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Liver Weight Response to Extended Chlordecone Exposure

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Chlordecone (Kepone) is a chlorinated hydrocarbon pesticide that has been shown to be both a neurotoxin and a reproductive toxin (Epstein 1978). Although it is no longer manufactured in the United States, since its use was banned in 1977, it is a useful agent to study, since much research has centered on this agent and since it possesses many of the characteristics of pesticides employed commercially today.

Most attention has been directed toward the effects of chlordecone on the reproductive and nervous systems, whereas only a comparatively few studies have reported its hepatotoxic effects. Hepatomegaly was reported in human males exposed to chlordecone (Lloyd 1975). Laboratory animal studies have reported enlarged livers in chlordecone-treated animals (Eroschenko and Wilson 1975). Chlordecone has been shown to impair biliary excretion function (Curtis and Mehendale 1979) and to inhibit liver mitochondrial ATPase (Desaiah et al. 1977). However, most of the liver studies have either been of short duration or have consisted of exposing the animals to chlordecone in the diet continuously throughout the duration of the experiment. This latter fact makes it difficult to determine the exact amount of pesticide ingested.

Of primary concern is whether a female, who is exposed to a chemical agent such as chlordecone in the workplace, not continuously, but for five consecutive days at a time for an extended period of time, would suffer ill effects. Swartz and Schutzmann (1986) have recently shown that mice exposed to chlordecone for either two or four weeks do exhibit an increase in liver weight and in the amount of Kepone incorporated into the liver, however, such effects are reversible once chlordecone exposure ceases. The purpose of this study is to determine the response of the liver to specific doses of chlordecone for five consecutive days/week for periods of up to ten weeks. An understanding of the liver response to this regimen of exposure is necessary because this timetable best reflects the occupational exposure that might occur.

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MATERIALS AND METHODS

Adult virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were used in this study. Mice were housed in animal quarters with a 14:10 light:dark cycle. Food and water were provided ad libitum. After a seven day period of acclimatization, mice were randomly distributed into three major treatment groups: a chlordecone-treated group, a group treated with estradiol-17B (E-17B) and a vehicle control group. The E-17B group also served as a control group. Chlordecone has been shown to exhibit estrogenic potencies (Gellert 1978; Eroschenko 1982; Uphouse et al. 1984). This group was employed to determine whether the effects elicited by chlordecone might be due to its estrogenic component.

Mice were exposed to these agents for either two, four, six, eight or ten weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This timetable was established to mimic an ordinary five day work week which would represent the maximum weekly exposure to which a female working with such a compound might be subjected.

Chlordecone (97% purity; Chem Service, West Chester, PA) was dissolved in sesame oil and administered in a 0.25 mg dose. E-17B (Sigma, St. Louis, MO) was administered in a dose of 0.1 mg dissolved in sesame oil. Control mice received the sesame oil vehicle only. All agents were administered by oral gavage in a 0.2 ml volume. There were a minimum of six animals in each treatment and control group at each time period. At the end of each of the time periods the animals were sacrificed, at which time the livers were removed and weighed. The livers were then immediately frozen until gas chromatographic analysis for chlordecone content was to be performed. The equipment and procedure used for gas chromatographic analysis were previously described (Swartz and Schutzmann 1986).

RESULTS AND DISCUSSION

Throughout all time periods examined in this study there were no significant differences between the weight gains of chlordecone-exposed animals when compared to E-17B and sesame oil animals at all time periods examined. This differs with the reports of Larson et al. (1979) and Curtis and Mehendale (1979) who reported weight loss in mice after treatment, but agrees with Cannon and Kimbrough (1979) who did not.

No deaths were reported in any of the chlordecone-treated groups. The total amount of chlordecone received by each of the groups of mice at two, four, six, eight and ten weeks were 2.5, 5.0, 7.5, 10.0 and 12.5 mg, respectively. Expressed on a mg/kg basis, the daily dosage of chlordecone was 8 mg/kg. Huang et al. (1980) gave daily doses of 50, 25 or 10 mg/kg of chlordecone and found 90% of the animals dead within 5, 9 or 24 days, respectively. Although the total amount of chlordecone administered to mice in

our study was much greater than that given by Huang et al. (1980), the reason that no deaths were recorded in this report is due to the fact that the daily exposure was much less and there was a two day hiatus from exposure each week which provided time for elimination of some of the pesticide from the system. In our study, those mice exposed to chlordecone for the six, eight and ten week periods did show some evidences of tremor, but it was not incapacitating. No data is presented for that group of mice exposed to E-17B for ten weeks due to the fact that all animals failed to survive because of an event totally unrelated to the experiment.

As reported previously (Swartz and Schutzmann 1986) the weight of the liver expressed as % of total body weight was significantly higher in chlordecone-exposed mice when compared to both E-17B and vehicle controls with as little as two weeks of treatment (Fig. 1). Livers from chlordecone-exposed animals increased linearly in % total body weight up through six weeks of exposure at which time they comprised 13.2 $\pm$ 0.3% (SEM) of the total body weight. Increased exposure times of eight and ten weeks did not result in a continued increase in liver weight but rather in a plateauing of this phenomenon. At eight and ten weeks these livers comprised 12.6 $\pm$ 0.4 and 12.7 $\pm$ 0.3 % of total body weight, respectively (Fig. 1). The weights of the livers of both E-17B and sesame oil controls differed little, not only throughout the time periods examined, but also little from each other at each of the times selected (Fig. 1). These values ranged between 5.3 $\pm$ 0.2 at two weeks and 6.3% at ten weeks for the sesame oil control and 5.6 $\pm$ 0.4 and 6.1 $\pm$ 0.1% at two and eight weeks, respectively, for the E-17B-treated mice.

Similarly, gas chromatographic analyses of the livers obtained from chlordecone-treated mice revealed a rapid incorporation of this pesticide into this organ. At two and four weeks there were 184 and 185 ppm of chlordecone, respectively, in the liver. Increasing the time of exposure past four weeks did not further increase the incorporation, rather there was a slight decrease to 167 ppm at six weeks, and 138 ppm at eight weeks and 146 ppm at ten weeks (Table 1). Both livers of E-17B-treated controls and the vehicle controls elicited merely background levels of chlordecone.

That the liver undergoes hypertrophy as a result of chlordecone exposure has previously been demonstrated. Huber (1965) found liver weights to double in 60-90 days when mice were fed a diet of chlordecone at 40 ppm. Liver enlargement has also been observed in chicks (Eroschenko and Wilson 1975) and in quail (McFarland and Lacy 1969). This hepatic enlargement was also associated with fatty infiltration.

From data obtained from both the assessment of liver weight and the gas chromatographic analyses it appears that by six weeks the livers of chlordecone-exposed mice attain their maximum size and can no longer incorporate additional amounts of the pesticide.

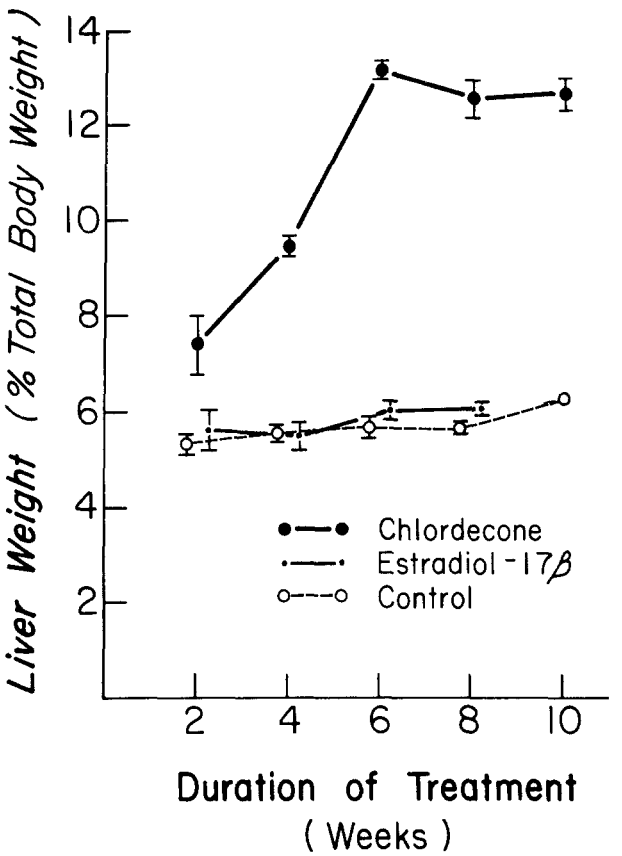

Figure 1. Graph depicting mean liver weights expressed as per cent body weight following either exposure to chlordecone, E-17B or sesame oil at all time periods examined. Values are means with the indicated SEM.

Table 1. Accumulation of chlordecone in mouse livers (ppm) after varying exposure times

Treatment	Duration of experiment (weeks) 2	4	6	8	10
Chlordecone	184.0	185.0	167.0	138.0	146.0
Estradiol-17B	0.09	0.18	0.01	0.09	____
Sesame oil (control)	0.32	0.02	0.68	0.14	0.20

This may occur as a result of hepatic dysfunction or it may a reflection of the liver adjusting to the toxic overload. Since the liver is positioned directly in the pathway of blood vessels which transport substances absorbed from the digestive tube, it is the first organ after the G-I tract to be exposed to toxic compounds administered by oral gavage. This organ possesses the ability to degrade these toxic compounds but it can be overloaded and subsequently damaged. Induced alterations in liver structure and/or function can adversely affect some or all of the activities attributed to this organ. These include protein synthesis and secretion, bile formation, metabolism of lipid-soluble drugs and steroids, lipoprotein synthesis and carbohydrate metabolism (Ross and Reith 1985).

On the other hand, the liver response observed here may be similar to the situation in which drug tolerance occurs. Administration of some drugs induces a marked rise in smooth endoplasmic reticulum (ER) which is accompanied by an increase in drug metabolizing enzymes within the smooth ER. Guzelian et al. (1980) reported an accumulation of smooth ER in chlordecone-exposed humans. This suggests that the hepatic microsomal drug metabolizing system (cytochrome P-450 dependent monooxgenase system) is induced. These changes represent an adaptive response of liver cells which results in increased efficiency in eliminating the inducing agent.

It has been shown in short term studies that the effects on the liver induced by chlordecone are reversible. Mehendale (1981) reported increases in liver weight of rats exposed to chlordecone for 35 days to return to control levels when treatment ceased. Swartz and Schutzmann (1986) observed that the tremendous increase in liver weight and incorporation of chlordecone by the liver following a four week exposure to this agent returned to normal levels three weeks following termination of exposure.

Whether the livers of animals exposed for periods of up to ten weeks in this study would return to normal size is not known. It would appear that since they were not experiencing severe weight loss and only manifested minimal tremors, that it would be possible. Another important factor in this study is the treatment protocol that provides for exposure to chlordecone for five consecutive days per week followed by two days of no exposure. It may be that the two day respite from exposure may allow the animal time to eliminate chlordecone from the body and thus dilute its toxic effects even over a period as long as ten weeks. This time framework would appear to be more apropos than constant daily exposure, since most workers are on this type of schedule. Thus, a person exposed for five days at a time may have a reduced risk to this agent than has been shown in studies employing continuous exposure.

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