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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY
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Dear Candy:

I have completed the laboratory study we initiated last winter to determine if dimethoate exposure of pheasants results in detectable tissue residues. As you recall, this was designed as a verification study after we detected what appeared to be dimethoate in tissues of pheasants harvested by Don Wilt in Umatilla County. I first conducted an experiment using bobwhite quail from our in-house colony to be sure that our analytical techniques were correct and as a range-finder to decide what doses and time-frame to use for the pheasant study. The quail study was followed by a definitive test using 40 hen pheasants procured by the ODF&W from a pheasant farm near The Dalles.

Quail were fed dimethoate-treated feed at either 0, 20, 100, or 500 ppm. Pheasants were fed 0, 100, or 500 ppm treated feed. Treated-feed was presented for 5 days. Quail were killed at 6 hr, 24 hr, 3 days, or 5 days posttreatment and pheasants were killed at 0 hr, 6 hr, 24 hr, and 7 days posttreatment. Liver and kidney tissues were analyzed for dimethoate residues by gas chromatography. Brain cholinesterase activity was assayed in all birds to verify that exposure had occurred. Food consumption also was measured. In addition, Don Wilt collected 7 pheasants during November to January from areas where he knew that dimethoate had been applied within a week to 10 days of collection and 5 birds from nontreated areas. Tissue residue analysis and brain cholinesterase activity were performed on these birds as well.

No dimethoate residues were found in any tissue from any of the laboratory of field-collected birds. We did find a peak on our chromatograms analogous to the peak seen from last summer's field-collected birds. Although this peak appeared in the same place on the chromatogram as a peak generated by dimethoate, its shape was unusual, prompting us to do some further exploration of the cause of the peak. Using different techniques on the Gas Chromatograph, we determined that the peak definitely was not caused by dimethoate or its metabolites. Additionally, peaks were found in tissues from both treated and untreated birds (both quail and pheasant) and the size of the peaks had no relationship to the amount of dimethoate consumed by the birds. At this time,

we do not know what is causing these peaks. We have submitted samples for analysis on a GC-mass spectrometer which should help us identify what is causing the anomaly, but have not yet received the results.

Brain cholinesterase activity of pheasants collected from the Pendleton area in November to January are shown in table 1.

Table I. Brain Cholinesterase Activity from Field-Collected Pheasants (Nov. 1989 - Jan. 1990)

TREATED FIELDS		UNTREATED FIELDS	
BIRD NUMBER	AChE[1]	BIRD NUMBER	AChE
1F	4.6	3F	4.8
2F	5.0	4F	3.8
6F	4.4	5F	4.8
7F	4.8	9F	4.0
8F	4.8	10F	5.4
11F	---		
12F	5.4		
MEAN	4.8 (0.2)[2]	MEAN	4.6 (0.3)

[1] Cholinesterase activity expressed as umole of substrate hydrolyzed per minute per gram of tissue.

[2] standard error of the mean.

It is obvious from these data that the pheasants collected from areas where dimethoate was sprayed do not have depressed cholinesterase activity compared to controls collected from the field. Table 2 shows brain cholinesterase activity values of the captive pheasants. The values of the control birds (0ppm) are slightly higher than the values of the field collected birds. This likely is due to difficulty in freezing the brains of field-collected birds at -70 C as quickly as we were able to for our laboratory birds. The data in Table 2 also show that brain cholinesterase activity remained depressed following dimethoate feeding for at least 7 days after starting on clean feed. Table 3 shows similar data for the bobwhite quail study.

In summary, these laboratory studies failed to confirm our suspicions from last summer that the pheasants in the Pendleton area are being exposed to dimethoate. The peaks seen on our

Table II. Brain Cholinesterase Activity of Pheasants Following Exposure to Dimethoate-treated Feed.[1]

DIMETHOATE	TIME POST TREATMENT			
(ppm)	0 HR	6 HR	1 DAY	7 DAYS
0[2,3]	5.3 (0.2)[4]	5.3 (0.1)	5.0 (0.2)	--
100	4.7 (0.3)	4.6 (0.1)	4.6 (0.1)	--
500	4.4 (0.3)	4.4 (0.2)	4.2 (0.2)	4.8 (0.2)

[1] Cholinesterase activity expressed as umoles of substrate hydrolyzed per minute per gram of tissue.

[2] Control (0 ppm) values significantly different than treated values (100 and 500 ppm) (P=0.0001). No difference due to time within each treatment.

[3] N=4 for all groups except 500 ppm at 7 days where N=3.

[4] Numbers in parentheses represent standard error of the mean

Table III. Brain Cholinesterase Activity in Bobwhite Quail Following Exposure to Dimethoate Treated Feed.[1]

DIMETHOATE		TIME POST TREATMENT			
(ppm)	N	6 HR	1 DAY	3 DAYS	5 DAYS
0	2	6.4 (0.6)[2]	5.8 (0.2)	5.4 (0.1)	5.7 (1.2)
20	3	5.6 (0.1)	5.6 (0.2)	6.0 (0.1)	6.2 (0.3)
100	3	4.1 (0.2)[4]	5.2 (0.2)	4.8 (0.2)	5.3 (0.3)
500	-[3]	3.6 (0.3)[4]	3.4 (0.8)[4]	5.0 (0.4)	5.4 (-)

[1] Cholinesterase activity expressed as umole substrate hydrolyzed per minute per gram of tissue.

[2] Standard error of the mean.

[3] N=3 for 6 HR, N=4 for 1 DAY, N=2 for 3 DAYS, N=1 for 5 DAYS

[4] Cholinesterase activity significantly different from control values (P<0.05).

chromatograms last summer were the same anomalies that we saw in our intensive laboratory studies. Brain cholinesterase activity patterns of birds collected from areas of known dimethoate treatment in the late fall and winter failed to substantiate significant exposure.

The most signficant result of this study is that we discovered that dimethoate was being used throughout the year in the Pendleton area, including winter and early spring. This differs from the usual use pattern of late spring and early summer and is related to the growing of winter wheat crops in the area. Don Wilt reports that the intense spraying regime probably will be discontinued as the farmers now believe that they have gotten the upper hand on the Russian wheat aphid problem.

Recommendations: The results of this year's work have shown that we have no evidence that exposure to environmental contaminants caused the decline of the pheasant population in the Pendleton area. There may be some contaminant that we did not analyze for (e.g., heavy metals) or an exposure that we were unable to detect that is causing a reproductive problem. However, I think that, at this point, we should examine other possible causes. I am sure that John Crawford could speak to the many possibilities of habitat insufficiency or the effects of the wet/drought cycles of the past decade. There are, of course, many infectious or parasitic diseases that can cause infertility. It is my recommendation that, if there is still interest in pursuing the cause of the decline of this population, we hold another meeting to discuss all the possibilities and what it would take in terms of time and resources to address the problem from a holistic point of view.

Please feel free to give me a call to discuss the results I presented here. I hope to find out soon what the "mystery peak" is and write a brief manuscript so other analytical chemists will not be led astray in a similar fashion when analyzing bird tissues for organophosphate compounds or their metabolites. I enjoyed to opportunity to work on this puzzle of pheasant recruitment with you and am only sorry that I was not able to find a simple, easy solution to the problem.

Sincerely,

Anne Fairbrother

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