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Organophosphorus Insecticide Exposure in Hawks Inhabiting Orchards during Winter Dormant-spraying

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The Sacramento and San Joaquin Valleys of California contain over 400,000 acres of almond orchards sprayed during the winter dormant season with a mixture of oil and mainly one of three organophosphorus (OP) insecticides; parathion; O-O-diethyl O-p-nitrophenyl phosphorothioate, Diazinon; O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate, or methidathion; O,O-dimethyl phosphorodithioate, S-ester with 4-(mercaptomethyl)-2-methoxy- ^{2}-1,3,4-thiadiazolin-5-one) (Van Steenwyk, et al., 1983).

Reports of red-tailed Hawk (Buteo jamaicensis, RT) losses during the dormant season have been made to the California Department of Fish and Game (CDFG, Ed Littrell, pers. comm.) by wildlife rehabilitation groups in heavy almond production areas in California's Central Valley. Hawks were brought to their centers with signs of OP exposure that responded to treatment with atropine, an antidote to OP poisoning.

Evaluation of OP exposure in wild birds often requires measurement of brain (ChE) activity and OP residues from the gastro-intestinal tract (Ludke et al. 1975, Hill and Fleming 1982). Even when plasma ChE levels of living birds are used, the values must be compared to tables of "normal" values with high inherent variability. Our lab (Hooper et al. 1987) and others (Karlog and Poulson 1963, Martin et al. 1981) have applied a ChE reactivation method to determine OP exposure. Increase in plasma or brain homogenate ChE activity following in vitro 2-PAM (2-pyridinealdoxime methochloride) treatment indicates the presence of OP-inhibited ChE. An advantage of this method is it's ability to detect the presence of OP-inhibited plasma ChEs in live birds independent of absolute ChE values.

We studied OP exposure in hawks caught in and around 40,000 nearly continuous acres of almond orchards in northern Butte County during the winter dormant spray season. Blood and excreta samples were evaluated for the presence of OP-inhibited ChEs and for OP excretory metabolites, respectively.

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MATERIALS AND METHODS

Hawks were trapped opportunistically within and adjacent to almond orchards. Blood was collected from the tibialis vein of the leg. Each bird was banded with a U.S. Fish and Wildlife Service band and either released or placed in a holding cage lined with plastic to collect an excreta sample.

Blood and excreta samples from nine RTs were collected from birds housed at the Raptor Center of the School of Veterinary Medicine at the University of California, Davis (UCD), using methods identical to those in the field. These samples served as controls for blood ChE levels and for OP metabolite spiking and recovery trials in the excreta analysis.

Heparinized blood samples were centrifuged, the plasma removed and assayed for ChE activities and reactivatability within 24 hours of sampling. Plasma and tissue ChE and acetylcholinesterase (AChE) activities were determined using the method of Ellman et al. (1961) modified for use with a BioTek EL309 Microplate Reader. Acetylthiocholine iodide (AThCh, $5x10^{-4}$ M, Sigma) was the substrate in a final reaction volume of 0.250 ml. Means and standard deviations of triplicate runs were expressed in umoles AThCh hydrolyzed/min (or units)/ml plasma or /gram brain weight. AChE was determined by incubating the samples for five minutes in the presence of 10^{-5}M iso-OMPA (tetraisopropylpyrophosphoramide, Sigma; Aldridge 1953), a BChE inhibitor.

Reactivatability of OP-inhibited ChEs was tested on plasma samples and brain homogenates. Duplicate ChE-containing samples were incubated 40 minutes at 38 C in the presence and absence of 2-PAM (Sigma). Reactivation concentrations of 2-PAM were 10^{-4} M for plasma and 10^{-3} M for brain with a final assay concentration of $1.2x10^{-5}$ M for both. Pre- and post-incubation samples were assayed concurrently in triplicate. Samples containing 2-PAM were corrected for absorbance changes induced by 2-PAM. Changes in AChE and total ChE activity following 2-PAM treatment were compared to the pre-treatment values using the student's T-test (Snedecor and Cochran 1967) for single-tailed evaluation. Positive values with $p < 0.05$ were taken as a strong indication that the sample contained OP-inhibited ChE.

Analysis for excretory OP residues was performed using a modification of the method of Weisskopf et al. (in press) on a Varian 1700 gas chromatograph fitted with a DB-1701 megabore column. Alkyl phosphate residues were methylated on-column with trimethylanilinium hydroxide and detected with an alkali flame ionization detector. Operating conditions were: injector, 300°C; column, 110°C; and detector, 250°C. The specific residues measured were diethyl- and dimethyl dithiophosphate (DEDTP, DMDTP), diethyl- and dimethyl thiophosphates (DETP, DMTP) and diethyl phosphate (DEP). These compounds include the parent phosphate groups and their oxidation products from the OPs parathion, diazinon and methidathion. Minimum levels of detection

of the metabolites were 0.02 ng for DEP, and 0.01 ng for the remaining four. Correlations between blood ChE levels and excretory residue levels in the RTs were determined using Spearman's coefficient of rank correlation (Snedecor and Cochran 1967). Values from captive and wild RTs were combined for the correlation determination.

Feather parent OP levels were obtained by extracting 20 pooled feathers in methylene chloride, evaporating the solvent, resuspending the sample in iso-octane and analyzing the residues on a Varian 3700 gas chromatograph equipped with a DB-1 Megabore column. Residues were detected with a thermionic detector.

Brain, blood and excreta samples were obtained from some of the RTs brought to the Bidwell Nature Center, Chico, CA (BNC) which died or were showing ataxia and general depression.

Table 1. Red-tailed hawk plasma cholinesterase values collected during January and February 1987.

		Total ChE		AChE	
		units/min/ml			
	N	Mean	S.D.	Mean	S.D.
UCD Raptor Center Controls (Range)	9	0.790 (0.566-1.009)	0.162	0.302 (0.194-0.433)	0.078
Wild-caught (Range)	12	0.427 (0.025-0.796)	0.231	0.130 (0.007-0.204)	0.071
BNC (Range)	4/3	0.158 (0.071-0.222)	0.068	0.015 (0.000-0.027)	0.014

RESULTS AND DISCUSSION

Plasma ChE activities from captive control RTs averaged 0.790 $\pm$ 0.162 units/ml for total ChE and 0.302 $\pm$ 0.078 units/ml for AChE. Mean plasma total ChE and AChE activities from the wild RTs were 0.427 $\pm$ 0.231 and 0.130 $\pm$ .071 units/ml plasma, respectively (Table 1).

Total ChE in six, and AChE in seven of the 12 wild birds were more than two standard deviations below the levels for the captive hawks (under 59% and 48% of control, respectively). Eight of the 12 RTs had depressed values for one or both ChE activities by this definition.

Reactivation of OP-inhibited total ChE was successful in four of the 14 wild-caught birds (Table 2). Three of the four showed reactivation of AChE activity with initial values of more than two standard deviations below the mean of the controls.

Table 2. 2-PAM reactivation of total ChE and AChE from wild red-tailed hawks.

Bird	Total ChE Pre	Total ChE Post	AChE Pre	AChE Post
Wild-caught Hawks: Serum units/min/ml				
RT 1	0.599	0.587	0.238	0.212
RT 2	0.633	0.616	0.189	0.173
RT 3	0.515	0.482	0.119 a	0.112
RT 4	0.201 a	0.264 b	0.053 a	0.062 b
RT 5	0.634	0.636	0.194	0.180
RT 6	0.191 a	0.193	0.071 a	0.064
RT 7	0.796	0.745	0.119 a	0.116
RT 8	0.567	0.547	0.179	0.169
RT 9	0.025 a	0.106 b	0.008 a	0.037 b
RT 10	0.316 a	0.322 b	0.138 a	0.140
RT 11	0.267 a	0.315 b	0.058 a	0.078 b
RT 12	0.383 a	0.390	0.196	0.187
BNC Hawks: Serum units/min/ml				
RT 13	0.222 a	0.239	nd	nd
RT 14	0.201 a	0.219 b	0.019 a	0.030 b
RT 15	0.138 a	0.128	0.027 a	0.025
RT 16	0.071 a	0.179 b	0.000 a	0.040 b
BNC Hawks: Brain Values units/min/gram				
RT 16	5.39 a	5.33	nd	nd
RT 17	4.93 a	4.60	nd	nd

Means of triplicate assays.
a: Value more than two standard deviations below mean control levels. b: Activity increased significantly ($P < 0.05$) by 2-PAM incubation.
nd: not determined.

Blood and brain samples of five RTs found incapacitated in the area of the orchards and brought to the BNC (Table 2) indicated that they had been exposed to intoxicating levels of OPs. Plasma enzyme levels of these birds were severely depressed compared to those of the UCD captive birds (means of total ChE and AChE activities were 0.158 and 0.015 units/ml plasma, respectively). Brain ChE levels from two of the birds which died were 5.39 and 4.94 units/gram brain tissue, 25%-30% of the activities found in wild RTs (Henny et al. 1985, Hill 1988 and Hooper, unpublished data). Total ChE and AChE activities from plasma of two of the birds were reactivatable with 2-PAM.

Table 3. Alkyl phosphate levels in excreta samples from wild-tapped or rehabilitated hawks

	Enzyme Activity units/min/ml		Alkyl Phosphates ug per hawk						
			Total			Components			
BIRD	ChE	AChE	OPs	DIE	DIM	DETP	DEP	DMDTP	DMTP
RT 9	0.025r	0.008	d2.93	1.89	1.04	1.47	0.421	0.554	0.487
			d1.14	0.75	0.39	0.66	0.087	0.105	0.290
		Total	4.07	2.64	1.44				
RT 16	0.071r	0.00r	d1.45	1.36	0.09	1.18	0.182	0.033	0.057
			u5.56	4.49	1.07	4.23	0.236	0.703	0.370
			f0.03	0.02	0.00	0.026	nd	nd	nd
		Total	7.04	5.88	1.16				
RT 15	*0.138	0.027	d0.102	0.054	0.048	0.033	0.021	0.026	0.022
		Total	0.102	0.054	0.048				
RT 06	0.191	0.071	u0.066	0.017	0.049	0.017	nd	0.016	0.033
			f0.00	0.00	0.00	nd	nd	nd	nd
		Total	0.066	0.017	0.049				
RT 14	*0.201r	0.019r	d0.149	0.149	0.00	0.086	0.063	nd	nd
		Total	0.149	0.149	0.00				
RT 11	0.267r	0.058r	u1.47	1.47	0.00	1.09	0.382	nd	nd
			f0.06	0.06	0.00	0.055	nd	nd	nd
		Total	1.53	1.53	0.00				
RT 10	0.316r	0.138	u0.082	0.082	0.00	0.044	0.038	nd	nd
		Total	0.082	0.082	0.00				

DIE: Diethyl OP residues, DIM: dimethyl OP residues. Ranked according to ascending levels of ChE. r:plasma OP-inhibited ChE, reactivated by 2-PAM. u, f and d are residues from urate, fecal or diarrhea samples. * is minimum level estimated from partial samples. nd: not determined.

OP residue analysis of feathers from two dead hawks from the BNC demonstrated the presence of parathion on both birds (0.9 and 0.16 ppm), with no detectable amounts of diazinon, methidathion or other OPs.

Excretory analysis provided quantitative OP residue concentrations for nine control RTs and nine wild RTs (Table 3). Samples from two wild hawks lost volume due to evaporation and absorption and are reported on a semi-quantitative basis only. No alkyl phosphate residues were detected in the excreta of the control birds. All of the nine wild RTs from which excreta samples were

collected contained detectable residues.

OP metabolite residues were recovered from both the urate and solid (fecal) portions of the samples, though in two birds they were found only in the urate portion. Diethyl-substituted residues were present in the excreta of all of the exposed hawks at levels from 0.017 to 5.876 ugrams/bird. Dimethyl residues were found in all but three of these birds at concentrations from 0.035 to 1.436 ugrams/bird. DETP was the most abundant residue and was found in all exposed birds. DEDTP was found in only one bird, RT 18.

Spearman's coefficient of rank correlation showed significant inverse correlations ($P < 0.01$) between plasma total ChE and excretory alkyl phosphate levels (initial fecal sample only from birds sampled multiply) ($r = -0.700$, $n = 16$) and also between plasma AChE activity and initial fecal alkyl phosphate levels ($r = -0.650$, $n = 16$).

Plasma total ChE and AChE activities two standard deviations or more below control levels are considered good evidence of OP exposure (Hill and Fleming, 1982). However, many factors influence plasma ChE levels, increasing the difficulty of diagnosing OP poisoning based on plasma ChEs alone. The reactivation of OP-inhibited plasma ChEs proved to be a useful method for confirming OP exposure given the wide variations found in plasma ChE levels. The inverse correlations between ChE activities and the alkyl phosphate levels strengthened the conclusions.

Hawks brought to the BNC showed typical signs of OP poisoning; general malaise, ataxia, anorexia and diarrhea. Only one of the wild-trapped hawks (Number 9) showed similar signs of OP toxicosis. Though we were able to sample only five of the twelve hawks brought to the center, of the five, two died with brain ChE depressions of 65% to 75%. The other three had extremely depressed plasma ChEs (one of which was reactivatable) and excretory residues of all three types of OP parent compounds. The success of atropine therapy with many of these hawks confirmed the diagnosis of OP intoxication.

Plasma ChE activities have been shown to rapidly return to normal levels following the termination of OP exposure, whether acute or chronic (Ludke et al. 1975, Fleming 1981 and Westlake et al. 1981). Hill and Mendenhall (1980) found a direct correlation through time between the amount of OP-poisoned prey consumed and the resulting depression of plasma ChE, indicating that sampling plasma ChEs is a sensitive method for evaluating recent OP exposure. Hooper et al. (1988) have further shown that reactivation of parathion-inhibited plasma ChE from surviving, exposed birds is possible for only 24 hours following OP exposure. The low plasma ChE activities and their successful reactivation indicate that many RTs in this study had been exposed within 24 to 48 hours of trapping.

Reactivation of OP-inhibited ChE was successful in birds with primarily diethyl-substituted residues while hawks with a large proportion of dimethyl residues and low ChE activity had no reactivatable ChE. Martin et al. (1981) found a similar relationship in brain ChEs of quail poisoned with different diethyl- and dimethyl-substituted OPs. The dimethyl OPs were less susceptible to reactivation by 2-PAM, most likely due to their faster rate of aging, where dealkylation of the OP decreases the effectiveness of 2-PAM as a reactivator of ChE.

The toxicities of the three OPs used in the area during the study have been reviewed by Smith (1987). Methidathion was the least toxic of the three OPs when tested with mallards, pheasants and chukars, having LD50s of 23.6, 33.2, and 225 mg/kg respectively. Parathion and diazinon both showed greater toxicity to mallards (LD50s: 2.40 and 3.54 mg/kg, respectively) and to pheasants (LD50s: 12.4 and 4.33 mg/kg, respectively). Similarly, the five day dietary LC50s for Coturnix Quail fed methidathion, diazinon and parathion were 980, 167 and 238 ppm, respectively. The presence of primarily diethyl-substituted alkyl phosphates together with their greater toxicities in test species suggest that parathion and diazinon play major roles in the toxicity seen in the wild population. The potentially cumulative nature of the toxicity, however, would suggest that concurrent exposure to less potent OPs could certainly aggravate already compromised cholinergic processes.

The low levels of alkyl phosphates found in the excreta of the wild birds suggest that the exposure was chronic, with multiple doses causing a cumulative depression in ChEs. Studies of multiple OP exposures have shown that cumulative toxicity occurs when subsequent exposures further decrease ChE activity before complete recovery from earlier depressions (Okinaka et al. 1964, Busby et al. 1987). The relatively low fecal and high urate levels suggest an exposure route other than consuming contaminated prey. (Prey should contain both parent OPs and their metabolites. Since the metabolites are highly polar, they should be poorly absorbed, leading to increased fecal levels.) The route of OP exposure in wild RTs is not known. Secondary poisoning, dermal exposure and exposure via preening of feather residues deserve investigation and further research.

The dose-response relationships and pharmacokinetics of the OPs sprayed on the orchards is not known for RTs and other raptors. Indeed, little of this information is available for as common an experimental animal as the chicken, which has been used to study OPs for over fifty years (Smith and Lillie, 1931).

In conclusion, depression and reactivatability of blood ChEs and the presence of excretory and feather OP residues indicate that raptors in and around almond orchards are being exposed to significant levels of OPs during the dormant-spraying season. The results of documented exposures ranged from depression of blood

ChEs with no apparent symptoms to incapacitating toxicity requiring rehabilitation and leading to death in two RTs. The generally low levels of excretory alkyl phosphates indicate the exposure probably occurred chronically at sub-lethal doses leading to cumulative toxicity.

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