The substance isolated from feed extracts and authentic TUA had identical R_f values in a number of solvent systems, and both were hydrolyzed under autoclave conditions to yield similar ratios of tylosin and desmycosin as a function of pH. In addition, controlled regeneration of both substances at constant pH and different temperatures yielded equivalent rate constants.

SUMMARY

The evidence presented in this report indicates that tylosin reacts with urea to form an antimicrobially inactive compound, which has been identified as TUA. The formation of TUA does not represent an actual loss of available tylosin.

Elucidation of this reversible tylosin–urea reaction affords the opportunity to investigate the application of TUA as a stabilized form of tylosin in urea-containing mixtures. Investigation is continuing to determine if TUA would be a useful therapeutic agent for ruminant production and management. The results of on-station and feedlot experience with TUA in cattle, as well as *in vitro* and *in vivo* hydrolysis and rumen function data, will be reported elsewhere.

LITERATURE CITED

Hamill, R. L., Haney, M. E., Jr., Stamper, M., Wiley, P. F., *Antibiot. Chemother. (Washington, D. C.)* **11**, 328 (1961).
Kavanagh, F., Ed., *Anal. Microbiol.* 371 (1963).
Kline, R. M., Golab, T., *J. Chromatogr.* **18**, 409 (1965).
Morin, R. B., Gorman, M., "Encyclopedia of Chemical Technology," Kirk-Othmer, Ed., Vol. 12, 2nd ed., Wiley, New York, N. Y., 1967, p 632.
Morin, R. B., Gorman, M., Hamill, R. L., DeMarco, P. V., *Tetrahedron Lett.* 4737 (1970).
Zigeuner, G., Gardziella, E. A., Bach, G., *Monatsh. Chem.* **92**, 31 (1961).

Received for review June 23, 1972. Accepted October 12, 1972.

Effects of Different Classes of Pesticides on Pentobarbital Anesthesia and Toxicity in Japanese Quail

Helene C. Cecil,* Joel Bitman, and Susan J. Harris

Two hours after a single oral dose of DDT, methoxychlor, or malathion, Japanese quail responded with prolonged pentobarbital sleeping times. Prolonged sleeping times occurred 48 hr after DDT treatment. In contrast to DDT, sleeping times returned to control levels 24 hr after methoxychlor or malathion treatment. Abate reduced sleeping times, while Sevin had no effect. *Ad libitum* feeding of DDT or methoxychlor prolonged pentobarbital sleeping times, but malathion, Abate, or Sevin had little effect. Although very few birds died from the toxicity of the pesticide alone, birds died in many pesticide groups when pentobarbital was administered. Mortality was greatest during anesthesia when pentobarbibital had been administered 2 hr after a single oral dose of the pesticide. *Ad libitum* feeding of pesticides did not increase mortality during pentobarbital anesthesia.

In almost all species the liver is the major site for the metabolism of foreign chemicals (Bush, 1963). A standard dose of pentobarbital will produce a standard sleeping time, the duration of which is primarily dependent upon the detoxification of the barbiturate in the liver. An increase in liver microsomal enzyme activity will be reflected in greater metabolism of the barbiturate and a shorter sleeping time. An inhibition of liver microsomal enzyme activity would result in a longer barbiturate sleeping time.

We recently reported that *o,p'*-DDT and *p,p'*-DDT, as well as their metabolites, initially increased pentobarbital sleeping time in Japanese quail. After 2 weeks on the pesticide diet, sleeping times approached the control values, indicating that DDT effects on pentobarbital sleeping times were less, although pesticide lipid residues were accumulating (Bitman *et al.*, 1971).

In the present study we have compared compounds of several different pesticide classes (organochlorine, organophosphate, and carbamate) in their effects upon pentobarbital sleeping times in Japanese quail.

Animal Physiology and Genetics Institute, Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705.

EXPERIMENTAL PROCEDURE

Mature male and female Japanese quail, 40–80 days old, were housed individually on a schedule of 14 hr of light and 10 hr of dark. The quail were fed the pesticide in olive oil with a feeding needle or were fed *ad libitum* diets containing the pesticide. Two organochlorines (*p,p'*-DDT and methoxychlor), two organophosphates (malathion and Abate), and a carbamate (Sevin) were used. The pesticides were: *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethaned], 99+% pure, Aldrich Chemical Co.; technical DDT, Olin-Matheson Co. analyzed to be 70% *p,p'*-DDT; methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane]; technical methoxychlor, Grade II, 90% Sigma; Sevin [1-naphthyl *N*-methylcarbamate], Union Carbide; Technical Sevin, 50% wettable powder, Union Carbide; malathion [*O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl) dithiophosphate], 99.7%, American Cyanamid Co.; technical malathion, 92%, American Cyanamid Co.; Abate [*O,O,O',O'*-tetramethyl *O,O'*-(thiodi-*p*-phenylene) phosphorothioate], 99%, American Cyanamid Co.; technical Abate, 86.2%, American Cyanamid Co.

At appropriate times after administration of the pesticide, sodium pentobarbital was injected intramuscularly at a dosage rate of 50 mg/kg of male body weight or 60 mg/kg corrected female body weight. Body weights of the

females were corrected for the reproductive tract by subtracting 10 g and, if an egg was also present, an additional 10 g was subtracted. Birds were considered asleep as long as they could not right themselves when placed on their back. Control birds slept for 40 to 60 min. Duration of sleeping time for treated birds is expressed as percent of control. *p,p'*-DDT and methoxychlor residues in the body fat were determined by gas–liquid chromatography after Florisil cleanup (Bitman *et al.*, 1971).

RESULTS

DDT and Methoxychlor. DDT was selected as an example of a persistent and methoxychlor as a degradable organochlorine pesticide. Technical DDT or *p,p'*-DDT increased sleeping times to the same extent in male and female quail and these data, therefore, were combined. The persistent organochlorines, *p,p'*-DDT or technical DDT, increased sleeping times for more than 48 hr after a single oral feeding of 100 mg/kg of body weight (Figure 1). Sleeping times were 230 to 245% of control 2 hr after a single oral dose and decreased to 149% of control 48 hr after a single oral dose of DDT (treated *vs.* control: 2 hr, $p < 0.001$; 48 hr, $p < 0.001$).

The birds would not tolerate 100 mg of technical methoxychlor/kg of body weight; consequently the single oral dose was reduced to 50 mg of technical methoxychlor per kg of body weight. The treated birds (100 mg/kg) that survived the pentobarbital sedation had sleeping times comparable to the birds receiving 50 mg/kg, therefore the data were combined (Figure 1). The initial action of methoxychlor was similar to that of DDT and sleeping times increased immediately. In contrast to DDT, sleeping time values returned to control levels 24 hr after methoxychlor treatment.

Methoxychlor-treated male quail had a greater increase in pentobarbital sleeping time than females (male *vs.* female: 2 hr, $p < 0.001$; 5 hr, $p < 0.001$). Two hours after methoxychlor treatment, male quail had sleeping times 257% of control ($p < 0.001$), an increase equivalent to the magnitude of response in male and female quail treated with DDT, while female quail treated with methoxychlor had sleeping times 170% of the control ($p < 0.001$).

Ad libitum feeding DDT or methoxychlor (Figure 2) did not increase sleeping times as much as a single oral feeding. Feeding DDT to male quail increased sleeping times to 150 and 170% of the control (treated *vs.* control $p < 0.001$). Sleeping times of female quail were not affected by *ad libitum* feeding of 100 or 300 ppm of DDT. *Ad libitum* feeding of methoxychlor had little effect on sleeping times in male quail. However, sleeping times were prolonged in methoxychlor-fed female quail, being 65% longer than the controls (treated *vs.* control $p < 0.001$).

DDT accumulated in the body fat to a much greater extent than methoxychlor. At 3 and 7 days of *ad libitum* feeding, the methoxychlor concentration in the fat was 14–15 μg/g of lipid, while DDT was 500 μg/g at 3 days and 1000 μg of DDT/g of lipid at 7 days.

Organophosphates. The two organophosphates studied did not have the same action (Figure 3A). Malathion prolonged sleeping times, while Abate shortened sleeping times after a single oral feeding. Pure malathion had a greater effect than technical malathion (technical *vs.* pure: 2 hr, $p < 0.025$; 5 hr, $p < 0.001$). Since the technical malathion was 92% malathion, the single dose of technical malathion was equivalent to 74 mg of malathion/kg of body weight. A greater amount of technical malathion could not be used because the birds would not survive the pentobarbital anesthesia 2 hr after higher malathion treatment (Table I). *Ad libitum* feeding of malathion or Abate had no effect on sleeping times (Figure 2).

Carbamate. The carbamate, Sevin, had no effect on pentobarbital sleeping times, with results with technical Sevin being comparable to pure Sevin (Figure 3B). *Ad libitum* feeding of Sevin (Figure 2) had no effect on sleeping times.

Mortality Data. All pesticides were administered at doses well below their LD_{50} (Tucker and Crabtree, 1970). The only deaths from the pesticides occurred in the Abate-treated groups (2/105 in the short term single oral

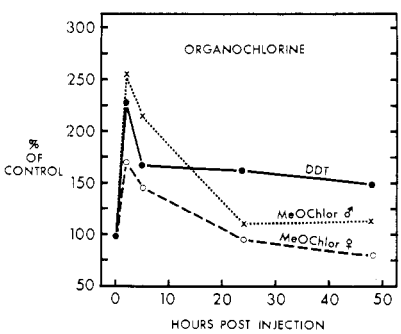

Figure 1. Pentobarbital sleeping times of male and female Japanese quail after a single oral injection of 100 mg of DDT or 50 and 100 mg of methoxychlor per kg of body weight. The DDT curve represents the combined data for *p,p'*-DDT and technical DDT feeding to male and female quail. Each point is the mean of 19 to 31 birds for DDT, 6 to 20 birds for methoxychlor-injected males, and 13 to 27 birds for methoxychlor-injected females.

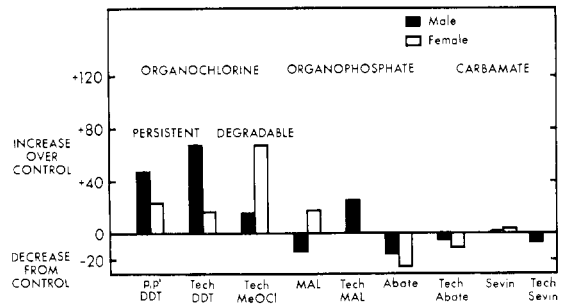

Figure 2. Pentobarbital sleeping times of Japanese quail after *ad libitum* feeding of pesticides for 3 and 7 days. Bars represent combined data after feeding 100 ppm and 300 ppm of the pesticide for 3 and 7 days. Each solid bar is the mean of 24 to 57 males and each open bar is the mean of 10 to 15 females.

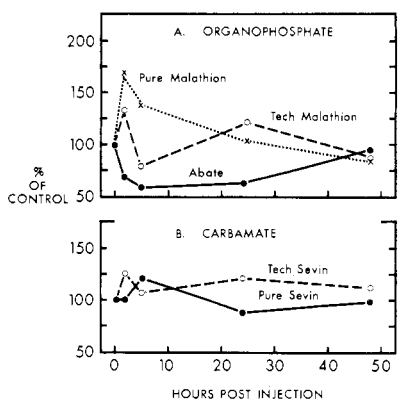

Figure 3. Pentobarbital sleeping times of male and female Japanese quail after a single oral injection of malathion, Abate, or Sevin. All curves are the combined data for male and female quail. The Abate curve represents the combined data for pure and technical Abate. Administered dose and number of birds per point are: pure malathion, 80 mg/kg of body weight (n = 10–15); technical malathion, 80 mg/kg of body weight (n = 10–15); Abate 50 mg/kg of body weight (n = 10–15); pure Sevin, 100 mg/kg of body weight (n = 12–35); technical Sevin, 200 mg/kg of body weight (n = 5–10).

Table I. Mortality of Pesticide-Treated Japanese Quail during Pentobarbital Anesthesia

Treatment	% mortality: Single oral feeding, Dose, mg/kg of body weight	Time post-feeding: 2 hr	Time post-feeding: 5–48 hr	*ad libitum* feeding,[b] 3 and 7 days, 100 and 300 ppm
Tech DDT	100 (70)[a]	23	3	2
p,p'-DDT	100	18	19	7
Tech MeOChlor	100(60)	55	23	7
Tech MeOChlor	50 (30)	18	5	
Tech Malathion	100 (92)	83	N.D.	4
Tech Malathion	80 (74)	40	7	
Pure Malathion	80	11	12	9
Tech Sevin	200 (100)	9	6	4
Pure Sevin	100	19	17	0
Tech Abate	100 (82)	0	28	3[c]
Pure Abate	100	0	7	6[c]

Control mortality = 4% during pentobarbital anesthesia.

[a]Values in parentheses are the calculated amount of the pure compound fed. [b]Food intake = 16 g/bird/day. [c]Data for 3 and 7 days at 100 ppm only. Birds fed 300 ppm of Abate *ad libitum* had a 100% mortality during anesthesia.

dose of 100 mg of Abate per kg of body weight; 7/78 in 3 and 7 day feeding of 100 and 300 ppm of Abate). Although very few birds died from the toxicity of the pesticide alone, birds died in many pesticide groups when pentobarbital was administered (Table I). There was a much higher mortality during pentobarbital anesthesia after a single oral dose of 100 mg of pesticide per kg of body weight than after *ad libitum* feeding of 100 or 300 ppm of pesticide for 3 or 7 days. Mortality during pentobarbital anesthesia was less than 10% after *ad libitum* feeding 100 or 300 ppm of pesticide. Mortality was generally greatest during anesthesia, when pentobarbital had been administered 2 hr after a single oral dose of the pesticide. Only 12 out of 273 controls died during pentobarbital anesthesia.

Two hours after oral injection of 100 mg of DDT/kg of body weight, mortality was 18–23% during pentobarbital anesthesia. A single oral dose of 100 mg of methoxychlor per kg of body weight appeared to be too high for quail. When sleeping times were conducted 2 hr after a single oral dose of 100 mg of methoxychlor per kg of body weight, 55% of the treated birds died during pentobarbital sedation (Table I). At later times after methoxychlor treatment, mortality was only 23% during pentobarbital anesthesia. Mortality was greatly reduced with methoxychlor treatment when the dose was reduced to 50 mg of methoxychlor per kg of body weight.

The effects of technical malathion were similar to those of methoxychlor. When 100 mg of technical malathion per kg of body weight was administered, 83% of the birds died during pentobarbital anesthesia. Reducing the technical malathion dosage to 80 mg/kg of body weight reduced mortality to 40% when pentobarbital was administered 2 hr after malathion and to 7% at later times. Treatment with 80 mg of pure malathion per kg of body weight produced fewer deaths when pentobarbital was administered.

Mortality in Sevin-treated birds during pentobarbital anesthesia was not excessively high. With Abate treatment no deaths occurred during pentobarbital sedation 2 hr after administering 100 mg of Abate per kg of body weight. However, mortality increased at later times. With *ad libitum* feeding of 300 ppm of Abate for 7 days, all the birds died during pentobarbital anesthesia. With *ad libitum* feeding of 100 ppm of Abate, mortality during anesthesia was 3–6%, comparable to mortalities with *ad libitum* feeding of the other pesticides and controls.

DISCUSSION

There is little literature on the effects of pesticides on sleeping times of birds. DDT treatment has been shown to lengthen sleeping times in Japanese quail (Bitman *et al.*, 1971) and chickens (Stephen *et al.*, 1971). In the present studies, a single oral dose of DDT, methoxychlor, and malathion prolonged pentobarbital sleeping times. However, feeding for 3 or 7 days may have altered the pentobarbital detoxification pattern, since the sleeping times were not as prolonged as after a single feeding.

The lengthening of pentobarbital sleeping time in quail after the administration of DDT, methoxychlor, or malathion is similar to the effects these pesticides have in mice (Hart and Fouts, 1965; Rosenberg and Coon, 1958). No lengthening of sleeping time by these pesticides has been seen in rats, but rather shortened sleeping times occur (Hart and Fouts, 1965).

Although pesticide doses were selected which would not kill the birds, birds in certain pesticide groups died when pentobarbital was administered. Apparently the pentobarbital induced too deep an anesthesia for survival. This effect was most pronounced 2 hr after the administration of DDT, methoxychlor, or malathion. Also, at 2 hr after pesticide injection, the greatest prolongation of sleeping time occurred. However, none of the pesticides hastened the onset of action of pentobarbital, indicating that they did not increase the rate of entrance of pentobarbital to the brain nor depress the central nervous system.

In our studies, DDT, methoxychlor, and malathion prolonged sleeping times and we concluded that these pesticides interfered with the metabolism of pentobarbital. Lengthening of barbiturate sleeping time reflects decreases in the levels of liver enzyme activity which detoxify barbiturates, although the absolute amount of enzyme may be unchanged. This prolonged anesthesia may be due to a general inhibition of hepatic detoxifying enzymes or could be due to a loss of activity in some cells with maintenance of activity in others. Sell *et al.* (1972) have concluded that the action of DDT in reducing hepatic microsomal enzyme action in the bird is one of competitive inhibition.

There have been no studies on the effect of pesticides on pentobarbital metabolizing enzymes in birds. Definitive proof may be difficult to obtain through enzyme analysis because of dilution of the tissue and the inhibitor.

The definitive effects of pesticides on avian liver microsomal enzymes in general have yet to be described and the literature presents conflicting findings. Although Stephen and coworkers (1971) found an increase in sleeping time in chickens, they were unable to show any consistent change in liver microsomal enzyme activity. Contrary to finding a decrease in liver microsomal activity, Abou-Donia and Menzel (1968) found that liver microsomal oxidative activity was increased in chicks hatched from eggs which had been injected with DDT. Peakall (1967, 1970) observed an increase in the rate of steroid metabolism by hepatic microsomes of pigeons or doves treated with DDT, also suggesting a stimulation of liver microsomal activity. However, reduction in liver enzyme activity has also been reported. Sell *et al.* (1971) found reduced hepatic hydroxylase activity after feeding DDT to White Leghorn hens or Japanese quail (Sell *et al.*, 1972). Gillett and Arscott (1969) also found hepatic microsomal epoxidase activity markedly depressed in quail fed DDT. These depressions of hepatic enzyme activity are in accord with the prolonged sleeping times we observed in quail fed DDT.

Although lengthened sleeping time is an indication of reduced activity of liver microsomal enzymes which detoxify pentobarbital, it is misleading to imply that pesticides which lengthen sleeping time decrease "liver microsomal enzymes." It would be unusual and unexpected if all liver microsomal enzymes responded to pesticides in the same manner. Avoidance of the simplistic use of "liver microsomal enzymes" as a single class, all reacting in an identical fashion, and consideration of specific microsomal enzymes will provide a greater understanding of the biological effects of pesticides.

LITERATURE CITED

Abou-Donia, M. B., Menzel, D. B., *Biochemistry* **7**, 3788 (1968).
Bitman, J., Cecil, H. C., Harris, S. J., Fries, G. F., *J. Agr. Food Chem.* **19**, 333 (1971)
Bush, M. T., *Physiol. Pharmacol.* **1**, 206 (1963).
Gillett, J. W., Arscott, G. H., *Comp. Biochem. Physiol.* **30**, 589 (1969).
Hart, L. G., Fouts, J. R., *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **249**, 486 (1965).
Peakall, D. B., *Nature (London)* **216**, 505 (1967).
Peakall, D. B., *Science* **168**, 592 (1970).
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**, 58 (1971).
Sell, J. L., Davison, K. L., Poonacha, K. B., *J. Agr. Food Chem.* **20**(3), 553 (1972).
Stephen, B. J., Garlich, J. D., Guthrie, F. E., *Bull. Environ. Contam. Toxicol.* **5**, 569 (1971).
Tucker, R. K., Crabtree, D. G., U. S. Department of the Interior, Fish and Wildlife Service, Resource Publication No. 84, 1970.

Received for review July 25, 1972. Accepted October 10, 1972.

Long-Term Studies of Residue Retention and Excretion by Cows Fed a Polychlorinated Biphenyl (Aroclor 1254)

G. F. Fries,* G. S. Marrow, Jr., and C. H. Gordon

Nine cows were fed 200 mg per day of Aroclor 1254 (PCB) for 60 days. Milk and body fat samples were obtained during and for 60 days following feeding. Concentrations of PCB in milk fat approached equilibrium after 40 days. The average concentration in milk from 40 to 60 days was 60.9 ± 4.5 μg/g fat. Concentration in body fat was 41.7 ● 11.5 μg/g at 60 days. When feeding stopped, concentration in milk fat declined 50% within 15 days. After 15 days the rate of the first-order decline in concentration was much less. The average rate constant was 0.010 day^{-1} and varied among cows from 0.005 to 0.016 day^{-1}. The variation could not be related to such parameters as milk fat production or body weight change. Decline in concentration of PCB in body fat paralleled decline in concentration of PCB in milk fat.

Considerable interest has developed in polychlorinated biphenyls (PCB), a class of industrial organochlorine compounds. After many years of use, residues of PCB's have recently been found distributed widely in environmental and food samples (Kolbye 1972; Peakall and Lincer, 1970). The most extensive uses of PCB's have been as dielectrics and plasticizers (Nisbet and Sarofim, 1972). However, the minor use of the PCB Aroclor 1254 in a silo sealant poses a significant source of PCB contamination of milk.

Aroclor 1254 is a commercial mixture of PCB's containing an average 54% chlorine, with the major components ranging from tetrachlorobiphenyl to heptachlorobiphenyl (Sissons and Welti, 1971). A commercial sealant applied to the interiors of concrete stave silos contained about 15% Aroclor 1254. This source has caused PCB residues in milk exceeding the U. S. Food and Drug Administration guideline of 5 μg/g in milk fat (Kolbye, 1972).

Agricultural Environmental Quality Institute, Agricultural Research Center, Agricultural Research Service, Beltsville, Maryland 20705.

Residues of PCB in silage occur at high levels adjacent to the treated walls (Skrentny *et al.*, 1971). There is some migration of the residue in the silage, but residues seldom occur beyond 3 ft from the wall. Gas chromatographic examination of the residues from silage suggest little, if any, microbial or chemical change in the composition of Aroclor 1254 (Fries, 1972; Skrentny *et al.*, 1971).

We have compared the behavior of PCB and DDE residues in milk after environmental exposure (Fries *et al.*, 1972). Only the period after removal of the sources of both contaminants from the diet was studied. The rates of decline in milk concentrations of the two residues were identical. Platonow *et al.* (1971) have studied the distribution of residues in the milk of two cows that received a single dose of PCB. The average excretion in milk was 5% within 4 days. We are not aware of long-term controlled studies on the relationship of dietary intake of PCB to residue accumulation and excretion in the cow.

This study was carried out to determine milk and body fat residues while cows consumed a fixed level of Aroclor 1254 and to determine the rate of decline of milk and body fat residue levels after removing PCB from the diet.