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Residue Accumulation in Mice Chronically Fed Lindane (γ-HCH)

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Adult female swiss mice were treated with various doses of lindane (γ-Hexachlorocyclohexane). Residue analyses were performed by GLC from whole brain, fat, kidney, liver, adrenal, ovary and femoral muscle. There was a dose and time dependent increase of residue accumulation in the tissues in the order of abdominal fat > brain > kidney > femoral muscle > liver > adrenal > ovary. Despite minimum accumulation of lindane in the ovary, disturbed estrous cycle, reproductive failure and fetotoxicity were observed in the lindane-fed mice indicating adverse effects of this insecticide on reproductive success.

Key Words: Lindane, γ—HCH, Residue, Mice, Reproductive abnormalities, Estrous cycle

Introduction

Residue data from wild birds have been very useful to assess past exposure, lethality and susceptibility of a species to insecticides. Unfortunately, the knowledge of occurrence and effects of organochlorines in wild mammals is far less than that of birds. India is one of the major users of agricultural pesticides (Allen et al. 1984) and hexachlorocyclohexane compounds (HCH) constitute more than 50% of the total pesticide use (Anon 1984). Since the γ-isomer (lindane) is the most toxic ingredient of the HCH group, we studied the distribution of the residue of lindane in different tissues of mice (both virgin and pregnant) variously dosed with this insecticide.

Materials and Methods

Adult female Swiss mice 6-8 weeks old with average weight of 22g were used for this study. The animals were given standard pelleted food (Hindustan Lever, India) and tap water *ad libitum*. Lindane (99.8% pure, J J Chemicals, India) dissolved in olive oil was administered intragastrically, the LD_{50} of lindane in mice under study being 86 mg/kg body weight. The analyses of γ - HCH were performed from whole brain, ovary, adrenal gland, liver, kidney, abdominal fat and femoral muscle (anterior, medial and posterior femoral muscles). Such tissues from five mice were pooled for a single estimation.

The total intake of lindane remained either 43 mg (50% of LD_{50} in 2 weeks (Group II), 4 weeks (Group III) and 6 weeks (Group IV) period; or 86 mg (LD_{50}) in 4 weeks (Group V), or 129 mg/kg (150% of LD_{50}) in 6 weeks (Group VI) period as follows: *Group I*: Control, vehicle only; *Group II*: Lindane 3.07 mg/kg body weight daily for 2 weeks (total intake 43 mg/kg); *Group III*: Lindane 1.54 mg/kg body weight daily for 4 weeks (total intake 43 mg/kg); *Group IV*: Lindane 1.02 mg/kg body weight daily for 6 weeks (total intake 43 mg/kg); *Group V*: Lindane 3.07 mg/kg body weight daily for 4 weeks (total intake 86 mg/kg); *Group VI*: Lindane 3.07 mg/kg body weight daily for 6 weeks (total intake 129 mg/kg).

Residue Analysis

Tissues from five animals were pooled, weighed and homogenized with neutral sand and mixed throughly with acetonitrile and filtered through Whatman filter paper No. 4. This filtrate was then mixed thoroughly with n-hexane and saturated NaCl solution and the hexane layer was separated in a separating funnel. The aqueous acetonitrile layer was again washed with n-hexane twice and the hexane layer was again separated and collected. The hexane fractions were concentrated in rotatory vacuum evaporator and washed with chromatographic grade sulphuric acid (E.Merck, India) and then with distilled water. The neutral hexane layer was collected in a stoppered test tube and the amount of residue present was estimated by standard GLC technique (Pye Unicam, Model GCV, Cambridge, England) with an electron capture detector.

Results

There was no visible neurological symptoms in the lindane fed mice. The food intake was normal and the body weight remained unchanged.

The results of the residue analyses from various tissues of control and lindane fed mice are tabulated in Table-1. It is evident from the table that the tissues of control mice did not contain any trace of lindane residue, while residues of lindane accumulated in various tissues of mice fed lindane. There was also a progressive increase in the extent of accumulation of the residues in the tissues in a dose and time dependent manner. Interestingly, when the total intake of pesticide was kept constant, the accumulation increased with the increase in the duration of feeding the insecticide. For example, the accumulation of residues were in the order of Group IV < Group III < Group II (the total dose of the three groups being 43 mg/kg fed for 6 weeks, 4 weeks and 2 weeks respectively). Thus the degree of accumulation was more in 6 week period than in 2-or 4-week period. Also when lindane accumulation of the Groups II, V and VI are compared, it is found that the order of accumulation was Group II < Group V < Group VI (fed 3.07 mg/kg/day for 2-weeks, 4-weeks, and 6-weeks respectively). Here too, the amount of lindane accumulation increased with the increase in the dose and also with the duration of the insecticide exposure (table 1). Whatever the dose or duration of feeding, the concentration of lindane residue in the various tissues of the mice was always in the following order : Abdominal fat > brain > kidney > femoral muscle > liver > adrenal > ovary.

Discussion

The present study reveals that accumulation of lindane residue was highest in the abdominal fat and least in the ovary. The order of residue accumulation in various organs was as follows : abdominal fat > brain > kidney > femoral muscle > liver > adrenal > ovary. Comparable order of residue accumulation of γ-HCH was also reported by Srinivasan and Radhakrishnamurthy (1983) in lindane-fed albino rat. Because of the large total volume and percent fat content, it is not surprising that the adipose tissue accumulated by far the highest proportion of lindane residue. The degree of accumulation in various tissues increased proportionately with the dose and especially with the duration of treatment; nonetheless, the order of pesticide accumulation in the tissue remained the same in all experimen-

Table 1 *Lindane content (ppm) in various tissues of treated mice*

Tissues	Group I (Control)	Group II (3.07 mg/kg/day, 2 wks)	Group III (1.54 mg/kg/day, 4 wks)	Group IV (1.02 mg/kg/day, 6 wks)	Group V (3.07 mg/kg/day, 4 wks)	Group VI (3.07 mg/kg/day, 6 wks)
Fat	Nil	0.33	0.39	0.45	0.80	1.04
Brain	,,	0.23	0.30	0.34	0.56	0.63
Kidney	,,	0.15	0.19	0.26	0.37	0.48
Muscle	,,	0.10	0.16	0.20	0.24	0.32
Liver	,,	0.09	0.13	0.15	0.19	0.25
Adrenal	,,	0.01	0.02	0.05	0.15	0.19
Ovary	,,	0.008	0.009	0.02	0.10	0.12

Each value represents pooled samples from 5 mice.

tal groups. Under sublethal conditions of continuous exposure to an organochlorine insecticide, the concentrations of residues in different tissues are generally directly correlated with each other (Stickel 1973) Ordinarily the storage of residue is proportional to dose, but our results show that even when the same dose was given over varying periods (2, 4 and 6 weeks), the concentration of lindane increases with longer duration of intake, indicating that the dietary dosages of this organochlorine has still not reached an apparent equilibrium or plateau, which an insecticide generally attains during continuous exposure.

Though the most common site of insecticide attack is the nervous system, brain generally tends to accumulate lower levels of insecticide residues in the mammalian system (Matsumura, 1985). We however, found tha lindane accumulated in the brain of mice quite significantly, surpassing other organs save adipose tissue. Surprisingly in the domestic duck, we found that the residue build up of lindane was least in the brain (Mandal et al. 1988).

In the ovary, accumulation of lindane was least compared to other organs. Huber (1965) also noted that gonads in chronically kepone-fed mice accumulate less residues. Indeed, the level of residues of various organochlorines in the gonads does not appear to be particularly high on a whole organ basis (Matsumura 1976a), though this organ (especially the ovary) is quite vulnerable to insecticide toxicity (Sircar & Lahiri 1989, Chakraborty 1986).

Since there was no lethality in any group of lindane-fed mice, the accumulated residues were well tolerated. However, with least residue accumulation in ovary, we observed disturbed estrous cycle, reproductive failure and fetotoxicity in mice indicating adverse effects of this insecticide on reproduction (Sircar 1987, Sircar & Lahiri 1989). It is known, however, that reproduction is often affected at levels of insecticides which generally do not affect the wellbeing of the adults (Keplinger et al. 1968, 1970, Matsumura 1976b, Chakrabarti 1986, Sircar 1987, Sircar & Lahiri 1989).

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