of slight cytoplasmic fatty vacuolization. This change was not observed at 25 ppm and was not accompanied by adverse effects on growth, mortality, clinical parameters, organ weights, or reproductive capacity. Herban at 500 ppm produced no abnormalities among parental animals or their progeny but at a level of 3000 ppm, growth of parental animals was slightly depressed; increases in absolute liver weights and in the liver-to-body weight ratios were observed. The growth depression was attributed to unpalatability of the test diet. No other parameter was adversely affected except for a slight depression in weanling body weights which did not persist beyond the first litter of the second generation. No-effect levels are judged to be 10, 25, and 500 ppm for Delnav, toxaphene, and Herban, respectively. Reproduction was not affected at levels of 10, 100 and 3000 ppm of Delnav, toxaphene and Herban, respectively.

The objective of this study was to determine the effects of each of three pesticides upon the reproductive performance of three successive generations of albino rats. The materials investigated were an organic phosphate insecticide [2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithionate)][3], a polychlorinated bicyclic terpene insecticide (toxaphene), and a substituted urea herbicide [3-(hexahydro-4, 7-methanoindan-5-yl)-1,l-dimethylurea].[4]

For each material a feeding level which had been demonstrated to produce minimal toxic effects in rats and a lower level considered to be without toxic effects in other studies were selected. In the case of Delnav, Frawley *et al.* (1963) reported no adverse effects upon the mortality, growth, and pathologic findings of albino rats following 90 days of feeding at a dietary level of 100 ppm. However, inhibition of brain, plasma,

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[3] Delnav®, Hercules Incorporated.
[4] Herban®, Hercules Incorporated.

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and erythrocyte cholinesterase activity of male and female animals treated at this level was noted, and as light depression in plasma and erythrocyte cholinesterase activity occurred at 10 ppm. Based on these findings, dietary levels of 3 and 10 ppm were selected.

Results of oral toxicity studies with toxaphene in the rat, as reported by Lehman (1952), indicated that liver changes, primarily fatty degeneration with cytoplasmic vacuolization, could be produced by continuous feeding of toxaphene at 100 ppm. Animals at 25 ppm were not affected. Therefore, dietary levels of 25 and 100 ppm were selected for the present study.

A 2-year feeding study with Herban in the rat conducted in this laboratory at dietary levels up to 5000 ppm revealed slight liver enlargement and slight growth depression at the highest level. This was attributed to poor diet acceptance. The levels selected for the reproduction study were 3000 ppm, to diminish the effects of unpalatability, and 500 ppm, which was a no-effect level in the chronic study.

METHODS

Two control and 6 test groups of Sprague Dawley albino rats, each consisting of 8 male and 16 female animals, were utilized. The animals were received as weanlings and were grouped and started on the experimental diets after 1 week of acclimation (28 days of age).

Technical grades of the pesticides were employed, and diets were prepared by adding corn oil[5] solutions of the pesticides to a commercial rat ration.[6] All diets, including the control diet, were adjusted to contain 2% corn oil. Diets were prepared fresh weekly and were available ad libitum without interruption until sacrifice of the animals.

The F_0 parental animals were mated at 100 days of age, 2 females to 1 male. The male was removed upon evidence of copulation (sperm-positive vaginal examination) or after 2 estrous cycles (10 days). If mating did not occur, each unmated female was exposed to another male from the same group during 2 additional estrous cycles.

First litters were retained for 21 days, litters of more than 10 pups were reduced to that number on day 5 post-partum; after a 10-day rest after weaning, the parental animals were again mated to obtain second litters. At weaning of the second litters, 8 males and 16 females were randomly selected from each group as parental animals for the next generation. This procedure was continued through 3 successive 2-litter generations.

For parental animals, weight gains prior to first mating and weight gains up to time of sacrifice, after 42 weeks on test diets for F_0 parents and after 39 weeks for F_1 and F_2 parents, were determined. Reproductive indices were calculated, and gestation times were recorded. All parental animals were examined for gross pathologic changes at sacrifice; organ weights and organ-to-body weight ratios were determined. Tissues (34) were retained and subjected to histologic examination.

Total and free cholesterol concentrations were determined for 8 animals of each sex from each group of third generation parents after 39 weeks of testing, using the methods of Caraway and Fanger (1955). At the same time, the following determinations were made on 3 male and 3 female rats from 1 control group and from each high-level test

[5] Mazola Oil-Heat Treated, Corn Products, Inc., Argo, Illinois.
[6] Wayne Mouse Breeder Diet, Allied Mills, Inc., Chicago, Illinois.

group: complete blood counts (hemoglobin, hematocrit, erythrocyte, total and differential leukocyte), cell indices (mean corpuscular volume of hemoglobin concentration and color, saturation and volume indices), reticulocyte count, platelet count, prothrombin time, serum glutamic–pyruvic transaminase and serum glutamic–oxaloacetic transaminase activities (Reitman and Frankel, 1957), fasting blood sugar (Hoffman, 1937), serum alkaline phosphatase (Bessey *et al.*, 1946), blood urea nitrogen (Marsh *et al.*, 1957) and icterus index. Urinalyses conducted at the same time included evaluation for glucose, albumin, and microscopic elements. Plasma, erythrocyte, and brain cholinesterase activity measurements were conducted for F_2 parental animals treated with Delnav, using the Williams (Williams *et al.*, 1957) modification of Michel's (1949) electrometric method.

All viable and stillborn progeny were counted, examined for external physical abnormalities, and records of progeny survival were maintained. Lactation indices were calculated, and at weaning (21 days *postpartum*) each pup was weighed and re-examined for physical abnormalities. Gross and microscopic pathologic studies, except for organ weights, were conducted upon F_{3b} weanlings. Plasma, erythrocyte, and brain cholinesterase determinations were made for 5 male and 5 female weanlings, selected randomly from second litters of each generation, from controls and from those groups treated with Delnav at levels of 3 and 10 ppm.

All data were analyzed by an analysis of variance and Student's *t* test was used for intergroup comparisons.

RESULTS AND DISCUSSION

Growth patterns for all parental animals were normal except for those treated with Herban at 3000 ppm, in which case a weight depression of approximately 10% at the time of first breeding was observed. This weight depression persisted until sacrifice and was associated with decreased food consumption.

No treatment resulted in increased mortality and no untoward behavioral effects were noted. Pathologic examination revealed that all deaths, with the exception of 6 female deaths which occurred as the result of uterine hemorrhage at parturition, were attributable to severe respiratory infection. No differences were observed among test and control groups of F_2 parental animals with regard to total, free and esterified cholesterol concentrations, hematologic parameters, urine analyses, and other clinical chemistry findings. Cholinesterase activity determinations for animals treated with Delnav failed to confirm the slight depressions in plasma and erythrocyte cholinesterase activity reported by Frawley *et al.* (1963) for younger rats fed 10 ppm.

In all 3 generations, the absolute liver weights and corresponding liver-to-body weight ratios were elevated among both male and female animals in the Herban 3000 ppm group. Organ-to-body weight ratio elevations ranged from 11 to 23%. Animals in the lower dietary group (500 ppm) did not exhibit an increase in either absolute liver weights or in the liver-to-body weight ratios. Liver weights and ratios for all Delnav and toxaphene test groups were comparable to those of control groups. Table 1 presents mean liver-to-body weight ratio (g/100 g) data for male and female parental animals in all 3 generations. No consistent statistically significant intergroup differences were found among organ weights of the kidneys, spleen, gonads, heart, brain, adrenal glands, and thyroid gland or among the corresponding organ-to-body weight ratios.

TABLE 1

MEAN LIVER-TO-BODY WEIGHT RATIO DATA IN PARENTAL ANIMALS

Treatment	Mean liver-to-body weight ratio (g/100 g)					
	Male			Female		
	F_0	F_1	F_2	F_0	F_1	F_2
Control 1	3.54	3.29	4.16	3.21	3.36	3.79
Control 2	3.69	3.32	3.77	3.53	3.31	3.83
Delnav, 3 ppm	3.41	3.47	3.87	3.62	3.28	3.61
Delnav, 10 ppm	3.29	3.58	3.67	3.34	3.08	3.78
Herban, 500 ppm	3.82	3.31	4.04	3.55	3.45	4.19
Herban, 3000 ppm	4.25[a]	4.17[a]	4.68[a]	3.97[a]	3.98[a]	4.89[a]
Toxaphene, 25 ppm	3.68	3.65	4.21	3.79	3.62	4.17
Toxaphene, 100 ppm	3.92	3.53	4.20	4.23[a]	3.34	4.20

[a] Statistically different from control, $p < 0.01$.

Pathologic findings, both gross and microscopic, revealed slight to moderate pneumonitis, mild hyperemia of the liver and kidneys, and other tissue alterations consistent with those diseases frequently observed in albino rats. The only finding among test animals which was not observed among control rats was cytoplasmic vacuolization of the livers of 63% of all animals examined in the toxaphene 100 ppm group. This condition, which has been reported previously by Lehman (1952), was mild in all cases and was not seen among toxaphene-treated animals at the 25 ppm dietary level. Findings among Delnav and Herban test animals were comparable to those of control animals in each of the 3 generations. The incidence of tumors was not influenced by ingestion of any of the 3 materials at any level fed. Ingestion of Delnav, Herban, and toxaphene did not interfere with the desire and ability to copulate or with the ability of the female to conceive and to carry the reproductive process to successful parturition. Mean gestation times ranged from 21 to 22 days. Lactation indices showed no deficiencies in the ability of test females to nourish their young. No significant differences in the number of pups delivered, number of stillborn animals, or number of pups viable at birth and at various intervals in the lactation period (day 0 to 21 post-partum) were noted among test and control animals in any of the 3 generations. Examinations of stillborn and dying pups failed to reveal any deaths which could be correlated with the administration of the test materials. Twenty-one day growth of pups at both dietary levels of Delnav and toxaphene compared favorably to that of untreated animals. Weanlings of both sexes from dams fed Herban 3000 ppm showed slight weight depressions in the first generation and in the first litter of the second generation. Third generation weanlings at this level and all pups from the 500 ppm Herban group showed body weights comparable to those of control animals. Data relating to reproductive effects are summarized in Tables 2–4.

No unusual reactions were observed among either test or control progeny at any phase of the testing program and values for erythrocyte, plasma, and brain cholinesterase activity among weanlings from Delnav-treated groups revealed no significant

TABLE 2

REPRODUCTIVE EFFECTS OF DELNAV IN RATS

Dose level (ppm)	Litter No.	Mating index[a]	Fertility index[b]	Pregnancy index[c]	Parturition index[d]	Mean litter size (viable)	Live birth index[e]	5-Day survival index[f]	Lactation index[g]	Weanling body weights (g)	
										Male	Female
None (control)[h]	F1a	55	87	100	100	10.4	93	96	91	50	49
	F1b	89	94	100	100	12.3	97	97	97	53	52
	F2a	60	92	100	100	9.7	93	98	86	51	50
	F2b	62	100	94	91	12.6	98	96	97	51	50
	F3a	35	100	94	87	8.9	92	90	73	48	44
	F3b	40	87	83	83	8.8	95	88	82	43	46
3	F1a	58	68	81	81	9.8	88	91	90	53	53
	F1b	84	100	100	100	12.0	93	88	100	56	54
	F2a	75	88	100	100	11.5	98	97	95	49	47
	F2b	62	100	100	100	12.7	100	97	97	53	50
	F3a	35	62	62	56[i]	8.3	81	95	77	43	40
	F3b	43	100	94	88[i]	10.4	94	94	77	44	43
10	F1a	58	79	94	94	10.9	94	96	95	51	47
	F1b	84	94	94	94	11.8	96	96	97	54	51
	F2a	75	89	100	100	10.5	98	96	86	48	47
	F2b	62	94	94	94	11.7	97	91	98	52	48
	F3a	35	93	87	87	9.7	98	94	89	41	39
	F3b	43	92	85	85	8.9	93	92	85	46	45

[a] Copulations/estrous cycles × 100.
[b] Pregnancies/copulations × 100.
[c] Pregnancies/females mated × 100.
[d] Deliveries/females mated × 100.
[e] Pups born viable/total pups delivered × 100.
[f] Pups viable at day 5/pups born viable × 100.
[g] Pups viable at day 21/pups retained at day 5 × 100.
[h] Data for 2 control groups are averaged.
[i] One female resorbed entire litter.

TABLE 3

REPRODUCTIVE EFFECTS OF TOXAPHENE IN RATS

Dose level (ppm)	Litter No.	Mating index[a]	Fertility index[b]	Pregnancy index[c]	Parturition index[d]	Mean litter size (viable)	Live birth index[e]	5-Day survival index[f]	Lactation index[g]	Weanling body weights (g)	
										Male	Female
None (control)[h]	F1a	55	87	100	100	10.4	93	96	91	50	49
	F1b	89	94	100	100	12.3	97	97	97	53	52
	F2a	60	92	100	100	9.7	93	98	86	51	50
	F2b	62	100	94	91	12.6	98	96	97	51	50
	F3a	35	100	94	87	8.9	92	90	73	48	44
	F3b	40	87	83	83	8.8	95	88	82	43	46
25	F1a	66	79	100	100	11.4	94	92	79	49	48
	F1b	100	100	100	100	12.4	99	90	96	54	53
	F2a	48	89	100	94	12.0	97	96	88	45	44
	F2b	75	100	100	87[i]	12.1	96	95	98	61	51
	F3a	31	100	88	88	9.9	96	93	83	45	42
	F3b	42	87	87	87	11.6	98	96	91	44	43
100	F1a	76	73	100	100	10.9	97	94	86	49	49
	F1b	56	100	94	94	11.5	96	95	89	54	54
	F2a	54	87	87	87	9.2	89	99	98	51	47
	F2b	75	100	100	100	10.6	99	98	98	51	47
	F3a	37	100	94	88[i]	9.3	97	97	86	53	53
	F3b	39	93	81	81	9.7	96	93	94	45	45

[a] Copulations/estrous cycles × 100.
[b] Pregnancies/copulations × 100.
[c] Pregnancies/females mated × 100.
[d] Deliveries/females mated × 100.
[e] Pups born viable/total pups delivered × 100.
[f] Pups viable at day 5/pups born viable × 100.
[g] Pups viable at day 21/pups retained at day 5 × 100.
[h] Data for 2 control groups are averaged.
[i] One female resorbed entire litter.

TABLE 4

REPRODUCTIVE EFFECTS OF HERBAN IN RATS

Dose level (ppm)	Litter No.	Mating index[a]	Fertility index[b]	Pregnancy index[c]	Parturition index[d]	Mean litter size (viable)	Live birth index[e]	5-Day survival index[f]	Lactation index[g]	Weanling body weights (g)	
										Male	Female
None (control)[h]	F1a	55	87	100	100	10.4	93	96	91	50	49
	F1b	89	94	100	100	12.3	97	97	97	53	52
	F2a	60	92	100	100	9.7	93	98	86	51	50
	F2b	62	100	94	91	12.6	98	96	97	51	50
	F3a	35	100	94	87	8.9	92	90	73	48	44
	F3b	40	87	83	83	8.8	95	88	82	43	46
500	F1a	69	80	100	94	11.7	95	92	92	50	48
	F1b	85	82	93	87	12.2	92	96	93	55	54
	F2a	48	100	100	100	10.7	97	99	98	48	48
	F2b	76	100	100	100	11.5	94	95	96	55	53
	F3a	57	94	100	100	10.3	95	97	80	41[i]	40
	F3b	53	94	94	81	11.1	97	93	90	45	45
3000	F1a	58	68	87	87	11.2	99	97	100	44[i]	42[i]
	F1b	75	100	100	100	10.3	98	96	100	47[i]	44[i]
	F2a	67	88	88	88	8.7	94	98	100	45[i]	43[i]
	F2b	74	88	94	94	9.5	90	98	97	47	46
	F3a	50	88	93	93	8.4	96	81	79	43[i]	41
	F3b	37	91	71	71	8.1	94	91	81	46	46

[a] Copulations/estrous cycles × 100.
[b] Pregnancies/copulations × 100.
[c] Pregnancies/females mated × 100.
[d] Deliveries/females mated × 100.
[e] Pups born viable/total pups delivered × 100.
[f] Pups viable at day 5/pups born viable × 100.
[g] Pups viable at day 21/pups retained at day 5 × 100.
[h] Data for 2 control groups are averaged.
[i] Statistically different from simultaneous control, $p < 0.01$.

depressions. No evidence of teratogenicity was noted among test progeny at any phase.

No-effect levels are judged to be 10, 25 and 500 ppm for Delnav, toxaphene and Herban, respectively.

REFERENCES

BESSEY, O. A., LOWRY, O. H. AND BROCK, M. J. (1946). Method for the determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.* **164**, 321–329.

CARAWAY, W. T. AND FANGER, H. (1955). Ultramicroprocedures in clinical medicine. *Amer. J. Clin. Pathol.* **25**, 317–326.

FRAWLEY, J. P., WEIR, R., TUSING, T., DUBOIS, K. P. AND CALANDRA, J. C. (1963). Toxicologic investigations on Delnav. *Toxicol. Appl. Pharmacol.* **5**, 605–624.

HOFFMAN, W. S. (1937). Glucose determinations using automatic procedures. *J. Biol. Chem.* **120**, 51–55.

LEHMAN, A. J. (1952). Oral toxicity of toxaphene. *Quart. Bull. Ass. Food Drug Offic. U.S.* **16**, 47.

MARSH, L. T., FINGERHUT, B. AND KIRSCH, E. (1957). Blood urea nitrogen determination using the auto analyzer. *Amer. J. Clin. Pathol.* **28**, 681–688.

MICHEL, H. O. (1949). Cholinesterase activity determinations using an electrometric method. *J. Lab. Clin. Med.* **34**, 1564–1568.

REITMAN, S. and FRANKEL, S. (1957). Colorimetric method for the determination of the serum glutamic oxalacetic and glutamic pyruvic transaminase. *Amer. J. Clin. Pathol.* **28**, 56–63.

WILLIAMS, M. W., FRAWLEY, J. P., FUYOT, H. N. AND BLAKE, J. R. (1957). Modification of the Michel electrometric technique for dog and rat blood cholinesterase. *A.O.A.C.* **40**, 1118–1120.