Failure of Hexachlorophene to Alter Hepatic Detoxification Enzyme Activity

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Hexachlorophene HCP ; 2,2'-methylene-bis (3,4, 6-trichlorophenol) has been widely used as an antibacterial agent in many soap and cosmetic formulations. Until recently HCP was considered to be completely devoid of toxic effects.

GUMP (1969) has reviewed the general toxicology of HCP and studies by HERTER (1959) and LARSON (1968) have reported acute toxic effects in humans, but little attention has been given to the effects of subacute exposure. Studies by KIMBROUGH and GAINES (1971), KENNEDY et al. (1972) and NAKAUE et al. (1973) have described pathologic lesions caused by HCP in the white matter of the central nervous system. The underlying mechanism for the pathologic effects have not yet been determined. It has been reported by CALDWELL et al. (1972) that subtle biochemical effects may be responsible for the lesions seen in cases of HCP toxicity. Other parameters which would affect the toxicity or metabolism of HCP itself or other chemicals have not been investigated. Since the activities of enzymes located in the microsome function of the liver are affected by a large variety of chemicals, including aryl chlorines, it was thought worthwhile to investigate the effects of HCP exposure on hepatic microsomal enzyme activities in 2 rodent species.

MATERIALS and METHODS

Hexachlorophene (G-11 Brand, Givaudan Corp., Clifton, N.J.) was added to stock ration (Special Mix Mouse Chow, Ralston Purina Co., St. Louis, Mo.) of albino rats or Swiss white mice (both species obtained from Charles River Breeding Laboratories, Wilmington, Mass.) to give final concentrations of 100 or 200 parts of HCP per million parts of stock ration.

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Groups of 5 male and 5 female rats were fed each HCP containing diets ad libitum for 14 days. Groups of 15 female mice were fed the diets for 30 days. A negative control group consisting of the same numbers of either rats or mice were maintained on a diet of untreated stock ration. Similar numbers of rats and mice were treated daily with 50 mg/kg pentobarbital for 3 days by the intraperitoneal route.

Livers from the rats were used to assay the activities of 2 hepatic microsomal enzymes which are responsible for the enzymatic oxidative detoxification of O-ethyl-O-(4-nitrophenyl) phenyl-phosphonothioate (EPN) and the O-demethylation p-nitroanisole using the method of KINOSHITA et al. (1966).

All animals were sacrificed by exsanguination and the total liver was quickly excised, weighed, and placed in an ice/water bath. A 1 g portion was removed and homogenized in 1.15% aqueous KCl solution containing 0.25% nicotinamide. Twenty percent (w/v) homogenates were prepared. Three aliquots of the liver homogenates equaling 50, 75, and 100 mg from the rats pretreated with pentobarbital were assayed. Fifty mg of liver tissue from HCP treated and negative control animals were used. All assays were conducted in duplicate. Results are expressed as enzyme activity per 50 mg of fresh liver tissue per 50 minutes. The values obtained were treated by an Analysis of Variance; significant findings were further examined using the "t"-test.

For sleeping time determinations mice fed HCP for 30 days, untreated mice, and mice given 3 daily injections of 50 mg pentobarbital/kg were each given a single intraperitoneal injection of pentobarbital (55 mg/kg). The time from dose administration to regaining of the righting reflex was recorded and defined as the sleeping time according to the criteria defined by FUJIMOTO (1972). Trials were conducted in duplicate.

All animals were sacrificed within 1 hour of the sleeping time determinations, and the brain and spinal cord of each mouse were removed, fixed in 10% neutral buffered formalin, stained with hematoxylin-eosin, and examined microscopically for evidence of compound induced lesions.

Results

The results of the hepatic microsomal enzyme activity determinations are presented in Table I.

The growth of rats, as measured by body weights was unaffected during 2 weeks of exposure to HCP in the diet. A

TABLE I
Microsomal Enzyme Activity in
Livers of Rats Fed Hexachlorophene

Group	Sex	Body Weight Change (g)	Liver Weight (g)	Detoxification of EPN (μg p-nitrophenol formed/50 mg liver/60 min.)	O-Demethylation of p-nitroanisole (μg p-nitrophenol formed/50 mg liver/60 min.)
Negative Control	M	93	11.33	6.72 ± 0.30	3.04 ± 0.17
	F	36	7.92	2.62 ± 0.19	2.34 ± 0.40
Positive Control Pentobarbital	M	102	11.15	9.42* ± 0.69	4.53† ± 0.65
	F	48	9.29*	3.47* ± 0.38	3.40* ± 1.03
Hexachlorophene 100 ppm	M	118	13.34	7.25 ± 1.58	3.08 ± 0.25
	F	40	8.22	2.87 ± 0.06	2.24 ± 0.16
Hexachlorophene 200 ppm	M	116	14.43	5.58 ± 0.74	2.67 ± 0.14
	F	43	9.22*	3.13 ± 0.20	2.72 ± 0.42

* Statistically significant difference at the 95% confidence level.
† Statistically significant difference at the 99% confidence level.

slight increase in absolute liver weight, considered to be at the upper part of the range of normal for larger control populations, was observed among animals receiving 200 ppm HCP. Liver weights of animals fed 100 ppm HCP were slightly greater than the weights observed in negative controls but were considered normal. Pentobarbital treatment did not result in an increase in liver weights in male rats. A slight increase in liver weight was seen in the female rats treated with pentobarbital.

The activity of the enzyme catalyzing the oxidative detoxification of EPN was not affected by pretreatment with HCP. Pentobarbital treatment resulted in a significant increase in this enzyme activity of male rat liver and a lesser increase in female rat livers (Table I). The response was linear with activity per mg liver being similar at all three tissue levels examined.

The results of the O-demethylation of p-nitroanisole assays also indicate that HCP did not cause an increase in the activity of this enzyme system in either sex. Pentobarbital did stimulate this enzyme activity.

The results of the sleeping time determinations on mice are presented in Table II. Statistically significant findings were observed with 100 ppm HCP. However, the response was not

TABLE II
Sleep Time of Female Mice Treated with Pentobarbital

Group	Sleep Time (min.) Trial I	Sleep Time (min.) Trial II
Negative Control	78 ± 47	72 ± 12
Positive Control	26† ± 14	24† ± 20
Hexachlorophene 100 ppm	52* ± 13†	52* ± 13
Hexachlorophene 200 ppm	68 ± 38	65 ± 38

* Statistically significant difference at the 95% confidence level.
† Statistically significant difference at the 99% confidence level.

dose-related and the magnitude of the depression in sleep time was small. Pretreatment with pentobarbital resulted in a significant reduction in sleeping time.

The results of the sleeping time determinations on mice are presented in Table II. Statistically significant findings were observed with 100 ppm HCP. However, the response was not dose-related and the magnitude of the depression in sleep time was small. Pretreatment with pentobarbital resulted in a significant reduction in sleeping time.

Histopathologic evaluation of brain and spinal cord showed a mild to moderate diffuse pericapillary edema within the brain of 11 of 15 mice fed 200 ppm HCP. In 3 of these animals, there was focal vacuolation of the white tracts of the cerebrum, cerebellum, or brain stem. The vacuolar lesion in the white matter was attributed to edema rather than demyelination and was confided to those animals with the most severe perivascular edema. Lesions were not present in mice fed 100 ppm HCP.

Discussion

Activities of two enzyme systems found in the hepatic microsomal fraction of rats responsible for the oxidative detoxification of EPN and for the O-demethylation of p-nitroanisole were not induced by treatment with HCP. The activity of these liver microsomal enzymes were, however, stimulated by pretreatment of rats with pentobarbital.

The duration of pentobarbital induced sleep in mice pretreated with HCP was not biologically significantly greater than in untreated mice. Mice pretreated with pentobarbital had significantly shorter sleep times.

Microscopic examination of the nervous system tissues of mice fed 200 ppm HCP revealed mild to moderate edema within the grey matter of the brain. Focal vacuolization of the white tracts of the cerebrum, cerebellum, and brain stem was seen in 3 of 15 treated mice. The lesions appeared to be attributable to edema rather than to demyelination. Mice fed 100 ppm showed no evidence of pathologic change. The dose response to HCP in terms of focal brain vacuolization in the mouse is similar to that seen in the rat.

References

GUMP, W.S.: J. Soc. Cosmetic Chemists 20, 173 (1969).

HERTER, W.B.: Kaiser Foundation Med. Bull. 7, 228 (1959).

LARSON, DL.: Hospitals J. Amer. Hosp. Assoc. 42, 63 (1968).

KIMBROUGH, R.D., and T.B. GAINES: Arch. Environ. Health 23, 114 (1971).

KENNEDY, GL., Jr., I.A. DRESSLER, W. R. RICHTER, M. L. KEPLINGER, and J.C. CALANDRA: Toxicol. Appl. Pharmacol. 22, 276 (1972).

NAKAUE, H.D., F.N. DOST, and D.R. BUHLER: Toxicol. Appl. Pharmacol. 24, 239 (1973).

CALDWELL, R.S., H.S. NAKAUE, and D.R. BUHLER: Biochem. Pharmacol. 21, 2425 (1972).

KINOSHITA, F.K., J.P. FRAWLEY, and K.P. DuBOIS: Toxicol. Appl. Pharmacol. 9, 505 (1966).

FUJIMOTO, J.M.: Toxicol. Appl. Pharmacol. 23, 623 (1972).