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Liver and Brain Nucleic Acids and Brain RNA Synthesis in Suckling Rats Fed Dieldrin[1] (38082)

WERNER G. BERGEN,[2] DORICE M. CZAJKA-NARINS,[3] ELAINE L. FINK,[2] AND ELIZABETH S. RIMPAU[2]

Pesticide Research Laboratory, Department of Animal Husbandry and Department of Human Development, Michigan State University, East Lansing, Michigan 48824

Chlorinated hydrocarbon insecticides are generally neurotoxic at high dose levels (1). At low dose levels, over a prolonged exposure period, chlorinated hydrocarbon insecticides also may adversely influence other physiological processes in a manner unrelated to their primary pharmacological effects (2). Compounds which may interfere with normal organ (liver, brain, and muscle, etc.) development are potential hazards to health and well being (3). Previous work from this laboratory (4) has shown that dieldrin (HEOD)[4] when fed to weanling rats at 1 and 5 ppm for a 2 or 4 wk period did not affect body weight gain and did not alter the normal pattern of brain and liver development (based on tissue RNA and DNA analysis).

Since the suckling rat has been shown to be more sensitive than either the weanling or adult rat to many toxins it was decided to extend the studies with dieldrin to the suckling rat.

The work reported here was initiated to study the effect of oral intake of dieldrin in suckling rats (age 1–16 days) on brain and liver nucleic acid levels, brain RNA synthesis and liver aminopyrine demethylase activity.

Materials and Methods. Pregnant rats (Sprague–Dawley) were placed in plastic maternity cages 1–3 days before parturition. After birth, the litter size was adjusted to 12 and the pups were allowed to nurse the dams. The lactating females were maintained on an agar gel diet containing (in grams) casein (19.3); glucose monohydrate, (33.8), cornstarch, (31.5); salt mix, (4); corn oil, (5); vitamin mix, (1); non-nutritive fiber, (2) and agar, (3.4). The nursing pups were fed a dried skim milk–corn oil preparation containing HEOD once daily by oral gavage. Based on the known intake of nursing pups (5), preparations containing appropriate amounts of the HEOD were fed by gavage to equal a daily intake of 0, 1, 5, 10, and 50 ppm HEOD.

Pups were sacrificed at birth, and 8 and 15 or 16 days after birth. For nucleic acid analysis, liver and brains were rapidly removed from the pups, weighed, frozen, and stored at −70°C. DNA and RNA concentrations were determined by a modified Schmidt and Thannhauser (6) procedure as outlined by Munro and Fleck (7). Protein was determined with a Folin–phenol procedure (8).

For the aminopyrine demethylase assay, livers were removed from the pups and immediately homogenized with a Potter-Elvejhem homogenizer in 4 vol of a 0.25 *M* mannitol, 0.07 *M* sucrose, 0.001 *M* EDTA, 0.01 *M* Tris, (pH 7.4) buffer with one slow stroke. The homogenates were centrifuged at 10,000*g* for 10 min, the supernatants removed and used as enzyme source for the aminopyrine demethylase assay as described by Cook and Wilson (9). The enzyme re-

[1] Michigan Agricultural Experiment Station Journal Article No. 6559.

[2] Department of Animal Husbandry.

[3] Department of Human Development.

[4] (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.

action was terminated with 10% trichloroacetic acid (TCA) after 30 min. The precipitate was removed by centrifugation and formaldehyde concentration was assayed in the supernatant according to Nash (10). Protein in the liver homogenates was determined with a Folin–phenol procedure (8). The specific enzyme activity was expressed as ng formaldehyde formed/mg protein/30 min.

To study RNA synthesis, brains were rapidly removed from the pups, blotted, and weighed. Thin slices (approx 0.3 mm) were cut by hand from the whole brain. The slices (approx 200 mg) were then incubated in 5 ml Krebs–Ringer bicarbonate buffer (pH 7.4) containing [2-^{14}C]uridine (52 mCi/mmole) under O_2/CO_2 (95/5) at 37°C for 60 min similar to the procedure outlined by Guroff *et al.* (11). The incubation was terminated with the addition of an equal volume of ice cold 20% TCA, and the slices were then homogenized. The precipitates were isolated by centrifugation and washed three times with 5 ml 10% TCA. The pellets were washed with acetone, ether, dried, and finally solubilized with 1 *N* NaOH. Radioactivity was determined with a liquid scintillation spectrometer. RNA synthesis in the brain sliced was expressed as counts per minute [2-^{14}C]uridine incorporated/100 mg fresh brain slice/hr.

Analysis of variance was used to determine significance and Duncan's multiple range test was then used (as needed) to determine differences among means (12).

Results and Discussion. Growth rates of the suckling rats were not affected by the daily intake of 1, 5, 10, 50 ppm HEOD in their diet. The pups exhibited normal behavior throughout the experimental period. Occasional losses of pups were related to handling and the mothering ability of the dams. Further, neither liver weight nor brain weight (g/100 g body wt) was affected by the HEOD treatments.

Intake of the various concentrations of HEOD did not affect liver aminopyrine demethylase activity (within age group) in the pups at either 8 (overall mean for all HEOD levels, 76.8 ± 10.0 ng HCHO formed/mg protein/30 min) or 15 (overall mean for all HEOD levels, 163.2 ± 19.2 ng HCHO formed/mg protein/30 min) days of age. The potential for induction of microsomal detoxifying enzymes is low in young rats; however, the activity of these microsomal enzymes increases markedly with age (13). An increase in liver aminopyrine demethylase was seen in this study between pups of 8 and 15 days of age. Liver aminopyrine demethylase activity in four 200 g male rats not exposed to any pesticide or other inducing agents ranged from 400 to 600 ng formaldehyde formed/mg protein/30 min.

Brain RNA synthesis ([2-^{14}C]uridine incorporation into brain slices) was studied in 8 and 15 day old pups. The level of HEOD administration did not influence brain RNA synthesis. The overall mean [2-^{14}C]uridine incorporation at all levels of HEOD was 1122.4 ± 215.0 cpm/100 mg fresh brain/hr at 8 days of age and 3871.2 cpm/100 mg/fresh brain/hr at 15 days of age. The specific activity of the uridine in the incubation system was approximately two-fold higher in the experiment with the 15 day old rats which accounts for the higher incorporation of radioactivity.

RNA, DNA, and protein concentration in the brains of suckling rats fed HEOD at 8 or 16 days of age were either similar to or higher than the concentrations noted in the unexposed (control) rats (Table I). Although there were some significant changes in RNA and DNA content, these changes cannot be easily related to any physiologically important developmental phenomena.

RNA, DNA, and protein concentrations in the livers of suckling rats fed HEOD at 8 or 16 days of age are presented in Table II. At 8 and 16 days of age, the various treatment groups had either similar to or higher levels of RNA, DNA, and protein than the controls, except the 16 day old rats fed 50 ppm HEOD. These rats had significantly lower ($p < .01$) RNA and DNA levels.

Liver and brain nucleic acid responses in suckling rats to feeding of 1, 5, 10, and 50 ppm HEOD do not suggest an impairment of organ development. Impairments of

TABLE I. Brain RNA, DNA, and Protein Content in Suckling Rats Fed HEOD.

HEOD (ppm)	Age (days)	No. of rats	mg/g wet tissue		
			DNA	RNA	Protein
0	8	6	2.36	4.21[a]	107.0
1	8	6	2.34	5.01[b]	114.9
5	8	6	2.43	5.06[b]	103.8
10	8	6	2.58	4.26[a]	122.9
50	8	6	2.16	4.33[a]	120.8
SEM			0.09	0.20	6.4
0	16	6	2.14[a]	4.32[a]	157.0[a,b]
1	16	6	2.90[c]	4.67[b]	137.4[a]
5	16	6	2.61[b,c]	4.54[a,b]	152.6[a]
10	16	6	2.52[b]	4.54[a,b]	183.3[b]
50	16	6	2.78[c]	4.40[a,b]	218.2[c]
SEM			0.07	0.10	9.3

[a,b,c] Values (within column) not sharing common superscript were significantly different at ($p < 0.1$).

organ development are usually associated with a marked decline in nucleic acid content of tissues (14) while elevated nucleic acid levels are generally associated with improvements in protein nutrition or tissue function (15).

This study and previous work (4) from our laboratory has been conducted to systematically relate the oral, chronic consumption of HEOD on brain and liver development in suckling and young growing rats. All studies showed that there was no impairment of brain and liver development. Furthermore, the results suggest that brain RNA synthesis is also not impaired by 1–50 ppm HEOD during one critical period of brain growth. Myhr (16) and Litterst *et al.* (17) have screened the effect of a number of chlorinated hydrocarbon insecticides on protein and RNA synthesis in HeLa cells. They found that HEOD did not influence RNA and protein synthesis in HeLa cells whereas

TABLE II. Liver RNA, DNA, and Protein Content in Suckling Rats Fed HEOD.

HEOD (ppm)	Age (days)	No. of rats	mg/g wet tissue		
			DNA	RNA	Protein
0	8	6	4.02	13.40[b]	—[a]
1	8	6	4.10	14.53[b,c]	207.0
5	8	6	4.55	15.77[c,d]	218.4
10	8	6	3.78	16.08[d]	205.8
50	8	6	4.24	15.06[c,d]	251.3
SEM			0.17	0.48	11.7
0	16	6	3.80[c]	15.15[c]	257.3
1	16	6	4.48[d]	15.77[d]	275.2
5	16	6	4.11[c,d]	14.38[b]	229.7
10	16	6	4.32[d]	14.88[c]	233.8
50	16	6	2.96[b]	13.82[b]	242.5
SEM			0.13	0.17	10.8

[a] Not determined.

[b,c,d] Values (within column) not sharing common superscript were significantly different at ($p < .01$).

with other chlorinated hydrocarbon compounds marked depressions were noted. Overall, it would appear that HEOD is not toxic to growing rats over the dose ranges and time periods studied.

Other workers (18) have also shown little effect of HEOD on the activity of myelinated nerve fibers, and HEOD to 100 ppm in the diet had no effect on weight gain, survival, and organ development in weanling rats (19), but chlorinated hydrocarbons (including HEOD) have been reported to interfere with estrus as well as interfere with testosterone binding in the prostate gland of rats (20). Dietary HEOD concentrations in excess of 150–200 ppm caused decreased weight gain, lowered survival, and fatty infiltration in the liver (19). Harr *et al.* (21) however showed that dietary dieldrin concentrations in excess of 40 ppm were lethal to growing female rats. Further, at sublethal levels, endothelial degeneration and polioencephalomalacia were noted. This latter report disagrees sharply with our findings (this paper, 4) and other work (19, 22) on HEOD toxicity. The oral LD_{50} of HEOD in rats has been shown to be 63.5 mg/kg (22) and 100 g rats consuming 12 g of a 100 ppm HEOD diet would only consume 1.2 mg HEOD daily. This represents one-fifth of the published oral LD_{50} value. The results of Harr *et al.* (21) are puzzling and may be ascribed to some error in calculations during the HEOD additions to their rations. The source or purity of HEOD can also not be ascertained from the report by Harr *et al.* (21). It is possible that a contaminant(s) in technical or commercial grade dieldrin (possibly photoisomers) may be highly toxic. Improper mixing of HEOD into rations (HEOD should be dissolved into the dietary oil) may cause spurious results in toxicological studies.

Summary. The effect of dietary dieldrin (1–50 ppm) on nursing pups' (rats) liver aminopyrine demethylase, brain RNA synthesis and liver and brain RNA and DNA concentrations was investigated. The feeding of dieldrin did not influence liver aminopyrine demethylase activity within age groups. Dietary dieldrin did not influence brain RNA synthesis. Brain and liver development (based on tissue nucleic acid levels) was not modified by dietary dieldrin in nursing pups. Overall, the pups grew normally and organ weights were not depressed by the pesticide treatment.

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