# The Effect of Hexobarbital on the Retention of DDT Analogs by the Rat

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A number of drugs induce the formation of hepatic microsomal enzymes which are involved in detoxification reactions. This mechanism has been advanced as a possible means of reducing insecticide accumulation in the tissues of animals and man (1). Several drugs have been effective in reducing dieldrin retention in rats under a variety of conditions (1, 2, 3). The barbiturates have been the most effective.

Several DDT analogs also induce the formation of microsomal enzymes (4, 5) and the liver has been implicated as a site of p,p'-DDT metabolism (6). Sanchez (7) and Morello (8) found that DDT induced metabolic changes in rat liver which increased both the reductive and phenolic type metabolites of DDT in vitro. Thus, it is possible that an enzyme inducing compound might reduce the retention of DDT and related compounds.

This study was carried out to determine the effect of hexobarbital on the retention of several DDT analogs by the rat.

## Experimental

The test animals were virgin female rats, aged 125 to 134 days, descended primarily from the Wistar strain. In each experiment 4 litters were used and one rat from each litter was randomly



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assigned to each treatment. The rats were maintained in individual cages. Rations and distilled water were supplied ad libitum and food consumption was recorded.

All experiments were 14 days in length. Several days prior to the beginning of the experiments, the rats were transferred from a pelleted stock ration[1/] to one consisting of the same pellets ground in a Wiley mill. The hexobarbital, when used, was mixed directly with the ration at the rate of 3 mg/g of feed. The pesticides were added to the ration at the rate of 5 µg/g. Acetone solutions of the pesticides were spread over the surface of an aliquote of the feed, and the solvent was allowed to evaporate by exposure to air before mixing with the remaining feed.

At the end of the feeding period the rats were killed with ether and the contents of the alimentary tract were removed. The entire animal, with approximately an equal weight of water and twice the weight of ice, was then homogenized in a Waring blender. A satisfactory preparation was obtained except for the skin, much of which was left in relatively large pieces and not included in the samples.

Duplicate 5 g samples of the homogenate were prepared for analysis by drying with a suitable amount of anhydrous sodium sulfate and were then extracted with petroleum ether. Florisil was used for clean-up and the pesticides were determined by electron capture gas chromatography (9).

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1/ Wayne Lab-blox Allied Mills, Inc., Chicago, Illinois.

## Results and Discussion

The effect of hexobarbital on the retention of dieldrin, o,p'-DDT, and p,p'-DDT was determined in the first experiment (Table 1). The treatments had no significant effect on body weight or feed intake and these factors do not complicate the interpretations of the results. The minor amounts of p,p'-DDE and p,p'-DDD, 3.8 and 3.1% of the total residue retained, are included in the value for p,p'-DDT.

The three compounds represent the different types of behavior found in our work. These are high retention by the control group and little or no response to hexobarbital (p,p'-DDT), high retention by the control group and significant reduction with hexobarbital (dieldrin), and low retention by the control group and significant reduction with hexobarbital (o,p'-DDT).

TABLE 1

The effect of hexobarbital and the retention of dieldrin, p,p'-DDT, and o,p'-DDT by rats

| Treatment | Body weight | Pesticide intake | Pesticide retention | Retention Intake | Hexobarbital Control |
|---|---|---|---|---|---|
| | (g) | (µg) | (µg) | (% ± SE) | (%) |
| Dieldrin | | | | | |
| Control | 231 | 1029 | 745 | 72.6 ± 5.4 | |
| Hexobarbital | 214 | 986 | 274 | 27.7 ± 3.1 | 38.2 |
| p,p'-DDT | | | | | |
| Control | 231 | 1066 | 642 | 59.9 ± 2.1 | |
| Hexobarbital | 218 | 1018 | 560 | 55.0 ± 1.5 | 91.8 |
| o,p'-DDT | | | | | |
| Control | 235 | 1089 | 113 | 10.3 ± 0.3 | |
| Hexobarbital | 229 | 1025 | 38 | 3.8 ± 0.6 | 36.7 |

TABLE 2

The effect of hexobarbital on the retention of the p,p'- and o,p'- isomers of DDE and DDD by rats

| Treatment | Body weight | Pesticide intake | Pesticide retention | Retention / Intake | Hexobarbital / Control |
|---|---|---|---|---|---|
| | (g) | (µg) | (µg) | (% ± SE) | (%) |
| p,p'-DDE | | | | | |
| Control | 244 | 1125 | 652 | 58.0 ± 0.8 | |
| Hexobarbital | 241 | 1104 | 556 | 50.3 ± 1.2 | 86.7 |
| o,p'-DDE | | | | | |
| Control | 230 | 1093 | 448 | 41.1 ± 2.9 | |
| Hexobarbital | 234 | 1098 | 137 | 12.5 ± 0.7 | 30.4 |
| p,p'-DDD | | | | | |
| Control | 233 | 1068 | 481 | 45.0 ± 1.4 | |
| Hexobarbital | 229 | 1018 | 152 | 14.9 ± 1.8 | 33.1 |
| o,p'-DDD | | | | | |
| Control | 226 | 1034 | 92 | 8.9 ± 1.1 | |
| Hexobarbital | 232 | 1058 | 26 | 2.5 ± 0.8 | 28.1 |

The second experiment was carried out to determine the response of the other environmentally important analogs, p,p'-DDD and p,p'-DDE, to the hexobarbital treatment. The results from these compounds, as well as the o,p'- isomers of these compounds, are presented in Table 2. The retention and response to hexobarbital of p,p'-DDE was similar to p,p'-DDT, while o,p'-DDE and p,p'-DDD were similar to dieldrin, and o,p'-DDD was similar to o,p'-DDT.

The differences in retention of the various compounds by the control groups may be caused either by differences in the rate of absorption from the gastro-intestinal tract or by differences in the rate of metabolic transformation within the animal. In other

work we have found that the relative retentions of the compounds are similar at the beginning of the experiments but the differences become greater as the length of the experiment increases. This suggests that the major difference among the compounds is the rate of metabolic transformation.

The high retention of p,p'-DDE and its failure to respond to hexobarbital is in agreement with the observations that p,p'-DDE is not transformed within the animal (5). The similarity of the behavior of p,p'-DDT to p,p'-DDE suggests that in spite of the possible transformations of p,p'-DDT to p,p'-DDE and p,p'-DDD as well as DDA (5), these reactions are not of great significance at the intake levels which we studied. This would not rule out the possibility that these reactions are more important under different conditions.

The behavior of dieldrin, p,p'-DDD and o,p'-DDE suggests that the capacity for metabolism of these compounds is present in the animal, but it is not of great significance until the formation of enzymes is induced in the liver. On the other hand, the ability of the rat to metabolize o,p'-DDT and o,p'-DDD is great even without the induction of liver enzymes.

Many of the compounds involved in this study induce the formation of liver microsomal enzymes. It is possible that for compounds such as o,p'-DDT and o,p'-DDD, 5 μg/g may have been above the "no effect" level for enzyme induction. This would account for the low retention by the control groups. To test

this possibility groups of rats were fed diets containing dieldrin, dieldrin + o,p'-DDT, or dieldrin + o,p'-DDT at 5 μg/g levels (Table 3). Dieldrin retention by the three groups was identical, indicating that the 5 μg/g is below the "no effect" level when this parameter is used.

While o,p'-DDT may comprise as much as 15 to 20% of technical grade DDT, it has not generally been reported in significant concentrations in animal tissues or products. This observation can be accounted for by the rapid rate of o,p'-DDT metabolism which prevents its accumulation by the animal. If, in the environment, o,p'-DDE and o,p'-DDD are formed from o,p'-DDT by methods analogous to the formation of p,p'-DDD and p,p'-DDE from p,p'-DDT, they would also not be accumulated for the same reason. The differences in behavior of the o,p'- compounds and the corresponding p,p'- isomers suggest that metabolic routes of the two groups of compounds differ greatly.

TABLE 3

The effect of 5 μg/g of o,p'-DDT and p,p'-DDT in the diet on the retention of dieldrin by the rat

| Treatment | Retention | | |
|---|---|---|---|
| | o,p'-DDT | p,p'-DDT | Dieldrin |
| | (% ± SE) | (% ± SE) | (% ± SE) |
| Control | ---- | ---- | 61.9 ± 2.1 |
| o,p'-DDT | 14.3 ± 0.6 | ---- | 61.2 ± 2.4 |
| p,p'-DDT | ---- | 50.2 ± 1.9 | 61.8 ± 1.3 |

The overall significance of the induction of liver microsomal enzymes in the animal has not been evaluated. With all other factors equal, enzyme induction must be considered an undesirable effect. On the other hand, when enzyme induction allows the animal to eliminate toxic compounds which would otherwise be retained, it could be considered beneficial. In any event, its practical use as a method of reducing insecticide accumulation is probably not great. On the basis of effectiveness there are some compounds, such as p,p'-DDT, which do not respond. Many of the compounds which do respond are rarely encountered as a residue. If these compounds are encountered at levels sufficient to warrant a drastic countermeasure, the levels would probably be sufficient to induce the enzyme formation by the compounds.

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