"No Effect" Level of DDT in Induction of Microsomal Epoxidation

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The induced enhancement by dietary DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] of rat liver microsomal epoxidation of aldrin (1,2,3,4,10,10-hexachloro - 1,4,4*a*,5,8,8*a* - hexahydro - 1,4:5,8-*endo-exo*-dimethanophthalene) to dieldrin (its 6,7-epoxide) was used as a measure of the minimal detectable effect of DDT on the rat. A comparison of litter mates established that the "no effect" level of dietary DDT is 2.0 p.p.m., and that 2.5 p.p.m. of DDT in the diet is the lowest dose producing a significant increase in microsomal epoxidation in 6-week-old male rats fed for 2 weeks. The extent of induction of microsomal epoxidation appears to be directly related to the concentration of DDT in the diet.

For a quarter of a century, DDT has served well in combating disease and increasing our production of food and fiber. Recently, the role of DDT and other persistent compounds has been questioned, particularly in regard to the effects of chronic, low-level exposure to DDT. In an attempt to assess the magnitude and nature of the public health hazard of DDT, the epoxidation of aldrin to dieldrin in adolescent male rat liver microsomes has been examined. Several chlorinated hydrocarbon insecticides have been shown to be inducers of drug-metabolizing enzymes of the microsomal fraction (Ghazal *et al.*, 1964; Gillett *et al.*, 1966; Hart and Fouts, 1963). The studies by Ortega *et al.* (1956) and Ortega (1966) demonstrated that DDT produces notable changes in the endoplasmic reticulum, which is isolated as the microsomal fraction, in rats fed 5 p.p.m. of DDT, but that no pathological lesions could be associated with these changes. More recently, Kinoshita *et al.* (1966) reported that 5 p.p.m. of dietary DDT increased the activity of several microsomal drug-metabolizing activities. Unspecified insignificant increases were found for lower levels, but results at these lower levels were not detailed.

Aldrin epoxidase, a typical microsomal mixed function oxidase, is particularly well suited for use as a tool in the determination of the effect of DDT. Under the assay conditions, only one product is formed, and the quantitative and sensitive measurement of this resultant dieldrin gives the assay a broad dynamic range. The increase in aldrin epoxidase also appears to be somewhat more sensitive to the concentration of dietary DDT than some other microsomal activities. A preliminary experiment (Gillett *et al.*, 1966) had suggested that an analytically detectable least significant dose would be about 1.0 p.p.m. of dietary DDT for two weeks in 6-week-old male rats. The purpose of this report is to consider more extensively the effects on microsomal epoxidation of diets containing from 0.1 to 100 p.p.m. of DDT.

EXPERIMENTAL

Treatment of Rats. Litter mate groups of male rats (Corvallis-Wistar strain) were weaned at 28 days of age onto a semisynthetic diet (Tinsley, 1965) to which pure DDT could be added in the corn oil used to constitute the diet. As available, the animals were distributed singly to diets and caged individually with free access to food and water. Each litter mate group contained at least one animal on the control (no added DDT) ration. Two groups were on 0, 2.5, 10, 25, and 100 p.p.m.; three groups were on 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 10.0 p.p.m; and two groups (one a litter of 10 males) were on 0, 1.0, 2.0, 5.0, and 10.0 p.p.m. of DDT. The litter group was sacrificed on the morning of the fourteenth day on the diet, and the microsomal fraction was isolated by differential centrifugation as previously described (Gillett *et al.*, 1966).

Preparation and Analysis of Diets. With the exception of the control diet, which is a basal ration used in nutritional experiments in this laboratory, each of the diets was prepared in batches large enough to complete the entire experiment, then stored at −20° C. until needed. The control rations were sampled as prepared, and the contents of the fortified diets were verified by analysis for chlorinated hydrocarbon residues by extraction and gas chromatography (Gillett *et al.*, 1966). Rockland laboratory chows, which were fed to the mothers, and to which the weanlings had access during their fourth week of growth, were examined by a similar procedure. Residues were corrected for recovery (about 90%) of standards added to control diets prior to extraction. The analytical method has a standard error of 7% at 1.0 p.p.m. and a sensitivity of 0.01 p.p.m. for DDT. The presence of DDT and its cogeners and of cyclodiene insecticides was of primary interest, and any questionable peaks were verified by chromatography on a different column with a microcoulometric detector.

Determination of Aldrin Epoxidase. The activity of the washed microsomal fraction was determined as before (Gillett *et al.*, 1966) at three or four protein levels (0.05 to 2.0 mg. per flask). The linear response over this range yielded the activity per milliliter of suspension, which could be converted to units per milligram of microsomal protein [determined by the modified biuret method (Fincham, 1954)] or units per gram of fresh liver tissue. One unit was taken as 10^{-12} mole of dieldrin formed per minute from 20 μg. (about 55 mμmoles) of aldrin incubated at 38° C. for 15 minutes. The resultant data were compared

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by Student's t test according to the methods of Li (1964), using $P < 0.05$ as the level of significance.

Materials. Pure *p,p'*-DDT was obtained from Nutritional Biochemical Corp. Cyclodiene insecticides were donated by the Shell Development Co., Modesto, Calif. NADP and Tris buffer were purchased from General Biochemicals. All solvents were purified by redistillation to remove interfering contaminants.

RESULTS AND DISCUSSION

The rats exhibited normal gains in growth, and all animals appeared healthy when sacrificed. Diets were found to contain the stated amount of DDT when corrected for recovery in the extraction. About 5% of the DDT was found to have been converted to DDD during storage. Pure *p,p'*-DDT did not break down to *p,p'*-DDD on the gas chromatographic column. The control diets contained 0.014 p.p.m. of DDE, 0.010 p.p.m. of DDT, and no other chlorinated hydrocarbons detectable at the level of 0.01 p.p.m. Laboratory chows showed a variable contamination of from 0.02 to 0.40 p.p.m. of total chlorinated hydrocarbons, mainly as DDE, DDT, heptachlor epoxide, and dieldrin.

The levels of aldrin epoxidase are shown in Table I. Litter variation can account for the broad deviation of the control value, although the litter of 10 males confirmed the internal consistency previously noted (Gillett *et al.*, 1966). The comparison of the differences between treated and control litter mates, also shown in Table I, suggests that there is a significant increase in epoxidation for the 0.2-p.p.m. diet and for diets containing 2.0 p.p.m. or more. However, there is no significant difference, for any of the diets from 0.1 to 2.0 p.p.m., between the means of the increase in either the specific activity or activity per gram of liver. The means of the diets from 1.0 to 5.0 p.p.m. overlap, in that the adjacent means are not significantly different, but alternate means are different. Regression analysis of the log of the mean increase in activity per gram of liver against the log of the concentration of DDT in the diet suggests that the induction of microsomal epoxidation is directly related to the level of DDT in the diet. The slope of the line is not significantly different from unity; hence, the relationship probably is that shown in Equation 1 and suggests the absence of rate-controlling steps in the penetration of DDT to its site of action, probably the nucleus of the liver cell (Hart and Fouts, 1965).

$$\Delta E = K(\text{DDT}) \qquad (1)$$

where ΔE = increase in epoxidase, K = rate of microsomal synthesis, and (DDT) = dietary concentration of DDT.

Variation between litters in the absolute amount of increase in epoxidase prompts the inclusion of the relative increase of epoxidation in Table I. The increases in specific activity and activity per gram of liver are comparable and significant for diets containing 2.5 p.p.m. or more of DDT. A doubling of activity seems well within the range of natural or endogenous control of epoxidation. Figure 1 illustrates the problem of establishing an exact basis for the "no effect" dosage. Diets containing from 1.0 to 2.0 p.p.m. of DDT form a line with a negligible slope; the diets containing from 1.0 to 100 p.p.m. yield a slope near unity. These lines intersect below 2.0 p.p.m., which occa-

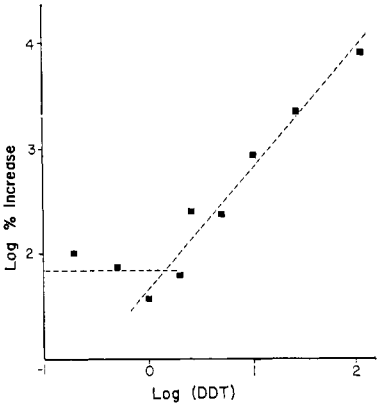

Figure 1. Percentage increase of microsomal epoxidation of aldrin, per gram of liver, relative to litter mate controls, as a function of dietary DDT concentration

Table I. Effect of Dietary DDT Concentration on Rat Liver Microsomal Epoxidase

DDT in Diet, P.P.M.	No. of Rats	Epoxidase Activity: Units[a] per mg. of microsomal protein	Epoxidase Activity: Units per g. of fresh liver	Changes in Epoxidation between Litter Mates: Increase in specific activity, units/mg. of protein	Changes in Epoxidation between Litter Mates: Fresh liver, increase in units per g.	% Increase Relative to Litter Mate Controls: Specific activity	% Increase Relative to Litter Mate Controls: Liver, activity per g.
0	9	47.2 ± 45.8	380 ± 260				
0.1	3	5.1 ± 2.25	120 ± 64	0.47 ± 2.99	83 ± 55	16 ± 77	87 ± 103
0.2	3	10.93 ± 3.14	140 ± 47	3.97 ± 1.62[b]	77 ± 51	68 ± 212	115 ± 86
0.5	3	8.43 ± 1.94	130 ± 8	0.90 ± 2.82	37 ± 58	31 ± 49	74 ± 83
1.0	6	25.8 ± 29.1	440 ± 310	5.52 ± 10.4	41 ± 95	14 ± 21	37 ± 55
2.0	5	97.8 ± 83.0	1010 ± 660	57.7 ± 61.8[b]	554 ± 580	67 ± 117	62 ± 113
2.5	2	60.1 ± 6.6	1290 ± 140	37.8 ± 0.9[b]	925 ± 177[b]	181 ± 64[b]	256 ± 29[b]
5.0	4	175.3 ± 79.2	2010 ± 570	123 ± 68[b]	1390 ± 530[b]	235 ± 49[b]	232 ± 67[b]
10.0	6	287 ± 191	3360 ± 1860	232 ± 211[b]	2930 ± 1800[b]	875 ± 659[b]	852 ± 435[b]
25	2	374 ± 113	7700 ± 1820	347 ± 137	7360 ± 1840	1260 ± 425[b]	2170 ± 541
100	2	933 ± 283	27,100 ± 10,000	904 ± 400	26,800 ± 14,100	3200 ± 1410	7870 ± 4160

[a] μμmoles of dieldrin formed per minute.
[b] $P < 0.05$.

sionally causes a significant increase in epoxidation. Hence the region from 1.0 to 5.0 p.p.m. presents a graded increase in the probability of a diet causing a significant increase in epoxidation, as opposed to a distinct threshold level lying between 2.0 and 2.5 p.p.m.

Ortega (1966) has characterized low-level DDT-induced changes in the endoplasmic reticulum and the constitutive microsomal enzymes as adaptive rather than pathological, since the responses were reversible and not accompanied by other deleterious changes in morphology and function. Yet the meaning of the least effective dose or "no effect" level can be altered by the nature of the interaction with other xenobiotics. Aldrin treatment produces a positive benefit by protection against parathion poisoning (Triolo and Coon, 1966). On the other hand, since epoxidation of aldrin yields the more toxic dieldrin, DDT would be expected to be deleterious to an animal chronically exposed to DDT and acutely exposed to aldrin. The complexity of this interaction is enhanced by the as yet undetermined extent of the DDT-induced increase in dieldrin metabolism and excretion, which has been shown by Street (1964) and his co-workers (Street *et al.*, 1966).

The available data suggest that human dietary DDT levels are substantially below those concentrations which would be expected to produce a sensible change in microsomal oxidation. Cummings (1967) reported that a total diet sample, based on the intake of a 16- to 19-year-old boy in the Baltimore, Md., area, contained 0.003 to 0.010 p.p.m. of DDT and about 0.010 to 0.017 p.p.m. of total chlorinated hydrocarbon pesticides, most of which are known to be inducers about as active as DDT (Ghazal *et al.*, 1964; Gillett *et al.*, 1966). Hence, the 100-to-1 safety factor seems to have been maintained, even though tolerances as high as 7 p.p.m. are allowed in several crops.

Although evaluation of the dosage of DDT producing a detectable change in the rat could allow establishment of certain limitations on the levels of dietary DDT, the picture of the entire microchemical insult on mammals, and especially people, remains blurred. Yet it can only be in the context of that insult that the beneficial or detrimental nature of microsomal induction can be placed. Microsomal induction is reversible and subject to a number of intrinsic control factors, such as sex, age, and nutritional status (Gillett *et al.*, 1966). In establishing standards and protocols for a safe environment, induction should be afforded further consideration, particularly in relation to other xenobiotics and casual chemicals now being introduced or already prevalent in that environment.

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NOMENCLATURE

DDD = 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane
NADP = nicotinamide-adenine dinucleotide phosphate
Tris buffer = tris(hydroxymethyl)aminomethane and its hydrochloride
G-6-P = glucose-6-phosphate
G-6-P DH = glucose-6-phosphate dehydrogenase

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