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A COMPARISON BETWEEN INTUBATION AND FOOD ADDITION AS ROUTES OF ORAL EXPOSURE FOR NORTHERN BOBWHITES TO DDT INSECTICIDE

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ABSTRACT: Our objective was to compare two methods of oral dosing of p,p'-DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane) on uptake of DDT metabolites and isomers (i.e., p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD) in livers and brains. p,p'-DDT was administered to northern bobwhite (*Colinus virginianus*) by intubation with corn oil or as a feed additive for 56 days. When adjusted for amount of DDT consumed, total DDT (ΣDDT, the summation of all DDT metabolites and isomers) and p,p'-DDE concentrations differed significantly ($P < 0.10$) in both brains and livers, whereas p,p'-DDD differed only in brains and p,p'-DDT differed only in livers. Paired comparisons between brains and livers differed significantly for ΣDDT, ($P < 0.05$), p,p'-DDE ($P < 0.05$) and p,p'-DDT ($P < 0.1$) for both intubated and food-dosed treatment groups, whereas p,p'-DDD ($P < 0.05$) differed only in the intubated group. We concluded that method of oral exposure affected the uptake of DDT in livers and brains for northern bobwhites.

Key words: *Colinus virginianus*, contaminant uptake, DDT, oral exposure, intubation, food addition, organochlorine pesticides, comparative toxicology.

INTRODUCTION

Two commonly used techniques to administer a toxicant orally are food addition and intubation. Each method has certain advantages in experimental situations. Food addition eliminates confounding effects of a carrier, better simulates contamination of an animal's food source, is more continuous because the animal will receive a comparable dose every time it eats, and causes less stress associated with handling. In comparison, intubation allows greater precision in dosing, allows oral administration of less palatable chemicals, and minimizes worker exposure to chemicals during mixing, daily feeding, and clean-up of unused food. Also, peak concentrations in blood or at target organs resulting from bolus doses are more likely to exceed an animal's ability to detoxify a substance than the sustained lower levels resulting from dosage in the diet (Nutrition Foundation, 1983).

This study uses northern bobwhite (*Colinus virginianus*) females to compare effects on tissue retention of food addition and corn oil intubation as oral administration techniques for the toxicant, p,p'-DDT [1, 1, 1- trichloro- 2, 2- bis (p- chlorophenyl)ethane; Sigma Chemical Co., St. Louis, Missouri 63178, USA]. Only females were used because other work in our laboratory was focussing on effects of contamination on female reproduction.

METHODS

Female northern bobwhites were purchased in mid-April 1987 from a commercial breeder. They were used in a behavioral study of food choices and kept in an open-air polebarn on the campus of Virginia Polytechnic Institute and State University (Blacksburg, Virginia 24061, USA) before use in the present study. They were brought inside in early October 1987 and acclimated to their surroundings for 3 wk before the start of the experiment. Bobwhites were housed individually in galvanized steel cages measuring 51 × 56 × 35 cm and maintained at approximately 21 C with a 9L:15D light cycle.

Thirty non-laying bobwhites were randomly assigned to three groups of 10 bobwhites each. A control group (C) received no DDT. A second group (DDT-F) received food treated with DDT dissolved in acetone at a concentration of 25 mg DDT/kg food. The third group (DDT-I) received intubated corn oil containing 6622 mg DDT/ml corn oil. Food for C and DDT-I bobwhites was treated with comparable amounts of acetone. We assumed acetone treatment did not affect food intake. All bobwhites were given corn oil and weighed at 7-day intervals. Indi-

vidual bobwhites in all groups received corn oil via intubation in amounts adjusted for body mass. Corn oil doses were adjusted so that DDT-I group members each received a dose of 25 mg DDT/kg body mass. The C and DDT-F bobwhites received equivalent amounts of corn oil on a body mass basis. Food and water were provided ad libitum. Amount of food provided and orts were weighed daily. Spillage was similar among bobwhites and ignored. The study lasted 56 days with eight dosing periods.

All bobwhites were killed on day 56 by cervical dislocation. Carcasses were refrigerated overnight at 4 C, and livers and brains were removed the next day. The tissues were freeze-dried and weighed. Fat, with associated DDT and metabolites, was extracted from the liver and brain samples with petroleum ether, using a Soxhlet apparatus (Pyrex®, Fisher Scientific Co., Norcross, Georgia 30091, USA). Samples were extracted for 8 hr. Mean recoveries, based on internal spikes using Aldrin, were 96.7% for brain and 101.8% for liver tissues. Sample clean-up, using activated florisil columns (U.S. Silica Company, Berkeley Springs, West Virginia 25411, USA), was performed with 150 ml 6% ethyl ether-petroleum ether.

Concentrations of o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, and p,p'-DDT were analyzed on a Tracor 540® gas chromatograph (Tracor Corporation, Austin, Texas 78721, USA), equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, and a 1.83 m × 4 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport® (Supelco, Inc., Bellefonte, Pennsylvania 16823, USA). All samples were verified on either a Hewlett Packard 5820A® gas chromatograph (Hewlett Packard, Greensboro, North Carolina 27420, USA), equipped with a Ni_{63} electron capture detector, using a carrier gas of 95% argon-5% methane, and a 2.44 m × 6.4 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport®, or on a Tracor 550® gas chromatograph, equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, and a 1.83 m × 4 mm i.d. glass column packed with 3% OV-1 on 80/100 mesh Supelcoport®. Limits of detection for each isomer in either brain or liver tissue were determined as the smallest concentration of that isomer measured in that tissue. Liver tissue had smaller limits of detection for each isomer because more tissue was available for analysis. In determining total DDT (ΣDDT), all isomers and metabolites were added; isomer values below the limit of detection were assigned midpoint values between zero and the limit of detection. For comparisons between tissues or between treatment groups, the program UNCENSOR V2.0m (Newman et al., 1989) was used to generate means and standard errors when some samples contained values for some metabolites or isomers below the limit of detection.

Statistical analyses were performed using the SAS® statistical package (SAS Institute, Inc., 1985a, b). General linear modelling (SAS, PROC GLM) was used to analyze the amount of food eaten, and for differences in body masses among dose groups and over time. Least-squares mean analyses were performed to determine which days were significantly different from others for both body mass and food consumption. Data were tested for normality using the Shapiro-Wilk statistic (SAS, PROC UNIVARIATE). Comparisons for each isomer in livers and brains within each treatment group were made using paired t-tests. Comparisons between DDT-F and DDT-I groups for adjusted residue concentrations were performed using Student's t-tests. Unless otherwise noted, statistical significance was chosen at the level of $\alpha = 0.1$.

During a previous behavioral study, food intake for these bobwhites was approximately 19 g/day under natural light conditions in May and June (Stinson, 1989). Food consumption of < 16 g/day, possibly influenced by the 9 h day-length, and decreasing food intake during the first part of the experiment led to lower DDT exposure in the DDT-F group than anticipated. To account for this, adjusted residue concentrations were calculated by dividing concentrations found in brain or liver (μg/g) by the total amount of DDT (μg) which the individual received. DDE/DDT ratios were calculated by dividing the concentrations of p,p'-DDE by the sum of p,p'-DDD plus p,p'-DDT (used because p,p'-DDT is known to convert to p,p'-DDD under anoxic conditions as cold as −20 C (Walker and Jefferies, 1978)). ΣDDT is the summation of all DDT isomers and metabolites measured in the samples.

RESULTS

Two DDT-F bobwhites died from unknown causes during the experiment. They had no overt signs of DDT poisoning, such as tremors, and their food did not contain unusual amounts of DDT. Because they did not complete the experiment, data relevant to them were dropped from all statistical analyses.

Body masses differed significantly among groups throughout the entire experiment ($P = 0.0452$). However, initial masses (Day 0 of Fig. 1) of bobwhites randomly assigned to the three dose groups

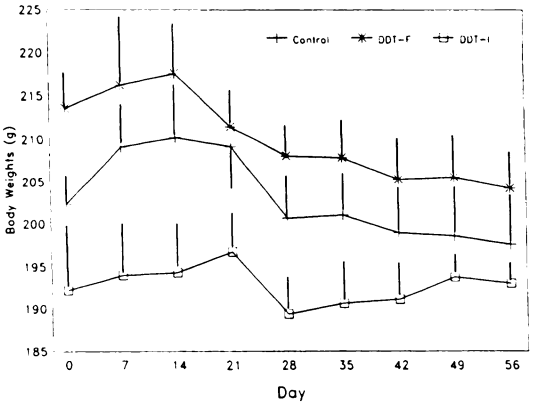

FIGURE 1. Mean body masses of bobwhites receiving no DDT (Controls), or 25 ppm DDT in food (DDT-F) or intubated in corn oil (DDT-I) measured every 7 days.

were marginally significantly different ($P = 0.0725$). When initial body masses were used as covariants, body masses no longer were found to differ among dose groups ($P = 0.5356$). Thus it appears that differences in body mass resulted from chance, rather than from a treatment.

When body masses were analyzed through time, a significant effect for daily body mass was noted ($P < 0.0001$). Least squares means analyses within general linear modelling showed that masses from Days 7, 14, and 21 differed significantly from masses on Days 28, 35, 42, 49, and 56. No other combination of days showed significance.

The amount of food consumed by bobwhites (Fig. 2) did not differ significantly among dose groups ($P = 0.1267$). The amount of food consumed did change through time ($P < 0.0001$). Least squares means analyses found food consumption differed among days. Days 28, 35, and 42 were significantly different from the greatest number of other days (≥50 days). Bobwhites ate less on these days presumably because they received corn oil on these days.

Brain concentrations of DDT isomers and metabolites did not differ significantly from liver concentrations in the C bobwhites (Table 1). In DDT-F bobwhites, ΣDDT ($P < 0.0001$), p,p'-DDE ($P =$

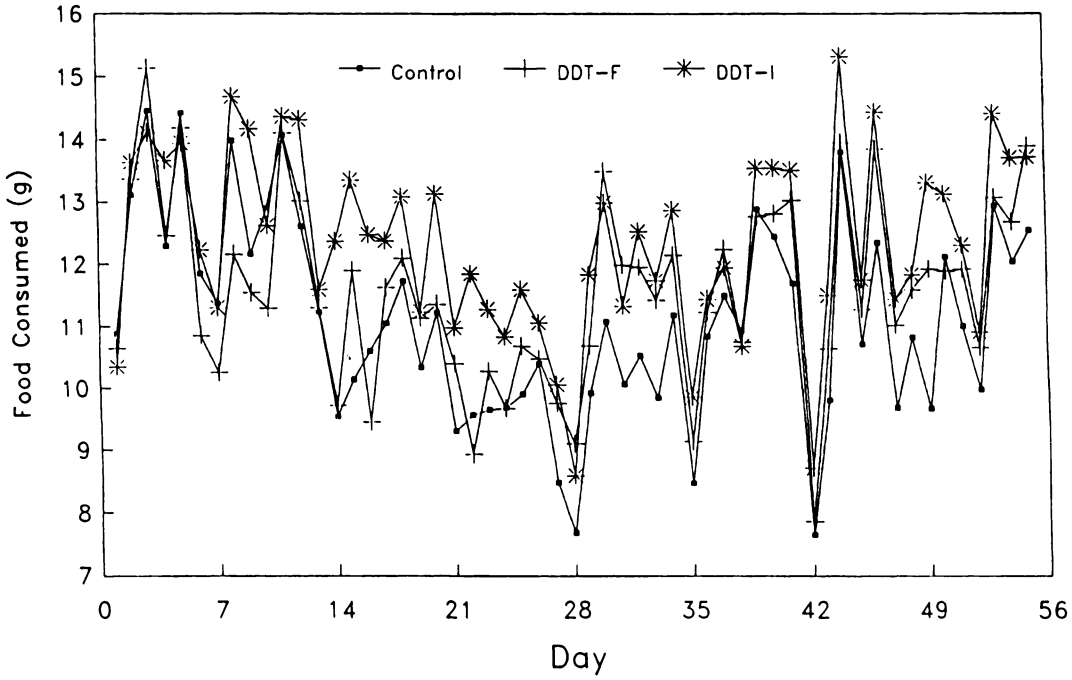

FIGURE 2. Mean daily food consumption of bobwhite receiving no DDT (Control), or 25 ppm DDT in food (DDT-F) or intubated in corn oil (DDT-I).

TABLE 1. Concentrations (μg DDT/g tissue) of DDT isomers and metabolites found in brains and livers of northern bobwhite receiving no DDT (C) or 25 ppm DDT as a food additive (DDT-F) or in corn oil (DDT-I). Results of paired t-tests comparing concentrations of each metabolite measured in both the brain and liver of the same individual are reported.

		Brain			Liver				
Compound	Treatment	n[a]	Mean	SE	n	Mean	SE	t	P
ΣDDT	C		ND[b]	0	10	0.8384	0.0753		
	DDT-F	8	2.6498	0.1744	8	10.6011	0.9094	9.45	<0.0001
	DDT-I	9	14.7329	1.8998	8	33.4725	2.9662	7.05	<0.0001
o,p'-DDE	C		ND	0		ND	0		
	DDT-F		ND	0	7	0.3481	0.0162		
	DDT-I	1	0.3822	0	9	0.8836	0.2094		
o,p'-DDT	C		ND	0		ND	0		
	DDT-F		ND	0	1	0.3623	0		
	DDT-I		ND	0		ND	0		
p,p'-DDD	C		ND	0	1	0.0053	0		
	DDT-F	2	0.2045	0.0058	8	1.8418	0.1104	4.13	0.1513
	DDT-I	9	0.8339	0.1878	10	6.2249	1.0201	5.25	0.0008
p,p'-DDE	C		ND	0	10	0.5164	0.0350		
	DDT-F	8	1.5430	0.1329	8	7.5087	0.9638	6.82	0.0002
	DDT-I	9	10.9421	1.4007	10	23.3799	1.8544	6.65	0.0002
p,p'-DDT	C		ND	0	1	0.5138	0		
	DDT-F	7	1.2949	0.0887	6	0.9628	0.1505	−2.48	0.0679
	DDT-I	9	4.7825	0.9974	9	3.2101	0.6666	−2.47	0.0388

[a] n = number of samples containing measurable amounts of the compound.
[b] ND = none detected.

0.0002), and p,p'-DDT (P = 0.0679) concentrations all differed significantly between brains and livers. Concentrations in brains and livers of the DDT-I bobwhites differed significantly for ΣDDT (P < 0.0001), p,p'-DDD (P = 0.0008), p,p'-DDE (P = 0.0002), and p,p'-DDT (P = 0.0388). When measurable amounts were found, the liver contained greater concentrations of DDT metabolites and isomers, except for p,p'-DDT, than the brain from the same individual.

Significant differences occurred between DDT-treatment methods for adjusted brain concentrations (i.e., concentration measured in the brain/total amount of p,p'-DDT received during the experiment) (Table 2) for ΣDDT (P = 0.0022),p,-p'-DDD (P = 0.0125), and p,p-DDE (P = 0.0016). Significant differences of adjusted liver concentrations (Table 2) were found for ΣDDT (P = 0.0191), p,p'-DDE (P = 0.0931), and p,p'-DDT (P = 0.0588).

DDE/DDT ratios from brains were significantly different (P = 0.0055) between DDT-F and DDT-I bobwhites (Table 3), but ratios from livers showed no difference. Comparisons of DDE/DDT ratios between brain and liver tissues showed that tissues from DDT-F bobwhites (P = 0.0017) and DDT-I bobwhites (P = 0.0188) were significantly different.

DISCUSSION

The amount of food consumed was not altered by addition of DDT to the food indicating little or no taste aversion. However, handling the bobwhites and/or giving them corn oil via intubation temporarily affected the amount of food consumed for all groups on some days when corn oil was given. Significantly less food was consumed on Days 28, 35, and 42 when oil was given, subsequently followed by increased food consumption (Fig. 2). Day 28, the first day that showed a severe reduction in food consumption, was also the

TABLE 2. Adjusted (concentration in organ/total amount fed or dosed) DDT isomers and metabolites found in brains and livers of northern bobwhite receiving 25 ppm DDT as a food additive (DDT-F) or in corn oil (DDT-I). The results of Student's *t*-tests comparing DDT-F and DDT-I groups within either brains or livers are reported.

Compound	Treatment	Brain				Liver			
		Mean	SE	*t*	*P*	Mean	SE	*t*	*P*
ΣDDT	DDT-F	0.0001610	0.0000110	4.306	0.0022	0.0006314	0.0000580	2.605	0.0191
	DDT-I	0.0003991	0.0000542			0.0008805	0.0000716		
o,p'-DDE	DDT-F	ND*	0			0.0000228	0.0000001	0.640	0.7309
	DDT-I	0.0000101	0			0.0000190	0.0000053		
o,p'-DDT	DDT-F	ND	0			0.0000288	0		
	DDT-I	ND	0			ND	0		
p,p'-DDD	DDT-F	0.0000011	0.0000003	2.686	0.0125	0.0001203	0.0001151	1.228	0.1253
	DDT-I	0.0000046	0.0000012			0.0001552	0.0000236		
p,p'-DDE	DDT-F	0.0000213	0.0000019	3.962	0.0016	0.0004877	0.0000692	1.431	0.0931
	DDT-I	0.0006206	0.0000095			0.0006039	0.0000472		
p,p'-DDT	DDT-F	0.0000121	0.0000009	1.118	0.1463	0.0000403	0.0000153	1.730	0.0588
	DDT-I	0.0000158	0.0000030			0.0000810	0.0000182		

* ND = none detected.

beginning of the period of significantly lower body masses. Food consumption after Day 28 was more erratic than early in the study. The change in feeding behavior is the likely cause of the reduction in body masses.

To simulate continual exposure to a contaminant, the chemical must be administered more often than once weekly. In this study, all bobwhites were handled similarly each week when given corn oil. Even with such an intermittent dosing regime, handling and/or administration of corn oil disrupted eating patterns and led to body mass loss in all treatment groups including controls, possibly confounding potential effects of interest caused by the contaminant. Reducing the amount of corn oil or using a different carrier (i.e., non-nutritional carrier) may alleviate this potential problem. If the problem is related to handling, fewer doses would be indicated, but fewer doses would constrain experimental design and testing protocols by making them more artificial.

As p,p'-DDE is the primary metabolite, it reflects "background" DDT exposure not related to treatment. Only p,p'-DDE was found in detectable amounts in C bobwhite brains. In DDT-treated groups (DDT-F and DDT-I), the liver had higher concentrations of DDT isomers and metabolites after adjustments for amount of exposure. p,p'-DDT was an exception. From this, it appears that when exposure is relatively high, brains may have a greater tendency to store p,p'-DDT than the liver which has a greater ability to metabolize p,p'-DDT. Also, because livers of some C bobwhites had detectable concentra-

TABLE 3. DDE/DDT ratios found in both brains and livers of bobwhite quail receiving 25 ppm DDT in corn oil (DDT-I) or as a food additive (DDT-F). Results of Student's *t*-tests between treatment groups and paired *t*-tests between tissues are reported.

Treatment	Brain				Liver			
	Mean	SE	*t*	*P*	Mean	SE	*t*	*P*
DDT-F	0.6904*	0.1333	3.6004	0.0055	2.4256	0.4418	1.2407	0.2326
DDT-I	2.4448*	0.4696			3.5610	0.8014		

* Indicates a significant difference ($\alpha = 0.05$) between mean ratios of livers and brains for DDE/DDT ratios with that treatment group using paired *t*-tests.

tions of p,p'-DDD, p,p'-DDE, and p,p'-DDT, it appears that under normal conditions and low exposure, the liver is capable of metabolizing most DDT before it reaches the brain.

Methods of dosing did not change the distribution between tissues (i.e., the tissue with a greater concentration for the DDT-F bobwhites also had a higher concentration in DDT-I bobwhites), but did affect the concentrations found. Because adjusted ΣDDT values were greater for DDT-I bobwhite in both brains and livers, but adjusted p,p'-DDT concentrations did not differ between treatment groups in brain tissue, it would appear that the technique used to expose the bobwhite to DDT affected the amount absorbed through the gut wall but did not affect the amount passing through the liver, into systemic circulation, and being absorbed by the brain.

DDE/DDT ratios, a method to compare rates of metabolism, suggest that no difference existed in the rate of metabolism in livers between the techniques but a difference did exist in brains. This, at least partially, may be an artifact of the experimental design. The DDT-I bobwhites had not received any DDT for 7 days when killed whereas the DDT-F bobwhites were continually exposed to treated food until killed. Smaller differences in the DDE/DDT ratio between tissues for DDT-I bobwhites suggests that concentrations in the body had equilibrated, but because the DDT-F bobwhites were continually dosed until the end of the experiment, they had not yet been able to equilibrate between the brains and livers. This could be avoided in future studies by withdrawing treated food from the DDT-F group at the time of the last intubation for the DDT-I group.

CONCLUSIONS

When designing experiments that require oral dosing of a toxicant, food addition and oil intubation should not be considered equivalent. Greater quantities of DDT administered via corn oil intubation were measured in brains and livers. Intubation of oil and/or associated handling disrupted feeding patterns and may have been the cause of body mass losses. DDE/DDT ratios indicated that a period of contaminant withdrawal will allow equilibration among tissues within a bobwhite's body.

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