tion of photodieldrin and its metabolites in orally treated animals thereby causing low levels of ^{14}C-labeled residues in tissues.

Analyses of organic extracts of liver and kidney show a number of metabolites. Most of the metabolic products found in liver extract are also observed in the urine and feces extracts with the exception of the two metabolites designated C and I. It is assumed that metabolite C might have formed or accumulated in the urine and feces during the process of excretion and elimination. Only two metabolic products are detected in the kidney.

Our attempts in identifying the above metabolites will help to understand the detoxication mechanisms involved in the metabolism of photodieldrin in rabbits.

ACKNOWLEDGMENT

We thank J. P. Bederka, Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, for providing metabolic cages. We thank H. B. Matthews and J. R. Bend of the National Institute of Environmental Health Sciences for a critical review of the manuscript.

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Received for review February 10, 1975. Accepted May 15, 1975. This study was supported by Grant No. ES00808 from the National Institute of Environmental Health Sciences.

Effect of Hexachlorophene on Reproduction in Rats

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Administration of hexachlorophene to three successive generations of albino rats at dietary levels of 12.5, 25, and 50 ppm failed to produce any changes with respect to mating, fertility, length of gestation, and number of deliveries. Numbers of progeny produced, survival of the young, and weight of the animals at weaning were not altered by exposure to the chemical. All progeny obtained from test and control groups were free of gross external abnormalities and displayed no unusual behavior during the experiment. Histopathologic evaluation of parental animals from each of the three generations and from weanlings of the final (F3b) generation failed to reveal any lesions which could be attributed to the ingestion of hexachlorophene.

Hexachlorophene, 2,2′-methylenebis(3,4,6-trichlorophenol), has been widely used as an antibacterial agent in a wide variety of products. The toxicity of the material has been recently reviewed (Kimbrough, 1974). Since the material is a polychlorinated polycyclic compound, concern has been expressed over the possible long-term effects of repeated, low-level exposures to the chemical.

Thorpe (1967) reported that the oral administration of hexachlorophene (HCP) to male rats as well as to sheep produced degeneration of spermatogenic cells. Gaines and Kimbrough (1971) reported reduced survival in F1 generation offspring fed 100 ppm. Studies with hamsters (Alleva, 1973) have indicated that HCP did not interfere with reproduction in that species. The present study assesses the influence of feeding HCP on reproduction in rats fed over three consecutive generations.

Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois 60062.

Table I. Reproductive Performance of Rats Fed Hexachlorophene

Dietary level, ppm	Litter no.	Mating index[a]	Fertility index[b]	Pregnancy index[c]	Parturition index[d]
None (control)	F1a	59	94	100	94
	F1b	75	100	100	100
	F2a	33	87	100	92
	F2b	59	92	100	100
	F3a	27	61	69	100
	F3b	34	70	78	71
12.5	F1a	63	94	100	100
	F1b	89	100	100	100
	F2a	64	100	100	100
	F2b	58	100	100	100
	F3a	38	88	94	100
	F3b	57	88	93	100
25.0	F1a	52	100	100	86
	F1b	60	93	100	100
	F2a	33	87	100	100
	F2b	54	100	100	92
	F3a	36	77	88	100
	F3b	50	93	100	100
50.0	F1a	52	88	100	93
	F1b	87	100	100	100
	F2a	36	100	100	93
	F2b	48	100	100	100
	F3a	33	74	88	100
	F3b	44	100	100	100

[a] Copulations/estrus cycle × 100. [b] Pregnancies/copulations × 100. [c] Pregnancies/females mated × 100. [d] Deliveries/females mated × 100.

Table II. Progeny Survival for Rats Fed Hexachlorophene

Dietary level, ppm	Litter no.	Mean litter size (viable)	Live birth index[a]	Survival index[b] days 1–5	Survival index[c] days 6–21
None (control)	F1a	10.4	95	87	85
	F1b	11.3	99	75	82
	F2a	10.8	97	97	75
	F2b	11.5	96	99	91
	F3a	9.7	99	90	51
	F3b	11.2	100	82	92
12.5	F1a	11.6	96	63	81
	F1b	12.7	98	81	90
	F2a	11.6	98	79	65
	F2b	11.5	99	82	92
	F3a	11.3	95	89	71
	F3b	13.7	97	84	71
25.0	F1a	11.4	98	65	81
	F1b	10.0	91	90	93
	F2a	11.3	99	84	74
	F2b	13.0	97	82	92
	F3a	10.6	98	95	85
	F3b	11.7	97	96	87
50.0	F1a	11.0	97	58	91
	F1b	13.2	99	80	100
	F2a	10.1	95	88	82
	F2b	11.5	99	84	93
	F3a	10.7	97	59	90
	F3b	10.5	90	88	70

[a] Pups born viable/total pups delivered × 100. [b] Pups viable at day 5/pups born viable × 100. [c] Pups viable at day 21/pups retained at day 5 × 100.

EXPERIMENTAL SECTION

The study employed a control and three test groups. Each group consisted of 8 male and 16 female CD rats (Charles River Breeding Laboratories, North Wilmington, Mass.). All the animals were grouped and started on the experimental diets at the age of 21 days.

Diets were prepared by adding acetone solutions of HCP to a commercial ration (Special Mix Mouse Chow, Ralston Purina Co., St. Louis Mo.) to attain final concentrations of either 0 (control), 12.5, 25.0, or 50.0 ppm. Diets were prepared fresh weekly and were available ad libitum without interruption until sacrifice of the parental animals after weaning of their second litters.

The parental animals were housed for breeding, 2 females per male, at 100 days of age. The male was removed upon evidence of copulation of each female or at the end of 2 estrus cycles (10 days). If mating did not occur, each unmated female was housed with another male of the same group for 2 additional estrus cycles.

First litters were retained for 21 days, litters of more than 10 pups being reduced to that number on day 5 postpartum. After the weaning of first litters the females were allowed a 10-day rest period before being remated to obtain second litters. At weaning of the second litters, 8 males and 16 females were randomly selected from each group to serve as parental animals for the next generation. This procedure continued for three successive two-litter generations.

Weight gains prior to the first mating and weight gains up to the time of sacrifice were determined. Reproductive indices were calculated and gestation times were recorded. All parental animals were examined for gross pathologic changes at sacrifice and organ weights and organ to body weight ratios were determined. Tissues were retained and subjected to histologic examination.

All viable and stillborn progeny were counted and examined for external physical abnormalities, and records of progeny survival were maintained. Indices of survival during the pre-weaning period 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 F3b weanlings.

All data were analyzed by an analysis of variance and a student's "t" test was applied for evaluation of intergroup differences.

RESULTS AND DISCUSSION

Growth patterns for animals in all groups were normal and there were no deaths attributable to ingestion of HCP. Pathologic examination revealed that all deaths, except for those of two females which resulted from severe uterine hemorrhage during parturition, were attributable to severe respiratory infection. No abnormal reactions or untoward behavioral reactions were noted among treated animals.

Gross and microscopic pathologic findings from examination of tissues and organs of parental animals of each generation revealed only those changes consistent with diseases frequently observed in albino rats. No evidence of central nervous system impairment, either functionally or histologically, was obtained during the three generations.

The mating, fertility, pregnancy, and parturition indices (Table I) indicate the ingestion of HCP did not affect the desire or ability to copulate or the ability of the male to impregnate or of the female to conceive and to carry the reproductive process to successful parturition. Mean gestation times ranged from 21 to 22 days with extended periods

Table III. Body Weights of Progeny from Rats Fed Hexachlorophene

Dietary level, ppm	Litter no.	Weanling body wt, g	
		Male	Female
None (control)	F1a	50	46
	F1b	53	53
	F2a	45	39
	F2b	49	49
	F3a	46	40
	F3b	47	44
12.5	F1a	42[a]	42[a]
	F1b	48	45[a]
	F2a	47	45[a]
	F2b	55[a]	49
	F3a	42	43
	F3b	47	46
25.0	F1a	47	44
	F1b	50	48
	F2a	45	45[a]
	F2b	48	48
	F3a	45	44
	F3b	49	44
50.0	F1a	49	45
	F1b	49	47
	F2a	41[a]	42
	F2b	55[a]	51
	F3a	53	49[a]
	F3b	50	49[a]

[a] Differ significantly from control weights; $P < 0.05$.

of 23 and 24 days observed in one F1b animal fed 50 ppm and in one F3a control female, respectively.

The number of live born young (Table II) exceeded 10 per litter (colony norm 11.2 ± 1.2) except for the F3a control litters. The number of pups stillborn or cannibalized was not elevated in any of the groups. The live birth index was lower than 95% in only two cases, F1b litters at 25 ppm and F3b litters at 50 ppm. The low values resulted because of 1 female at each period delivering a complete litter of stillborn pups (11 in the 25 ppm litter and 13 in the 50 ppm litter). Pup survival during lactation was not reduced by exposure to HCP. Values lower than 75% (colony norm 83 ± 8%) were seen among control animals in the F3a generation, 12.5 ppm animals in the F2a, F3a, and F3b generations, 25 ppm animals in the F2a generation, and 100 ppm animals in the F3b generation. The greatest reduction (to 51%) was observed in the control F3a litters.

Body weights of progeny at weaning (21 days postpartum) showed no consistent differences which could be attributed to HCP (Table III). Male weanling weights were reduced (as compared to the concurrent control weights) in the 12.5 ppm F1a litter and the 50 ppm F2a litters and were increased in the 12.5 and 50 ppm F2b litters. Female weights were lowered in the 12.5 ppm F1a and F1b litters and were increased in the 12.5 ppm F2a, 25 ppm F2a, and 50 ppm F3a and F3b litters.

None of these variations could be related to level of treatment or to cumulative duration of exposure through three generations and are consistent with the random variations encountered in control populations.

All progeny obtained during the experiment were free of external abnormalities and a complete, histopathologic evaluation of weanlings randomly selected from the F3b generation failed to reveal any treatment-related lesions. No unusual reactions were observed among either test or control progeny at any phase of the testing program.

It is concluded that HCP fed to rats throughout three generations at feeding levels of 50 ppm or less does not interfere with reproductive parameters and that signs of a pharmacotoxic response to the chemical are not produced.

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Received for review March 24, 1975. Accepted June 6, 1975.