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Orchard Dormant Sprays and Exposure of Red-Tailed Hawks to Organophosphates

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A pilot study of raptors live-trapped in the Central Valley of California suggested that they were being exposed to organophosphate insecticides (OPs) in winter dormant-season sprays (Hooper **et al.**,1989). Eight of 12 raptors trapped in the vicinity of orchards in Butte County had low blood cholinesterase (ChE); 4 responded to oxime reactivation, and OP metabolites were found in their excreta. This paper presents preliminary results of an enlarged study from a large number of hawks live-trapped in the Sacramento and San Joaquin Valleys of California during the winter of 1987-88. ChE levels of birds from rehabilitation centers and from hawks kept at the University of California, Davis (UCD) Raptor Center are also reported.

MATERIALS AND METHODS

Hawks perching within and adjacent to orchards were trapped using a Bal Chatri noose trap (Berger and Mueller, 1953). More than 100 birds were trapped in Butte County ("Northern Region"). Several dozen more were trapped in Stanislaus, Merced and Kern counties ("Central Region"). Most were Red-tailed Hawks (*Buteo jamaicensis*); also studied were Red-shouldered Hawks (*Buteo lineatus*), American Kestrels (*Falco sparverius*), and a Western Bluebird (*Sialia mexicana*). Birds were restrained, body measurements taken, blood drawn into heparinized tubes, feet were washed with alcohol and the samples stored on ice. Feather clippings were taken, US Fish and Wildlife leg bands were fitted, and the birds released. In the Northern Region, birds were placed in teflon-lined cages for several hours to collect excreta. Location of capture, weight, tail length, other body characteristics, proximity to orchards (within or greater than 1/4 mile) and approximate age were recorded.

Assays were generally those given in Hooper **et al.** (1989). ChE activities were usually determined within 24 hours by the method of Ellman **et al.** (1961) modified for a microplate reader (Biotek, Winooski, VT), a final reaction volume of 250 µl and 5mM acetylthiocholine (ACTC). Results are expressed in µmoles

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ACTC hydrolyzed/min/ml plasma or μmoles/min/gram wet weight of brain. Serum acetylcholinesterase (AChE) was determined by preincubating samples for 5 minutes with 0.1 mM isoOMPA (tetraisopropylpyrophosphoramide), to inhibit non-specific cholinesterases. Values more than 2 standard deviations below those of apparently normal wild birds were considered indicating exposure to OPs (Hill, 1988). Reactivatability of OP-inhibited ChEs was tested by incubating duplicate samples for 40 minutes at 38 °C with and without 0.1 mM 2-pralidoxime (2-PAM, final assay concentration of 12 μM). Corrections were made for absorbance changes induced by 2-PAM. Increases after oxime treatment greater than 5 percent and differing significantly by $P<0.05$ (Student's t-test) indicated the presence of OP-inhibited ChEs. Excretory OP metabolites were analyzed according to Weisskopf **et al.** (1988) on a Hewlett-Packard 5890 GLC fitted with a 30 m DB-1701 megabore column operated at 130 °C, injector held at 300 °C, flow rate of 11 ml/min and a flame photometric detector in the phosphorus mode at 250 °C. Alkyl phosphate metabolites were propylated on-column with tetra-propylammonium hydroxide (Aldrich Chemical Co.). Specific residues determined were diethyl- and dimethyl-dithiophosphates, diethyl- and dimethyl-thiophosphates and diethyl- and dimethyl-phosphates. The dimethylphosphates were those expected from the methidathion and the diethyl products from the ethyl parathion, diazinon and chlorpyriphos used in the orchards. Twenty pooled feather clippings from each bird were extracted in methylene chloride, the solvent evaporated, the sample resuspended in iso-octane and the residues analyzed on a Varian 3700 GLC with a DB-1 Megabore column and a thermionic detector by California Department of Fish and Game. OPs from foot washes were determined by evaporating the ethanol solvent, suspending the sample in ethyl acetate, and analyzing it on a Hewlett Packard 5890 GLC with a megabore DB-1701 column

Table 1. Plasma ChE of Red-tailed hawks

Captive Hawks From UCD Raptor Center		
	Mean	Range
ChE	0.892 ± 0.180	0.659 - 1.22
AChE	0.324 ± 0.065	0.236 - 0.405
N=8		
Hawks Trapped In November and December Northern Region		
	Mean	Range
ChE	0.769 ± 0.160	0.471 - 1.22
AChE	0.210 ± 0.066	0.111 - 0.401
N=48		

μmoles/min/ml. Means ± standard deviations. N is sample number. Wild birds trapped between 11-8-87 and 12-29-87.

and a flame photometric detector. Blood and excreta were obtained from hawks showing signs of OP poisoning brought to the Bidwell Nature Center, Chico, CA. Brain samples were taken from those that died.

RESULTS AND DISCUSSION

Study of more than 150 Red-tailed Hawks and other birds demonstrated that exposure to OPs occurred during the winter dormant spray season. Total ChE levels for 8 hawks from the UCD Raptor Center were similar to those found in 48 Red-tailed Hawks sampled before or shortly after spraying began in the Northern Region. However, AChE levels in field-sampled birds were 65% the levels of Raptor Center birds (Table 1). Nine (ChE) and 10 (AChE) of the 13 Red-tailed Hawk samples from rehabilitation centers either had ChE activities 2 standard deviations below field controls, were reactivated by 2-PAM, or both (Table 2).

Table 2. Plasma ChE of hawks from rehabilitation centers in northern and central regions

	Activity	Range	Percent		
			Reactivated	Depressed	Inhibited
ChE	0.345 ± 0.210	0.058 - 0.663	31	69	69
AChE	0.054 ± 0.048	0.0005 - 0.153	62	69	77

μmoles/min/ml. Samples from 13 birds. Depressed: values 2 standard deviations below the mean of field controls (birds sampled in November and December). Inhibited: samples that were reactivated, depressed or both.

Five of the 11 birds found dead in the orchards or brought moribund to the rehabilitation center had very low and reactivatable brain AChE (Table 3). Mean activities of the rest were similar to the "apparently normal" control values (19.0 ± 3.2) reported by Hill (1988).

Table 3. Brain AChE of hawks from rehabilitation centers in northern and central regions.

	Activity	Range	Reactivated
All	10.3 ± 8.5	21.0 - 1.09	5/11
High	17.4 ± 3.4	21.0 - 12.4	0/6
Low	1.75 ± 0.65	2.7 - 1.09	5/5

μmoles/min/gm. All = 11 birds, separated into high (6) and low (5).

ChE and AChE levels of 20 birds from the Northern Region sampled during the spray season (January and February) and captured more than 1/4 mile from the orchards were similar to those sampled in November and December. Only 10-

15% were considered to be inhibited (Table 4). In contrast, both mean ChE and AChE levels were statistically depressed, and approximately 1/3 of the 34 birds captured within 1/4 mile of the orchards had either depressed ChE, reactivatable enzyme or both (Table 4). Similar results were found for birds in the Central Region (Table 5). Inhibitions ranged from 27% for ChE in birds > 1/4 mile to 43% for AChE of birds < 1/4 mile from the orchards.

Table 4. Plasma ChE of red-tailed hawks in the northern region during spray season

Birds Captured < 1/4 Mile from Orchards					
	Activity	Range	Percent		
			Reactivated	Depressed	Inhibited
ChE	0.644 ± 0.24	1.06 - 0.212	27	27	38
AChE	0.164 ± 0.079	0.338 - 0.031	29	15	35
N=34					
Birds Captured > 1/4 Mile from Orchards					
	Activity	Range	Percent		
			Reactivated	Depressed	Inhibited
ChE	0.791 ± 0.22	1.27 - 0.353	5	5	10
AChE	0.194 ± 0.064	0.33 - 0.066	10	5	15
N=20					

μmoles/min/ml. Hawks trapped between 12-30-87 to 2-21-88

Foot wash samples were obtained for 10 birds perched in, 6 birds captured within 1/4 mile of, and 5 birds captured greater than 1/4 mile from the orchards in the Northern Region during the spray season (Table 6). All birds taken in, and 5/6 birds within 1/4 mile of the orchards had measureable OP residues. Only 1 bird captured more than 1/4 mile from the orchards had measureable OP residues (parathion). Up to 74 μg of OP were detected per bird; highest was parathion, followed by diazinon, methidathion and chlorpyrifos. Feather residues supported the foot wash results. Parathion was found on the feathers of 60-80% and diazinon was found on 40-60% of the birds tested.

OP metabolites were found in 60-80 % of excreta of the Red-tailed Hawks tested, and in excreta from other birds captured during the study (Table 8). Twenty-five of 27 Red-Tailed Hawks captured < 1/4 mile from orchards had a mean of 426 ± 846 ppb and 2 of 6 hawks captured > 1/4 mile from orchards had a mean of 52 ± 103 ppb OP metabolites.

A combination of OP pesticides and light oils are sprayed during the winter in orchards of the Central Valley of California to control pests such as San Jose Scale and Peach Twig Borer. Approximately 400,000 acres of almonds plus large

acreages of other fruit crops are involved. Inauguration of dormant OP sprays has been estimated to have reduced the overall year-round application of OP sprays by up to 40 percent.

Table 5. Plasma ChE of red-tailed hawks in the central region during spray season.

Birds Captured < 1/4 Mile from Orchards					
	Activity	Range	Percent		
			Reactivated	Depressed	Inhibited
ChE	0.680 ± 0.35	1.38 - 0.149	36	29	36
AChE	0.145 ± 0.085	0.287 - 0.024	43	29	43
N=14					
Birds Captured > 1/4 Mile from Orchards					
	Activity	Range	Percent		
			Reactivated	Depressed	Inhibited
ChE	0.717 ± 0.32	1.26 - 0.173	18	27	27
AChE	0.116 ± 0.059	0.211 - 0.023	27	18	27
N=11					

μmoles/min/ml Hawks trapped between 1-5-88 to 2-23-88

Table 6. Organophosphate ester residues in red-tailed hawk foot washes

Birds Captured < 1/4 Mile From Orchards N=16					
	Et-Pt	Diaz	Met	Chlpyr	Total
Mean	6030	1220	681	1420	7200
STD	16800	1470	558	—	18100
Max	68800	4840	1550	1420	74400
Min	116	177	178	—	212
Percent Positive	94	69	31	6	94
Birds Captured > 1/4 Mile From Orchards N=5 One sample positive: 61 ng of ethyl parathion					

Nanograms/bird. Et-Pt: ethyl parathion; Diaz: Diazinon; Met: methidathion, Chlpyr: Chlorpyrifos.

Table 7. Organophosphate residues and red-tailed hawk residues on feathers (ppm)

	Captured < 1/4 Mile		Captured > 1/4 Mile	
	Et-Pt	Diaz	Et-Pt	Diaz
Mean	1.97	0.66	0.62	0.37
STD	4.17	1.94	0.78	0.30
Number	28	21	8	8
Maximum	17.0	9.10	2.50	0.84
Minimum	0.12	0.10	0.13	0.11
Incidence	28/34	14/35	8/12	8/13

Intact and outside portions of 20 feathers per bird.

Table 8. Combined organophosphorus metabolites in excreta (ppb)

Sample Number	Mean	Maximum	Percent Positive
	Red-tailed Hawks		
45	356 ± 687	3030	75
	Red-shouldered Hawks		
4	217	332	50
	Kestrel		
1	30	30	100
	Western Bluebird		
1	14000	14000	100

Mean of birds positive for metabolites

Nevertheless, winter sprays drift onto non-target crops and they have been implicated in the presence of OPs in the fog above the Central Valley (Glotfelty **et al.**1987).

The results of this, and a previous report (Hooper **et al.**,1988) demonstrate that hawks (and other birds) in the vicinity of orchards are exposed to OPs in dormant sprays. (Detailed analyses of the data will be presented in a future report.) The data suggest that repeated exposures may be involved. The phenomenon of aging restricts detectable oxime reactivation of OP-enzyme complexes to hours (methyl-OPs) or at best several days (ethyl-OPs) following exposure (Wilson BW, Hooper MJ, Hansen ME, Nieberg PS (1991) Reactivation of organophosphate inhibited AChE with oximes. In Chambers JE, Levi PE (ed) Organophosphates: Chemistry,

Fate and Effect. Academic Press, In Press). In general, blood enzyme levels, excreta metabolites and feather residues are consistent with both acute and chronic exposures to OPs. The levels of exposure to OPs that constitute a danger to hawks and other protected species in orchards are not known.

Data are needed to relate exposure to OPs with physiological or morphological damage and morbidity, to establish no-effect-levels and to assess risks to wildlife. Many of the orchards studied were almonds, since they make up a large proportion of the acreage where winter-dormant spraying occurs. But hawks and other birds also frequent vineyards and other fruit and nut orchards.

The results of our studies (Hooper **et al.** 1989; Wilson BW, Hooper MJ, Littrell EE (1988) Exposure of Red-tailed Hawks to Agricultural Chemicals during Dormant Spray Season in the Central Valley of California. Report to California Department of Fish and Game) plus other observations of California Department of Fish and Game led the California Department of Food and Agriculture to call for a reevaluation of OP dormant spraying (Jones, 1989, Letter to 14 registrants of dormant sprays. October 19, 1989). Supported by a consortium coordinated by the Almond Board of California, a study is underway to examine the movement and exposure of radio-tagged hawks in a large study area and the toxicity of parathion, diazinon and methidathion to surrogate birds such as pigeons and captive-bred kestrels.

Several important factors must be considered if exposure to OPs of wild birds and other non-target species is to be reduced. These include determination of primary route(s) of exposure, and developing spray application methods that provide spray levels below no-effect levels (NOELS) to non-target species and effective pest control.

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