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Acute, Subchronic, and Chronic Toxicity of Chlordecone¹

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Acute, Subchronic, and Chronic Toxicity of Chlordecone. LARSON, P. S., EGLE, J. L., JR., HENNIGAR, G. R., LANE, R. W., AND BORZELLECA, J. F. (1979). *Toxicol. Appl. Pharmacol.* 48, 29-41. Oral LD50 values were 132 mg/kg for male rats, 126 mg/kg for female rats, 71 mg/kg for male rabbits, and approximately 250 mg/kg for dogs. The percutaneous LD50 for male rabbits was 410 mg/kg. Deaths were preceded by the development of severe tremors. Gross autopsy findings were essentially negative. Rats were fed chlordecone in concentrations of 1, 5, 10, 25, and 80 ppm for up to 2 years. All rats on 50 and 80 ppm died during the first 6 months. Depressed growth occurred at concentrations as low as 10 ppm for females and 25 ppm for males. Food consumption tended to increase as the dietary concentration of chlordecone became greater. This effect was associated with a measured increase in the metabolic rate. Concentrations of 5 ppm and higher accelerated and intensified the rate of development of proteinuria. Increased liver-to-body weight ratios were consistently observed. Elevated ratios for other organs measured (kidney, heart, spleen, and testes) were found at certain periods, but at higher chlordecone concentrations than required for liver. Principal histopathologic findings in rats were degenerative changes in liver cells (fatty changes, hyperplasia), kidney lesions (primarily glomerulosclerosis), and testicular atrophy. Hematocrit and hemoglobin values were reduced in rats receiving concentrations of 25 ppm and above. There was no evidence of a clotting defect, although some rats receiving 50 and 80 ppm showed a bleeding tendency. Beagle dogs fed chlordecone at concentrations of 1, 5, and 25 ppm for periods up to 127 weeks exhibited few gross or histopathologic signs of toxicity.

Chlordecone (decachlorotetracyclodecanone) is a polycyclic chlorinated compound also known by the commercial name, Kepone. It has been useful in controlling various insects such as roaches, ants, the banana root borer, and the tobacco wireworm. It is lipophilic and very persistent in the environment (Sietz et al., 1973). The production of this

pesticide in Hopewell, Virginia led to an environmental contamination problem of considerable magnitude. Neurological signs and symptoms and abnormalities of the liver and testes appeared in workers involved in the manufacturing of this substance (Cohn et al., 1976).

It has been difficult to devise rational therapy for acute or chronic chlordecone intoxication due to the relatively few studies of the mammalian toxicity of chlordecone.

The subchronic toxicity of chlordecone has been studied in the mouse (Huber, 1965). McFarland and Lucy (1969) have observed

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acute toxic effects in Japanese quail fed chlordecone. The latter study dealt with reproductive effects of the pesticide, as did the investigations of Naber and Ware (1965) in the hen and Good *et al.* (1965) in the mouse.

Other studies have focused upon cellular changes in the liver, gonads, and adrenal gland of Japanese quail (Atwal, 1973; Eroschenko and Wilson, 1974, 1975), hepatic mixed-function oxidase induction in mice (Fubacher and Hodgson, 1976), inhibition of ATPase activity in the catfish brain (Desai and Koch, 1975) and rat liver (Desai *et al.*, 1977a,b), and fetal toxicity in rats and mice (Chernoff and Rogers, 1976). The National Cancer Institute has reported that chlordecone is carcinogenic in mice and rats (Anonymously, 1976).

More recent studies have dealt with the effects of the pesticide on biliary drug excretion (Mehendale, 1977) and adrenal catecholamine content (Buggett *et al.*, 1977).

The present investigation was carried out to fill the need for a comprehensive study of the acute, subchronic, and chronic toxicity of chlordecone in two mammalian species, the rat and the dog.

METHODS

Chlordecone Solution and Diets

The material used in these studies was supplied by Allied Chemical Company and contained 94% chlordecone. All doses and concentrations are expressed as active ingredient. Solutions were made by dissolving chlordecone in warmed corn oil. The final dietary concentrations were obtained by making appropriate dilutions of a 1000-ppm concentration which were prepared by thoroughly mixing 20 ml of a 5% (w/v) corn oil solution of chlordecone with 980 g of finely ground Purina Chow.

Acute Toxicity

Using a 5% solution of chlordecone in corn oil the acute oral LD50 was determined by the method of Litchfield and Wilcoxon (1949) for male and female Wistar albino rabbits (four dosages with 10 animals each). The mean weights were: male rats, 180 g; female rats, 150 g; rabbits, 2 kg. The range of body weights for the dogs was 4.5 to 7.5 kg. The rats were

fasted for 5 hr prior to dosing. The dogs were fasted overnight and given 20 mg/kg morphine sulfate 1 hr before dosing to retard emesis.

An acute percutaneous LD50 was obtained for male albino rabbits (four dosages with 10 animals each, mean weight 2.2 kg) using a 20% (w/v) solution of chlordecone in corn oil. The hair was clipped from the trunk, which was then fitted with a Druize-type rubber girdle and the material introduced under the girdle; absorption appeared to be complete.

Subchronic and Chronic Toxicity

Rats. Six groups of 40 young male (mean weight, 64 g) and 40 young female (mean weight, 60 g) Wistar rats were randomly selected and placed on each of the following dietary concentrations of chlordecone: 0, 5, 10, 25, 50, and 80 ppm. The rats were weighed weekly and food consumption was measured over a 3-day period at the end of 1, 3, 6, 12, and 24 months.

Hematologic studies (hematocrit and hemoglobin, total and differential white cell counts) were made on five rats (randomly selected) of each sex at each diet level at 3-month intervals. Additional blood studies conducted at the end of the third month included platelet counts, serum calcium, prothrombin clotting time, and Factor V time.

Pooled urine samples (five animals, randomly selected) from each sex at each dietary level were analyzed for reducing substances (Urmitz method) and protein (Shevky and Stafford, 1923) at 3-month intervals. Oxygen consumption determinations were made by spirometry at 9 months on diet.

During Week 13, five rats (randomly selected) of each sex at each feeding level were sacrificed. This was repeated at 1 year for rats on 0 through 25 ppm (there being no survivors on 50 and 80 ppm). Additionally, three to five rats of each sex on 5 and 10 ppm diets and three males on 25 ppm were returned to control diet for 4 weeks and then sacrificed. The study was terminated by sacrifice of surviving animals after 2 years on diet. Organ to body weight ratios were determined at each sacrifice period for liver, kidneys, heart, spleen, and testes. The following tissues were taken for histopathologic study: brain, spinal cord, heart, lung, liver, kidney, spleen, gut, urinary bladder, bone marrow, skeletal muscle, skin, pancreas, thyroid, adrenal, pituitary, and gonad.

In another study, 40 male and 40 female rats were placed on diets containing 0 and 1 ppm of chlordecone. Except for omission of oxygen consumption measurements, fat analyses, special blood studies, and withdrawal studies at 1 year, the experimental details were the same as in the first study described above.

Dogs. Sixteen purebred beagle dogs immunized against distemper and infectious hepatitis were divided into four groups of two males and two females each. At an average age of about 6 months

they were placed on diets of finely ground Purina Dog Chow Kibbled Meal containing 0, 1, 5, and 25 ppm of chlordecene. Weighed quantities of food moistened with an equal weight of water were offered to the dogs once a day for a period of about 30 min and the amount eaten was recorded. The dogs were weighed once a week.

Hematologic determinations (hemoglobin and hematocrit and total white and differential cell counts) were made prior to placing the dogs on diet, at 2, 4, and 13 weeks on diet and at 3-month intervals thereafter. Urine concentrations of reducing substances and protein were determined on each dog at the same time periods except for 2 weeks.

During Week 108, two liver function tests were performed on the dogs receiving 0 and 25 ppm chlordecene. In one test, the sulfobromophthalein method was used, 10 mg/kg being given iv and blood drawn 15 min later for estimation of the serum concentration of the dye. The second test consisted of measurement of serum cholinesterase activity.

Two dogs on 25 ppm were sacrificed at the end of Week 124 and remaining surviving animals were sacrificed during Week 128. The same organ-to-body weight measurements were made and tissues taken for histopathologic study as in the study with rats described above.

RESULTS

Acute Toxicity

Oral LD50 values obtained for rats were 132 ± 8 mg/kg for males and 126 ± 12 for females. Deaths ranged from 2 to 7 days following dosing. Gross autopsy findings were essentially negative. The oral LD50 found for male rabbits was 71 ± 6 mg/kg. Deaths ranged from 2 to 11 days following dosing, all but one occurring within 6 days. Gross autopsy findings were essentially negative. The oral LD50 for dogs was estimated at about 250 mg/kg, deaths ranging from 2 to 8 days following dosing. Gross autopsy findings were essentially negative. The percutaneous LD50 found for male rabbits was 410 ± 65 mg/kg, deaths ranging from 3 to 13 days following dosing. There was little or no evidence of skin irritation.

In all of the species reported above, the outstanding sign of intoxication was the development of severe DDT-like tremors. These reached maximum intensity in 2 to 3 days following exposure and gradually sub-

sided over a period of a week or more in survivors. Exacerbation of the tremors occurred whenever the animal became excited, for example, by handling.

Subchronic and Chronic Toxicity: Rats

Tremors in rats receiving 80 and 50 ppm developed at the end of 2 and 3 weeks, respectively, and became progressively more severe with time. Slight tremor became evident in some rats on 25 ppm at about 3 months, developed to a moderate degree in a few by 5 to 6 months, and tended to regress rather than worsen with further passage of time. Rats on lower feeding levels showed no tremors at any time.

All rats on 80 ppm died within 17 weeks. Rats on 50 ppm survived well through 13 weeks, but all had died by week 25. One- and 2-year survivals were adversely affected in females on 25 ppm.

Body weights. Body weight data at representative periods for rats on 5 through 80 ppm chlordecene diets are summarized in Figs. 1 and 2. Depressed growth was evident by Week 3 in females (Fig. 1). Analyses of regression of the data with 95% confidence limits (Larson *et al.*, 1960) indicate the effective concentrations for this to be about 31 ppm at 3 weeks, 14 ppm at 6 weeks, 8 ppm at 13 weeks, 6 ppm at 26 and 52 weeks, 8 ppm at 78 weeks, and 23 ppm at 104 weeks. For males (Fig. 2), significant

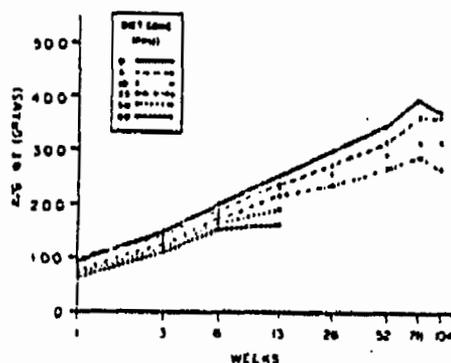


FIG. 1. Growth data for female rats fed chlordecene.

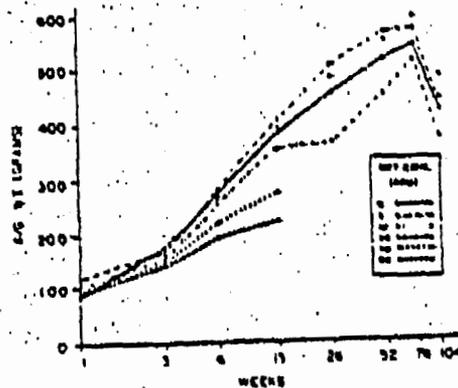


FIG. 2. Growth data for male rats fed chlordecone.

depression in growth did not appear until after Week 3. Thereafter, for the periods listed excepting 72 weeks, group analyses showed growth to be depressed at 25 ppm and any higher concentrations. At 5 and 10 ppm, treated males grew more rapidly than the controls. Thus, at 26 weeks, regression analysis indicated that male rats on 5 ppm weighed significantly ($p < 0.05$) more than the controls; this was true at both 5- and 10 ppm at 52 weeks.

Rats on experimental diets for 1 year that resulted in a depression of growth were then returned to control diet for 4 weeks prior to sacrifice. No definite tendency toward accelerated weight gains was observed during this 4-week period.

Food consumption. Food consumption data are summarized in Figs. 3 and 4. A trend toward increased consumption (per body weight) with increasing concentrations of chlordecone was observed. This tended to become accentuated with time.

Metabolic rate. Body weight and food consumption data and the development of muscle tremors were suggestive of an increased metabolic rate. Measurements were made during the ninth month and these are summarized in Table 1. Analysis of regression of the data with 95% confidence limits indicated that the upward trend in females did not reach statistical significance, but in males the

concentration for a significant elevation was about 12 ppm.

Hematology. In the hematologic data there was some suggestion of depression in hematocrit on 80 ppm at 3 months, and 25 ppm at 15 months, but the changes were not significant. Total white and differential white cell counts were within normal ranges.

An unusual development appeared during collection of blood for the hematologic studies at 3 months and was also noted sporadically at later test periods. The blood was obtained by cutting off the tip of the tail. Instead of only a few drops being obtained as is usual, some of the rats on the 50- and

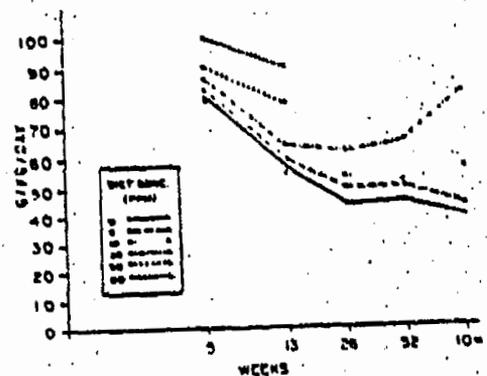


FIG. 3. Food consumption of female rats fed chlordecone.

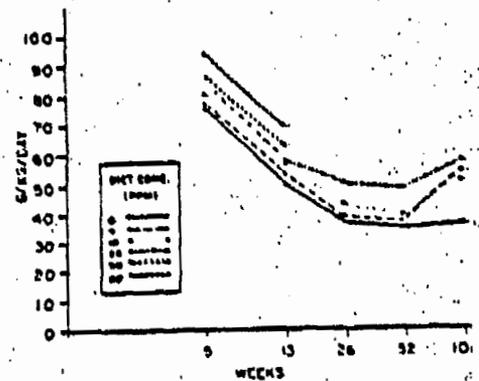


FIG. 4. Food consumption for male rats fed chlordecone.

TABLE 1

METABOLIC RATE VALUES (AS DETERMINED BY OXYGEN CONSUMPTION) OBTAINED DURING THE NINTH MONTH OF FEEDING OF CHLORDEZONE TO RATS

Sex	Dietary concentration (ppm)	Calories/m ² body surface/hr					Averages
		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	
Female	0	25.2	32.1	33.0	26.8	34.0	30.2
	10	34.8	26.3	33.5	31.9	32.4	31.8
	25	38.2	28.3	34.4	34.1	42.3	35.5
Male	0	29.7	32.6	27.4	30.2	29.3	29.9
	5	23.6	26.8	37.1	36.1	29.7	30.7
	10	48.4	34.5	35.5	35.7	34.5	37.7
	25	46.5	47.9	33.1	39.4	42.3	41.9*

* Significantly different from control ($p < 0.05$) by regression analysis.

TABLE 2

MEAN URINARY PROTEIN CONCENTRATIONS IN RATS RECEIVING CHLORDEZONE IN THEIR DIETS FOR 2 YEARS

Sex	Dietary concentration (ppm)	Urinary protein concentration (mg/100 ml)*							
		3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
Female	0	30	70	50	60	180	120	140	150
	5	30	90	70	100	500	650	650	98
	10	20	80	130	190	390	650	650	445
	25	30	160	180	280	430	500	620	590
Male	0	30	90	130	80	360	450	550	400
	5	40	110	320	620	640	620	650	540
	10	30	280	450	550	630	650	630	580
	25	30	420	350	500	650	650	650	1115

* Average of determinations on two samples.

0-ppm diets bled so profusely that it was necessary to cauterize to check the bleeding. Before this was realized, three rats subsequently bled to death after being returned to their cages. This prompted some additional studies. Platelet counts, serum calcium concentrations, prothrombin times, and Factor V times were not significantly different from controls. An alternative possibility may be that the profuse bleeding resulted from an unusual degree of dilatation of the tail vessels. Visual observation of the tail vessels suggested vasodilation. This could reflect an effect on the thermoregulatory mechanism secondary to elevated metabolic rates.

Urinalysis. The tests for urinary concentrations of reducing substances showed no dose-related effects from the chlordane at any period in the studies. Determinations of urine protein concentration are summarized in Table 2. It appears that chlordane, in concentrations of 5 ppm and above, accelerated and intensified the development of proteinuria. There appeared to be some regression of this effect in animals from the 5-ppm diet withdrawn from chlordane for 4 weeks after 12 months on diet. In the separate study in which 1 ppm was fed in the diet, no definite effect on urine protein concentration was observed.

TABLE 3
LIVER-TO-BODY WEIGHT RATIOS ON RATS RECEIVING CHLORDEBONE IN THEIR DIETS
FOR PERIODS OF UP TO 2 YEARS*

Sex	Dietary concentration (ppm)	Ratios $\times 10^3$			
		3 months	12 months	12 months + 4 weeks withdrawal	24 months
Female	0	35.7 \pm 3.0 (5) ^b	35.4 \pm 6.1 (5)	37.3 \pm 5.7 (4)	35.5 \pm 0.1 (14)
	5	42.2 \pm 3.4 (4)	37.0 \pm 5.1 (5)	36.8 \pm 1.8 (4)	43.6 \pm 10.3 (10)
	10	52.9 \pm 3.5 (4) ^a	39.4 \pm 10.7 (5)	46.9 \pm 5.5 (4)	64.1 \pm 17.5 (9) ^a
	25	65.4 \pm 5.4 (5)	70.8 \pm 17.2 (5) ^a	—	165.3 \pm 3.0 (3) ^a
	50	91.5 \pm 12.4 (5) ^a	—	—	—
	80	85.4 \pm 5.7 (5) ^a	—	—	—
Male	0	42.7 \pm 5.5 (5)	33.0 \pm 6.1 (5)	34.0 \pm 4.4 (4)	40.8 \pm 5.8 (7)
	5	46.8 \pm 2.2 (5)	41.3 \pm 4.8 (5)	36.4 \pm 6.6 (5)	56.4 \pm 10.7 (3)
	10	53.9 \pm 1.8 (5)	44.2 \pm 10.0 (5)	44.5 \pm 2.8 (5)	50.2 \pm 16.8 (4)
	25	60.0 \pm 5.9 (4) ^a	39.5 \pm 10.6 (5) ^a	59.4 \pm 3.4 (3)	75.3 \pm 18.9 (3) ^a
	50	82.4 \pm 9.1 (4) ^a	—	—	—
	80	89.1 \pm 17.7 (5) ^a	—	—	—

* Values are means \pm SD.

^a Number of rats indicated in parentheses.

^b Significantly different from control ($p < 0.05$) by regression analysis.

TABLE 4
KIDNEY-TO-BODY WEIGHT DATA ON RATS RECEIVING CHLORDEBONE IN THEIR DIETS
FOR PERIODS OF UP TO 2 YEARS*

Sex	Dietary concentration (ppm)	Ratios $\times 10^3$			
		3 months	12 months	12 months + 4 weeks withdrawal	24 months
Female	0	6.9 \pm 0.7 (5) ^b	7.6 \pm 1.0 (5)	7.9 \pm 0.5 (4)	8.9 \pm 3.5 (14)
	5	7.7 \pm 0.5 (4)	8.1 \pm 0.7 (5)	7.6 \pm 0.4 (4)	8.8 \pm 1.1 (10)
	10	8.3 \pm 0.8 (4)	7.4 \pm 0.5 (3)	7.7 \pm 0.9 (4)	9.6 \pm 1.7 (9)
	25	8.3 \pm 0.5 (5)	7.8 \pm 0.6 (5)	—	12.8 \pm 1.6 (5)
	50	9.2 \pm 0.8 (5)	—	—	—
	80	8.6 \pm 0.7 (5)	—	—	—
Male	0	8.0 \pm 0.3 (5)	6.8 \pm 1.3 (5)	7.6 \pm 1.0 (4)	10.1 \pm 4.6 (7)
	5	8.1 \pm 0.9 (5)	7.5 \pm 1.1 (5)	7.3 \pm 1.3 (5)	20.2 \pm 11.1 (3)
	10	8.0 \pm 0.4 (5)	8.0 \pm 0.8 (5)	8.2 \pm 1.2 (3)	13.0 \pm 5.7 (4)
	25	8.8 \pm 0.7 (4)	8.8 \pm 0.5 (5)	11.4 \pm 2.6 (3)	13.7 \pm 3.0 (3)
	50	9.2 \pm 0.4 (4)	—	—	—
	80	9.0 \pm 1.1 (5)	—	—	—

* Values are means \pm SD.

^a Number of rats indicated in parentheses.

Organ weight ratios. Liver-to-body weight ratio data are summarized in Table 3. Regression analyses of regression with 95% confidence limits indicated the chlordecone concentrations for significant increases in females and males, respectively, were: 5.3 and 15 ppm at 3 months, 8.7 and 9 at 12 months, 5.4 and 5 at 24 months. In rats returned to control diet for 4 weeks after 1 year, the difference in ratios for control and 10-ppm females barely reached significance at $p = 0.05$ and regression analysis for males placed the concentration for significant elevation at 3.7 ppm. In a second study at 1 ppm, a significant increase was found only for females at 3 months, the ratios for controls being 32.9 ± 1.1 and for experimentals 36.0 ± 2.6 (five rats each case).

Kidney-to-body weight ratio data are summarized in Table 4. Regression analyses placed the concentrations for significant increases at 12 ppm for females and 41 ppm for males at 3 months, 17 ppm for males at 12 months (no trend apparent for females), and 10 ppm for males returned to control diet for 4 weeks after 1 year (no trend apparent for females). Group comparisons of 24-month data revealed a significant increase only for males on 25 ppm. In the second study at 10 ppm, a significant increase was found only for females at 3 months, the ratios for controls being 6.7 ± 0.3 and for experimentals 11.1 ± 0.6 (five rats in each case).

Heart ratio data placed values for significant increases at 24 ppm for females and 10 ppm for males at 3 months (regression analyses), 25 ppm for males (group analysis). There was no trend for females at 12 months, no significant differences in either sex in rats returned to control diet for 4 weeks after 1 year, 25 ppm for females (group analysis) and no trend for males at 2 years. No significant difference existed between the 10-ppm rats and their controls.

Testes-to-body weight ratios were significantly depressed at 50 and 80 ppm at 3 months ($4.0 \pm 3.4 \times 10^{-3}$ and 2.6 ± 2.3 versus 5 ± 0.9 for controls) and significantly greater

at 25 ppm at 1 year ($8.3 \pm 1.2 \times 10^{-3}$ versus 6.7 ± 0.7). No significant differences appeared at the other time periods or at any time period in the 1-ppm study.

Spleen ratios showed no trend except for a significant increase over controls in the 25-ppm females at 2 years.

Histopathology. Histopathologic findings in rats sacrificed at 3 months on diet are summarized in Table 5. Congestion seen in the liver at 10 ppm was minimal in degree. Although degenerative changes in liver cells (swollen with margination of granules) occurred at the higher concentrations, no frank necrosis was seen in any of the sections in the entire series. For some unknown reason, the liver changes in rats receiving 80 ppm were less severe than in those receiving 50 ppm. Testicular atrophy, seen at the higher feeding levels in most severe form, appeared not to be related to alterations in the liver, since in four of the animals in which there was severe atrophy of the testes, the liver was not severely affected.

Histopathologic findings in rats on 1 through 25 ppm chlordecone sacrificed after 12 months on diet may be summarized as follows. Enlarged livers, indicated in Table 3, may be explainable on the basis of congestion. Examination of the nervous system did not reveal any foci of necrosis. The kidney lesions found were not markedly different from those normally seen in a certain percentage of rats of this age and did not provide a clear morphologic basis for the finding of elevated protein excretion.

Regarding findings on rats sacrificed after 12 months on diet followed by 4 weeks on control diet, none of the lesions seen appeared relatable to treatment.

Of the tissues submitted to microscopic examination from rats sacrificed after 2 years on diet, or dying during Year 2 in which tissues were not too autolyzed for meaningful interpretation, only kidney and liver presented lesions that appeared possibly related in incidence and degree to treatment. In kidney, the predominant lesion was glo-

TABLE 5

HISTOPATHOLOGIC FINDINGS IN RATS RECEIVING CHLORDEZONE IN THEIR DIETS FOR 3 MONTHS

Sex	Dietary concentration (ppm)	Histopathologic findings*
Female	0	Focal nonspecific myocarditis in 1. Eosinophilic infiltration of endometrium in 1.
	1	Focal nonspecific myocarditis, eosinophilic myometritis plus eosinophilic portal infiltration in liver in 1.
	5	Minimal tubular fatty nephrosis in 1.
	10	Congestion in liver in 2.
	25	Congestion in liver in 2. Loss of lipoid in adrenal gland in 2.
	50	Minimally swollen liver cells in 3. Swollen liver cells plus loss of lipoid in adrenal gland in 2.
Male	80	Swollen liver cells in 3.
	0	Minimal atrophy of testes in 1. Congestion in spleen in 1.
	1	Chronic bronchitis in 1. Congestion in spleen in 1.
	5	No lesions found.
	10	Congestion in liver in 3. Congestion in liver plus atrophy of testes in 1.
	25	Congestion and pale-staining swollen cytoplasm in liver cells plus atrophy of testes in 1. Swollen pale liver cells with margination of cytoplasmic granules plus atrophy of testes in 1. Swollen pale liver cells with margination of cytoplasmic granules plus minimal atrophy of testes in 1. Minimally swollen liver cells plus minimal atrophy of testes in 1.
	50	Swollen liver cells with margination of cytoplasmic granules plus adrenal atrophy with lipoid loss plus extreme atrophy of testes in 2. Swollen liver cells plus marked atrophy of testes in 1. Swollen liver cells plus some loss of lipoid in adrenal plus slight atrophy of testes in 1.
	80	Swollen liver cells plus atrophy of testes in 4. Swollen liver cells plus atrophy with fibrosis of testes in 1.

*Ten controls of each sex and five of each sex per diet level of chlordane.

merulosclerosis and this was analyzed as to degree according to the following criteria:

Grade 1: Minimal thickening of the capillary basement membrane of all of the glomeruli with protein in Bowman's space and hyaline protein casts in the tubular portion of the nephron, with slight dilatation of the tubules. No evidence of chronic pyelonephritis (interstitial nephritis).

Grade 2: Moderate degree of thickening of the capillary basement membrane of all of the glomeruli with protein in Bowman's space and considerable numbers of hyaline casts in the tubular portion of the nephron, leading to a moderate degree of dilatation. No or minimal accompanying pyelonephritis.

Grade 3: Kidneys enlarged and grossly

brown or tan color with numerous small cystic structures presenting themselves beneath the capsule (dilated tubules with protein). Microscopically, marked thickening of the capillary basement membrane of all of the glomeruli with accumulation of hyaline material in the intercapillary space. Adhesions sometimes obliterating the Bowman's space. Marked dilatation of the tubular portion of the nephron filled with protein so as to form cystic structures. Some degree of chronic pyelonephritis was invariably present.

Findings according to this grading system are summarized in Table 6. Rats receiving 1 ppm did not differ appreciably from the controls, but at higher feeding levels chlordane appeared to exert some nephrotoxic effect.

It is possible that lesions seen in the livers of three females on 10 ppm and one female and two males on 25 ppm were carcinomatous in nature.

Subchronic and Chronic Toxicity: Dogs

Tremors evident in rats on 25-ppm diet were not observed in dogs on 25-ppm diet.

Three dogs died during the study, one on control diet during Week 71, one on 1 ppm during Week 48, and one on 5 ppm during Week 50. All had developed severe dermatitis early in the study and this continued in increasing degree until death.

Body weight gains were similar in all groups of dogs during the first year, but tended to be lower in 25-ppm animals during the second year. Food consumption data indicated decreased food efficiency (kg body wt gain/kg food consumed) in dogs receiving chlordecone.

There were no effects on hematocrit, hemoglobin, total and differential white cell counts, or urinary concentrations of protein and reducing substances.

Liver function tests (sulfobromophthalein retention and serum cholinesterase) performed during Week 180 on 0- and 25-ppm dogs showed no differences between the two groups.

Organ-to-body weight data are summarized in Table 7. Only three of the differences from the controls are statistically significant: livers of 25-ppm dogs ($p < 0.01$), and kidneys and hearts of 25-ppm dogs ($p < 0.05$). Histopathologic examination revealed no consistency or gradation relatable to the test material.

DISCUSSION

Acute oral LD50 values obtained in the present study were: 132 mg/kg for male rats and 126 mg/kg for female rats. This agrees with the value of 125 mg/kg for both sexes reported by Gaines (1969). The acute percutaneous LD50 obtained in rabbits was

410 mg/kg, which is considerably less than the >2000 mg/kg reported by Gaines (1969). Sherman and Ross (1961) found the acute oral LD50 of chlordecone to be 450 mg/kg for New Hampshire chicks. McFarland and Lacey (1969) fed 400 ppm chlordecone to male Japanese quail and found a median death time of 10 days with death characterized by extensive body tremors progressing through ataxia to complete paresis. DeWitt and George (1960) observed approximate chronic LD50 values of 500 mg/kg for quail and 100 mg/kg for pheasants.

In the chronic feeding experiments, rats on 50 and 80 ppm all died during the first 6 months and 1- and 2-year survivals were adversely affected in females receiving 25 ppm. Huber (1965) found that 80 ppm or higher was lethal to all adult mice within 32 days but no mortality occurred in animals fed 40 ppm in tests extending up to 12 months. Huber also reported a 20-50% increase in food and water consumption of mice fed 40 ppm or more with no additional increase in body weight. Sublethal levels had no effect on the body weights of adults, but lethal levels led to weight loss prior to death. Mehendale (1977) exposed rats to 50, 100 and 150 ppm in the diet for 16 days. Gain in body weight was reduced after chlordecone to 86, 62, and 33% of the control group for the three doses, respectively. Chernoff and Rogers (1976) found a decline in maternal weight gain during gestation in rats and mice fed chlordecone. Naber and Ware (1965) fed chlordecone at 75 and 150 ppm to laying hens over a 16-week period. Hens fed the higher level lost body weight, although food consumption was not altered; the control gained weight.

In the present study, depressed growth occurred at diet concentrations estimated by statistical treatment to be as low as 6 ppm for females and 25 ppm for males. Food consumption data indicated a trend toward increased consumption per kilogram body weight with an increasing dietary concentration.

Hematologic studies indicated a tendency toward depression in hematocrit and hemoglobin values, a bleeding tendency in some, but no evidence of a clotting defect. This suggested that excessive dilution of the tail vessels, possibly secondary to an elevated metabolic rate was responsible for this effect. The only related finding is a significant increase in the hematocrit in the quail reported by McFarland and Lacey (1969).

Tests for urine concentration of reducing substances showed no diet-related difference. Tests for urine concentration of protein indicated that chlordecone in a concentration 5 ppm and higher accelerated and intensified the rate of development of proteinuria in rats.

Liver-to-body weight ratios were increased in both male and female rats. This has also been observed by Chernoff and Rogers (1976) in pregnant rats and mice, by Fabacher and Hodgson (1976) in mice, and by Eroshenko and Wilson (1965) and McFarland and Lacey (1969) in the Japanese quail. Huber (1965) found that the liver was the primary organ to increase in size in all animals fed 30 ppm or more. At 40 ppm, liver weight doubled in 60-90 days, but it was also noted that enlarged livers decreased in size when standard diet was withdrawn.

In the present study, elevated ratios for other organs (kidney, spleen, heart, and testes) were found at certain periods, but the chlordecone concentrations required for these effects to occur were higher than for liver. Significantly decreased ratios occurred only for testes at 50 and 80 ppm at 3 months. In the Japanese quail, McFarland and Lacey (1969) found the testes of chlordecone-treated rats were much larger than controls, but there was no significant difference in average weight. The enlarged testes had dilated seminiferous tubules, apparently containing lipid. The tubular epithelium was almost devoid of spermatozoa and spermatids. They also reported an increased weight of the gastrointestinal tract in treated animals, but no change in the weight of lungs, kidneys, or

brain. Eroshenko and Wilson (1974) found that immature Japanese quail of both sexes fed 200 ppm chlordecone, when exposed to 16 hr of light/day, had significantly enlarged reproductive organs, livers, and adrenal glands. When the photoperiod was reduced, testicular regression was recorded much earlier in males fed chlordecone than controls.

Principal histopathologic findings in rats sacrificed at 3 months were degenerative changes in liver cells (swollen with margination of granules, no frank necrosis) and testicular atrophy in animals on 25 ppm and higher concentrations. Congestion in the liver was seen in some animals. Kidney lesions found were not markedly different from those normally seen in rats of this age and did not provide a clear morphologic basis for the finding of elevated protein excretion. There are other reports of hepatic changes induced by chlordecone. Huber (1965) observed focal necrosis, cellular hypertrophy, hyperplasia, congestion, liposphere formation, and fewer mitochondria in mice. McFarland and Lacey (1969) reported an enlarged fatty liver with plurivacuolar inclusions in the quail. Fabacher and Hodgson (1976) found an increase in hepatic microsomal protein, cytochrome *P*-450, Type I and Type II ligand binding to cytochrome *P*-450, and *O*- and *N*-demethylation of *p*-nitroanisole and aminopyrine in the mouse. Atwal (1973) administered daily injections of chlordecone in corn oil to Japanese quail and reported large lipid vacuoles in the cytoplasm of hepatic cells of dilation of the rough endoplasmic reticulum. These latter studies are suggestive of induction of the hepatic mixed-function oxidase system by chlordecone. This is further supported by the recent observations of Mehendale (1977) showing increases in cytochrome *P*-450, NADPH-cytochrome *c* reductase, and aniline binding.

In rats surviving between 1 and 2 years on diet, two tissues (liver and kidney) presented lesions that were subjected to special study and interpretation. In the liver, the problem

concerned a possible carcinomatous nature of the lesions seen in six rats (three females on 10 ppm, one female and two males on 25 ppm). Following independent review by four pathologists, this possibility remains an equivocal one.

In the National Cancer Institute study (Anonymous, 1976), a significant increase was found in the incidence of hepatocellular carcinoma in rats fed chlordecone at 24 ppm and in mice fed 20 and 40 ppm. Also, the time to detection of the first hepatocellular carcinoma observed at death was shorter for treated than control mice. In chlordecone-treated mice and rats, extensive hyperplasia of the liver was also found. The incidence of tumors in other tissues was not significantly different from controls.

Regarding the kidney lesions in the present study, primarily glomerulosclerosis, the incidence in rats fed 1 ppm did not differ significantly from the controls. At higher feeding levels, chlordecone appeared to exert some nephrotoxic effect.

Purebred beagle dogs were fed chlordecone in their diet in concentrations of 0, 1, 5, and 25 ppm for periods of up to 127 weeks with few effects observed attributable to the test compound. Organ-to-body weight ratio determinants showed three differences (increases) from the controls that were statistically significant: livers of 25-ppm dogs ($p < 0.01$) and kidneys and hearts of 25-ppm dogs ($p < 0.05$). It is felt that the kidney and heart differences may reflect the virtual absence of fat and consequently decreased body weight of the 25-ppm dogs, but the liver difference may reflect a more specific effect. However, this effect is less than that found in rats.

This investigation and related studies indicate that the nervous system, the reproductive system, and the liver are major targets of chlordecone toxicity. Experiments designed to elucidate the mechanisms of these effects, their reversibility, and consequences (e.g., reproductive studies) are required for a more complete understanding of the toxicology of the compound.

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