Histopathologic Changes Produced by Hexachlorophene in the Rat as a Function of Both Magnitude and Number of Doses

Gerald L. Kennedy, Jr., and Donovan E. Gordon
Industrial Bio-Test Laboratories, Inc.
1810 Frontage Road
Northbrook, Ill. 60062

INTRODUCTION

The ability of hexachlorophene [HCP, 2,2-methylenebis(3,4,6-trichlorophenol)] to produce hind limb paralysis along with vacuolar lesions in the brain and spinal cord in the rat has been well documented (KIMBROUGH and GAINES 1971; KENNEDY et al 1972, 1975; NAKAUE et al 1973; deJESUS and PLEASURE 1973). The onset of posterior paralysis was shown to be related to the magnitude of the exposure. The above studies, in all of which the chemical was administered in the diet, involved varying weekly doses (in terms of mg/kg), calculated on the basis of body weight and food consumption. This report covers a study designed to monitor the effects of HCP in the rat at each of 4 dose levels and at various lengths of exposure, using serial sacrifices at each of 3 time intervals.

MATERIALS AND METHODS

A total of 75 young (70-day old) albino rats (CD stain, Charles River Breeding Laboratories, Wilmington, Massachusetts) were divided into 5 equal sized groups so that the group mean body weights on the first treatment day were identical. Groups were selected to receive either 0, 10, 20, 40, or 80 mg HCP (G-11 Brand, Givaudan Corporation, Clifton, New Jersey) per kg body weight. Doses were administered daily via gavage with the HCP prepared as a 10 percent (w/v) suspension in a 0.5 percent (w/v) carboxymethyl cellulose-water solution. Control (0 mg/kg) animals received the vehicle in volumes equivalent to those given the highest test group animals. Animals were housed individually, and food (Laboratory Rat Chow - Ralston Purina Inc., St. Louis, Missouri) and water were available ad libitum. The body weight of each animal was obtained daily to allow accurate mg/kg dosing and was recorded weekly to serve as an index of growth.

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Five animals from each group were sacrificed 4 hours following either the 7th, 14th, or 21st dose. The brain, spinal cord and optic nerve were removed from each animal immediately after sacrifice and placed in 10 percent formyl saline for 72 hours. The tissues were then processed and replicate sections stained with hematoxylin-eosin and solochrome cyanine (to demonstrate myelin). The stained sections were examined by light microscopy to determine the occurrence and severity of the pathologic changes.

Statistical evaluation of mean body weights were analyzed for significance of difference by Student's "t" test (SNEDECOR 1956). The 95 percent confidence level was selected to indicate significance.

RESULTS

Growth, as indicated by body weight data, was reduced at the 40 mg HCP/kg dose (Table 1). The magnitude of the observed change was minimal and statistical significance was seen only at the 2 and 3 week points. No signs of a pharmacotoxic response to HCP were seen during the experiment at dose levels of either 10 or 20 mg/kg. Weakness of the hind limbs, followed by restricted movements, was observed in animals treated at 40 mg/kg. The first signs of a response in this group were observed after the 5th dose with the majority of the animals displaying hind-limb immobility following the 10th dose. Among the 5 animals treated for 21 consecutive days, paralysis of both hind-limbs, along with diarrhea, was observed in 4 of the animals. The fifth animal appeared free of any untoward reaction to HCP.

All animals given 80 mg HCP/kg died following 2 to 5 doses, displaying anorexia and severe weight reduction, but no sign of paralysis were evident prior to death.

Histopathologic changes (Table 2) were present in the brain, spinal cord, and optic nerve. There was generalized edema or status spongious of the white matter which involved the entire central nervous system. Histologically, the lesion was characterized by the separation of myelin strands or vacuolation of the white matter. The brain (especially the cerebellum) was more severely affected than the spinal cord or optic nerves. There was no evidence of demyelination or of a cellular reaction in the neural tissues of affected animals.

The scores, presented as average grade, indicate the exent of involvement in the sections examined. Absence of change is rated as 0, and severe vacuolation (at least 75 percent of the viewing area consists of numerous individual to coalescent vacuoles of various sizes) is rated as grade 3. Grades 1 and 2 refers to vacuolation of intermediate severity. The mean values presented indicate the average grade for each group of affected animals. Animals with a grade of 0 have been eliminated.

A dose of 10 mg HCP/kg produced slight vacuolation in the brain of some rats, the spinal cord of a few, and the optic nerve of 1. The degree of involvement from week 1 through week 3 shows no trend toward increased severity. Twenty mg HCP/kg resulted in slight to mild vacuolation. This lesion was observed in brain and spinal cord tissues from all animals sacrificed at 3 weeks, in 2 animals per interval at 1 and 2 weeks, and optic nerves of 2 or 3 animals at each interval.

Exposures of 40 mg HCP/kg produced mild to severe vacuolation in the brain which was evident throughout the study. All animals examined showed evidence of the change, including the asymptomatic rats mentioned previously. Spinal cord and optic nerve tissues were likewise affected in all animals (excepting 1 at the first week) with the relative degree of involvement not being any different at week 3 than at week 1.

Some animals died following 2 or 3 doses of 80 mg HCP/kg. However, severe vacuolation was seen in the brain and spinal cord of all rats. Changes graded as moderate were observed in 3 of the 5 postmortem animals. Due to the consistency in results obtained in the postmortem animals of this group, only 5 of the 25 animals were studied microscopically.

DISCUSSION

HCP, given by daily gavage at exposures of either 10, 20, or 40 mg/kg, leads to a dose-related accumulation of fluid (edema) in the white matter characterized by vacuoles or cystic spaces in the myelin of the brain, spinal cord, and optic nerve. The vacuolar lesion resulted from separation of the myelin sheaths by focal accumulation of fluid with no evidence of demylination. In this experiment, with continued HCP treatment, no consistent increase in relative severity was observed either in terms of number of animals affected, or in the involved area of the altered tissue. Findings among animals treated for 1 week were not significantly

different than those of animals treated for either 2 or 3 weeks. Although the degree of vacuolation in the 40 mg/kg was greater than that of the 20 mg/kg group, there was no real sharp demarcation between the histopathologic changes observed in these 2 groups. However, hind-limb paralysis was observed in the 40 mg/kg but not the 20 mg/kg group.

A dose of 80 mg/kg, equal to or exceeding the rat LD_{50} values reported by FLORESTANO (1949), CHUNG (1963), and NAKAUE (1973), but less than that reported by GUMP (1969), resulted in the deaths of all treated animals following 2 to 5 doses. No outward signs of a central nervous system response were observed but histopathologic evaluation revealed extensive damage in the CNS tissues.

TABLE 1
Mean Body Weights of Female Rats Treated with HCP Orally

Dose (mg/kg)	Body Weight (g) Test Week Number: 0	1	2	3
0	164	189	211	231
10	164	184	206	220
20	164	186	202	225
40	164	186	200*	216*
80	164	X	X	

*$p < 0.05$

TABLE 2
Histopathologic Changes in Rats Given HCP

Tissue	Dose Level (mg/kg)	Week 1 Incidence	Week 1 Average Grade	Week 2 Incidence	Week 2 Average Grade	Week 3 Incidence	Week 3 Average Grade
Brain	0	0	-	0	-	0	-
	10	4	1.0	3	1.0	2	1.5
	20	5	1.8	5	1.5	5	2.1
	40	5	2.3	5	2.4	5	2.6
	80	5	3.0	X	X	X	X
Spinal Cord	0	0	-	0	-	0	-
	10	0	-	3	1.0	2	1.0
	20	2	2.0	2	2.0	5	1.6
	40	5	1.6	5	1.9	5	2.1
	80	5	3.0	X	X	X	X
Optic Nerve	0	0	-	0	-	0	-
	10	0	-	0	-	1	1.0
	20	3	1.5	2	2.2	2	2.5
	40	4	2.2	5	2.1	5	2.6
	80	3	2.0	X	X	X	X

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