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Placental Transport of Lindane during Early and Late Stages of Gestation in Rats

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Lindane (gamma-isomer of hexachlorocyclohexane, gamma-HCH), an organochlorine pesticide, is widely used as an agricultural pesticide especially in developing countries (Grey et al. 1983). Human exposure is likely because of its use in some pharmaceutical preparations and in public health for pest control purpose (Lange et al. 1981; Reuber 1979). It has been detected in human milk and fat samples in India and in many developed countries (Conway et al. 1985; Jani et al. 1988; Muthanna et al. 1985; Solly and Shanks 1974). The accumulation of lindane over a long period in fat samples and its presence in milk suggests that the human fetus may be exposed to lindane at some time during gestation from the maternal tissue stores. The present study was, therefore, undertaken to determine the placental transfer of lindane in rats during early and late stages of gestation.

MATERIALS AND METHODS

Albino female wistar rats (weighing 175-200 gm) from Industrial Toxicology Research Centre animal house colony were mated with healthy male rats. The sperm positive day of the vaginal smear was taken as the day of conception (0 day of pregnancy). After mating, rats were divided into four groups and the each group was further subdivided into two groups. The experimental rats of group 1 and 2 were administered daily an acute oral dose (2.5 and 7.5 mg/kg, dissolved in groundnut oil) of lindane (99.5% pure, ICI, U.K.) on days 6-12 or 12-16 of gestation. The rats of group 3 and 4 were given a single cumulative treatment of lindane, on day 12 (17.5 and 52.5 mg/kg) and 16 (12.5 and 37.5 mg/kg), equivalent to 7 and 5 times the daily dose given during 6-12 or 12-16 days of gestation to group 1 and 2 rats. The control animals of each group received the same

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amount of oil. The body weights on the first day of treatment were used for calculating doses and the animals sacrificed 24 hr after the last treatment.

Samples of maternal tissues were weighed and frozen until assayed. The day 17 rat fetuses were dissected out and the liver, brain and remaining carcass analysed separately. The rat fetuses of gestational day 13 were analyzed whole. Tissues from non-treated pregnant rats were assayed for lindane. Since the pesticide was not detected, the data from these animals have not been included in the tables.

The analysis of lindane was carried out by the modified procedure of Litterst and Miller (1975). The tissues were homogenized in two volumes of 25% methyl alcohol-water and the compound was extracted successfully with 10, 5, 5 and 5 ml of hexane. The total hexane extract was cleaned up on a florisil column. Solvent extracts were concentrated, dried with anhydrous Na_2SO_4 and transferred to 5 ml volumetric flasks with hexane and made to volume.

Analysis was carried out by using a Varian Aerograph Series 2400 GC equipped with an electron capture detector (^{63}Ni). A glass column (6 x ¼") packed with 1.5% OV-17 + 1.95% QF 1 on 100-120 mesh chromosorb GH/P was used. The carrier gas was N_2 at 60 ml/min. The injector, detector and column temperature were maintained at 210°C, 210°C and 190°C, respectively. The chart speed was 0.5 cm/min and attenuation was fixed at 2×10^{-9}.

The recorder response of the compound was a single sharp peak and the height was found to be proportional to the concentration. The identification of the peak of the compound in tissues was based on its retention time and the absence of the peak in samples of control animals. For all concentrations, the recovery of lindane from tissues was 87.5% ± 6.5 (S.E.).

RESULTS AND DISCUSSION

The rats were studied at two dose levels at both middle and late gestation. The mid gestational rats were sacrificed on day 13 after seven treatments and the late gestational rats were sacrificed on day 17 after five treatments. The data on the effects of lindane on the body weights and tissues of rats were presented in Table 1. There was no significant difference in the maternal body and organ weights of the treated rats at either dose level or gestational period compared to the values of the non-treated rats, whose data have not been included in the Table.

Table 1. Body[a] and organ[b] weights of pregnant rats after daily oral administration of lindane

	Treated gestational days 6-12		Treated gestational days 12-16	
Dose (mg/kg)	2.5	7.5	2.5	7.5
Body (g)	170±08	165±10	176±08	172±06
Liver (g)	7.4±0.5	6.5±0.3	7.7±0.6	7.5±0.8
Thymus (mg)	363±15	348±18	419±24	404±20
Lung (mg)	984±38	1045±26	1013±17	1084±26
Fetus (mg)	81±03	82±07	84±11	88±14
Placenta (mg)	148±06	150±11	165±18	160±09

a: maternal weight at sacrifice
b: mean of 6 rats ± S.E.

Lindane was detected in blood at all gestational days and at all dose levels and the accumulation of lindane was dose dependent. Those groups receiving 5 administrations or a single treatment of lindane at late gestation had the highest blood levels (Table 2 and 3).

Table 2. Concentration[a] of lindane (ng/g or ng/ml) in maternal tissues of rats administered lindane at different period of gestation

	Treated gestational days 6-12		Treated gestational days 12-16	
Dose (mg/kg)	2.5	7.5	2.5	7.5
No. of rats	6	6	6	6
Liver	212±05	815±11	232±07	885±13
Thymus	1291±17	2073±21	1382±13	2145±15
Fat	7041±27	20824±106	7878±30*	25139±156*
Lung	98±04	322±07	140±06*	463±05*
Fetus	175±03	334±06	378±15**	617±11*
Placenta	344±14	770±10	682±18*	993±13*
Amniotic fluid[b]	152±03	531±07	222±06	546±09
Ovary	2471±17	3209±15	4847±38**	6847±21**
Blood	81±02	386±10	87±03	446±08
Column	A	B	C	D

a: Results are mean ± SE in wet tissues; b: 200 ul assayed for each rat; statistically significant at *p 0.01; **p 0.001 level when compared the values of column C to column A and column D to column B.

There was a dose related deposition of lindane in the liver and thymus at both stages of gestation. The concentration of lindane receiving 5 administration did not differ greatly from those receiving 7, suggesting that the liver and thymus was not a major depot for lindane (Table 2). The concentration of lindane in the lung was generally low in all groups and deposition was dose dependent (Table 2).

Table 3. Concentration[a] of lindane (ng/g or ng/ml) in maternal tissues of rats administered single cumulative dose equivalent to total dose of lindane given during 6-12 or 12-16 days of gestation.

	On day 13		On day 17	
	Lindane treatment on day 12 of gestation		Lindane treatment on day 16 of gestation	
Dose (mg/Kg)	17.5	52.5	12.5	37.5
No. of rats	6	6	6	6
Liver	144±3.5	652±10	154±1.7	696±8.9
Thymus	995±17.5	1510±19	1192±21*	1640±34
Fat	4984±34	10772±74	5856±47*	15139±89*
Lung	78±2.6	287±13	101±10	358±15
Fetus	88±04	225±12	305±10**	440±13
Placenta	312±07	623±23	353±11	684±30
Amniotic fluid[b]	96±03	403±14	154±06	436±17
Ovary	1821±29	2876±54	3490±40*	4809±36*
Blood	47±02	190±07	61±04	338±11
Column	A	B	C	D

a: Results are mean ± S.E. in wet tissues; b: 200 ul assayed for each rat. Statistically significant at *p 0.01; **p 0.001 level when compared the values of column C to column A and column D to column B.

There was proportionately more lindane in the fat of the rats in late gestation compared to those in mid gestation (Table 2). This was also seen in the rats receiving a single treatment on day 12 or 16 of gestation (Table 3). This might be possible either due to the change in the kinetics of lindane deposition in the fat or the maternal fat is mobilized towards the end of gestation creating a significantly higher concentration of lindane in the fat.

The fetuses receiving the higher dose of lindane had a

significantly greater deposition of lindane than those receiving the lower dose. This was noted at both stages of gestation (Table 2 & 3). The fetuses at the later stage of gestation (5 treatments) had a significantly greater concentration of lindane than those at comparable dose levels at mid gestation (7 treatments). A late gestation fetus has more fat than an earlier gestation fetus which might facilitate a build up of lindane. The lindane content of the fetuses of the single treatment (equivalent to total dose of lindane giving during 6-12 days of gestation) at day 12 was dose related (Table 3). As the dose increased three fold from 17.5 to 52.5 mg/kg, the content in the fetuses increased two to three fold. The day 17 fetuses of the single treatment (equivalent to total dose of lindane giving during 12-16 days of gestation) rats receiving comparable low dose of lindane had significantly greater lindane concentrations than the day 12 fetuses. Thus the lindane concentration in the fetus is related to dose levels, total amount received and stage of gestation.

There was a significant increase in the deposition of lindane in the placentas at the higher dose level compared to the lower dose level at both stages of gestation. The deposition of lindane in the placentas was greater at the later stage of gestation than the earlier stage even though these rats received two less

Table 4. Lindane content[a] of day 17 fetal tissues of rats treated on day 16 or days 12-16 of gestation

	Gestation days		Dose	Lindane (ng/g) of the fetus		
	Treated	Killed	(mg/kg)	Brain	Liver	Carcass
Row 1	12-16	17	2.5	67±2.5	278±4.2	183±6.7
2	12-16	17	7.5	165±5.1	361±10.0	225±7.6
3	16	17	12.5	103±3.9*	353±5.9*	249±10.2*
4	16	17	37.5	154±6.8	542±12.2*	374±12.5*
Column (comparison by row within column)				A	B	C

a: Results are mean ± S.E. in wet tissue
Statistically significant at *p 0.001 level when compared the values of row 3 to row 1 and row 4 to row 2.

treatment. In all groups, the concentration of lindane was greater in almost all the placentas compared to

their respective fetuses.

The day 17 rat fetuses were dissected in order to assay the fetal liver, brain and carcass. These results are presented in Table 4. At each dose level, the liver had the greatest concentration followed by the carcass and the brain. The lindane concentration of the fetal carcass of the single treatment rats was 70.5 and 69% of that of the fetal liver for the 12.5 and 37.5 mg/kg groups respectively. For the multiple treatment rats the lindane concentration of carcass was 65.8 and 62.3% of the liver concentration for the 2.5 and 7.5 mg/kg groups respectively. Villeneure and Hierlihy (1975) also reported the similar pattern for the distribution of hexachlorobenzene in fetal rats.

In general, the lindane from maternal depots readily crosses the placentas and is deposited in fetuses during gestation. The presence of lindane residues in breast milk also suggested the risk of fetal exposure (Conway et al. 1985; Jani et al. 1988; Muthanna et al. 1985). Curley et al. (1969) have also reported the presence of lindane in organs of still born and blood of new born babies and the concentration falls largely within the same range as that for general adult population. It is also possible that in addition to direct exposure to pesticide during pregnancy and lactation, residues accumulated in body fat due to previous exposure may get mobilized enhancing fetotoxicity. Hence more studies are needed to determine the magnitude of fetal accumulation, pattern of fetal deposition and the kinetics of placental transport for the extrapolation of this type of data from experimental animals to human beings.

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