Liver and Brain Nucleic Acids and Body Composition of Growing Rats Fed Dieldrin[1] (36654)

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While most chlorinated hydrocarbon insecticides (Cl–HCI) are neurotoxic at high dose levels (1), these compounds at low dose levels also may influence adversely other physiological processes in a manner unrelated to their primary pharmacological effects (2). Compounds that may interfere with normal organ (liver, brain and muscle) development in mammals are potential health hazards (3). The adverse effects of malnutrition on organ development in young growing mammals have been well documented (4, 5), however, it is not known whether low levels of Cl–HCI in the diet may modify organ development in young growing mammals.

To evaluate if Cl–HCI interfere with organ development, diets containing 1 or 5 ppm dieldrin (HEOD)[2] were fed to weanling male rats. Organ development was monitored (4) by DNA and RNA content of an early developing tissue (brain) and a later maturing tissue (liver). Since Cl–HCI primarily are deposited in body lipids (6) feeding of such compounds may promote lipid deposition. Therefore, total carcass fat was also determined.

Material and Methods. Twenty-one-day-old weanling, male rats (Sprague-Dawley) weighing 45–50 g were fed three rations (20 rats/ration) composed of 20% casein, 35% glucose monohydrate, 33% corn starch, 4% salt mix, 5% corn oil, 1% vitamin mix, 2% nonnutritive fiber and containing either 0, 1 or 5 ppm HEOD and water *ad libitum* for 2 or 4 weeks.

At the outset five weanling rats were sacrificed (by overdose of ether) and livers and brains were removed, blotted, weighed, frozen and stored at —70°. Similarly, five animals from each group were sacrificed after 2 and 4 weeks of the feeding period and tissues were collected as above. Separate groups of rats (5/treatment) were sacrificed for analysis of body composition. After rats had been killed by an overdose of ether, the gastrointestinal tracts were removed, flushed clean with distilled water and then replaced into the carcass. The carcasses were then frozen and stored at —70°.

Liver and brain DNA and RNA levels were determined by a modified Schmidt and Thannhauser (7) procedure as outlined by Munro and Fleck (8). Liver protein content was determined by difference between the nitrogen concentration in the whole liver homogenate and in the supernatant after sulfosalicylic acid (5%) precipitation of liver proteins. Body composition of whole carcass homogenates was determined as outlined by Mickelsen and Anderson (9). Analysis of variance was used to determine significance and Duncan's multiple range test was then used to determine differences among the means (10).

Results and Discussion. Growth rates of the rats were not affected by 1 or 5 ppm HEOD in the diet. The average weight gains in 4 weeks were 180 ± 4, 200 ± 2 and 195 ± 7 g/rat for 0, 1 and 5 ppm HEOD, respectively. The rats exhibited normal behavior throughout the feeding period and no mortality occurred. Further, neither liver weight (g/100 g body wt) nor brain weight was affected by the treatments.

Consumption of 1 and 5 ppm HEOD did

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[2] (HEOD) 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene.

TABLE I. Carcass Protein, Fat and Dry Matter Content of Growing Rats Fed HEOD.[a]

HEOD (ppm)	Interval after weaning (weeks)	(g/100 g dry carcass)		Dry matter (g/100 g wet carcass)
		Protein[b]	Ether extract	
0	2	57.1 ± 1.2	30.7 ± 1.3	30.8 ± 0.8
1	2	56.9 ± 1.0	31.1 ± 0.6	31.3 ± 0.6
5	2	56.5 ± 1.1	31.8 ± 1.8	31.8 ± 0.5
0	4	56.2 ± 1.2	32.6 ± 1.3	31.0 ± 1.2
1	4	54.7 ± 1.9	36.2 ± 1.4	31.3 ± 0.9
5	4	56.1 ± 3.1	33.5 ± 1.3	31.4 ± 0.9

[a] Mean ± SE for five rats.
[b] $N \times 6.25$.

not influence total carcass fat in young growing rats (Table I). The concentration of HEOD used may not have been sufficient to produce an effect of HEOD on body lipid content, however, concentrations of HEOD were chosen to represent a possible range of contamination in food supplies. Further, a longer feeding period may show different results.

Liver RNA concentration was significantly lower ($p < .01$) in weanling rats than all other groups (Table II). After 2 weeks, liver RNA concentration was significantly higher ($p < .01$) in rats fed 5 ppm HEOD than those receiving the 1 ppm HEOD or control ration, while after 4 weeks there were no differences in liver RNA concentration between the control and treatment groups. DNA concentration of livers in rats fed 5 ppm HEOD for 2 weeks was significantly ($p < .05$) higher than DNA levels observed for all the other treatments (Table II). Although there were differences in RNA and DNA concentrations between treatments, differences in liver RNA/DNA ratios and protein concentrations were not observed between the treatments.

Since the concentration of protein in the liver was relatively constant throughout the trial, variations in the liver protein/RNA ratios were a reflection of changes in liver RNA. Compared to weanling rats, at 2 weeks postweaning, protein/RNA ratios were significantly lower ($p < .01$) for all three treatment groups, but the control group protein/RNA ratio was significantly higher ($p <$

TABLE II. Liver RNA, DNA and Protein Content and RNA/DNA, Protein/RNA and Protein/DNA Ratios in Rats Fed HEOD.[a]

HEOD (ppm)	Interval after weaning (weeks)	(mg/g wet tissue)			RNA/DNA	Protein/RNA	Protein/DNA
		RNA	DNA	Protein[t]			
0	0	9.5 ± 0.9[b]	2.0 ± 0.2[g]	156.0 ± 21.1	4.8 ± 0.5	16.5 ± 1.3[e]	78.2 ± 3.6
0	2	12.5 ± 1.5[c]	2.5 ± 0.8[h]	159.7 ± 11.6	5.3 ± 1.3	12.9 ± 1.6[bch]	68.4 ± 19.7
1	2	13.0 ± 1.3[c]	2.9 ± 0.8[g]	157.2 ± 8.0	4.8 ± 2.0	12.2 ± 0.8[bg]	58.4 ± 22.6
5	2	14.2 ± 1.3[d]	3.5 ± 0.6[h]	168.3 ± 20.5	4.2 ± 0.6	12.0 ± 2.1[bg]	49.0 ± 6.5
0	4	11.7 ± 1.8[c]	2.6 ± 0.6[g]	154.5 ± 6.8	4.8 ± 1.2	13.1 ± 0.4[e]	63.0 ± 16.9
1	4	11.6 ± 0.6[c]	2.5 ± 0.7[g]	175.2 ± 3.2	4.8 ± 1.0	15.1 ± 1.0[d]	74.0 ± 20.5
5	4	11.2 ± 1.1[c]	2.3 ± 0.6[g]	169.0 ± 7.0	5.1 ± 1.1	15.3 ± 1.7[d]	78.2 ± 10.4

[a] Mean ± SD for five rats.
[bcdef] Values not sharing common superscript were significantly different at ($p < .01$).
[gh] Values not sharing common superscript were significantly different at ($p < .05$).
[t] $N \times 6.25$.

.05) than those observed for the 1 and 5 ppm HEOD groups at 2 weeks postweaning. By 4 weeks postweaning the protein/RNA ratios increased nearly to values observed for the weanlings. The liver protein/DNA ratios followed the general trend exhibited by the protein/RNA ratios.

Since total liver size and protein concentration was not affected by HEOD in young growing rats, the increases in RNA and DNA levels, especially after 2 weeks of HEOD consumption suggest that the liver was actively producing protein synthetic machinery possibly associated with drug metabolizing enzymes (11). In experimental systems based on changes in protein intake or nutritional quality, an increase or decrease in liver RNA concentration results in concomitant parallel changes in liver protein (12). Further, an increase in DNA signals an increase in cell numbers and an increase in RNA signals an increase in cytoplasmic mass (4). Since the liver contains polyploid nuclei, increases in DNA are not always directly related to increase in cell numbers (4), but cell mass usually is proportional to DNA in hepatocytes (8). Thus the observed changes in nucleic acid levels are probably related to the induction of drug metabolizing enzymes and since these enzymes are not likely to contribute much to the overall liver protein content, the induction signal from HEOD resulted in an overshoot response in RNA and DNA synthesis (11).

RNA and DNA concentrations in the brain of young growing rats were either similar to or higher than the concentrations found in the weanlings after feeding HEOD for 2 and 4 weeks (Table III). While at 2 weeks, the rats fed 5 ppm HEOD exhibited significantly higher ($p < .01$) RNA concentrations than the controls and the rats fed 1 ppm HEOD, consumption of 1 and 5 ppm HEOD resulted in significantly higher ($p < .01$) RNA and DNA concentrations compared to the control group at 4 weeks. RNA/DNA ratios were not affected by the various experimental treatments.

Liver and brain nucleic acid responses to feeding 1 and 5 ppm HEOD do not suggest an impairment of organ development. Impairment of organ development is usually associated with a marked decline in the nucleic acid content of tissues (5) while higher nucleic acid levels are generally associated with improvements in protein nutriture or tissue function (12). Since the transient overshoot of RNA and DNA synthesis in the liver apparently was associated with the induction of drug degrading enzymes (11), it can be concluded that HEOD under these experimental conditions did not induce any detrimental effects on nucleic acid metabolism and the protein synthesis system in the liver. The increases in RNA and DNA concentrations in brain are more difficult to explain, however, drugs apparently do not induce metabolizing enzymes in the brain (13). Whether increased brain nucleic acid levels can be deemed advantageous is not certain,

TABLE III. Brain RNA and DNA in Rats Fed HEOD.[a]

HEOD (ppm)	Interval after weaning (weeks)	(mg/g wet tissue) RNA	DNA	RNA/DNA
0	0	3.4 ± 0.3[b]	1.2 ± 0.1	3.0 ± 0.3
0	2	3.8 ± 0.3[c]	1.8[f]	—
1	2	3.5 ± 0.2[b]	1.4 ± 0.3[b]	2.6 ± 0.5
5	2	4.1 ± 0.3[d]	1.2 ± 0.4[b]	3.8 ± 1.6
0	4	3.6 ± 0.3[bc]	1.2 ± 0.5[b]	3.7 ± 2.2
1	4	4.4 ± 0.3[e]	1.9 ± 0.3[c]	2.3 ± 0.4
5	4	4.2 ± 0.2[d]	1.9 ± 0.3[c]	2.2 ± 0.3

[a] Mean ± SD for five rats.

[bcde] Values not sharing common superscript were significantly different ($p < .01$).

[f] Single value.

however, a recent report indicated a facilitation of central excitatory processes in rats orally dosed with low amounts of DDT (14).

Summary. Liver and brain nucleic acid concentrations and carcass composition were studied in young growing rats fed 0, 1 and 5 ppm Dieldrin (HEOD). HEOD did not change body fat and protein concentrations after a 2 or 4 week feeding period. Growth rates, and liver and brain weights were not affected by the consumption of 1 and 5 ppm HEOD. Liver RNA and DNA concentrations were elevated in rats fed 1 and 5 ppm HEOD after a 2 week feeding period, but declined nearly to levels noted for weanling rats after a 4 week feeding peroid. The consumption of HEOD elevated brain RNA levels after 2 and 4 week feeding periods, but DNA was elevated only after a 4 week feeding period. Since interference in organ development results in a decline in tissue nucleic acid concentrations, these results indicate that 1 and 5 ppm HEOD do not affect overall liver and brain development in young growing rats.

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