Comparison of DDT Effect on Pentobarbital Metabolism in Rats and Quail

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In rats, the *o,p'*- and *p,p'*-isomers of DDT and the environmentally important analogs, DDE and DDD, induce increased levels of liver microsomal enzymes. A standard dose of pentobarbital, therefore, produced a shorter period of hypnosis and sleeping time in pesticide treated rats than in controls. In complete contrast to this situation in the mammalian species, the chlorinated pesticides inhibit liver metabolism of pentobarbital in Japanese quail, and longer sleeping times are observed. In both rats and quail, *p,p'*-DDT and *p,p'*-DDE accumulate in body lipid linearly with time on the diet to levels of 150 ppm in rats and 1500 ppm in quail. This tenfold difference in accumulation may represent a species difference. In both rats and quail, *o,p'*-DDT and *o,p'*-DDE only accumulate to a level of 15 ppm. In spite of very low accumulations in body lipid, the *o,p'*-isomers were very effective in stimulating or inhibiting pentobarbital metabolism, indicating that toxicological effects are more closely related to intake than to body burden.

In almost all species the liver is the major site for the metabolism of foreign chemicals (Bush, 1963). The barbiturates are a class of foreign chemicals which are strong depressants of the central nervous system and induce sleep in the treated individual. A standard dose of pentobarbital will produce a standard sleeping time, and the duration of this period is primarily dependent upon the detoxification of the barbiturate by the liver. An increase in those liver microsomal enzymes which metabolize the barbiturate will be reflected in a shorter sleeping time. Conversely, any inhibition of the activity of these liver microsomal enzymes should be reflected in a longer duration of barbiturate sleeping time.

Hart *et al.* (1963) accidently discovered that spraying of animal rooms with organochlorine pesticides reduced sleeping time in the rat, suggesting that the liver microsomes had been stimulated. Subsequent studies by these investigators and others (Hart *et al.*, 1963; Hart and Fouts, 1965; Conney *et al.*, 1967) have demonstrated clearly that the microsomal enzyme systems exhibited increased metabolic activity *in vitro* for several foreign drugs and that *in vivo* the sleeping times were shortened with DDT, DDE, rhothane, perthane, methoxychlor, and chlordane.

It has recently been shown that the *o,p'*-isomer of DDT, which constitutes 15–20% of technical DDT, exerts an estrogen-like action on the reproductive tissues of mammals and birds (Levin *et al.*, 1968; Welch *et al.*, 1969; Bitman *et al.*, 1968). In contrast, *p,p'*-DDT has little if any estrogenic activity. Since *p,p'*-DDT shortens pentobarbital sleeping time, it seemed of interest to determine whether the *o,p'*-isomer induces a similar effect in rats. Accordingly, we studied in detail the time sequence of the effect upon pentobarbital sleeping time of the *o,p'*- and *p,p'*-isomers of DDT, and its two major metabolic products, DDE and DDD. The effects of treatment on mammals and birds were compared by conducting these experiments on rats and Japanese quail.

MATERIALS AND METHODS

Male and female rats (*Rattus norvegicus*) of an inbred Wistar strain (200–300 g of body weight) on a schedule of 12 hr of light and 12 hr of dark were fed diets containing the *o,p'*- and *p,p'*-isomers of DDT, DDE, and DDD (99+%, Aldrich Chemical Co.). Immature rats were placed on diets at 21 days of age (35–45 g body weight). Female rats were ovariectomized at 50 days of age; after a recovery period of 14 days they were placed on the appropriate pesticide-containing diet. Male rats were castrated when 36 days old; they were injected subcutaneously with 2 mg testosterone propionate/0.2 ml olive oil or 5 μg estradiol-17β three times a week for the duration of the experiment. The pesticides were fed after a hormone pretreatment period of 2 weeks.

Japanese quail (*Coturnix coturnix japonica*), on a schedule of 14 hr of light and 10 hr of dark, were fed *ad libitum* diets containing the pesticides. The quail were 1½ to 3 months of age when experiments were initiated. A corn-soybean meal type starter ration was used as the basal diet. Pesticides were dissolved in acetone and pipetted onto the diet, which was spread out in shallow pans. After evaporation of the solvent, the diets were thoroughly mixed.

The sleeping time was defined as the time between injection of the barbiturate and restoration of the righting reflex. Sodium pentobarbital was injected intraperitoneally in rats at a dosage rate of 32 mg/kg body weight. A dosage of 75 mg/kg of sodium pentobarbital was administered by the intramuscular route in Japanese quail.

Following florisil cleanup, concentrations of the pesticides in the lipid were determined by gas-liquid chromatography (glc). An F & M model 810 instrument equipped with an electron capture detector was used. The 4-ft × ¼ in. o.d. glass column was packed with 3.8% silicon gum rubber on 80-100 mesh Chromosorb W (acid-washed dimethylchlorosilane treated). A 95% argon–5% methane mixture was used as both carrier and purge gas. The column temperature was 185° C. Under these conditions the relative retention times were: *o,p'*-DDE, 1.00; *o,p'*-DDD, 1.30; *o,p'*-DDT, 1.75; *p,p'*-DDE, 1.26; *p,p'*-DDD, 1.66; and *p,p'*-DDT, 2.25. Thus, it was not possible to distinguish between *o,p'*-DDD and *p,p'*-DDE, or *p,p'*-DDD and *o,p'*-DDT by glc.

Representative samples from each experimental group were checked by thin-layer chromatography (tlc) on aluminum oxide G plates in which silver nitrate was incorporated for visualization (A.O.A.C., 1966). Parallel standards of the various analogs were also spotted. The R_f's of the six analogs were: *o,p'*-DDE, 0.51; *o,p'*-DDD, 0.23; *o,p'*-DDT, 0.49; *p,p'*-DDE, 0.54; *p,p'*-DDD, 0.20; and *p,p'*-DDT, 0.39. The spots were scraped from the thin-layer plates and the pesticides recovered from the adsorbent by elution with petroleum

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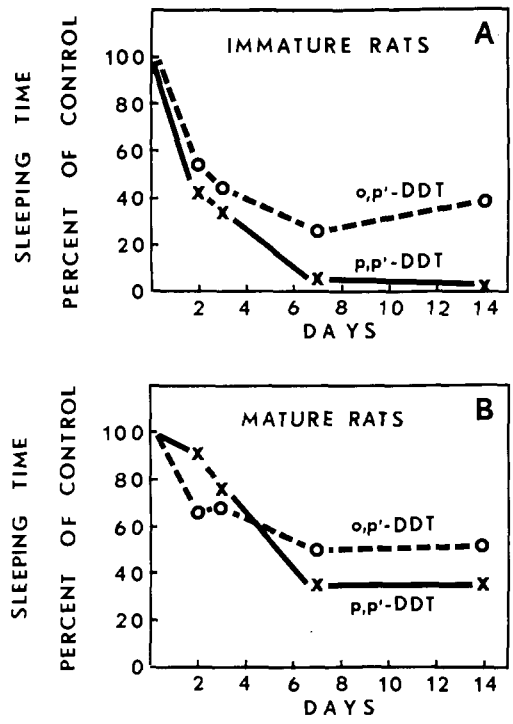

Figure 1. Sleeping times of rats fed 50 ppm *o,p'*- or *p,p'*-DDT. A. Immature male and female rats. Each point represents 8–12 rats. B. Intact cycling and ovariectomized female rats. Each point represents 8–12 rats

Table I. DDT Analogs and Pentobarbital Sleeping Time in Rats

Group	Percent of Control Sleeping Time After Feeding for 7 Days	14 Days
	Immature Females	
p,p'-DDT	10 ± 2	4 ± 1
o,p'-DDT	37 ± 3	39 ± 2
p,p'-DDE	1 ± 0.2	7 ± 2
o,p'-DDE	13 ± 6	14 ± 1
p,p'-DDD	28 ± 4	65 ± 7
o,p'-DDD	40 ± 9	44 ± 4
	Mature Females	
p,p'-DDT	51 ± 5	41 ± 5
o,p'-DDT	63 ± 4	60 ± 5
p,p'-DDE	47 ± 5	24 ± 3
o,p'-DDE	31 ± 5	24 ± 3
p,p'-DDD	77 ± 1	57 ± 5
o,p'-DDD	68 ± 2	61 ± 7

Immature Females: Each group consisted of five rats treated with 50 ppm of the pesticide.
Mature Females: Each group contained ten rats treated with 100 ppm of the pesticide.

ether. The identity of each pesticide was then reconfirmed by glc.

The treated birds were fed only a single compound. Tlc gave no evidence that both members of the pairs that were indistinguishable by glc were present within the same sample. Thus, no complications arose from using this glc method for the quantitative determinations.

RESULTS AND DISCUSSION

Two days after immature 21-day old rats were placed on a diet containing 50 ppm of the DDT isomer, the duration of pentobarbital anesthesia was reduced to only half that of controls (Figure 1A). There were no differences between immature male and female rats, and the line shown represents combined data from both sexes. After 1 week on the DDT diet, the *p,p'*-fed rats had sleeping times which were only 5% of controls, while the *o,p'*-DDT fed rats showed a pentobarbital anesthesia of about 30–40% of controls. In immature rats it appears that *p,p'*-DDT induces liver microsomal enzyme activity which almost completely metabolizes the standard dose of pentobarbital, while *o,p'*-DDT fed at the same level induces liver enzyme activity to a lesser extent, and sleeping time reductions of only 60–70% are observed.

Figure 1B shows the sleeping time reduction produced by *o,p'*- and *p,p'*-DDT in mature female rats, either intact cycling females or ovariectomized females. There were no differences between these two groups and their sleeping time data were combined. The 50 ppm *p,p'*-DDT diet has less effect in the mature than in the immature rats, reducing sleeping time to only 75% at 2–3 days and achieving a maximal reduction to about $^1/_3$ of controls. The *o,p'*-DDT fed rats showed a similar reduction to about 50% of control levels.

The effects upon pentobarbital sleeping time of the isomers of the DDT analogs, DDE and DDD, were also studied in immature and mature female rats. Table I shows the determination of sleeping time performed on rats after being on the diet for 1 and 2 weeks. With both the immature and mature rats, DDE appeared to elicit a greater reduction than DDT, which in turn was more effective than DDD. All compounds, however, stimulated pentobarbital metabolism and sleeping times were reduced to 5–60% of controls. It appears that the *o,p'*-isomer was about as effective as the *p,p'*-isomer for all compounds except DDT, in which case the *p,p'*-isomer lowered sleeping time to a much greater extent.

Although the immature male and female rats showed little or no differences in sleeping time, and the data shown in Figure 1A represented combined data, there were differences in male rats as they matured. Mature male rats showed much less response to 100 ppm *o,p'*-DDT in reduction of sleeping time than did the immature male rats fed 50 ppm *o,p'*-DDT (Figure 2). Conney and Burns (1962) have also found that foreign chemicals were able to stimulate drug-metabolizing enzymes to a greater extent in the immature male than in the adult male rat. If the mature rats were castrated and placed on a 100 ppm *o,p'*-DDT diet, they behaved somewhat like immature male rats, showing much greater reductions in pentobarbital sleeping time. When these castrates were treated with estrogen, they still acted like castrates as far as sleeping time was concerned, indicating that the female sex hormone does not affect the liver microsomal enzyme system.

If on the other hand the castrates were treated with testosterone, they reverted to the mature male rat state, and *o,p'*-DDT had less effect in reducing sleeping time in these testosterone-treated rats than in the castrates. The presence of the male sex hormone induces an elevated level of liver microsomal enzyme activity. Consequently, pesticide feeding is unable to stimulate liver microsomal induction further.

When the accumulation of pesticides in the rats was examined, we found that *p,p'*-DDT increased in an almost linear fashion in both immature male and female rats (Figure 3A) and in mature cycling or ovariectomized rats (Figure 3B). After 2 weeks feeding, pesticide concentrations were in the

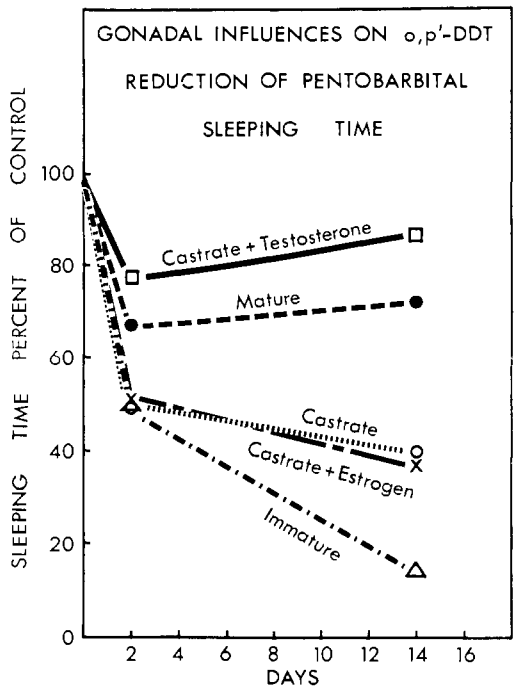

Figure 2. Gonadal influences on *o,p'*-DDT reduction of pentobarbital sleeping time. Each point represents five male rats fed 100 ppm *o,p'*-DDT in the diet. At 0 time, the immature, castrate, and castrate + estrogen slept 98, 143, and 188 min, respectively, while the castrate + testosterone and mature male rats slept 51 and 52 min. Sleeping times are expressed as percentages of these control values

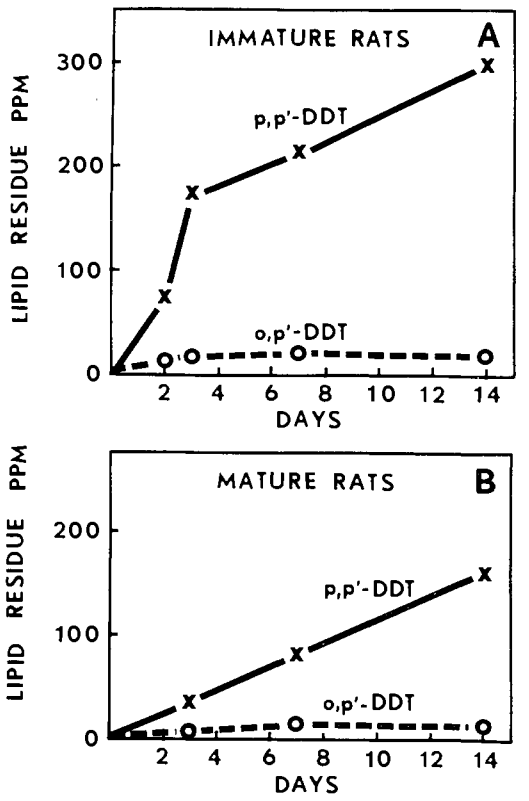

Figure 3. Accumulation of pesticides in rat lipid. A. Immature male and female rats. Each point represents ten rats. B. Mature ovariectomized and cycling female rats. Each point represents ten rats

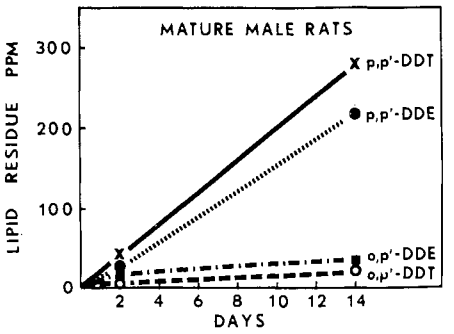

Figure 4. Accumulation of pesticides in rat lipid. Each point represents ten mature male rats fed 100 ppm of the pesticide

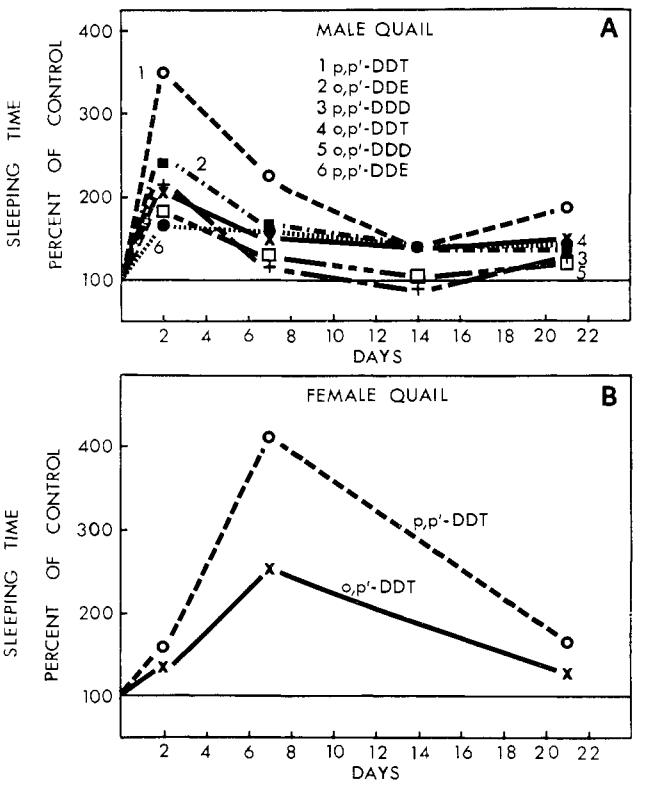

Figure 5. Sleeping times of quail fed pesticides. A. Male quail. Each point represents six quail. B. Female quail. Each point represents seven quail

range of 200–300 ppm in the adipose tissue of immature rats, and were about 150 ppm in the mature rats, showing an almost exact relationship between total intake of the pesticide and body weight of the rats. The *o,p'*-DDT residues in either immature or mature rats were only about $^1/_{10}$ of the *p,p'*-DDT levels, being in the range of 15–20 μg/g in the lipid.

o,p'- and *p,p'*-DDE were also extremely effective in reducing sleeping time (Table I), and their accumulation in rat lipid is compared to that of the *o,p'*- and *p,p'*-isomers of DDT in Figure 4. A very similar pattern was seen, the *p,p'*-isomers accumulating and the *o,p'*-isomers being lost from the body.

In spite of this great disparity in the accumulation of the two isomers, indicating that the *o,p'*-compounds were almost completely metabolized and excreted from the body, the similar sleeping times observed with the two isomers indicated that the *o,p'*-analog was about as effective as *p,p'* in stimulating liver microsomal activity to degrade pentobarbital.

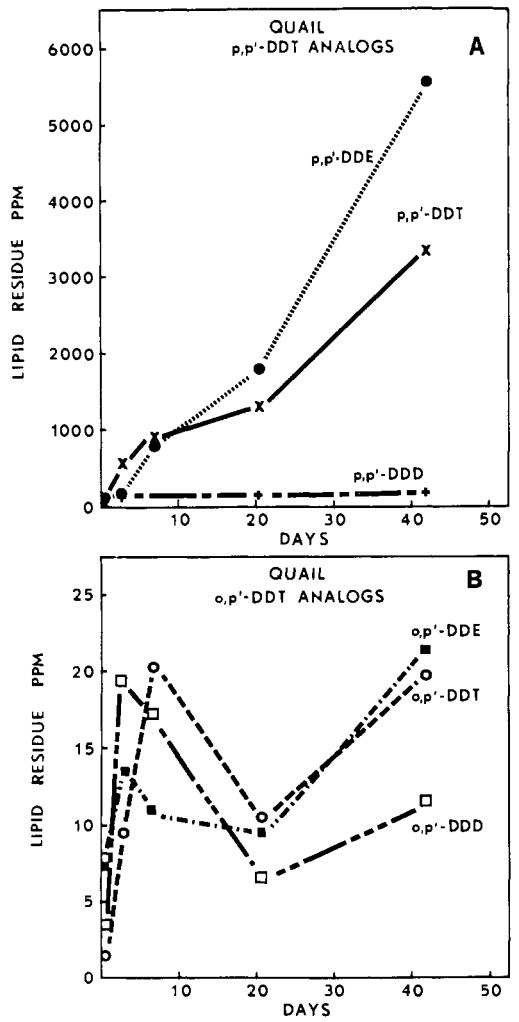

Figure 6. Accumulation of pesticides in quail lipid. Each point represents four quail

Table II. Toxicity to Pentobarbital: Percent Mortality

Days on Diet	Control	100 ppm *o,p'*-DDT	100 ppm *p,p'*-DDT
	Male Quail		
2	9	64	82
6	17	0	33
14	0	17	50
	Female Quail		
7	33	29	86

Average group size was seven quail.

These data suggest that toxicological effects in the case of this rapidly metabolized isomer are more closely related to intake than to body burden.

Figure 5 shows the effects of 3 weeks feeding of 100 ppm of *o,p'*- and *p,p'*-isomers of DDT, DDE, and DDD on pentobarbital sleeping time in quail. In complete contrast to the shortening of sleeping time observed in rats, the DDT isomers and metabolites prolonged sleeping time in quail. Two days after being placed on the feed, the male quail slept 1.5 to 3.5 times longer than untreated control quail in response to a standard dose of pentobarbital (Figure 5A). After 7 days the increase in sleeping time was not as great, and at 2 weeks the quail fed *o,p'*- and *p,p'*-DDD were not different from controls in the sleeping time test. The quail fed DDT and DDE still showed an increased sleeping time about 150% of control, but the increases were considerably below the increases measured at 2 days.

The female quail (Figure 5B) also showed an increase in pentobarbital sleeping time, but the time course was somewhat different. Two days after being placed on DDT containing feed, sleeping times were only about 50% greater than controls. At 7 days, however, liver microsomal activity was inhibited to the extent that pentobarbital metabolism was 2.5 to 4 times less than that of controls, as judged by increased pentobarbital hypnosis. Again, this increase in sleeping time was not maintained, and after 3 weeks feeding, the sleeping time was about 50% higher than that of untreated control birds. The decline in sleeping time after an early increase, in both male and female quail, suggested that an adaptation was taking place in response to the chlorinated hydrocarbon stimulus. In order to determine whether the inhibition of liver metabolism of the barbiturate observed at 21 days would be maintained at longer times on a pesticide diet, sleeping times were determined on quail fed pesticide-containing diets for 6 weeks. At 42 days, quail fed 100 ppm of *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, or *p,p'*-DDE still showed an increased mean sleeping time of 153% of control.

An adaptation of this magnitude should markedly affect the acute toxicity of an administered drug. In male quail if the increase of sleeping time at 2 days reflects a lowered metabolism of pentobarbital, the acute toxicity to this drug should be different, and a dose which the control can tolerate might be reflected in a greater mortality in the pesticide treated group. Then, at later times when the adaptation takes place, this same lethal dosage should be tolerated by the body and lowered mortality should be observed.

Pentobarbital at a dose rate of 105 mg per kg of body weight was injected intramuscularly (Table II). This dose killed only 1 out of 11 control birds but was lethal to 64% and 82% of *o,p'*- and *p,p'*-DDT treated male quail. At 6 and 14 days the male quail metabolized this dose to a greater extent and showed a lower mortality than at 2 days. This same dosage was more lethal in female quail and killed about $^1/_3$ of the control females. Female quail fed *p,p'*-DDT for 7 days could not effectively metabolize the pentobarbital and 86% died in response to this dose. These mortality experiments thus also demonstrated the adaptation in liver metabolism induced by the pesticides.

In order to determine whether this sleeping-time adaptation pattern might be a reflection of differing accumulation levels of the pesticides, samples of lipid from quail were analyzed for chlorinated hydrocarbon residues. *p,p'*-DDE and *p,p'*-DDT increased almost linearly with increasing time on the diet (Figure 6A). After 3 weeks on the diet, pesticide lipid concentrations were in the range of 1000–2000 ppm and after 42 days were in the 3000–5000 ppm range. *p,p'*-DDD rapidly increased to a level of about 100 ppm of lipid and remained in the 100–200 ppm range during the entire 42 day period. In marked contrast, the *o,p'*-isomers of DDT and the environmentally important analogs rapidly increased to concentrations of only 10–15 ppm in lipid and essentially remained at these levels during the entire feeding period (Figure 6B). Again, as observed previously in the rats, these extremely low accumulation levels of *o,p'*- analogs indicated that these compounds were almost completely metabolized and excreted from the body. In spite of this, however, they were about as effective as *p,p'*-compounds in influencing liver microsomal enzyme metabolism of pentobarbital. This again

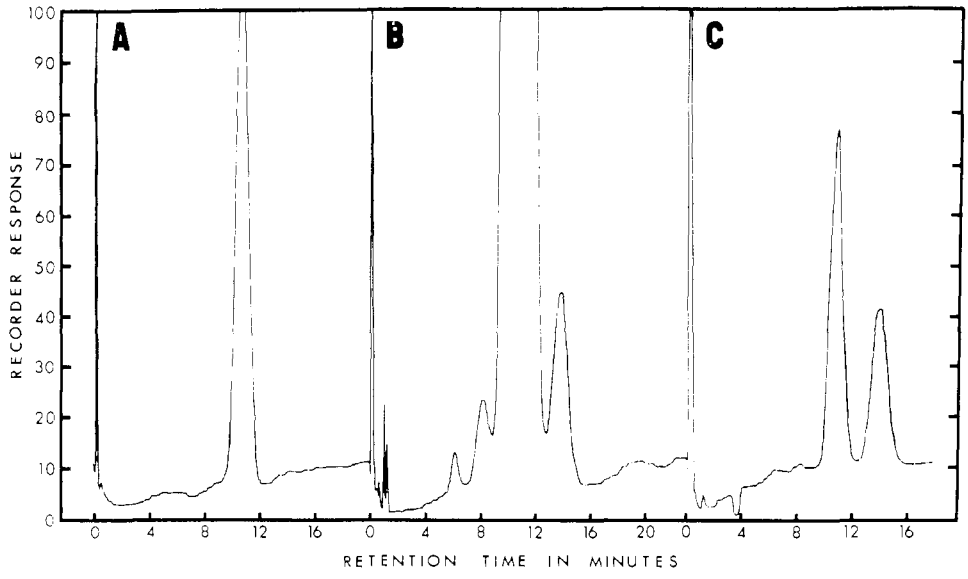

Figure 7. A. Glc recorder tracing of 3 μl of 1 μg/ml *o,p'*-DDT. B. Glc recorder tracing of 3 μl of 100 μg/ml of *o,p'*-DDT. The three minor peaks have retention times correspondng to *o,p'*-DDE, *p,p'*-DDE, and *p,p'*-DDT, respectively. C. Glc recorder tracing of 3 μl of the material eluted from tlc at the R_f corresponding to *p,p'*-DDT. Volume of the eluate was equivalent to that required for a concentration of 100 μg/ml of the starting *o,p'*-DDT

suggested that toxicological effects were more closely related to intake than to body burden.

The concentrations of the pesticides shown in Figures 2, 3, and 6 refer only to parent compounds that were fed to the individual groups. Each group had minor amounts of some of the other analogs present in the lipid residues. A summary of lipid accumulations for immature rats fed *o,p'*- or *p,p'*-DDT for 14 days and for quail fed for 42 days is presented in Table III. Both the rats and quail fed *p,p'*-DDT had the expected accumulation of *p,p'*-DDE and *p,p'*-DDD. In the case of *o,p'*-DDT feeding, both rats and quail showed minor concentrations of *p,p'*-DDE and significant concentrations of *p,p'*-DDT. These findings are in agreement with the report of Klein *et al.* (1964, 1965).

Klein *et al.* (1964, 1965) have interpreted their data to indicate that *o,p'*-DDT is converted to *p,p'*-DDT in the rat. However, in view of the large differences in the relative retentions of the two compounds in the animal body, we were concerned about the possibility of a minor contamination of the *o,p'*-DDT with *p,p'*-DDT. If the fractional retention of *p,p'*-DDT at low intakes is the same as at high intakes, the *p,p'*-DDT observed in rat lipid could be accounted for by a 0.48% contamination of the *o,p'*-DDT administered. The same value for quail would be 0.41%.

A recorder tracing of the *o,p'*-DDT at 1 μg/ml is shown in Figure 7A. In agreement with the findings of Klein *et al.* (1964), there was no evidence of *p,p'*-DDT. However, when a 100 μg/ml solution was used, several minor peaks were observed, including one with the same retention time as *p,p'*-DDT (Figure 7B). The identity was confirmed by tlc separation and subsequent recovery of the peak at the R_f corresponding to *p,p'*-DDT (Figure 7C). The tlc separation established the presence of *p,p'*-DDT (Figure 7C), although the spot was not completely free of *o,p'*-DDT due to tailing of the starting material which contained over 99.5% *o,p'*-DDT.

It is difficult to precisely quantify the amount of *p,p'*-DDT contaminant in the commercially pure (99+%) preparation of *o,p'*-DDT that was fed. The average of several replicate determinations was 0.39 μg/ml in a 100 μg/ml solution of *o,p'*-DDT. Thus, the animals fed 100 μg of *o,p'*-DDT/g diet would also receive 0.39 μg of *p,p'*-DDT/g diet. This value is in the same range as the amount of *p,p'*-DDT that would be required to produce our observed *p,p'*-DDT accumulations. Thus our results would not provide any evidence that *o,p'*-DDT is converted to *p,p'*-DDT in either rats or quail.

Table III. Concentration of Analogs in the Lipid of Rats and Quail Fed *o,p'*-DDT or *p,p'*-DDT

Fed	Found[a]			
	p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	*o,p'*-DDT
	μg/g			
	Immature Rats[b]			
o,p'-DDT	1.15	...	1.33	21.7
p,p'-DDT	18.8	2.84	279	...
	Quail[c]			
o,p'-DDT	1.93	...	13.7	19.4
p,p'-DDT	680	50.4	3310	...

[a] All values are an average of four animals. [b] Fed 100 μg/g diet for 14 days. [c] Fed 100 μg/g diet for 42 days.

We have found no previous literature references to pesticide-sleeping time interactions in birds. In mice, Rosenberg and Coon (1958) have reported an increase of hexobarbital sleeping time when fed certain organophosphorus insecticides. Azarnoff *et al.* (1966) have reported that *p,p'*-DDD shortens hexobarbital sleeping time in rats and dogs but prolongs pentobarbital sleep in dogs. Rosenberg and Coon (1958) have suggested that the mechanism of this action was a competition for the enzyme systems which are responsible for destruction of hexobarbital.

There have been several previous investigations on the effects of pesticides in Japanese quail. Shellenberger *et al.* (1965, 1966) have studied the effects of several organophosphate pesticides on Japanese quail. Recently, Gillett and Arscott (1969) reported that DDT, fed at 100 ppm for several months, causes a depression of hepatic microsomal epoxidase

activity in mature quail when aldrin and heptachlor were the substrates. In other experiments in which dieldrin was fed to young Japanese quail, aldrin epoxidase activity of hepatic microsomes was increased while cytochrome P-450 or NADPH-neotetrazolium reductase levels were not affected. Walker *et al.* (1969) have also studied the toxicity of dieldrin to Japanese quail. In studies on the effects of DDT and dieldrin on liver microsomal activity of hens, Sell *et al.* (1971) also found that the liver microsomal enzymes exhibited a marked independence. *p,p'*-DDT reduced liver microsomal aniline hydroxylase activity but did not significantly affect *N*-demethylase activity or cytochrome P-450 concentrations.

The concentrations of *p,p'*-DDT in the body fat of quail was approximately ten times that of rats (Figures 3, 4, and 6). This finding appears to be in conflict with older evidence summarized by Müller (1959), who concluded that "all species store DDT in their body fat at rates of the same order of magnitude when exposed repeatedly at the same dose rate." The apparent discrepancy is probably a result of two factors. We have found that quail consume more feed per unit body weight and have a lower body fat concentration than rats. As a result, the fraction of the consumed DDT retained does not differ greatly for the two species.

An interesting finding was the low amount of *o,p'*-isomers relative to *p,p'*-isomers in birds. In the rats, *o,p'*-isomers accumulated to about 15 ppm and *p,p'*-isomers were present at about 150 ppm, a ratio of about 1 to 10. In the quail, *o,p'*-isomers were also present at about 15 ppm in the lipid, but *p,p'*-concentrations were about 1500 ppm, a ratio of about 1 to 100. The fact that a similar proportion of the ingested *p,p'*-isomers is retained in both species suggests that the *o,p'*-isomers are more readily metabolized by the quail. These differences in the accumulation and metabolism of the persistent organochlorine pesticides in birds suggest a species difference and might fit well with the suggested greater adverse biological effects in avian as compared to mammalian species (Mrak, 1969).

LITERATURE CITED

Association of Official Agricultural Chemists, "Changes in Official Methods of Analysis," *J. Ass. Offic. Agr. Chem.* **49,** 227 (1966).

Azarnoff, D. L., Grady, H. J., Svoboda, D. J., *Biochem. Pharmacol.* **15,** 1985 (1966).

Bitman, J., Cecil, H. C., Harris, S. J., Fries, G. F., *Science* **162,** 371 (1968).

Bush, M. T., "Sedatives and Hypnotics. I. Absorption, Fate and Excretion," p. 206, *Physiol. Pharmacol.* Vol. I, W. S. Root and F. G. Hofmann, Eds., Academic Press, New York, 1963.

Conney, A. H., Burns, J. J., *Advan. Pharmacol.* **1,** 31 (1962).

Conney, A. H., Welch, R. M., Kuntzman, R., Burns, J. J., *Clin. Pharmacol. Therap.* **8,** 2 (1967).

Gillett, J. W., Arscott, G. H., *Comp. Biochem. Physiol.* **30,** 589 (1969).

Hart, L. G., Shultice, R. W., Fouts, J. R., *Toxicol. Appl. Pharmacol.* **5,** 371 (1963).

Hart, L. G., Fouts, J. R., *Arch. Exp. Pathol. Pharmakol.* **249,** 486 (1965).

Klein, A. K., Laug, E. P., Datta, P. R., Watts, J. O., Chen, J. T., *J. Ass. Offic. Agr. Chem.* **47,** 1129 (1964).

Klein, A. K., Laug, E. P., Datta, P. R., Mendel, J. L., *J. Amer. Chem. Soc.* **87,** 2520 (1965).

Levin, W., Welch, R. M., Conney, A. H., *Fed. Proc.* **27,** 649 (1968).

Mrak, E. M., Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, U.S. Dept. HEW (1969).

Müller, P., "DDT, the Insecticide Dichlorodiphenyl Trichloroethane and its Significance," p. 68, Birkhauser, Basel, 1959.

Rosenberg, P., Coon, J. M., *Proc. Soc. Exp. Biol. Med.* **98,** 650 (1958).

Sell, J. L., Davison, K. L., Puyear, R. L., J. AGR. FOOD CHEM. **19,** (1971).

Shellenberger, T. E., Newell, G. W., Bridgman, R. M., Barbaccia, J., *Toxicol. Appl. Pharmacol.* **7,** 550 (1965).

Shellenberger, T. E., Newell, G. W., Adams, R. F., Barbaccia, J., *Toxicol. Appl. Pharmacol.* **8,** 22 (1966).

Walker, A. I. T., Neill, C. H., Stevenson, D. E., Robinson, J., *Toxicol. Appl. Pharmacol.* **15,** 69 (1969).

Welch, R. M., Levin, W., Conney, A. H., *Toxicol. Appl. Pharmacol.* **14,** 358 (1969).

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