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Reaction of the Mouse Liver to Kepone Exposure

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Kepone (decachlorooctahydro-1,3,4-metheno-2H-cyclobuto (cd) pentalen-2-one) is a chlorinated hydrocarbon insecticide that received notoriety in 1975 when male workers were exposed to this pesticide in a production plant in Hopewell, Virginia. Major symptoms observed were nervousness, anorexia, ataxia and tremors (Epstein 1978). Oligospermia, abnormal sperm morphology and decreased sperm motility were also found in some of these workers (Cannon et al. 1979).

The liver was also affected in that some of these workers presented with hepatomegaly (Lloyd, 1975). Experiments with laboratory rats fed a Kepone diet for three months revealed a significant increase in liver weight over the duration of the experiment (Cannon and Kimbrough, 1979). Since the liver is an organ important for such activities as protein synthesis, steroid metabolism and toxic compound degradation, it is necessary to ascertain the role of such a pesticide as Kepone in altering these activities. This study was designed to determine whether exposure to Kepone over a short period of time would induce an increase in liver size and/or the incorporation of Kepone and whether such factors might be reversible once Kepone exposure ceases. This data was then compared to a group receiving estradiol-17B for similar periods of time in order to determine whether the effects elicited by Kepone might be due to its estrogenic component.

MATERIALS AND METHODS

Virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were used in this study. Mice, aged 7-10 weeks, 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 Kepone-treated group, a group treated with estradiol-17B(E-17B) and a vehicle control group.

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Mice were exposed to these agents for either two or four weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This time table 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, either in its synthesis, manufacture, shipping, etc. might be subjected.

Kepone (Chem Service, West Chester, PA) was dissolved in sesame oil and administered in a 0.25 mg dose. Preliminary studies revealed that a 0.25 mg (8 mg/kg) daily dose of Kepone did not cause any deaths for the duration of the experiment whereas animals receiving a 0.5 mg daily dose failed to survive the four week exposure. Estradiol-17B (Sigma, St. Louis, MO.) was administered at a dose of 0.1 mg dissolved in sesame oil. Control mice received the sesame oil vehicle only. All compounds 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 the end of the two and four week periods the animals were sacrificed. At the time of sacrifice livers were removed from the mice and weighed. They were then immediately frozen until gas chromatographic analysis for Kepone content was to be performed. The electron capture gas chromatographic method of Hansen et al. (1977) was employed. Preparations for analysis of Kepone incorporation began by extracting 1 gm homogenized samples of liver with 5 ml acetonitrile. The material was then partitioned with 2.0% aqueous sodium sulfate and 1:1 diethyl ether-petroleum ether. The material was then cleaned up in a Chromaflex column containing 3 gm Florisil topped with 2.0 gm of anhydrous sodium sulfate. The column was eluted with 20 ml of 5% diethyl ether in hexane to remove PCB and pesticides. Kepone was eluted in a second elution of 40 ml of 1% methanol in benzene.

Two separate gas chromatographic columns were used, one packed with 3% SP2100 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA) and the other with 3% SE 30 on 80/100 mesh Gas Chrom Q. The temperature of the ovens were 190°C and 230°C, respectively. The temperature of the injection ports were 200°C and 250°C. The temperature of the ^{63}Ni detectors was 300°C. The carrier gas was 5% methane in argon at a flow rate of 30 ml/min.

Recoveries of Kepone from samples fortified at the 0.10 and 100 ppm levels prior to extraction were 75-85%. The minimum quantifiable level was 0.005 ppm.

RESULTS AND DISCUSSION

Throughout both the two and four week treatment groups there were no significant alterations in body weights among the Kepone, E-17B and control groups. After the four week exposure period to either Kepone or E-17B the mean weight change was 2.1 $\pm$ 0.2 gm. (SEM) and 1.9 $\pm$ 0.4 gm. compared to 2.2 $\pm$ 0.3 gm. for the controls. These

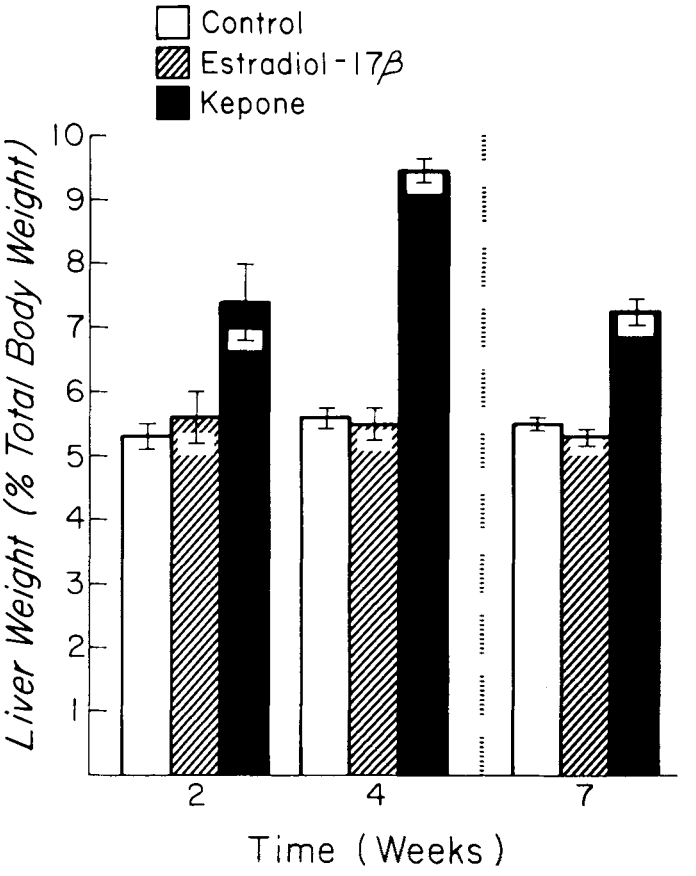

Figure 1. Histogram depicting the liver weight expressed as percent body weight for Kepone, estradiol-17B and sesame oil control mice exposed for either two or four weeks. The seven-week data presented at the far right of the histogram represents that for the groups of mice exposed to the above agents for four weeks and then maintained for three weeks with no further exposure (seven weeks total).

Table 1. Accumulation of Kepone in mouse livers (ppm) after exposure for two and four weeks and three weeks after treatment ceased

Treatment	Duration of experiment (weeks)		
	2	4	7
Kepone	184.0	185.0	70.0
Estradiol-17B	0.09	0.18	0.23
Sesame oil (control)	0.32	0.02	0.91

differences were not significant. Treated animals sacrificed three weeks after the cessation of treatment also did not differ significantly in body weight from controls.

When expressed as percent of body weight, the liver weights of Kepone-exposed mice were found to be significantly larger than those of E-17B treated animals and controls as early as the end of the second week of treatment (Fig. 1). Livers of Kepone-exposed animals comprised 7.4 ± 0.6% (SEM) of the total body weight compared to 5.6 ± 0.4% and 5.3 ± 0.2% for E-17B and control animals respectively (Fig. 1).

After four weeks of experimentation, the disparity in liver weights of Kepone-treated animals compared to the other groups is even more obvious. Livers of Kepone-exposed mice ranged from 2.2-2.9 gm comprising a mean percent of body weight of 9.5 ± 0.2%. The livers of E-17B treated mice comprised 5.5 ± 0.3% of total body weight and those of controls comprised 5.6 ± 0.1%. This was statistically significant at the $P < 0.05$ level (Fig. 1).

In order to assess whether the observed effects were reversible, groups of mice exposed to either the Kepone, E-17B or sesame oil for four weeks were removed from this regimen for three weeks. After this time, the animals were sacrificed, livers removed and the same parameters investigated as described above.

In those animals that were continued on the experiments for three weeks following cessation of treatment, the mean percent of body weight that the livers of Kepone-treated animals comprised decreased from the four week level to 7.3 ± 0.2%. E-17B and sesame oil control livers comprised 5.3 ± 0.1 and 5.5 ± 0.1% respectively of the total body weight. This is statistically different at the $P < 0.05$ level (Fig. 1).

Livers were assayed for Kepone content to verify the incorporation of this pesticide into the liver. After two weeks of Kepone exposure the amount of Kepone found in the liver was 184.0 ppm (Table 1) and this value varied very little after four weeks (185 ppm). With the cessation of exposure after four weeks followed by a three week period of no exposure, the amount of Kepone in the liver dropped to 70 ppm. At all time periods examined, both the E-17B and sesame oil treated animals possessed livers with background levels of Kepone (Table 1).

The data presented here indicate that exposure to 0.25 mg Kepone for as little as two weeks results in a significant increase in the size of the liver when expressed as percent of total body weight when compared to that of E-17B-treated and control animals. The total cumulative dosage that the two week animals received was 2.25 mg and 4.75 mg for the four week exposed animals. These cumulative dosages are quite high; however, there were no deaths during any of the experiments. During the fourth week of the experiment the Kepone-exposed animals did exhibit slight tremors and an increased reactivity to noises. By this

time the animals had received a much larger cumulative dosage than Uphouse et al. (1984) had administered in a single exposure. These investigators found tremors in mice as early as 24 hours following a single exposure to 50 mg/kg of Kepone. As would be expected, the lower multiple dosages of Kepone and the two consecutive days of freedom from exposure each week employed in this study, contributed to this delay in the external manifestation of toxicity.

There were no alterations in weight gain of Kepone-exposed animals during the duration of the study when compared to both E-17B treated animals and sesame oil controls. McFarland and Lacy (1969) reported no weight gain in mature female quail fed 200 ppm Kepone for 30 days; however, they did find a significant weight gain in immature female quail fed Kepone.

Huber (1965) found that liver weights doubled in 60-90 days when mice were fed a daily diet of Kepone at 40 ppm. In the present study there was a significant increase in liver weight as early as two weeks of exposure and the livers doubled their size within four weeks. Liver enlargement has not been observed in chicks fed Kepone (Sherman and Ross, 1961).

The cause of this Kepone-induced increase in liver weights remains unknown. Recent evidence indicates that Kepone inhibits ATPase activity, ATP production and energy metabolism in mitochondria in the rat liver and that such inhibition alters cell function and reduces ionic transport across cell membranes (Desaiah et al., 1977). It has also been reported that, following ingestion of Kepone by immature quail, the oviduct contained swollen epithelial cells which in turn contained swollen mitochondria with disrupted cristae (Eroschenko and Palmiter, 1980; Eroschenko, 1982). It may be that these abnormal increases in liver and oviduct size are the result of the inhibition of mitochondrial function which in turn reduces ionic balance within the cells and produces the abnormal cellular swellings (Eroschenko, 1982).

The accumulation of Kepone within the liver reached its maximum within two weeks and continued administration of this pesticide for another two weeks increased this incorporation minimally. However, when the Kepone exposure was halted for three weeks, the storage of this chemical in the liver was reduced by 60%. This rapid decrease in Kepone residues in the liver after cessation of exposure was similar to that reported by Huber (1965) using mice exposed to Kepone in the diet.

The fact that the E-17B treated mice failed to induce any alteration in the size of the liver following either two or four weeks of exposure, indicates that the observed increase in liver size induced by Kepone is apparently not due to its estrogenic component but rather to the toxic nature of this compound. The present data provide the first indication that the liver can be significantly affected with a daily exposure to Kepone for as little as two weeks. This pesticide is readily incorporated into

the liver and may be altering specific hepatic functions such as protein synthesis and steroid metabolism. Therefore, a person working in industry, where exposure to such an agent is a distinct possibility, may be subjecting himself to serious physical consequences. Whether there is a complete return to normal hepatic activities following cessation of Kepone exposure cannot be ascertained from this study.

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REFERENCES

Cannon SB, Veazey Jr JM, Jackson PS, Burse VW, Hayes C, Straub WE, Landrigan PJ, Liddle JA (1978) Epidemic kepone poisoning in chemical workers. Am J Epidemiol 707:529-536.

Cannon SB, Kimbrough RD (1979) Short-term chlordecone toxicity in rats including effects on reproduction, pathological organ changes, and their reversibility. Toxicol Appl Pharmacol 47:469-476.

Desaiah D, Ho IK, Mehendale HM (1977) Inhibition of mitochondrial Mg^{2+} ATPase activity in isolated perfused rat liver by Kepone. Biochem Pharmacol 26:1155-1159.

Epstein SS (1978) Kepone-hazard evaluation. Sci of Total Environ 9:1-62.

Froschenko VP (1982) Surface changes in oviduct, uterus and vaginal cells of neonatal mice after estradiol-17B and the insecticide chlordecone (kepone) treatment: a scanning electron microscope study. Biol Reprod 26:707-720.

Eroschenko VP, Palmiter RD (1980) Estrogenicity of kepone in birds and mammals. In: McLachlan JA (ed) Estrogens in the Environment. Elsevier/North Holland, New York, pp. 305-325.

Hansen DJ, Goodman LR, Wilson Jr AJ (1977) Kepone: chronic effects on embryo, fry, juvenile, and adult sheepshead minnows. Chesapeake Sci 18:227-232.

Huber JJ (1965) Some physiological effects of the insecticide Kepone in the laboratory mouse. Toxicol Appl Pharmacol 7:516-524.

Lloyd JW (1975) Contact with Allied Chemical Corporation representative concerning toxicity of kepone. Memorandum to Felsing WA, NIOSH, August 29.

McFarland LZ, Lacy PB (1969) Physiologic and endocrinologic effects of the insecticide kepone in the Japanese quail. Toxicol Appl Pharmacol 15:441-450.

Sherman M, Ross E (1961) Acute and subacute toxicity of insecticides to chicks. Toxicol Appl Pharmacol 3:521-533.

Uphouse L, Mason G, Hunter V (1984) Persistent vaginal estrus and serum hormones after chlordecone (kepone) treatment of adult female rats. Toxicol Appl Pharmacol 72:177-186.

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