AGE DIFFERENCES IN SUSCEPTIBILITY OF SWISS WHITE MICE TO HEXACHLOROPHENE TOXICITY

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PREFACE

This study is concerned with the toxicity of hexachlorophene, a long-used disinfectant that, until recently, was found in many soaps and cosmetics. The primary purpose is to show that young animals, in this case mice, are much more susceptible to the toxic effects of hexachlorophene than are adult animals. The study was prompted by the recent ban by the United States Food and Drug Administration on the use of hexochlorophene in all products except prescription items. It is hoped that this experimental data will provide evidence that hexachlorophene may be safe for use in products available to adult humans.

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LIST OF SYMBOLS

d.p.m.	- Disintegrations per minute
FDA	- United States Food and Drug Administration
gm	- Gram
HCP	- Hexachlorophene
^{14}C-HCP	- Hexachlorophene containing the radioisotope carbon-14, in the molecule
L.D.$_{50}$	- The minimal lethal dose to 50% of the animals.
L.D.$_{100}$	- The minimal lethal dose to 100% of the animals.
mg/kg	- Milligrams of drug per kilogram of body weight
ml	- Milliliters

CHAPTER I

INTRODUCTION

Hexachlorophene (HCP) has been widely used since the mid-1940's as a powerful antibacterial agent. It has been incorporated in antiseptic agents, baby powders, after-shave lotions, deodorants, tooth paste, and many other non-prescription items available to the general public. Other uses included broad spectrum fungicides and bactericides used on food crops. Perhaps the most important use of HCP during the past thirty years was its incorporation into germicidal soaps used for surgical scrubs and infant care. The drug was found to be quite effective in controlling populations of gram-positive organisms in hospital nurseries (18,19,44,50,51).

For many years HCP was thought to be essentially non-toxic to humans. It was used as 1-3% concentrations in soap in most of the hospital nurseries in the country. Many surgeons used it several times daily for antibacterial scrubs prior to surgery (60). It was thought to be so safe and effective as an antibacterial agent that it was included in many cosmetics.

Numerous toxicity studies had been conducted in laboratory animals. Toxic reactions were shown in rats, mice, guinea pigs, dogs, sheep, and cattle (8,13,14,20,21,25,27,52,53). However, recognition of significant degrees of HCP toxicity in humans was slow in forthcoming. Gradually, over a 27-year period, evidence of human toxicity began to emerge.

Reports of cutaneous reactions, neurologic episodes and death due to HCP toxicity began to accumulate (1,4,21,27,28,30,33,36,40,59). In December, 1971, the United States Food and Drug Administration (FDA) sent a drug bulletin to 600,000 doctors and other health professionals warning them to discontinue use of HCP products in routine bathing of infants and adults (55). On January 6, 1972, the FDA announced in a press release that all skin cleansing products containing more than 0.75% HCP would be restricted to prescription use only (56).

To the general public, the almost complete removal of HCP from the public marketplace seemed to be an over-reaction on the part of the FDA. Most studies to determine the irritating and sensitizing properties of various topical HCP preparations on human skin had produced no evidence of lesions (21). Hexachlorophene was used in hundreds of products by a very large number of people in the United States and foreign countries, but remarkably few cases of irritation and allergy were reported. Several million cakes of deodorant soap were used by the general public with no reports of proven cases of sensitization.

The reported cases of toxicity of HCP in humans have been, in almost all cases, associated with misuse of the drug. Accidental ingestion of the drug caused some morbidity and rare fatalities (3,30,40,59). Applications of higher-than-recommended concentrations to the skin or failure to rinse the chemical from the skin accounted for most other reported cases of toxicity (4,22,28,33,42).

The majority of deaths associated with HCP toxicity have been in infants (40,41). In 1972, in France, thirty-nine babies died from applications of a baby powder accidentally containing 6% HCP, which was double the usual level (3,41,42). The deaths of fifteen persons in the United

States due to HCP during the period 1954-1971 were reported in September, 1972, by the FDA and publicized in the newspapers across the United States in an article syndicated by the Associated Press (3).

In imposing the restrictions on HCP use, the FDA used as its basis three recent reports. The first was in August, 1971, in which Kimbrough and Gaines (26) reported that rats fed HCP in their diet developed leg weakness which progressed to paralysis. The second study was conducted by Curley, et al. (11) on 50 newborn infants and showed that the infants could absorb HCP through the intact skin after being bathed with hexachlorophene soap. The third report was from Sterling Drug, Inc., the manufacturer of the most widely used 3% HCP solution, pHisoHex® (41). This study showed that newborn monkeys bathed in 3% HCP developed lesions in the brain similar to those produced in rats, as reported in the first study mentioned above. After considerable weighing of risks and benefits of HCP, the FDA decided to place restrictions on the use of the drug. In their decision, the FDA called for additional research to clarify and further delineate HCP toxicity effects.

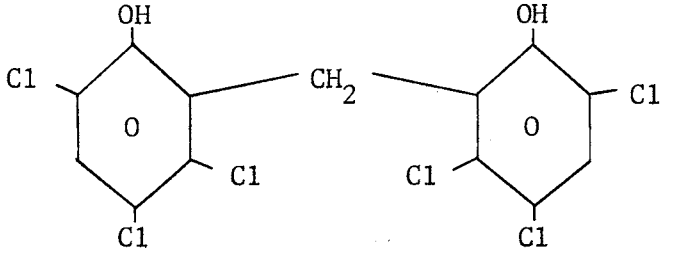
This thesis reports the results of studies designed to determine the effect of age difference in susceptibility to acute HCP toxicity in mice. The histopathological lesions observed with acute toxicity in different ages of mice are reported. Results are also reported on a third study to determine the concentrations of HCP in brain, liver and blood in different age groups of mice after a single oral administration of ^{14}C-labeled HCP.

CHAPTER II

LITERATURE REVIEW

Description of Hexachlorophene

The official chemical name of hexachlorophene is 2,2'-methylenebis (3,4,6-trichlorophenol). Other reference code terms which have been used for HCP include G-11 and AT-7. As can be determined from the chemical name, it is a phenolic compound. Hexachlorophene is practically insoluble in water, but is soluble in alcohol, acetone, ether, chloroform, propylene glycol, vegetable oils, and dilute aqueous solutions of alkalies (34). Pure HCP is a white, crystalline powder. It must be stored in light-resistant containers, as light will cause chemical breakdown (39).



$C_{13}H_6Cl_6O_2$

Figure 1. Hexachlorophene

Antibacterial Properties

Hexachlorophene was synthesized in 1939 and patented by the Guvaudan Corporation in 1941 (20). Commercial production began after World War II. The antibacterial qualities of this drug have been well documented (6,17,20,37,44,54). Hexachlorophene reduces the bacterial flora of the skin through continued daily use of soaps containing this drug in levels of 1-3%. Repeated applications at these concentrations allow the build-up of effective antibacterial levels of the compound on the skin. This compound is strongly bacteriostatic and bacteriocidal against Staphylococcus aureus and other gram-positive bacteria, but it has considerably lower activity against gram-negative micro-organisms (11,17,44, 54). If the compound is used routinely in a regular bathing program, a significant reduction in the bacterial flora of the skin occurs.

In recent years, there has been some controversy over the actual effectiveness of HCP. Sarkarny and Arnold (44) observed suppression of resident skin flora with no appreciable effect on streptococci and Escherichia coli, but an increased frequency of proteus species. Forfar (17) found that, although daily use of HCP on newborn infants and on the hands of attendant staff reduced neonatal staphyloccal infections, there was an accompanying absolute rise in the frequency of infections caused by gram-negative bacilli. A study by Evans, et al. (15), using twice daily scrubs with HCP soap on the toe web areas of medical student volunteers, showed an absolute decrease in the number of aerobic bacteria to approximately one third the original level within one week. The main reduction was in staphylococci and diptheroids, while at the end of one week there was an absolute rise in the number of gram-negative organisms. The gram-negative organisms were primarily bacilli, predominantly

pseudomonas, enterobacter, and klebsiella. These data suggest that one effect of the judicious daily use of HCP-containing soap is to increase the amount of gram-negative organisms on the skin, these organisms increasing with the decrease of staphylococci and diptheroids. The question that arises is whether the use of such soaps on patients during several days of hospitalization may actually be increasing the risk of hospital-associated infections due to gram-negative bacilli (15).

White and Duncan (60) explored the comparative effectiveness in preventing post-surgical infections of iodophor versus HCP scrub solutions for presurgical scrubbing of the surgeon's hands and operative area of the patient. Theoretically, the bacteriocidal effects of iodophor should be superior to HCP, which is bacteriostatic, when used on a one-time basis, such as in the presurgical preparation of a surgical patient. The results of the studies by White and Duncan (60) indicated that there was no difference in the incidence of infection when either of these two scrub solutions were used on a large number of patients and that both were acceptable. Custer, et al. (12) studied the comparative effectiveness of HCP and providine in preventing infection in contaminated wounds. These authors stated both antiseptic agents exerted a favorable influence on the contaiminated wound but their beneficial effects were outweighed by the harmful effects of the detergent in the scrub solutions.

Noone, et al. (37) found that a water soluble ointment containing 2% HCP was very effective in eliminating epidemic strains of Staphylococcus aureus from nasal passages of known carriers of this organism in a hospital. The ointment was applied twice daily for seven successive days to the nasal passages of individuals who were found to be carrying the resistant strains of staphylococcus in their nasal passages. The nasal

treatment was accompanied by bathing and washing with HCP soap. At the end of the seven day period, no resistant Staphylococcus aureus organisms could be cultured from the nasal passages. It was felt that this procedure was better for eliminating the resistant organisms than the use of antibiotic ointments, which may cause drug sensitivities to develop in the carriers and drug resistance to develop in the bacteria.

Other Uses of Hexachlorophene

Hirschler (23) and Dorsman (14) reported on the use of HCP as an anthelmintic drug against liverflukes (Fasciola hepatica) in sheep and cattle. The use of HCP for this purpose has not gained widespread use in the United States, but the reports by these investigators prompted toxicological studies of HCP in the ovine and bovine species (24,43,49,53,61).

In China, HCP has been used in the treatment of infestation by the liver fluke, Chlornorchia sinensis, in humans (9,29). This fluke infestation is transmitted to humans by the eating of infected raw fish. There has not been an effective treatment for this disease in man.

In 1969, the use of HCP as a broad-spectrum fungicide and bactericide on food crops was reported (35). Its use was also recommended for control of rust mites on citrus crops and rhizoctonia in soil. The application to the FDA for permission to use HCP in this role prompted the toxicological studies which eventually caused the FDA to greatly restrict the use of this drug (42).

Bacteriostatic Effects of Hexachlorophene on the Skin

The reduction in the number of bacteria on the skin achieved through the continued use of HCP preparations has been well established. This is attributed to the buildup and retention of effective levels of the compound on the skin (16). Manowitz and Johnston (32) reported on the concentrations of HCP retained by the skin under controlled washing conditions. In single applications, the amount of residue left on the skin after rinsing depended on the percentage of the drug in the soap. There was also a clear indication that the amount of HCP left on the skin increased with increased application time of the soap. Approximately equivalent amounts of HCP were retained on the skin whether bathing was by shower or by tub bath. Their data also indicated that substantial quantities of HCP were deposited on the skin from bathing with soaps containing relatively low concentrations of HCP. It was also found that the amount of HCP residue on the skin increased with the number of washings in a single day or over a four-day period. No plateau effects were noted in any of these tests, i.e., the concentration on the skin continued to rise with additional washings.

In contrast, Shemano and Nickerson (47) found that HCP accumulated on the skin during the first three or four washes and remained relatively constant thereafter. Compeau (10) showed that HCP built up during the first five to ten minutes of scrubbing but then accumulated no further with additional scrubbing time. He suggested that HCP was adsorbed on the skin through an anionic reaction with the cationic proteins of the skin. Manowitz (32) disagreed with this, however, and offered the suggestion from his evidence that the quantity of HCP on the skin is due to

its physical entrapment by the skin. He stated that the quantity of HCP on the skin, and therefore the number of bacteria on the skin, is affected more by the mechanics of the washing process and is subject to great variation due to individual washing habits.

Carroll, et al. (8) reported on the absorption of ^{14}C-labeled HCP through the intact and injured skin of the tail of rats. ^{14}C-HCP was absorbed through the intact tail skin of the rat at an average rate of 1.7 micrograms per square centimeter of exposed surface per hour. The tails of the rats were immersed for 3 hours in 15 ml of 1% (weight:volume) ^{14}C-HCP. Immediately after the development of second degree burn, the average absorption rate increased to 2 1/2 times that of normal skin for 24 hours and then dropped to normal or subnormal rates. A similar pattern of HCP absorption was noted with traumatized rat tails, except that the average rate of penetration of the isotope went up to 7 times that of normal skin.

A study undertaken by Curley, et al. (11) showed that infants bathed in HCP soap absorbed a significant quantity of HCP through the normal skin. Umbilical cord blood was drawn for electron-capture gas-liquid chromatography at the time of discharge from the hospital. During their 1-11 day stays in the hospital, the infants were washed once daily with 3% HCP detergent solutions diluted to varying degrees. Some were rinsed and some were not, but 45 of 50 infants showed an increase in HCP concentration in the blood at the time of discharge. Neither weight nor sex seemed to influence the HCP blood levels.

Larson (28) showed that HCP passed readily through the burned skin of humans bathed with 3% solutions. Other data which he presented indicated that HCP passed readily through the burned or intact skin of rats

and pigs.

Dermal Toxicity

By far the major use of HCP was in soaps, cosmetics, powders, and other preparations which were used on the skin. Before the FDA banned its use, about 4 million pounds of HCP a week were being used by American cosmetic and pharmaceutical companies (2). In spite of this vast amount of HCP being used on the skin of literally millions of people of all ages each day, there were surprisingly few cases of skin reaction to the drug.

Baker, et al. (4) reported on ten patients who suffered primary irritant contact dermatitis of the scrotum due to various types of HCP preparations used on the skin. The patients in this series used the drug according to directions furnished them but apparently failed to adequately agitate the water to thoroughly mix the solution before bathing the affected areas. After withdrawal of the HCP treatment, the lesions regressed rapidly. McDonald and Woodruff (33) reported twelve cases of acute scrotal dermatitis following washing of the scrotum with a detergent containing 3% HCP prior to genito-urinary surgery. This did not occur if the scrotum was rinsed throughly after the wash.

Herter (22) described central nervous disturbance and skin reactions in a newborn infant who, after being discharged from the hospital, was treated by the mother with a 3% HCP lotion after bathing. The lotion was left on the skin. After four days excoriations appeared on the face and buttocks. Nervous involvement was also present. Removal of the lotion resulted in remission of all signs.

Newcomer, et al. (36), in describing a melanosis of the face termed "chloasma", stated that the etiologic factors varied from time to time

and from place to place, but are generally caused by externally applied cosmetics or are of occupational origin. In their study, HCP was the only active ingredient common to the cosmetics employed by each patient. However, they were unable to produce any pigmentation experimentally which could be attributed to HCP.

Gump (21) cites evidence of skin irritation and systemic toxicity associated with experimental topical application of HCP preparations in rats, rabbits, and guinea pigs. When 600 humans were patch tested with 0.5 and 1% HCP in petrolatum, the results were all negative. The patch test was repeated in the patients after 10 days to determine whether sensitivity might have been produced. All 600 tests were negative, indicating HCP was non-irritating to the skin and was not a sensitizer. Another series of 50 subjects were found to develop erythema when subjected to patch testing with HCP in 80% aqueous propylene glycol (21). In another group of 50 persons patch tested with 5% HCP in dimethyl phthalate, only one subject showed a continuous reaction in the form of slight erythema. Photosensitization due to HCP has rarely been observed (21).

Systemic Toxicity

Since the synthesis of HCP in 1939 and its commercial introduction after World War II, much data has been accumulated on the toxicological properties of this drug. Studies have been reported on many different animals, and observations have been reported on humans that have experienced toxic systemic reactions (21,27).

A review of the different reports concerning the acute oral $L.D._{50}$ of HCP in mice indicated that most values lie in a narrow range between

160 and 210 mg/kg (21). The carrier of the HCP in these studies seemed to make some difference in the $L.D._{50}$, since no two reporters used the same carrier. A single report on the toxicity of HCP in guinea pigs indicated the $L.D._{50}$ to be somewhere between 250 and 300 mg/kg (20).

Rats showed a similar $L.D._{50}$ range for acute oral toxicity as for mice. The range appeared to be between 146 and 250 mg/kg depending on the carrier (21). Oral administration of HCP to adult rats at the rate of 25 mg/kg/day for 2 weeks caused paralysis and vacuolation in cerebral white matter (26). Similar findings accompanied the oral administration of HCP to weanling rats at the rate of 100 mg/kg (11). Hexachlorophene given orally to adult female rats at the rate of 5 mg/kg/day for 100 days caused brain damage in the adults and decreased the survival of their offspring (11).

Dogs are somewhat more susceptible than rats and mice. All of 12 dogs died after receiving single doses of 140 mg/kg of HCP in gelatin capsules (21). In further tests all dogs survived doses of 100 to 160 mg/kg. Delak, et al. (13) reported that 10 to 20 mg/kg was tolerated without toxic symptoms. Administration of 30 mg/kg produced toxic signs and doses of 40 to 50 mg/kg caused death of most dogs.

Sheep and cattle are also more susceptible than rodents. Since Hirschler (23) reported on the anthelmintic properties of HCP against liver flukes, many papers have been published on the subject (14,21,24, 43,49,53,61). Doses of 20 mg/kg were well tolerated by cattle, doses of 30 and 40 mg/kg gave toxic signs, and death may occur at the higher level (14). Sheep tolerated doses of 100 mg/kg in one study, but showed signs of toxicosis in another study when given doses of 70 to 80 mg/kg (21). Jack (24) reports suspected HCP toxicity in 20 calves that were

being fed milk from polythene buckets that were also being used for udderwash in the milking parlour. Hexachlorophene was used in the udderwash. Seven of 20 affected calves died.

Lusting (30) reported a fatal case of poisoning in a six-year-old child who drank 4 or 5 ounces of HCP lotion and died nine hours later. It was estimated that the ingested lotion contained approximately 250 mg of HCP per kilogram of body weight. Liu, et al. (29), as cited by Wear (59), observed some toxic reactions to HCP in eight patients who received three successive doses of 20 mg/kg/day. One of these became comatose the fourth day, but recovered after three days. The child reported by Herter (22) developed twitchings of the arms and legs and later progressed into convulsions. This infant developed toxic signs after cutaneous absorption of the drug. It recovered fully within a week after discontinuance of the drug. Another infant was accidentally given HCP orally daily for seven days (40). Neurologic signs developed and persisted for one month after withdrawal of the drug. It was estimated that the child received a total amount of 250 mg/kg of HCP in divided doses over a period of one week or roughly 37 mg/kg/day. In a report by Wear, et al. (59), ten persons were known to have ingested HCP accidentally during presurgical preparations. This occurred because the disinfectant (meant to be used as a preoperative scrub) was dispensed in a paper cup without adequate instructions and supervision by the nurse and because the suspension resembles milk of magnesia in color and viscosity. None of the persons died as a direct result of the ingestion, but all showed severe gastrointestinal aberrations with one person dying of cardiac arrest the following day in surgery.

In a study by Larson (28), blood levels of HCP were determined in

burned patients to whom 3% HCP detergents had been applied. He found that the drug passed readily through the burn wound. When serum levels of HCP became sufficiently high, convulsions could result in either the child or the adult. Larson also discussed "burn encephalopathy", which he said has been cited frequently in the literature to describe the burned patient who developed stupor, coma, confusional status, muscle twitching, convulsions and cerebral edema. The literature cites many etiologic possibilities, but Larson thought HCP might be the inciting factor. He highly recommended thorough rinsing of burned patients when HCP is used to control infection in the burned areas.

The signs of systemic HCP toxicity can be divided into two broad groups: those associated with disturbances in the nervous system and those associated with gastrointestinal tract disturbances. In rodents, the signs noted were predominantly neurological. The affected animals showed weakness of the rear legs progressing to paralysis, convulsions, dyspnea and respiratory paralysis (21,26,27). No gastrointestinal signs other than anorexia have been reported in rodents. Neurological signs in humans include generalized weakness, muscular twitching, nystagmus, coma and convulsions (30,40). Gastrointestinal signs in humans include severe vomiting and diarrhea with accompanying dehydration, abdominal cramps and a burning sensation in the throat and stomach (30,40,59). Similar signs were noted in dogs (46).

Gross and Histopathologic Lesions Associated With Systemic Toxicity

The most striking histopathologic lesion noted with HCP toxicity was edema and vacuolation of the white matter of the brain. This has been

seen in rats, mice, guinea pigs, dogs, and humans (21,26,27,38,40). The edema and vacuolation of the white matter of the brain appeared to be quite similar to that caused by triethyltin compounds (31). Scott, et al. (46) found no brain changes in dogs given HCP per os, but Ward (58) reports a litter of puppies which showed neurological signs and vacuolation of the white matter of the brain after dermal application of a HCP preparation which was not rinsed off. Chung, et al. (9), as noted by Gump (21), reported the brain had, at most, small perivascular inflammatory infiltrations. These authors also reported mild degenerative and inflammatory changes of the liver, kidney and lungs in rats. Thorpe (52, 53) reported findings of periportal fatty change in the sheep liver and also reported on testicular degeneration in sheep and rats caused by HCP. All lesions, if they did not cause death of the animals, appeared to be reversible rather quickly after withdrawal of the drug (25,26,27,31,40, 46,58).

Kimbrough and Gaines (26) found that the brains of rats receiving HCP at the rate of 25 mg/kg/day for 14 days weighed more than the brains of control rats. The average for the treated rats was 2.60 gm, with the average for the untreated rats being 1.99 gm. This increase in brain weight was statistically significant ($P < 0.001$), and helps support the theory of edema of the brain tissue.

A review of autopsies since 1966 of all children under 5 years at the University of Washington revealed a total of 21 infants with a similar specific vacuolar lesion of the brainstem, including the reticular formation (38). A statistically significant association was demonstrated between the occurrence of vacuolation of the reticular formation (VRF) and three or more exposures to 3% HCP. The lesions were found more

frequently in premature infants who were bathed three or more times in HCP than in larger infants bathed an equal number or fewer times.

Lusting (30) reported the gross findings in his fatal case of HCP ingestion were "congestion of organs and severe inflammation of the stomach".

There is no known specific antidote for HCP toxicity (30). Treatment consists of inducing vomiting quickly if oral ingestion occurs and, thereafter, to give supportive therapy as needed with special emphasis on fluid and electrolyte replacement (30,59).

Biochemical Effects of Hexachlorophene

Until recently, little had been published concerning the biochemical effects of HCP at the cellular and subcellular levels. Work done by Cammer and Moore (7) indicated that HCP uncouples mitochondrial oxidative phosphorylation in the liver and brains of rats. Blockus, et al. (5) agreed with Cammer and Moore and stated that HCP toxicity appeared to be quite similar to toxicity caused by 2,4-dinitrophenol. In work on dogs, he found an elevated body temperature, increased respiratory rate, and decreased carbon dioxide in expired air. Thorpe (53) reported loss of alkaline phosphatase and adenosine triphosphatase activity from the periportal zones of the hepatic lobules. He also found reduced succinic dehydrogenase and glutamate dehydrogenase activity. He correlated these findings with the inhibitory action of HCP on various oxidative enzymes.

Fate of Hexachlorophene in the Body

With only a small amount of work having been reported in this area, it appears that orally administered HCP is eliminated primarily in the

feces with only minor urinary excretion in rats and cows, but with a significant excretion of HCP in the urine of rabbits (49,61). In one study conducted by St. John and Lisk (49), it was found that no HCP was excreted in the milk of dairy cows. The findings of Wit and Van Genderen (61) were in agreement with this. St. John (49) found that HCP did not decompose in ruminal fluids, and that there was no detectable breakdown of the drug when it was incubated with fresh beef liver. No breakdown products of HCP were found in the urine or feces of these cows.

Wit and Van Genderen (61) also used rats and rabbits to study the fate of HCP in the body. In rabbits they found that approximately one third was excreted unchanged in the urine, one third was excreted unchanged in the feces, and one third appeared as unidentified metabolites in the feces. These latter products could not be extracted from the feces with ethanol. Approximately five days were required for the entire dose to be eliminated from the rabbits. Practically no HCP was excreted in the urine of rats. In rats and dairy cows, not all of the HCP could be recovered.

CHAPTER III

AGE DIFFERENCES IN SUSCEPTIBILITY TO HEXACHLOROPHENE

Mortality Differences From Hexachlorophene Toxicity in Different Aged Mice

Materials

Hexachlorophene, U.S.P. (39), was obtained from Sigma Chemical Company. The solvent used was peanut oil, commercial grade (Planter's). Hexachlorophene was added to the peanut oil to make a 1% (weight:volume) solution. Approximately 24 hours were required for the HCP to become completely dissolved in the peanut oil. Peanut oil was used as the solvent because preliminary studies indicated that peanut oil was non-toxic to mice.

Methods

Five age groups of Swiss white mice were used: 5, 10, 15, 30 and 60 days of age. In each age group there were subgroups with ten or more mice in each subgroup. Each subgroup was given a different dosage level of 1% HCP solution in an attempt to establish a single oral minimal lethal dose for all mice ($L.D._{100}$), a dosage which would kill approximately 50 percent of the mice ($L.D._{50}$), and the maximum single oral dosage which would kill none of the mice in that age group. Five mice in

each age group were given only peanut oil in the same volume as the subgroup in that age group receiving the highest HCP dosage rate. The mice were observed for one week after treatment. During this observation period, any signs of toxicity were recorded. Administration of the HCP solution was made via a 2-centimeter long polyethylene tube (.043 inch outside diameter) passed into the esophagus of the mice and connected to a microliter or tuberculin syringe delivery unit. The tuberculin syringe was used to deliver doses of solution larger than could be contained in the microliter syringe.

Preliminary studies indicated that the $L.D._{50}$ for the 60-day-old mice was approximately 150 milligrams of HCP per kilogram of body weight (150 mg/kg). Similar studies in 10-day-old mice indicated the $L.D._{50}$ was in the range of 30 mg/kg. Subsequent experimental dosage levels were designed to investigate the intermediate range of approximate $L.D._{50}$'s. One common dosage level of 90 mg/kg was used in all age groups.

The $L.D._{50}$ for each age group was derived by using proportions according to the equation A:B = C:D. In this equation, A is the unknown difference in dosage (mg/kg) between the dosage that would kill 50% of the mice and the highest dosage that killed less than 50% of the mice in that age group. B is the difference in dosage (mg/kg) between the lowest dosage that killed more than 50% of the mice in that age group and the highest dosage that killed less than 50% of the mice in that age group. C is the difference between 50% and the percentage of mice killed by the highest dosage killing less than 50% of the mice in the age group. D is the difference between the lowest percentage of mice killed above 50% and the highest percentage of mice killed below 50%. This method gives an approximate $L.D._{50}$ which was satisfactory for comparing differences in

susceptibility to HCP between the age groups of this experiment.

When dosing these mice with the peanut oil solution of HCP, it was found that the esophageal tube occasionally was accidentally passed into the trachea with the solution being deposited in the trachea and lungs. These mice were immediately removed from the experiment, as they inevitably died within 1-2 hours from asphyxiation.

Insofar as possible, each subgroup was made up of an individual litter of mice, but in some groups, two or more litters were used. No attempt was made to classify the individual animals according to size, sex or pregnancy. All animals were dosed on the basis of body weight. The solution was administered between 10:00 and 12:00 A.M. to all groups. Food and water were available to the animals at all times both pre- and post-treatment. Unweaned mice were left with their mothers at all times.

Results

Five Day Mice. The youngest mice (5 days old) were originally given 30, 60 and 90 mg/kg of HCP. It was found that HCP at the rate of 60 mg/kg killed all these mice, and 30 mg/kg killed only one. Two additional subgroups receiving 40 and 50 mg/kg were added in a further effort to determine the $L.D._{50}$ more closely. The experimental data is summarized in Table I. This data shows that the $L.D._{50}$ for 5-day-old mice lies near 35 mg/kg. The controls were dosed with an amount of peanut oil equal to that received by the 90 mg/kg group. One of the controls died, but this death was on the fifth day after treatment and was attributed to a leaking water dispenser which wet the bedding, causing chilling and death of this mouse. It did not appear that the control mouse died as a result of the peanut oil treatment.

TABLE I

MORTALITY RATE OF FIVE-DAY-OLD MICE TO SINGLE DOSES OF HEXACHLOROPHENE AT DIFFERENT LEVELS[1,2]

Dose of HCP	Litter 1		Litter 2		Fraction Killed	% Killed
	No. Killed	Total Dosed	No. Killed	Total Dosed		
Controls 0%	1	5	0	5	0/10	0%
30 mg/kg	1	12	0	5	1/17	6%
40 mg/kg	3	4	4	4	7/8	87.5%
50 mg/kg	4	4	5	5	9/9	100%
60 mg/kg	7	7	3	3	10/10	100%
90 mg/kg	9	9	1	1	10/10	100%

[1] $L.D._{50} \approx 35$ mg/kg.

[2] $L.D._{100} \approx 50$ mg/kg.

This group of mice showed very few signs of HCP toxicity prior to death. The baby mice continued to nurse in an apparently normal manner until immediately prior (1 hour or less) to their demise. Most mice died approximately 22-28 hours after treatment. In almost all cases, mice which did not die within 48 hours after treatment survived the HCP toxicity.

Terminally, the mice became hyperpneic, dyspneic, cyanotic, and died in convulsions. These signs were seen only in those mice that died and occurred less than one hour prior to death. No signs of toxicity were seen in those mice which survived.

Ten Day Mice. The 10-day-old mice were originally dosed with HCP at 30, 60 and 90 mg/kg. It was found that these dosage rates killed 27 of 28 mice (Table II). As this was not the anticipated result, another series of mice was treated with the HCP solution at the 30 mg/kg dosage. The results were similar. A fourth subgroup of mice was then given HCP at the rate of 20 mg/kg. This dosage rate killed 20% of the mice. From this data, an $L.D._{50}$ near 24 mg/kg for 10-day-old mice can be extrapolated. The $L.D._{100}$ would be slightly over 30 mg/kg.

The control mice were given the same volume of peanut oil as the mice receiving 90 mg/kg of 1% HCP solution. One of these controls was found dead due to undetermined causes 129 hours after being given the peanut oil. At the time the dead control was discovered, it was too autolyzed to determine the exact nature of its death.

Signs shown by these mice were similar to those in the 5-day-old mice in that the baby mice appeared normal until shortly before their time of death. About 30 to 60 minutes before death, the mice would become quite inactive, show difficulty in breathing and then progress into

TABLE II

MORTALITY RATE OF TEN-DAY-OLD MICE TO SINGLE DOSES OF HEXACHLOROPHENE AT DIFFERENT LEVELS[1,2]

Dose of HCP	Litter 1		Litter 2		Fraction Killed	% Killed
	No. Killed	Total Dosed	No. Killed	Total Dosed		
Controls 0%	1	6			1/6	17%
20 mg/kg	1	5	1	5	2/10	20%
30 mg/kg	10	11	4	5	14/16	87.5%
60 mg/kg	8	8	3	3	11/11	100%
90 mg/kg	9	9	3	3	12/12	100%

[1] $L.D._{50} \approx 24$ mg/kg.

[2] $L.D._{100} \approx 60$ mg/kg.

terminal convulsions. No gastrointestinal disturbances were noted. The mice continued to nurse and act normally until the terminal episode, which usually occurred 18 to 24 hours after administration of HCP. The maximum time from administration of the drug until death was 38 hours. As with all other age groups, individual mice that lived past 48 hours usually made a full recovery.

Fifteen Day Mice. Four subgroups were used in the 15-day-old age group, these being treated with HCP at the rates of 30, 60, 90 and 120 mg/kg, respectively. The data in Table III shows that the $L.D._{50}$ lies between 30 and 60 mg/kg. Extrapolation gives a figure near 45 mg/kg as the dose which would kill 50 percent of the mice. An $L.D._{100}$ appears to be slightly over 90 mg/kg of HCP.

Signs shown by the fifteen-day-old mice were more noticeable than in the previous two groups. These mice died more quickly than the two younger groups, most of them dying in a period 16-20 hours after administration of the drug. One mouse died after 32 hours, and some died as early as six hours after treatment. These mice showed lethargy and weakness within a few hours after drug administration. They developed a peculiar "flattening" of the rear quarters, with the rear legs abducted in a spread-eagle fashion. The rear legs were functional, as were the front legs, but there was a definite weakness in use of the rear legs. This weakness may have been neurological or muscular in origin. Dyspnea, hyperpnea and convulsions developed during the one to two hours immediately prior to death. Some mice seemed to become extremely weak and died with no apparent respiratory involvement or convulsions.

The controls received the same volume of peanut oil as the group which received 120 mg/kg of 1% HCP solution. The controls showed no

TABLE III

MORTALITY RATE OF FIFTEEN-DAY-OLD MICE TO SINGLE DOSES OF HEXACHLOROPHENE AT DIFFERENT LEVELS[1,2]

Dose of HCP	Litter 1		Litter 2		Litter 3		Fraction Killed	% Killed
	No. Killed	Total Dosed	No. Killed	Total Dosed	No. Killed	Total Dosed		
Controls 0%	0	4	0	3			0/7	0/7
30 mg/kg	2	10					2/10	20%
60 mg/kg	7	9	2	3	3	3	12/15	80%
90 mg/kg	6	6	3	4			9/10	90%
120 mg/kg	10	10	2	2			12/12	100%

[1] $L.D._{50} \approx 45$ mg/kg.

[2] $L.D._{100} \approx 120$ mg/kg.

adverse affects.

Thirty Day Mice. The thirty-day-old mice were divided into four subgroups which received 90, 120, 150 and 180 mg/kg of HCP in a 1% peanut oil solution, respectively. The data from this part of the study made it difficult to draw definite conclusions concerning an $L.D._{50}$. There was much variability in susceptibility to HCP between the litters of mice used. The data in Table IV shows that the $L.D._{50}$ lies between 90 and 150 mg/kg. Preliminary studies indicated the $L.D._{50}$ of HCP for thirty-day-old mice to be within a range of 120 to 150 mg/kg. As seen in Table IV, the problem appears to be with the second litter of 30-day-old mice used in the subgroup treated with 120 mg/kg. All of this litter (nine of nine) died from a dose of 120 mg/kg of HCP, possibly indicating some unknown factor in this litter making them more susceptible to HCP toxicity. The results of the subgroups receiving 90, 150 and 180 mg/kg supported the previous data that the $L.D._{50}$ of HCP for 30-day-old mice was in the approximate area of 130 to 140 mg/kg. By omitting the subgroup receiving 120 mg/kg of HCP, an $L.D._{50}$ near 132 mg/kg was obtained by extrapolation.

Definite neurological signs were noted in this group. "Flattening" or abduction of the rear legs was noted in almost all mice. The front legs were not involved. Weakness and inactivity were common signs. The mice became depressed and developed rough hair coats within a few hours after administration of HCP. Diarrhea was noted in some mice. Ptosis was noted in affected mice and the eyes appeared duller than in non-affected mice. Some of these mice died as early as three hours after receiving the HCP. Most deaths occurred 12 to 20 hours after administration, but a few mice lingered as long as 144 hours, showing paraparesis,

TABLE IV

MORTALITY RATE OF THIRTY-DAY-OLD MICE TO SINGLE DOSES OF HEXACHLOROPHENE AT DIFFERENT LEVELS[1,2]

Dose of HCP	Litter 1		Litter 2		Fraction Killed	% Killed
	No. Killed	Total Dosed	No. Killed	Total Dosed		
Controls 0%	0	5			0/5	0%
90 mg/kg	3	4	0	7	3/11	27%
120 mg/kg	0	2	9	9	9/11	81%
150 mg/kg	6	10			6/10	60%
180 mg/kg	9	10			9/10	90%

[1] $L.D._{50} \approx 132$ mg/kg. The $L.D._{50}$ obtained by omitting the subgroup treated with 120 mg/kg.

[2] $L.D._{100} \approx 120$ mg/kg.

rough hair coats, diarrhea, and hyperpnea before dying. Convulsions were seen terminally in about one-half of the animals. Again, as in previous groups, if the animals survived 48 hours, in almost all cases they made a complete recovery and appeared normal by the end of the seven-day observation period.

Controls were given peanut oil at the same dosage as those mice receiving 180 mg/kg of 1% HCP solution. There were no fatalities in the control group, and all mice in this group were normal throughout the seven-day holding period.

Sixty Day Mice. Sixty-day-old mice were divided into four treatment groups receiving 90, 120, 150 and 180 mg/kg of HCP. The extrapolated $L.D._{50}$ of HCP is 142 mg/kg, with the $L.D._{100}$ being slightly over 180 mg/kg. Table V summarizes the results of HCP toxicity in this age group.

Signs associated with this group were similar to the thirty-day-old mice. Abduction of the rear legs with typical flattening of the rear quarters was the most constant and obvious sign. This usually was seen starting 8 to 12 hours after drug administration. Rough hair coats, weakness, inactivity and diarrhea were other commonly observed signs. Rapid respiration was seen in most animals in the few hours immediately prior to death. Convulsions were seen only in a few animals. Terminally, the animals appeared to become progressively weaker and more inactive until death. No deaths occurred after 47 hours post-treatment, and all survivors were normal and active at the end of the seven-day observation period. No residual effects of toxicity were noted.

Controls received peanut oil at the same dosage as the mice receiving 180 mg/kg of 1% HCP solution. No controls died, and they appeared normal and active throughout the one-week observation period.

TABLE V

MORTALITY RATE OF SIXTY-DAY-OLD MICE TO SINGLE DOSES OF HEXACHLOROPHENE AT DIFFERENT LEVELS[1,2]

Dose of HCP	Litter 1		Litter 2		Litter 3		Fraction Killed	% Killed
	No. Killed	Total Dosed	No. Killed	Total Dosed	No. Killed	Total Dosed		
Controls 0%	0	6					0/6	0%
90 mg/kg	1	11					1/11	9%
120 mg/kg	0	5	0	3	3	5	3/13	23%
150 mg/kg	6	10					6/10	60%
180 mg/kg	9	11					9/11	82%

[1] $L.D._{50} \approx 142$ mg/kg.

[2] $L.D._{100} > 180$ mg/kg.

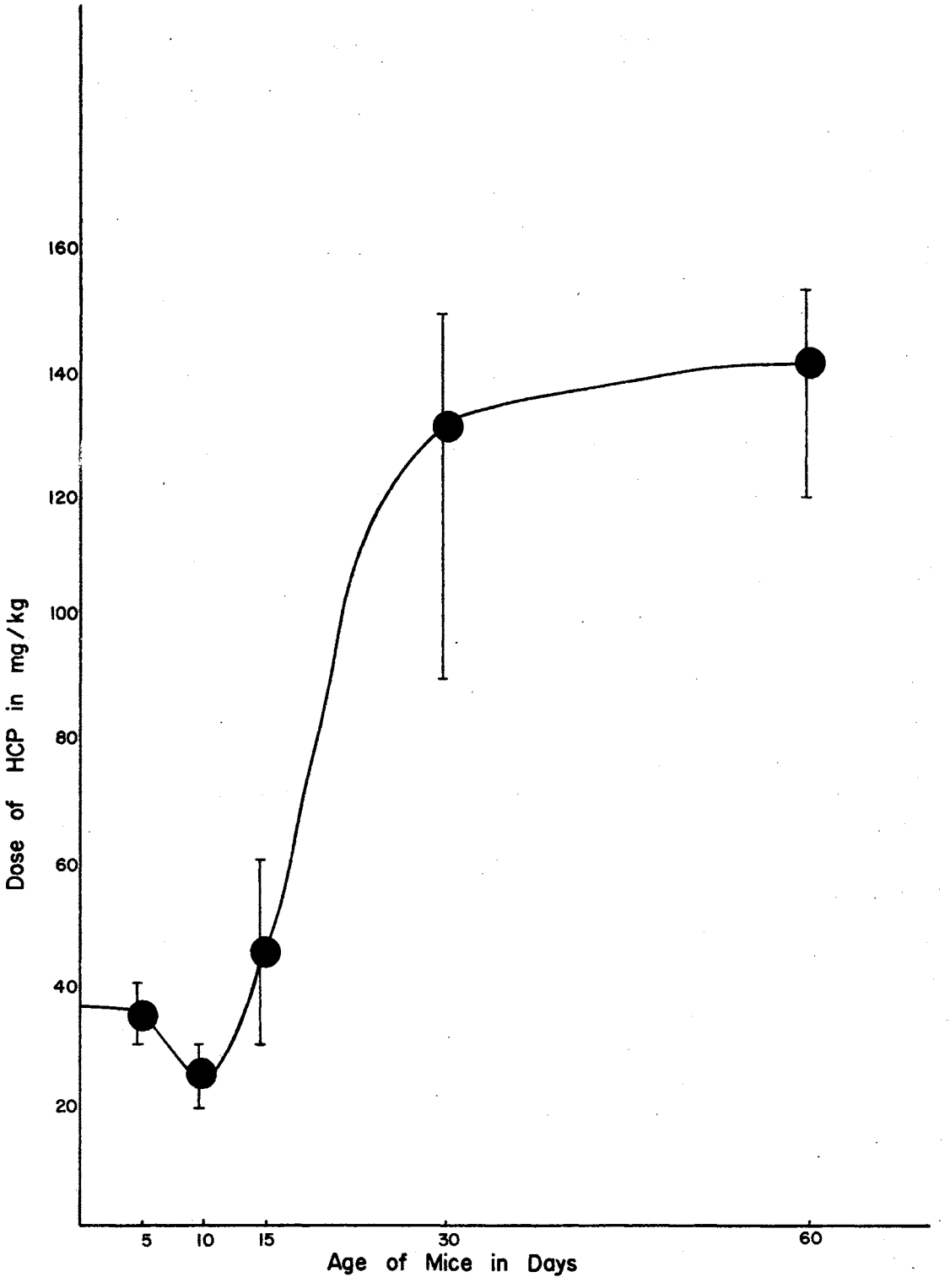


Figure 2. Approximate $L.D._{50}$ of Hexachlorophene for Different Aged Mice

Brain Weights and Pathological Lesions in Mice Treated With a Single Oral Dose of Hexachlorophene

Methods

This part of the experiment was designed to determine gross and histopathological lesions and brain weights of mice treated with a single oral dose of a solution of HCP in peanut oil (1% weight:volume). A 1% solution of HCP in peanut oil from the same stock solution that was used in the first study was used for this portion of the experiment.

The same age groups of Swiss white mice used in the previous study were used in this experiment, i.e., 5, 10, 15, 30 and 60-day-old mice. At least eleven mice in each age group were utilized. A minimum of five mice in each age group were given 1% HCP solution at dosage levels that would produce signs of toxicity and at least six mice were given peanut oil alone at the same dosage rate. When the treated mice started showing toxic signs, usually 10 to 24 hours after administration of HCP, the treated mice and the controls were killed by severing the neck with scissors. Because of the desire to use fresh tissues, only mice that were killed were used for this study. Mice that died from toxicity were not used. The brains were removed immediately and weighed, then transferred to 10% formalin solution. Representative samples of liver, intestine, lung and myocardium were also removed and fixed in 10% formalin. The tissues were allowed to fix in the formalin solution for at least 48 hours. Tissue sections at five microns thickness were made and stained with hematoxylin-eosin for histopathologic evaluation. The slides were coded and studied the first time without knowing whether the sections

were from treated or control mice.

Five-day-old mice were given 20 mg/kg of HCP in the peanut oil solution. Six mice were given HCP, but one died from toxicity. The five remaining treated mice were killed 24 hours after HCP administration, the brains weighed, and tissues saved for section. Six controls having received peanut oil alone were killed at 24 hours and tissues were saved for comparison with the HCP treated mice. The data is presented in Table VI.

Results

In the five-day-old mice, no detectable signs of toxicity were noted at 20 mg/kg except for the one mouse that died. The five treated mice had an average brain weight of 0.214 gm (standard deviation ± 0.0055). The six control mice had an average brain weight of 0.220 gm (standard deviation ± 0.0303). There was no significant statistical difference between the brain weights of the two groups in the five-day mice.

The ten-day-old mice were given HCP at the rate of 20 mg/kg (four mice) and 30 mg/kg (three mice). Twelve controls were given peanut oil at an equivalent dosage to that received by the 30 mg/kg group. The average brain weight of the HCP-treated group was 0.3314 gm (standard deviation ± 0.015). Of the control mice, the average brain weight was 0.3308 gm (standard deviation ± 0.012). This data is summarized in Table VII. As with five-day mice, there was no significant statistical difference between the group receiving HCP and that group receiving only peanut oil.

A group of nine 15-day-old mice were given HCP at the rate of 30 mg/kg and one received a dose of 60 mg/kg. Eight control mice received

TABLE VI

BRAIN WEIGHTS AND HISTOPATHOLOGICAL INTERPRETATION OF EDEMA OF THE WHITE MATTER OF THE BRAIN IN 5-DAY-OLD MICE

Mouse Number	Dose of HCP (mg/kg)	Brain Weight[1,2,3] (gm)	Received HCP	Received No HCP	Relative Degree of Vacuolation in Brain
1	30	.22	X		-
2	30	.21	X		+
3	30	.21	X		+
4	30	.22	X		+
5	30	.21	X		+
6	0	.28		X	-
7	0	.20		X	-
8	0	.22		X	-
9	0	.21		X	-
10	0	.20		X	-
11	0	.21		X	-

[1]Mean brain weight of HCP mice = .214 gm; standard deviation, ± .0055.

[2]Mean brain weight of control mice = .220 gm; standard deviation, ± .0303.

[3]The difference is not statistically significant. $t_{cal} = -.4327$; $t_{.05} = 2.262$.

TABLE VII

BRAIN WEIGHTS AND HISTOPATHOLOGICAL INTERPRETATION OF EDEMA OF THE WHITE MATTER OF THE BRAIN IN 10-DAY-OLD MICE

Mouse Number	Dose of HCP (mg/kg)	Brain Weight[1,2,3] (gm)	Received HCP	Received No HCP	Relative Degree of Vacuolation in Brain
1	30	.32	X		+
2	30	.32	X		+
3	30	.32	X		+
4	0	.33		X	-
5	0	.33		X	-
6	0	.32		X	-
7	0	.35		X	-
8	0	.33		X	-
9	0	.32		X	-
10	20	.33	X		+
11	20	.36	X		+
12	20	.33	X		+
13	20	.34	X		+
14	0	.35		X	-
15	0	.33		X	-
16	0	.32		X	-
17	0	.35		X	-
18	0	.32		X	-
19	0	.32		X	-

[1]Mean brain weight of HCP mice = .3314 gm; standard deviation, ± .015.

[2]Mean brain weight of control mice = .3308 gm; standard deviation, ± .012.

[3]The difference is not statistically significant. t_{cal} = .0952; $t_{.05}$ = 2.110.

peanut oil at an equivalent dosage to that received by the 30 mg/kg mice. Brain weights for the treated mice averaged 0.395 gm (standard deviation ± 0.0108). For the control mice, average brain weight was 0.379 gm (standard deviation ± 0.0113). Data for this group is given in Table VIII. The brain weights of mice receiving HCP were significantly heavier ($p < .025$) than the control group.

Six 30-day mice received 1% HCP in peanut oil at the dosage of 150 mg/kg. Seven mice of the same age group received peanut oil at an equivalent dosage, as shown in Table IX. The controls and HCP-treated mice were sacrificed 12 hours after drug administration. Mean brain weight in the treated group was 0.4233 gm (standard deviation ± 0.0234). Mean brain weight in the control group was 0.4214 gm (standard deviation ± 0.0212). There was no statistical difference in the mean brain weights between the two groups.

Six 60-day mice received 150 mg/kg of HCP and six mice of the same age received peanut oil at an equivalent dosage level. Average brain weight in the treated group was 0.483 gm (standard deviation ± 0.0075). This data, presented in Table X, shows a statistically significant heavier brain weight for the group receiving HCP ($p < .001$) than for the controls.

Tissues for microscopic evaluation were taken transversely through the brain in three areas: anterior one third of the cerebrum, posterior one third of the cerebrum and through the area of the pons.

Histopathologic examination of the hematoxylin and eosin stained sections of the brain revealed numerous small, irregularly distributed, sometimes confluent, interstitial spaces in the white matter. No lesions were noted in the gray matter. The most consistent lesions were found in

TABLE VIII

BRAIN WEIGHTS AND HISTOPATHOLOGICAL INTERPRETATION OF EDEMA OF THE WHITE MATTER OF THE BRAIN IN 15-DAY-OLD MICE

Mouse Number	Dose of HCP (mg/kg)	Brain Weight[1,2,3] (gm)	Received HCP	Received No HCP	Relative Degree of Vacuolation in Brain
1	30	.40	X		+
2	30	.40	X		+
3	30	.39	X		+
4	30	.39	X		+
5	30	.40	X		+
6	30	.41	X		+
7	0	.37		X	-
8	0	.37		X	-
9	0	.37		X	-
10	0	.38		X	-
11	0	.40		X	-
12	60	.41	X		+
13	0	.38		X	-
14	30	.39	X		+
15	30	.38	X		-
16	30	.38	X		-
17	0	.39		X	+
18	0	.37		X	-

[1]Mean brain weight of HCP mice = .395 gm; standard deviation, ± .0108.

[2]Mean brain weight of control mice = .379 gm; standard deviation, ± .0113.

[3]There is a statistically significant difference in the brain weight weights of the two groups ($p < .025$). $t_{cal} = 3.08$; $t_{.025} = 2.49$.

TABLE IX

BRAIN WEIGHTS AND HISTOPATHOLOGICAL INTERPRETATION OF EDEMA OF THE WHITE MATTER OF THE BRAIN IN 30-DAY-OLD MICE

Mouse Number	Dose of HCP (mg/kg)	Brain Weight[1,2,3] (gm)	Received HCP	Received No HCP	Relative Degree of Vacuolation in Brain
1	150	.47	X		+
2	0	.46		X	-
3	150	.42	X		+
4	150	.41	X		+
5	150	.41	X		+
6	150	.42	X		+
7	150	.41	X		+
8	0	.40		X	-
9	0	.41		X	-
10	0	.41		X	+
11	0	.41		X	+
12	0	.42		X	-
13	0	.44		X	+

[1]Mean brain weight of HCP mice = .4233 gm; standard deviation, ± .0234.

[2]Mean brain weight of control mice = .4214; standard deviation, ± .0212.

[3]The difference between the two groups is not statistically significant. $t_{cal} = 0.1538$; $t_{.05} = 2.201$.

TABLE X

BRAIN WEIGHTS AND HISTOPATHOLOGICAL INTERPRETATION OF EDEMA OF THE WHITE MATTER OF THE BRAIN IN 60-DAY-OLD MICE

Mouse Number	Dose of HCP (mg/kg)	Brain Weight[1,2,3] (gm)	Received HCP	Received No HCP	Relative Degree of Vaculoation in Brain
1	150	.48	X		+
2	150	.49	X		+
3	150	.50	X		+
4	0	.45		X	-
5	0	.46		X	-
6	0	.46		X	-
7	150	.48	X		+
8	150	.47	X		+
9	150	.48	X		+
10	0	.47		X	-
11	0	.45		X	-
12	0	.46		X	-

[1]Mean brain weight of HCP mice = .473 gm; standard deviation, ± .0105.

[2]Mean brain weight of control mice = .458 gm; standard deviation, ± .0078.

[3]The difference is statistically significant at the p = .001 level. $t_{cal} = 4.811$; $t_{.001} = 4.581$.

the brain stem. In all sections, this was the most severely affected area. Vacuolation was seen in the white matter tracts in the cerebellum. A similar vacuolated appearance was noted in the white matter of the cerebrum, especially noticeable in the fibers of the corpus callosum, lateral olfactory tract, and the internal capsule. The spaces were variable in size and shape and sometimes confluent one with another. They did not correspond to any of the perivascular spaces. No stainable material was seen in the spaces, and there was no cellular reaction around the spaces. Distribution of the lesions was similar in all except the younger age groups, in which lesions were more or less limited to the brain stem. Even though the vascular lesions responsible for the vacuolation is not apparent in the sections of tissue, it is interpreted to be edema because of its apparent lack of cellular or tissue fragment components and its definite space-occupying relationship.

No gross lesions were seen in any animal necropsied.

No histopathological lesions were found in the liver, kidney, intestine, lungs or myocardium.

In the blind study of histopathological lesions of 73 treated and control mice, 66 were correctly classified as to HCP-treated or normal. Of the seven brains that were incorrectly classified, four normals were classed as having lesions similar to treated mice. Three HCP-treated mice were classed as having no lesions. This is an overall success ratio of 90% for correctly classifying the sections as coming from treated or control mice.

Radioisotopic Studies on the Metabolic Fate of Hexachlorophene in Mice of Different Ages

Materials

Hexachlorophene (methylene-^{14}C) was obtained from Mallinckrodt Company. There were 15.01 mgs of ^{14}C-HCP in the sample with a total activity of 0.1 milliCuries. The purity was reported as greater than 98%. The solvent used was peanut oil.

Methods

The ^{14}C-HCP was diluted with peanut oil to 0.75% solution (weight: volume). Each 10 microliters of the solution contained .075 mg of ^{14}C-HCP or a calculated dose of 1.2×10^6 d.p.m. When a diluted sample of the radioactive solution was counted, the actual d.p.m. for this solution was determined to be 1.45563×10^6.

The same age groups of mice as used in the previous experiments (5, 10, 15, 30 and 60 days old) were used for this study. Five mice in each age group received ^{14}C-HCP and two controls in each age group received no medication. Mice that died less than 24 hours post-treatment were discarded. In the five-day-old and sixty-day-old mice, an additional group of five mice was dosed with ^{14}C-HCP in each age group. Two additional controls were used in both five-day and sixty-day mice. Each treated mouse received 10 microliters of the ^{14}C-HCP in peanut oil solution, or a dose of 1,455,630 d.p.m. The solution was administered with a 50-microliter syringe through a polyethylene tube inserted into the esophagus of the mouse.

In order to prevent "super-absorption" and "super-excretion" of such

a small dose of HCP in the older mice, the three older age groups were given an additional amount of non-radioactive or "cold" HCP approximately equal to one-half of the $L.D._{100}$, as previously established in the earlier experiments. The 5 and 10-day mice received only .075 mg of ^{14}C-HCP, while the 15, 30 and 60-day mice received .075 mg of ^{14}C-HCP, plus an additional dose of non-radioactive HCP.

The controls received no medication.

All mice were sacrificed by decapitation at 24 hours after administration of HCP. Twenty-five microliters (.0263 gm) of blood was taken from each mouse (48). The entire brain of each mouse was removed. In the 5, 10 and 15-day mice, the entire liver was removed, but in the 30 and 60-day mice, a weighed random sample of liver was taken. Liver weights are recorded in Appendix B.

The tissue samples were immediately placed in 2 ml of absolute ethanol to precipitate the protein and dissolve the HCP. Micro glass beads were added to each sample and the tissues were finely ground to enhance extraction of as much HCP as possible. The samples were then dried under lights and hot air. The dried samples were then resuspended in 15 ml of Bray's solution, and were allowed to stand for one week to insure maximum extraction of HCP.[1] Then the liquid portion of each sample was transferred into a clean glass scintillation vial to remove glass beads and tissue residue.

Radioactivity was determined in a Packard Tri-Carb Liquid Scintillation Counter. Three 10-minute counts were obtained on each sample.

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[1]Bray's solution formula: 0.8 gm of 1,4-bis-[2-(5-Phenyloxazolyl)]-benzene; 16.0 gm of 2,5-diphenyloxazol; 240 gm of napthalene; 400 ml of absolute methanol; 80 ml of ethylene glycol; dioxane sufficient to make 4 liters of solution.

Appropriate blanks and controls were used. A diluted (1:100) ^{14}C-HCP dose was counted. The counts for each sample were corrected for background count, instrument efficiency and quench correction (Appendix A).

Results

Appendices A and B present the detailed data and calculations involved in this part of the experiment. Table XI and Figure 3 summarize this information. Figure 3 shows that in general, as the age of mice increased, the amount of HCP in the tissues studied decreased. In this data, there is a striking similarity between mean blood and brain levels of HCP in each age group. In all cases, the concentration of HCP in the liver was higher than in either the blood or brain.

The values for the 10-day-old mice are quite different from what might be expected in that they are much lower than any of the other groups. This age group is the most susceptible to HCP toxicity, as shown in the previous experiments. Since the data indicate that they have the lowest levels of HCP in the tissues of any group, it seems rather likely that some experimental error may have been involved. There could be two possibilities as to the source of this difference. First and most likely, is that the dosing apparatus failed to deliver the full volume of ^{14}C-HCP solution to the mice. The second possibility is that this particular litter of mice did not absorb the HCP for some intrinsic reason or that they were able to excrete it at a much faster rate than other mice. The first possibility would seem to be the most probable.

Sample radiation counts that are less than four times that of background radiation are not usually considered significant. In this study, these figures and calculations have been shown and included in the data,

TABLE XI

STATISTICAL SUMMARY OF TISSUE LEVELS OF HEXACHLOROPHENE

Age (Days)	Number of Mice in Group	Tissue	Mean HCP Levels in Tissues (mg/kg x 10^{-6})	Standard Deviation (mg/kg x 10^{-6})
5	10	Blood	4,030	± 3,448
		Liver	13,793	± 9,630
		Brain	4,504	± 2,907
10	5	Blood	93	± 46
		Liver	885	± 519
		Brain	128	± 64
15	5	Blood	2,700	± 686
		Liver	20,385	± 6,728
		Brain	2,615	± 867
30	4	Blood	1,125	± 708
		Liver	5,201	± 2,336
		Brain	768	± 380
60	8	Blood	865	± 733
		Liver	3,661	± 3,713
		Brain	833	± 889

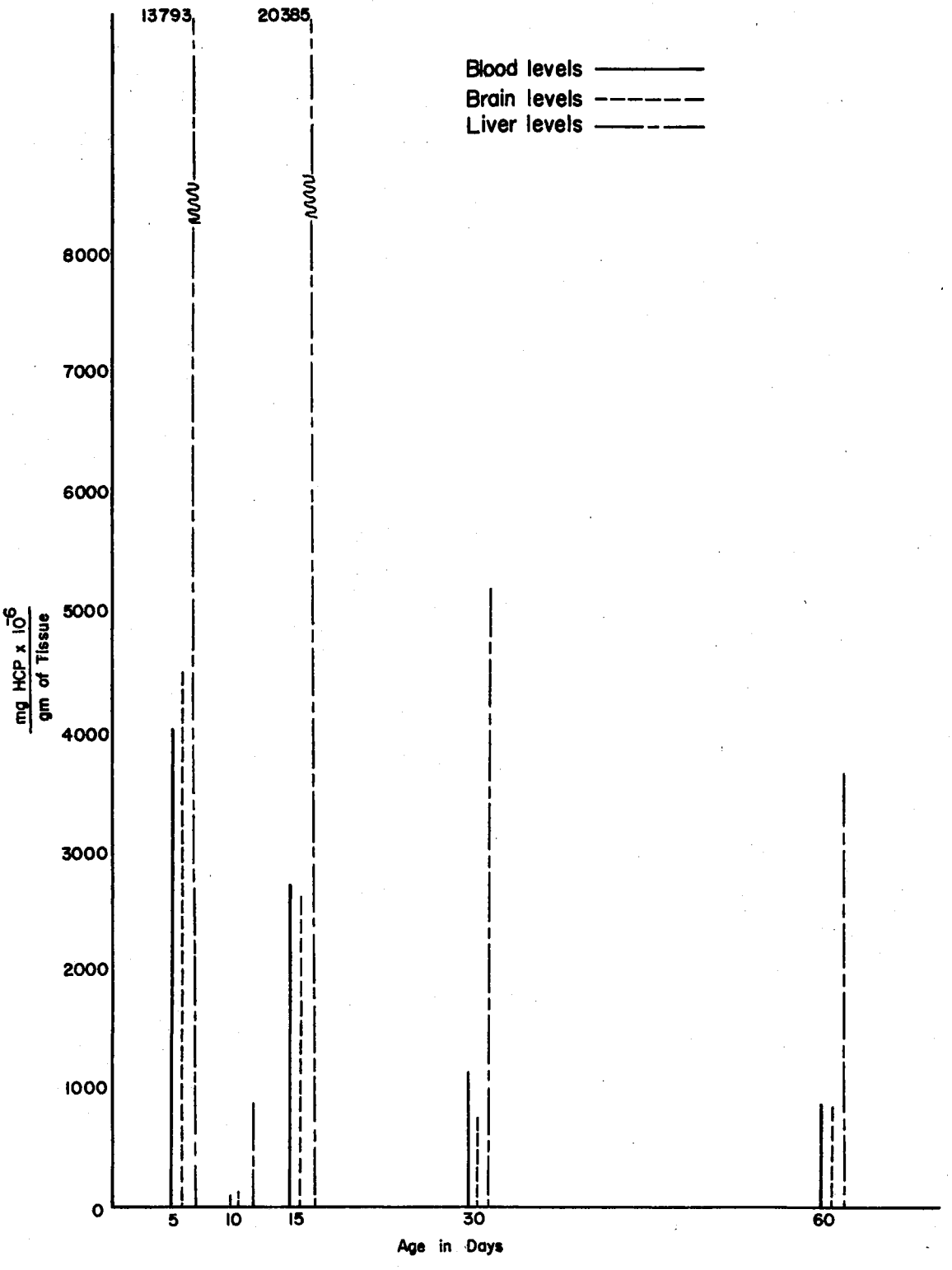

Figure 3. Tissue Levels of Hexachlorophene in Different Aged Mice

but net radiation counts less than 160 d.p.m. (four times background count) may not be significant.

Discussion

Hexachlorophene has been shown to have toxic properties by many investigators. Up until now no one had presented controlled experimental data to indicate that younger animals were more susceptible to the toxic effects of HCP than were adults. The data presented in this work clearly indicates that young mice are much more likely to die from ingestion of HCP than older mice. However, it appears that the youngest mice are not the most susceptible to toxicity, since 10-day mice died at lower dosage levels than the five-day mice (Figure 2). After 10 days of age, the amount of ingested HCP required to kill mice increased rapidly.

Several possible explanations are proposed to account for the increased susceptibility of young animals to HCP. One possibility is that the "blood-brain" barrier is not established in the infant mice, thereby allowing more HCP to enter the nervous tissue. If this were true, then there should have been a similar level of HCP in the blood and brain of younger mice, with a large difference in the blood and brain levels in older mice. The data from the third part of this experiment showed that the relative levels of HCP in blood and brain tissues were very similar in each age of mice. This evidence indicates that a blood-brain barrier for HCP may not exist in mice.

A second possibility which may account for the increased susceptibility of young mice is that they are capable of absorbing HCP in larger quantities or at a faster rate than older mice. If this were true, then the levels of HCP in all tissues would have been expected to be higher

for the young mice than the older mice. The third part of the experiment gave support to this idea in that the quantity of HCP in the brain, blood and liver was much higher for the 5 and 15-day mice than for the 30 and 60-day mice. The extremely low level of HCP in all tissues examined in the 10-day mice is discussed on page 42. It seems likely that this data is the result of experimental error.

A third reason that young mice are more severely affected by lower levels of HCP than older mice may be that the relative absorption rate is similar for all ages but that older mice are able to excrete or metabolize the HCP at a much higher rate than the younger mice. The enzyme systems necessary to metabolize the absorbed HCP may be more functional in older individuals. Experiments by Wit and Genderen (61) indicated that very little HCP is excreted in the urine of rats. It would seem likely then that the liver is the primary organ of detoxification of HCP, either by metabolism or excretion through the biliary system into the intestinal tract. The results of this experiment neither confirmed nor rejected this possibility, although the extremely high level of HCP in all samples of liver tissue in relation to blood and brain levels suggests that the liver is the excretory or detoxification organ for HCP in mice.

As noted in Figure 3, the blood levels of HCP were strikingly close to the brain levels. This would indicate that the passage of HCP from the blood into and out of the brain is by a simple process of diffusion. If there were an active process involved which either pumped HCP into neural tissue or inhibited its passage from the blood into the brain, then there should be a marked difference in the levels of HCP in the blood and brain.

The histopathologic lesions of HCP in the brain are easily recognized. In studying the sections of mouse brains without reference to whether the individual mouse was treated or untreated, there was a 90% success rate in correctly labeling the sections as being from treated or untreated mice. In the routine fixation and staining of hematoxylin and eosin slides of central nervous tissue, some vacuolation is produced as an artifact. This was seen primarily in the immediate vicinity of the ventricles or near the periphery of the brain. The vacuolation of the brain produced by HCP was much more extensive and recognizable than the artifactual vacuolation in most cases. Vacuolation was found in all parts of the brain in the white matter. The gray matter appeared to be histologically normal in all cases. Even small bundles of white fiber tracts which passed through gray matter, such as in the caudate-putamen nucleus, showed the "looseness" or separation of the fibers. Magee, et al. (31) described this change as interstitial edema of the white matter. He reported an increase in the weights and water content of rat brains showing this histological feature and interpreted these findings as evidence of edema. Kimbrough (26) also reported an increase in the brain weights of rats given HCP.

In two of the five age groups of mice, i.e., 15 and 60-day mice, a statistically significant increase was noted in the brain weights of HCP-treated animals as compared to controls. It is possible that there would have been significant brain weight increases in other groups, especially 30-day mice, if the brain weights had been recorded to three or four decimal places or if more mice had been used.

The exact nature by which HCP produces edema of the white matter is not known. The lack of cellular response is not supportive of an

inflammatory lesion. The data from this study and that presented by others (31,57) indicate that a blood-brain barrier plays little, if any, part in the process. The pattern of distribution of lesions does not appear to be oriented around blood vessels, and it has been shown that the gray matter of the rat is more vascular than the white matter (31). The evidence suggests that a vascular lesion appears to be unlikely. The only biochemical effect of HCP suggested in the literature indicates HCP uncouples oxidative phsophorylation (5,7,53).

> It is possible that the production and removal of free fluid from the central nervous system is of peculiar importance in its over-all metabolism and that a general disturbance, insufficient to impair the specific activities of the neurones themselves, may first show itself by a failure to deal efficiently with such an essential fluid turnover (31).

This area needs much further study.

Another finding in this experiment that requires additional study is the indication that 10-day mice are more susceptible to HCP toxicity than either 5 or 15-day mice. The radioisotope studies indicated that the 10-day mice actually absorbed less HCP than 5 and 15-day mice, but this could be in error. If the 10-day mice actually absorbed less HCP and yet were still more susceptible to toxicity than any of the other age groups, then the mechanisms involved in HCP toxicity are probably exceedingly complicated.

The results from this experiment also raised questions as to whether HCP is absorbed from the gut to a greater extent in young animals or is excreted or metabolized faster in older animals. The mode of excretion should be studied further to determine if the liver is the detoxification organ, either by metabolism or by excretion into the biliary system.

Another area which needs clarification is the mechanism by which HCP causes selective edema of the white matter of the brain.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Single doses of HCP administered orally in a 1% peanut oil solution to 5, 10, 15, 30 and 60-day-old Swiss white mice were found to cause death of the younger animals at a much lower dosage level than in the older animals. The $L.D._{50}$ for 10-day-old mice was approximately 24 mg/kg, while that for 60-day-old mice was near 142 mg/kg. The 10-day-old mice appeared to be more susceptible to toxicity from HCP than were 5-day-old mice.

Brain weights were recorded from mice receiving single doses of HCP in 1% peanut oil solution and from mice receiving only peanut oil at the same dosage rate. The 5, 10 and 30-day-old mice showed no statistically significant differences in brain weights between the treated and control mice, while the 15 and 60-day-old mice showed statistically significant differences in brain weights between the treated and control groups. Brain weights in the treated mice in the latter two age groups were heavier.

Histopathological examination of the brains of mice receiving orally administered HCP revealed vacuolation of the white matter of sufficient degree that was readily distinguishable, in most cases, from the brains of mice receiving no HCP. This vacuolation was similar to that described in previous HCP toxicity reports in humans and animals. The gray matter was not detectably affected. There were no signs of inflammation near

the areas of vacuolation.

^{14}C-Labeled HCP was given orally to 5, 10, 15, 30 and 60-day-old mice to determine the relative distribution of HCP in the tissues. Blood and brain levels at 24 hours after administration were, on the average, very similar when compared in mg of HCP per gm of tissue. Liver levels were much higher than brain or blood levels. Young mice had more HCP in their tissues than older mice, even though older mice were given larger doses of HCP.

Future investigations concerning HCP toxicity should include studies to determine how HCP is metabolized and excreted. Another area that needs to be explored is the difference in gastrointestinal absorption rates of HCP in various age groups of animals. Finally, an effort should be made to determine the process by which HCP causes vacuolation of the brain.

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APPENDIX A

RESULTS AND CORRECTIONS FOR ^{14}C-HEXACHLOROPHENE LEVELS IN TISSUES OF DIFFERENT AGED MICE

Five-Day Mice, Group 1

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (45 CPM)	Quench Correction	Net DPM Per Sample
Control 1	Heart	53.2	8.2	.82	10.0
	Liver	78.0	33.0	.82	40.2
	Brain	57.2	12.2	.82	14.9
Control 2	Heart	121.6	76.6	.82	93.4
	Liver	94.2	49.2	.82	60.0
	Brain	56.4	11.4	.82	13.9
1	Heart	4,190.4	4,145.4	.82	5,055.4
	Liver	36,522.4	36,477.4	.82	44,484.6
	Brain	26,112.3	26,067.3	.82	31,789.4
2	Heart	4,198.7	4,153.7	.82	5,065.5
	Liver	35,083.9	35,038.9	.82	42,730.4
	Brain	32,240.8	32,195.8	.82	39,263.2
3	Heart	574.2	529.2	.82	645.4
	Liver	6,751.2	6,706.2	.82	8,178.3
	Brain	4,215.0	4,170.0	.82	5,085.4

Five-Day Mice, Group 2

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (41 CPM)	Quench Correction	Net DPM Per Sample
Control 3	Heart	45.9	4.9	.82	6.0
	Liver	65.7	24.7	.82	30.1
	Brain	64.9	23.9	.82	29.1
Control 4	Heart	41.8	0.8	.82	1.0
	Liver	149.2	108.2	.82	132.0
	Brain	119.6	78.6	.82	95.9
4	Heart	254.3	213.3	.82	260.2
	Liver	1,196.4	1,154.4	.82	1,409.6
	Brain	4,821.7	4,780.7	.82	5,832.5
5	Heart	2,480.8	2,439.8	.82	2,976.6
	Liver	31,339.1	31,298.1	.82	38,173.9
	Brain	20,646.5	20,605.5	.82	25,138.7
6	Heart	1,646.5	1,605.5	.82	1,958.7
	Liver	31,107.6	31,066.6	.82	37,901.3
	Brain	25,937.4	25,896.4	.82	31,593.6
7	Heart	1,053.6	1,012.6	.82	1,235.4
	Liver	12,984.7	12,943.7	.82	15,791.3
	Brain	10,609.8	10,568.8	.82	12,893.9
8	Heart	533.8	492.8	.82	601.2
	Liver	7,000.2	6,959.2	.82	8,490.2
	Brain	5,467.4	5,426.4	.82	6,620.2
9	Heart	1,031.6	990.6	.82	1,208.5
	Liver	23,857.1	23,816.1	.82	29,055.6
	Brain	22,323.7	22,282.7	.82	27,184.9
10	Heart	1,324.3	1,283.3	.82	2,565.6
	Liver	16,163.0	16,122.0	.82	19,661.0
	Brain	9,290.4	9,249.4	.82	11,284.3

Ten-Day Mice

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (45 CPM)	Quench Corrections	Net DPM Per Sample
Control 1	Heart	43.7	0	.82	0
	Liver	68.2	23.2	.82	28.3
	Brain	50.4	5.4	.82	6.6
Control 2	Heart	45.2	0.2	.82	0.2
	Liver	62.9	17.9	.82	21.8
	Brain	49.7	4.7	.82	5.7
1	Heart	83.6	38.6	.82	47.1
	Liver	3,481.5	3,436.5	.82	4,190.9
	Brain	827.4	782.4	.82	954.1
2	Heart	78.3	33.3	.82	40.6
	Liver	1,356.1	1,311.1	.82	1,598.9
	Brain	507.8	462.8	.82	564.4
3	Heart	59.7	14.7	.82	17.9
	Liver	870.5	825.5	.82	1,006.7
	Brain	336.6	291.6	.82	355.6
4	Heart	112.8	67.8	.82	82.7
	Liver	4,142.2	4,097.2	.82	4,996.7
	Brain	1,204.9	1,159.9	.82	1,414.5
5	Heart	84.7	39.7	.82	48.4
	Liver	1,797.0	1,752.0	.82	2,136.6
	Brain	670.4	652.4	.82	762.7

Fifteen-Day Mice

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (45 CPM)	Quench Correction	Net DPM Per Sample
Control 1	Heart	51.0	6.0	.82	7.3
	Liver	490.2	445.2	.82	542.9
	Brain	79.5	34.5	.82	42.1
Control 2	Heart	47.5	2.5	.82	3.0
	Liver	217.5	172.5	.82	210.4
	Brain	59.4	14.4	.82	17.6
1	Heart	630.8	585.8	.82	714.4
	Liver	32,088.9	32,043.9	.82	39,077.9
	Brain	9,282.1	9,237.1	.82	11,264.8
2	Heart	316.0	271.0	.82	330.5
	Liver	16,546.9	16,501.9	.82	20,124.3
	Brain	3,867.1	3,822.1	.82	4,661.1
3	Heart	443.9	398.9	.82	486.5
	Liver	18,000.1	17,955.1	.82	21,896.5
	Brain	4,444.8	4,399.8	.82	5,365.6
4	Heart	584.6	539.6	.82	658.0
	Liver	31,725.0	31,680.0	.82	38,634.1
	Brain	7,707.0	7,662.0	.82	9,343.9
5	Heart	504.8	459.8	.82	560.7
	Liver	30,724.6	30,679.6	.82	37,414.1
	Brain	7,290.5	7,245.5	.82	8,836.0

Thirty-Day Mice

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (45 CPM)	Quench Correction	Net DPM Per Sample
Control 1	Heart	52.3	7.3	.82	8.9
	Liver	86.4	41.4	.82	50.5
	Brain	56.7	11.7	.82	14.3
Control 2	Heart	49.0	4.0	.82	4.9
	Liver	86.6	41.6	.82	50.7
	Brain	54.0	9.0	.82	11.0
1	Heart	56.2	11.2	.82	13.7
	Liver	393.5	348.5	.82	425.0
	Brain	210.5	165.5	.82	201.8
2	Heart	84.1	39.1	.82	47.7
	Liver	948.4	903.4	.82	1,101.7
	Brain	447.2	402.2	.82	490.5
3	Heart	56.7	11.7	.82	14.3
	Liver	1,979.9	1,934.9	.82	2,359.6
	Brain	56.7	205.7	.82	250.9
4	Heart	69.4	24.4	.82	29.8
	Liver	689.3	644.3	.82	785.7
	Brain	220.4	175.4	.82	213.9

Sixty-Day Mice, Group 1

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (45 CPM)	Quench Correction	Net DPM Per Sample
Control 1	Heart	47.4	2.4	.82	2.9
	Liver	56.4	11.4	.82	13.9
	Brain	48.6	3.6	.82	4.4
Control 2	Heart	43.8	0	.82	0
	Liver	48.3	3.3	.82	4.0
	Brain	43.6	0	.82	0
1	Heart	44.4	0	.82	0
	Liver	124.7	79.7	.82	97.2
	Brain	55.6	10.6	.82	12.9
2	Heart	52.7	7.7	.82	9.4
	Liver	316.9	271.9	.82	331.6
	Brain	91.0	46.0	.82	56.1
3	Heart	48.0	3.0	.82	3.7
	Liver	204.8	159.8	.82	194.9
	Brain	75.2	30.2	.82	36.8
4	Heart	44.0	0	.82	0
	Liver	57.6	12.6	.82	15.4
	Brain	48.6	3.6	.82	4.4

Sixty-Day Mice, Group 2

Mouse Number	Tissue	Average CPM Per Sample	Minus Background (41 CPM)	Quench Correction	Net DPM Per Sample
Control 3	Heart	43.8	2.8	.82	3.4
	Liver	57.6	16.6	.82	20.3
	Brain	41.3	0.3	.82	0.4
Control 4	Heart	42.0	1.0	.82	1.2
	Liver	202.5	161.5	.82	197.0
	Brain	50.0	9.0	.82	11.0
5	Heart	57.9	16.9	.82	20.6
	Liver	933.0	892.0	.82	1,088.2
	Brain	225.0	184.0	.82	224.5
6	Heart	62.7	21.7	.82	26.5
	Liver	2,554.3	2,513.3	.82	3,066.2
	Brain	331.4	290.4	.82	354.3
7	Heart	57.4	16.4	.82	20.0
	Brain	505.1	464.1	.82	566.2
8	Heart	51.4	10.4	.82	12.7
	Liver	1,267.2	1,226.2	.82	1,496.0
	Brain	277.2	236.2	.82	288.2

APPENDIX B

^{14}C-HEXACHLOROPHENE LEVELS IN BLOOD, LIVER AND BRAIN OF DIFFERENT AGED MICE

Five-Day Mice, Group 1

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
Control 1	2.8	0	0	0	Heart[1]	96	.0066	19
					Liver[2]	40	.0027	23
					Brain[3]	15	.0010	3
Control 2	2.6	0	0	0	Heart	831	.057	183
					Liver	60	.0041	38
					Brain	14	.0010	3
1	2.6	.075	.075	29	Heart	44,980	3.090	9,904
					Liver	44,480	3.056	28,650
					Brain	31,789	2.184	7,280
2	2.6	.075	.075	29	Heart	45,069	3.096	9,924
					Liver	42,730	2.936	27,520
					Brain	39,263	2.697	8,991
3	2.6	.075	.075	29	Heart	5,742	.394	1,264
					Liver	8,178	.562	5,266
					Brain	5,085	.349	1,165

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Average liver weight was 3.06% of body weight. The entire liver was used.

[3]Average brain weight was .225 gm. The entire brain was used.

Five-Day Mice, Group 2

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($\times 10^{-6}$)
Control 3	2.9	0	0	0	Heart[1]	60	.0040	11
					Liver[2]	30	.0020	17
					Brain[3]	29	.0020	6
Control 4	3.2	0	0	0	Heart	11	.0008	2
					Liver	132	.0090	68
					Brain	96	.0070	22
4	3.8	.075	.075	20	Heart	3,384	.232	510
					Liver	1,410	.097	626
					Brain	5,833	.401	1,336
5	3.4	.075	.075	22	Heart	34,633	2.379	5,831
					Liver	38,174	2.623	18,912
					Brain	25,139	1.727	5,756
6	3.3	.075	.075	23	Heart	22,119	1.520	3,837
					Liver	37,901	2.604	19,335
					Brain	31,594	2.170	7,235
7	3.0	.075	.075	25	Heart	12,683	.871	2,420
					Liver	15,791	1.085	8,843
					Brain	12,894	.886	2,952
8	2.9	.075	.075	26	Heart	5,966	.410	1,178
					Liver	8,490	.583	4,915
					Brain	6,620	.455	1,516

Five-Day Mice, Group 2 (Continued)

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
9	3.4	.075	.075	22	Heart	14,060	.966	2,367
					Liver	29,056	1.996	14,395
					Brain	27,185	1.868	6,225
10	3.5	.075	.075	21	Heart	18,751	1.288	3,067
					Liver	19,661	1.351	9,467
					Brain	11,284	.775	2,584

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Average liver weight was 3.06% of body weight. The entire liver was used.

[3]Average brain weight was .225 gm. The entire brain was used.

Ten-Day Mice

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($\times 10^{-6}$)
Control 1	5.3	0	0	0	Heart[1]	0	0	0
					Liver[2]	28	.0019	9
					Brain[3]	7	.0005	1
Control 2	4.8	0	0	0	Heart	4	.0003	0
					Liver	22	.0015	7
					Brain	6	.0004	1
1	5.4	.075	.075	14	Heart	870	.060	92
					Liver	4,191	.288	1,349
					Brain	954	.066	150
2	5.0	.075	.075	15	Heart	695	.048	80
					Liver	1,599	.110	549
					Brain	564	.039	89
3	5.3	.075	.075	14	Heart	325	.022	35
					Liver	1,007	.069	324
					Brain	356	.024	56
4	5.6	.075	.075	14	Heart	1,585	.109	162
					Liver	4,997	.343	1,514
					Brain	1,415	.097	223

Ten-Day Mice (Continued)

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
5	5.3	.075	.075	14	Heart	878	.060	95
					Liver	2,137	.147	688
					Brain	763	.052	120

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Average liver weight was 3.03% of body weight. The entire liver was used.

[3]Average brain weight was .327 gm. The entire brain was used.

Fifteen-Day Mice

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($\times 10^{-6}$)
Control 1	5.4	0	0	0	0	Heart[1]	135	.009	14-36
						Liver[2]	543	.037	140-354
						Brain[3]	42	.003	5-14
Control 2	5.9	0	0	0	0	Heart	61	.004	6-15
						Liver	210	.014	51-130
						Brain	18	.001	2-6
1	5.3	.075	.110	.185	35	Heart	12,957	.890	3,452
						Liver	39,078	2.685	26,139
						Brain	11,265	.774	3,680
2	5.9	.075	.120	.195	33	Heart	6,673	.458	1,683
						Liver	20,124	1.382	12,837
						Brain	4,661	.320	1,605
3	6.0	.075	.120	.195	32.5	Heart	9,989	.686	2,478
						Liver	21,897	1.504	13,333
						Brain	5,366	.369	1,848
4	5.3	.075	.110	.185	35	Heart	11,934	.820	3,180
						Liver	38,634	2.654	25,842
						Brain	9,344	.642	3,053

Fifteen-Day Mice (Continued)

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
5	5.4	.075	.110	.185	34	Heart	10,361	.712	2,709
						Liver	37,414	2.570	23,775
						Brain	8,836	.607	2,887

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Average liver weight was 3.64% of body weight. The entire liver was used.

[3]Average brain weight was .389 gm. The entire brain was used.

Thirty-Day Mice

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	Sample Weight (gm)	Organ Weight (gm)	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
Control 1	16.1	0	0	0	0	Heart[1]	.0263	1.45	491	.034	17-372
						Liver[2]	.21	1.03	248	.017	12-264
						Brain[3]	.422	0.422	14	.001	2-37
Control 2	16.2	0	0	0	0	Heart	.0263	1.46	272	.019	10-205
						Liver	.26	0.77	150	.010	10-214
						Brain	.422	0.422	11	.001	1-28
1	18.8	.075	1.7	1.775	94	Heart	.0263	1.69	880	.060	635
						Liver	.21	1.06	2,145	.147	2,468
						Brain	.422	0.422	202	.014	583
2	18.0	.075	1.6	1.675	93	Heart	.0263	1.62	2,938	.202	2,087
						Liver	.18	1.01	6,182	.425	7,043
						Brain	.422	0.422	491	.034	1,337
3	16.0	.075	1.4	1.475	92	Heart	.0263	1.44	783	.054	551
						Liver	.33	0.92	6,578	.452	7,245
						Brain	.422	0.422	251	.017	602
4	16.4	.075	1.5	1.575	96	Heart	.0263	0.0263	1,677	.155	1,226
						Liver	.21	0.92	3,442	.236	4,048
						Brain	.422	0.422	214	.015	548

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]#C-1, C-2 and 3 are actual weights. #1, 2, and 4 are 5.62% of body weight.

[3]Average brain weight was .422 gm. The entire brain was used.

Sixty-Day Mice, Group 1

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	Sample Weight (gm)	Organ Weight (gm)	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue (x10^{-6})
Control 1	30.9	0	0	0	0	Heart[1]	.0263	2.78	307	.021	6-212
						Liver[2]	.33	1.80	76	.005	2-81
						Brain[3]	.474	.474	4	.0003	0-18
Control 2	33.8	0	0	0	0	Heart	.0263	3.04	0	0	0
						Liver	.33	1.96	24	.002	1-23
						Brain	.474	.474	0	0	0
1	30.7	.075	2.8	2.875	94	Heart	.0263	2.76	0	0	0
						Liver	.30	1.78	577	.040	640
						Brain	.474	.474	13	.001	53
2	30.3	.075	2.7	2.775	91	Heart	.0263	2.73	976	.067	681
						Liver	.35	1.76	1,667	.115	1,806
						Brain	.474	.474	56	.004	225
3	32.3	.075	2.9	2.975	92	Heart	.0263	2.91	409	.028	287
						Liver	.32	1.88	1,145	.079	1,245
						Brain	.474	.474	37	.003	158
4	32.5	.075	2.9	2.975	91	Heart	.0263	2.93	0	0	0
						Liver	.31	1.89	94	.006	101
						Brain	.474	.474	4	.0003	19

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Average liver weight was 5.81% of body weight. A weighed random sample of liver was used.

[3]Average brain weight was .474 gm. The entire brain was used.

Sixty-Day Mice, Group 2

Mouse Number	Weight of Mouse (gm)	^{14}C-HCP Adm (mg)	HCP Adm (mg)	Total HCP Adm (mg)	Dose of HCP (mg/kg)	Tissue	Sample Weight (gm)	Organ Weight (gm)	DPM per Total Organ	% of Dose of ^{14}C-HCP per Organ	Mg of HCP per gm of Tissue ($x10^{-6}$)
Control 3	28.6	0	0	0	0	Heart[1]	.0263	2.57	332	.023	7-231
						Liver[2]	.293	1.678	116	.008	4-124
						Brain[3]	.418	.418	0.4	.000	0-2
Control 4	27.9	0	0	0	0	Heart	.0263	2.51	115	.008	2-81
						Liver	.420	1.613	757	.052	24-838
						Brain	.395	.395	11	.0008	1-49
5	29.1	.075	2.6	2.675	92	Heart	.0263	2.62	2,052	.14	1,439
						Liver	.347	1.722	5,400	.37	5,763
						Brain	.461	.461	225	.015	895
6	29.9	.075	2.7	2.775	93	Heart	.0263	2.69	2,710	.19	1,921
						Liver	.576	1.578	8,400	.58	10,148
						Brain	.459	.459	354	.024	1,471
7	30.7	.075	2.8	2.875	94	Heart	.0263	2.76	2,099	.144	1,502
						Liver	.463	1.439	---	---	---
						Brain	.449	.449	566	.039	2,490
8	35	.075	3.2	3.275	94	Heart	.0263	3.15	1,521	.104	1,086
						Liver	.568	2.292	6,037	.415	5,926
						Brain	.478	.478	288	.020	1,356

[1]Blood weight of mouse is 9% of body weight (45). Blood specific gravity is 1.052 (48). Blood sample was 25 microliters.

[2]Actual weight.

[3]Actual weight.

VITA

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