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LIFESPAN CARCINOGENICITY STUDIES WITH HEXACHLOROPHENE IN MICE AND RATS

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SUMMARY

Hexachlorophene (HCP) was tested for carcinogenicity in lifetime feeding studies in male Sprague—Dawley rats that were fed on a protein- and vitamin-deficient diet and in C57B1 and XVII/G mice that received the chemical incorporated into a complete diet. After 2 years, no significant carcinogenic effects were observed in these animals. In XVII/G mice injected subcutaneously at birth or receiving HCP via the mother's milk, the incidence of tumors was not statistically increased. No carcinogenic effect was observed in (C57B1 × C3H) F1 hybrid mice that received HCP transplacentally.

INTRODUCTION

HCP, an antibacterial agent active against gram-positive bacteria, is used as a preservative in cosmetics and also as an anthelmintic against *Fascicla hepatica* in sheep. The acute toxicity of the compound is relatively high: the oral LD_{50} is 40 mg/kg body wt. in the rabbit, 60 mg/kg body wt. in the rat and 160 mg/kg body wt. in the mouse; a single dose of 25 mg/kg body wt. induces toxic effects in the nervous system of rats [7]. Kimmel et al. [8] observed teratogenic effects in 40% of offspring after intravaginal administration to pregnant rats. Kennedy et al [6] and Thorpe [15] observed no such lesions after subcutaneous administration in rats and rabbits.

One study has been published concerning the effects of long-term skin application of HCP in mice. Stenbäck [14] painted different concentrations of HCP in acetone onto the skin of Swiss mice and observed occasional swelling of the skin, ulcerations and hyperplastic proliferations of the epidermis, but no local tumors. A few hemangiomas of the liver were also observed in old animals, but such tumors also occurred in controls.

Abbreviations: HCP, hexachlorophene; 2-AAF, 2-acetylaminofluorene.

Since HCP is widely used in agriculture, cosmetics and veterinary medicine, it seemed relevant to carry out a series of experiments in several animal models in order to obtain a better knowledge of the effects of long-term administration of HCP to rodents.

MATERIALS AND METHODS

Especially pure HCP was purchased from FLUKA AG. Except when otherwise specified it was administered to the animals mixed with the diet during their entire lifespan.

Sprague—Dawley rats

Thirty males (IFFA-CREDO Soc.), aged 6 months at the start of the experiment and weighing 290 g (±8 g), were used. A group of 15 controls were fed on a synthetic diet deficient in protein and poor in vitamins; this diet has been used routinely in our Institute for several years in experiments on liver cancer induction by azo compounds. The other 15 rats received the same diet into which HCP at a concentration of 50 mg/kg diet was incorporated. The average daily food intake per rat was 30 g.

C57B1 mice

This substrain was first used in our laboratory in 1953 and has been bred by brother-to-sister mating for the last 25 years. Lymphoid leukemias of thymic origin develop spontaneously at the age of 30—40 weeks in 1% of mice. Reticulosarcomas (10—15%) and adenomas or adenocarcinomas of the liver (2—3%) arise late in life.

One hundred and eighteen mice, 25 days of age, were used: 50 (25 males and 25 females) were used as controls and were fed a complete standard diet (U.A.R. Morris diet); the other 68 (33 females and 35 males) received the same diet but containing 150 mg/kg diet HCP. The average daily food intake per mouse was 3 g.

XVII/G mice

This strain was started in 1928 and has been bred in our Institute for 50 years by brother-to-sister mating. These animals develop mammary tumors or leukemias or other spontaneous tumors only exceptionally. However, over 70% of the mice develop 1 to 3 lung adenomas late in life. Multiple pulmonary tumors are rare in untreated mice but develop after administration of carcinogenic compounds such as 2-acetylaminofluorene (2-AAF), urethane or polycyclic hydrocarbons, even at low doses.

One hundred and fifty-three mice, 25 days of age, were used: 75 controls (38 females and 37 males) received the standard diet; the others (40 females and 38 males) were fed the same diet but containing 150 mg/kg diet HCP.

XVII/G mice given HCP via the milk

Since HCP is excreted in the milk [16], and since administration of carcinogenic compounds to lactating mothers of this strain can produce multiple pulmonary tumors in the offspring [13], an experiment was carried out using this model.

Twenty two XVII/G pregnant females were used: 7 mice received 0.5 mg/animal HCP for 20 days, starting from the day of parturition; 8 others received 0.5 mg methylcholanthrene in the same way (positive controls); 7 mothers used as negative controls remained untreated. The 7 HCP-treated mothers had 41 offspring, 38 of which survived over the age of 20 days. The 8 methylcholanthrene treated had 40 offspring, 34 of which survived 20 days or more. The 7 negative controls had 42 offspring, 37 of which lived for 20 or more days.

XVII/G mice injected at birth

Fifty two newborn XVII/G mice received 3 subcutaneous injections of 0.05 mg, 0.05 mg and 0.1 mg/animal HCP on the first, second and 8th days of life and were observed for lifespan.

(C57B1 × C3H) F1 mice

C57B1 females aged 3 months were mated with C3H males in order to obtain F1 hybrids. The observation of a vaginal plug was taken to be day 0 of pregnancy. Over 80% of these hybrids develop late in life spontaneous hepatomas. Other tumors, such as mammary adenocarcinomas and leukemias, are exceptional.

Nineteen pregnant C57B1 females were used; 9 of them were injected on the 16th and the 18th days of pregnancy with 0.5 mg/animal HCP; the others remained untreated and were used as controls. The 9 mothers in the HCP treated group delivered 52 offspring; neonatal mortality was low. The 10 controls had 52 offspring. After weaning all offspring received standard diet and drinking water ad libitum. Most of the animals were killed when moribund. All tumors and the principal organs were fixed in Bouin-Holland fluid for microscopic examination.

RESULTS

In spite of the high doses administered in some experiments (10% of the LD_{50} in the feeding experiments in mice), the chemical did not produce obvious signs of toxicity. In Sprague—Dawley rats, weight gain was similar for controls and animals that received HCP at a daily dose of approx. 1.5 mg/rat, which represents 8—10% of the LD_{50}. The weights of various organs (liver, kidney, testis, adrenal, thyroid and pituitary) were identical in both groups. No neurotoxicity was observed in mice or rats. Lifespan and survival rates were similar in treated and control groups. Most mice of both the pure strain and hybrids lived for 90—100 weeks and some for over 2 years (see also Tables 1—4).

Sprague—Dawley rats

No tumors were observed in either group, with the exception of an adenoma of the pituitary in one of the HCP-treated animals aged 103 weeks. The same animal had hyperplastic thyroid glands. The adenomatous pituitary was formed largely of eosinophilic cells. The thyroid changes were similar to those which are observed in animals receiving thiourea. The mean survival of the rats of both groups was nearly the same, i.e., 95 weeks. Three of the rats fed the HCP diet lived over 104 weeks.

C57B1 mice (Table 1)

HCP only produced tumors that are normally seen in this strain of mice; the latent periods of tumor induction were similar in control mice and in animals that received HCP. Reticulosarcomas (mostly diffuse) and a few thymic lymphomas appeared in mice over the age of 100 weeks. One liver tumor was observed in one of the HCP-treated females killed at the age of 103 weeks and was classified as an adenoma.

XVII/G mice (Table 2)

Sixty to eighty percent of both control and treated mice developed 1—3 lung tumors, which were in most cases typical papillary adenomas. One of the males treated with HCP and sacrificed at the age of 101 weeks had a hepatoma, which was considered to be non-malignant.

XVII/G mice given HCP via the milk (Table 3)

Pulmonary adenomas developed in 50—60% of treated males and females and in 50—79% of male and female controls. No multiple tumors of the lungs were observed in the off-spring of mothers that received 0.5 mg/animal HCP daily; whereas multiple lung tumors developed in almost 100% of positive

TABLE 1

CARCINOGENESIS OF HCP IN C 57 BL MICE GIVEN 150 mg/kg DIET

Group	Number of mice	Number with tumors	Percentage	Types of tumors	Mean survival time (weeks)
Control females	25	7	28	6 Reticulo-sarcomas 1 Tumor of the ovary	95
Females given HCP	33	9	24	8 Reticulo-sarcomas 1 Hepatoma	86
Control males	25	4	16	3 Reticulo-sarcomas 1 Pulmonary tumor	85
Males given HCP	35	6	17	6 Reticulo-sarcomas	90

TABLE 2

CARCINOGENESIS OF HCP IN XVII/G MICE GIVEN 150 mg/kg DIET

Group	Number of mice	Number with tumors	Percentage	Types of tumors	Mean survival time (weeks)
Control females	38	22	58	21 Pulmonary tumors 1 Tumor of the ovary	76
Females given HCP	40	28	70	28 Pulmonary tumors	71
Control males	37	23	62	23 Pulmonary tumors	81
Males given HCP	37	30	81	30 Pulmonary tumors 1 Liver adenoma	88

controls treated with methylcholanthrene. In 3 of the males that received HCP during nursing, liver tumors were observed: one was an adenocarcinoma with well-differentiated cells forming trabeculae and large hemorrhagic spaces.

XVII/G mice injected at birth

Twenty seven females and 16 males lived for 12 or more months; 32 developed lung adenomas, but no multiple lung tumors were observed. Average

TABLE 3

CARCINOGENESIS OF HCP IN XVII/G MICE GIVEN HCP DURING LACTATION

Group	Number of mice	Number with tumors	Percentage	Types of tumors	Mean survival time (weeks)
Female negative controls (no treatment)	25	19	79	19 Pulmonary adenomas	78
Females given HCP	25	16	60	16 Pulmonary adenomas	79
Females given methylchol-anthrene	19	19	100	19 Multiple lung tumors	53
Male negative controls (no treatment)	10	5	50	5 Pulmonary adenomas	61
Males given HCP	18	9	50	9 Pulmonary adenomas 3 Hepatomas	82
Males given methylchol-anthrene	15	14	93	14 Multiple lung tumors	51

TABLE 4

CARCINOGENESIS OF HCP IN (C57BL × C3H) F1 MICE TREATED IN UTERO WITH HCP

Group	Number of mice	Number with tumors	Percentage	Types of tumors	Mean survival time (weeks)
Control females	27	26	99	2 Reticulo-sarcomas 3 Lung tumors 24 Hepatomas	104
Females given HCP	30	24	80	3 Reticulo-sarcomas 5 Lung tumors 1 Mammary tumor 16 Hepatomas	101
Control males	25	22	88	1 Lung tumor 22 Hepatomas	97
Males given HCP	22	20	91	2 Reticulo-sarcomas 3 Lung tumors 1 Subcutaneous sarcoma 15 Hepatomas	97

survival times in males and females were 72 and 74 weeks respectively. Over 70% of untreated mice of this strain develop 1—3 lung adenomas late in life.

(C57B1 × C3H) F1 mice (Table 4)

These hybrids develop spontaneous liver tumors after the age of 80 weeks. In our experiments injections to pregnant females on the 16th and 18th day of gestation produced no tumors in the offspring which are not normally seen in control mice. The average survival times of mice with hepatomas were identical for controls and for mice treated transplacentally.

DISCUSSION

In order to investigate the possible carcinogenicity of HCP, experiments were carried out for lifespan in rats and mice of different genetic origins. Under the conditions of these tests, HCP does not appear to be carcinogenic for rodents. It is particularly interesting to note that the compound produced no pathological lesions in rats that received a protein- and vitamin-deficient diet and some of which lived for more than 2 years. The pituitary adenoma which was observed can be considered to have been spontaneous. These results are in accordance with those obtained in a bioassay on HCP, carried out under the Carcinogenesis Testing Program of the US National Cancer Institute in male and female Fischer rats that were fed HCP in the diet for 106 weeks; no carcinogenic effects were observed [10].

Hepatotoxic effects due to HCP have been observed in sheep in an experiment of several weeks duration [12]. It was expected that rats maintained during long periods on a protein- and vitamin-deficient diet would at least present fatty degeneration or other injuries to the liver; however, in our experiments, no particular liver lesions were observed. Furthermore, of the several hundred mice that received the chemical under different conditions, no hemangiomas were seen in the liver. The absence of such lesions, especially in C57B1 mice, is significant, since in many experiments performed in the past, animals of this strain were found susceptible to hemangioma induction with carcinogenic compounds, such as 2-AAF, urethane and others. It is noteworthy that newborn mice did not develop an excess of tumors after subcutaneous administration of HCP: it is known [1,5,11] that infant mice are usually very susceptible to tumor induction by chemical carcinogens, even at low doses.

We did not repeat the experiment of Stenbäck [14], using skin painting, since we were more interested in the possible appearance of distant tumors. It is known, however, that after its ingestion or skin application, HCP enters the blood stream within a short period of time [2,3,4].

Consequent to the original report of Larsen [9], a large number of studies have been carried out on the transplacental induction of tumors in mice. In our experiments, HCP administered to pregnant females did not modify the incidence or the latent period of induction of tumors normally seen in animals used in our experiments.

Three unusual tumors, i.e., hepatomas occurred in experiment No. 4 in which XVII/G mice received HCP via the mother's milk. In animals of this strain spontaneous liver tumors are very rare and it is possible that these hepatomas can be considered to have been induced. It is questionable, however, if we have to classify HCP on the basis of these findings as a carcinogen potentially oncogenic for humans. Of course, the problem remains open if doubtful results obtained in rodents do allow a conclusion for the human users of a chemical. There are examples that compounds discovered as carcinogenic for humans, by the epidemiologists have only a low activity for rodents and vice versa.

REFERENCES

1 de Benedictis, G., Maiorano, G., Chieco Bianchi L. and Fiore Donati L. (1962) Lung carcinogenesis by urethane in newborn, suckling and adult Swiss mice. Br. J. Cancer, 16, 686—689.
2 Curley, A., Kimbrough, R.D., Hawk, R.E., Nathenson, G. and Finberg, L. (1971) Dermal absorption of hexachlorophane in infants. Lancet, ii, 296—297.
3 Curley, A., Kimbrough, R.D., Hawk, R.E., Nathenson, G. and Finberg, L. (1972) Hexachlorophene in the blood. Food. Cosmet. Toxicol., 23, 114—115.
4 Drake, J.P. (1974) Hexachlorophene. Food. Cosmet. Toxicol., 12, 563—568.
5 Fiore Donati L., Chieco Bianchi L., de Benedictis, G. and Maiorano, G. (1961) Leukemogenesis by urethane in newborn Swiss mice. Nature (Lond.), 190, 278—279.
6 Kennedy, G.L. Jr., Smith, S.H., Keplinger, M.L. and Calandra, J.C. (1975) Evaluation of the teratological potential of hexachlorophene in rabbits and rats. Teratology, 12 83—87.

7 Kimbrough, R.D. and Gaines, Th. B. (1971) Hexachlorophene effects on the rat brain. Arch. Environ. Health, 23, 114—122.
8 Kimmel, C.A., Moore, W. Jr. and Stara, J.T. (1972) Hexachlorophene teratogenicity in rats. Lancet, ii, 765.
9 Larsen, C.D. (1947) Pulmonary tumor induction by transplacental exposure to urethane. J. Natl. Cancer Inst., 8, 63—70.
10 National Cancer Institute (1978) Bioassay of Hexachlorophene for Possible Carcinogenicity, Technical Report Series No. 40, NCI-CG-TR-40, Department of Health, Education and Welfare.
11 Pietra, G., Rappaport, H. and Shubik, P. (1961) The effects of carcinogen chemicals in newborn mice. Cancer, 14, 308—317.
12 Pugh, D.M. and Crowley, J. (1966) Some observations on the toxicity of hexachlorophene for sheep. Vet. Rec., 78, 86—90.
13 Rudali, G., Yourkovski, N. and Julliard, L. (1962) Influence de l'hérédité sur l'oncogenèse pulmonaire spontanée et provoquée chez les souris. Bull. Cancer, 49, 270—277.
14 Stenback, F. (1975) Hexachlorophene in mice. Effects after long-term percutaneous applications. Arch. Environ. Health, 30, 32—35.
15 Thorpe, E. (1967) Some pathological effects of hexachlorophene. J. Comp. Pathol., 77, 137—144.
16 West, R.W., Wilson, D.J. and Schaffner, W. (1975) Hexachlorophene concentrations in humans milk. Bull. Environ. Contam. Toxicol., 13, 167—169.