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State of California
THE RESOURCES AGENCY
Department of Fish and Game

Oxime Reactivation of Cholinesterase: Monitoring Organophosphate Exposure in Endangered Species

by

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1987

Final report under contract number C-1523, funded by Environmental License Plate Fund (Condor Studies), FY 86/87.

PROJECT SUMMARY/ABSTRACT

Organophosphates (OPs) are important pesticides that pose dangers to humans, domestic animals and wildlife. OPs inhibit a group of enzymes known as cholinesterases (CHEs) whose activity is essential for neuronal control of many vital functions. Depressions below normal levels of plasma and red blood cell CHEs in live animals or brain CHE in dead animals are indicative of OP exposure. As normal blood values are quite variable within a species, the less variable brain enzyme levels are often used to evaluate wildlife exposure and mortality. However, the time when an exposure occurred is often difficult to infer from brain data and animals need to be sacrificed to determine the enzyme content of their nervous tissue. These shortcomings make monitoring OP exposure in endangered species difficult.

This project focused on identifying when an animal has been exposed to an OP using non-lethal methods which have minimal impacts on the test animal. We measured the OP-induced loss of CHE activities in blood plasma and the in vitro recovery of the activity of OP-inhibited enzymes by treatment with the oxime pyridine-2-aldoxime methiodide (2-PAM, 2-pralidoxime). The CHE enzymes in the plasma of birds were characterized, demonstrating that more than one CHE enzyme was present in the plasma of most species of birds tested. Acetylcholinesterase (ACHE), the CHE form of nervous and muscle tissues, was found in the plasma, often in very high levels, depending upon the species. For example, Peregrine and Prairie Falcon plasmas were virtually pure ACHE, whereas California Condor plasma had both ACHE and non-specific cholinesterase (BCHE) forms. Both ACHE and BCHE enzymes were sensitive to OPs and, once inhibited, could be reactivated by the addition of 2-PAM.

The techniques developed in the laboratory were tested in the field by examining blood from California Condors trapped from the wild and raptors brought to the UCD School of Veterinary Medicine and by performing a study in which raptors (mostly Red-tailed Hawks) were trapped in and around almond orchards during the dormant spraying season when OP pesticides are applied. The results of condor blood monitoring suggested that the birds had not recently been exposed to OPs. Of the fourteen birds sampled in the orchard study, more than half of the birds showed evidence of OP exposure. Plasma CHEs were depressed and some could be reactivated by oxime treatment. The results demonstrate that plasma CHE monitoring, accompanied by oxime reactivation provide a limited impact method for the determination of OP exposure. Work continues on the use of fecal samples for alkyl phosphate analysis with methods similar to those used in human urine testing.

INTRODUCTION

The widespread use of organophosphorus insecticides (OPs), their high acute toxicity due to their ability to inhibit cholinesterases (CHEs) in the nervous system and their adverse effects on non-target species make it important to be able to rapidly identify when an animal has been exposed to them. An accepted method for monitoring OP exposure in wild animals is to determine if brain tissue acetylcholinesterase (ACHE) activity has fallen below normal levels (Hill and Fleming, 1982). This necessitates collecting non-exposed specimens of the species in question to establish normal brain CHE levels or referring to tables of "normal" values in the literature. However, the variability in the methods for measuring CHE activity often makes correlation of normal values difficult. The use of brain material and the need for control values make the conventional monitoring methods difficult to use with species which are endangered or locally rare. The diagnosis of non-lethal exposures is particularly difficult when sacrificing an animal to obtain a brain sample is not an option. Improved techniques are needed to rapidly establish whether wildlife, particularly endangered species, have been exposed to OPs. The current study focuses on blood cholinesterases and a novel reactivation method to alleviate some of these difficulties.

There have been many papers describing the CHEs in the blood and tissues of man and other animals (see Massoulie and Bon, 1982 for review). In general, there are two major types; specific ACHE which preferentially hydrolyzes the neurotransmitter acetylcholine (ACH) and is found in the nervous system, muscle and red blood cells of many mammals, and non-specific cholinesterase (or BCHE) found in liver, muscle and the plasma of vertebrates. ACHE tends to be insensitive to the organophosphate iso-OMPA and is inhibited at high concentrations of ACH. Non-specific BCHEs tend to hydrolyze substrates other than ACH such as butyrylcholine as rapidly or more rapidly than they do ACH and are more sensitive to iso-OMPA than are ACHEs.

Methods for determining CHE activity include assays of radiolabelled acetylcholine (Johnson and Russell, 1975), pH measurement of the acetate released from the hydrolysis of acetylcholine (Michel, 1949) and spectrophotometric assays which measure the hydrolysis of acetyl- or butyryl-thiocholine esters coupled to the breakdown of a coloring reagent by the resulting thiocholine (Ellman, 1961). We have adapted the thiocholine assay method for a 96 well optical reader.

Inhibition of CHEs by OPs involves a reaction in which an OP phosphorylates the active site of the enzyme and thus blocks its activity. The oxime, pyridine 2-aldoxime methiodide (2-PAM), can remove the OP from the site, restoring the activity of the enzyme as long as a second reaction known as "aging" has not occurred (Fleisher and Harris, 1965). Aging involves the loss of an alkyl residue from the OP, creating a charge in the OP moiety. This charge prevents the approach of the similarly

charged oxime, thus blocking reactivation. The rate at which an OP ages is dependent on the type of OP and can take from hours to weeks. 2-PAM is best known for its use as an antidote in the treatment of OP toxicosis. This lab (Hooper et al. 1986) and others (Karlog and Poulson, 1963 and Martin et al. 1981) have employed 2-PAM for the detection of OP inhibited CHEs in brain tissue of poisoned birds. Significant increases in CHE activity in tissue homogenates following treatment with 2-PAM provide positive evidence that the animal has been recently exposed to an OP, regardless of the initial level of activity of the enzymes.

The overall goals of this study were to examine the applicability of the 2-PAM reactivation assay in conjunction with blood CHE monitoring methods for use in identifying OP poisoning in wild birds and to establish the conditions under which the method could be applied to endangered species as well as to other wild animals. In so doing, we obtained data on the exposure of wild birds to OPs in California and demonstrated species-specific differences in the blood CHEs of birds.

GENERAL METHODS

Pesticides with which we have been especially concerned in this study include parathion (phosophorothioic acid O,O-diethyl O-p-nitrophenyl ester), diazinon (phosophorothioic acid O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester) and methidathion (phosphorodithioic acid O,O-dimethyl ester S-ester with 4-(mercaptomethyl)-2-methoxy-2-1,3,4-thiadiazolin-5-one).

In addition to three endangered species, California Condor (Gymnogyps californianus), Bald Eagle (Haliaeetus leucocephalus) and the Peregrine Falcon (Falco peregrinus), other avian species studied included the Burrowing Owl (Speotyto conicularia), a species of special concern, as well as the, Northern Harrier (Circus cyaneus), Cooper's Hawk (Accipiter cooperi), Red-tailed Hawk (Buteo jamaicensis), Red-shouldered Hawk (Buteo lineatus), Rough-legged Hawk (Buteo lagopus), American Kestrel (Falco sparverius), Prairie Falcon (Falco mexicanus), Golden Eagle (Aquila chrysaetos), Turkey Vulture (Cathartes aura), Common Barn Owl (Tyto alba), Chukar Partridge (Alectoris chukar) and the Domestic Chicken (Gallus gallus).

Blood samples were collected in heparinized syringes, placed in heparinized containers and kept on ice until processed within 24 hours. The plasma was removed following centrifugation and the samples were immediately assayed for cholinesterase activities and reactivatability.

Plasma and tissue CHE activities were determined in triplicate using the method of Ellman et al. (1961) modified for use with a BioTek Model EL309 Automated Microplate Reader. The assay was carried out in a pH 8.0, 0.1 M phosphate buffer. Final reagent concentrations were 3.2x10-4 M DTNB (5,5'-

dithiobis(2-nitrobenzoic acid) and 5.0x10-4 M acetylthiocholine iodide (AThCh). Plasma samples required a ten-fold dilution and brain samples required a 500-fold dilution to fall within the sensitivity range of the reader. The final reaction volume was 0.250 ml. Following the subtraction of a blank value (buffer + DTNB + AThCh), means and standard deviations of triplicate runs were determined, converted to umoles AThCh hydrolysed/min (units) and corrected for dilutions.

The activity of BCHE was separated from that of ACHE by incubation for 5 minutes in the presence of the specific inhibitor iso-ompa (tetra-isopropylpyrophosphoramide; Aldrich, 1952) at 10-5M unless otherwise indicated. At the end of the incubation period, substrate was added to the assay mixture and the activity was determined.

Multiple molecular forms of plasma cholinesterases were separated from each other using sucrose density gradient separation (Sketelj et al., 1978). Plasma samples were added to 5-20 percent sucrose gradients prepared in high salt detergent buffer (1 mM NaCl and 0.5% Triton X-100) and centrifuged for 18 hours at high speed (35,000 rpm) in a Model SW-40 swinging bucket rotor and Sorvall Model OTD50 ultracentrifuge. The gradients were divided into 36 equal portions with an ISCO fractionator and Gilson fraction collector and each fraction was assayed for ACHE and BCHE activity.

Reactivation of OP-inhibited CHEs was performed on 10-fold diluted plasma samples or on 50-fold diluted brain homogenates. The homogenization and dilution buffer was the same as that used in the Ellman assay. Duplicate CHE-containing samples were incubated 40 minutes at 38 C in the presence and absence of 2-PAM. A 2-PAM containing blank was incubated with all samples. Reactivation concentrations of 2-PAM were 10-4 M for plasma and 10-3 M for brain while the final assay concentration was 1.2x10-5 M for both. Pre- and post-incubation samples were assayed concurrently in triplicate. Samples containing 2-PAM were corrected for 2-PAM induced absorbance changes. The activities of ACHE and total CHE following incubation with or without 2-PAM were compared to the pre-treatment values using the Student's t-test with values for single-tailed evaluation. Positive values with $p \leq 0.05$ were taken as a strong indication that the sample contained OP-inhibited cholinesterase.

A pilot project to analyze residues from fecal urate samples using the method of Weiskopf et al. (1987) is in progress. Fecal urate samples are suspended in water, saturated with salt and adjusted to pH 3 with acetic acid. Alkyl phosphates are extracted using a disposable cyclohexyl resin column and eluted with methanol followed by acetone. Once concentrated, the samples are analyzed on a gas chromatograph and detected with an alkali flame ionization detector.

RESULTS AND DISCUSSION

Plasma Cholinesterases of Birds

A striking finding of the study was that the relative activities of specific ACHE to non-specific BCHE differed greatly in different species of birds. This was determined by measuring total CHE activity and ACHE activity in the presence of the selective BCHE inhibitor iso-OMPA. Figure 1 is an example of a titration of CHE activity in the plasma of a California Condor (UN 1) in which the hydrolysis of AThCh (expressed as percent of total activity) is plotted versus increasing concentrations of iso-OMPA. Approximately 60 percent of the total CHE activity (representing the BCHE activity) was inhibited by the agent with an I50 of approximately 10-7M. The remainder of the activity (the specific ACHE activity) was resistant to iso-OMPA to concentrations as high as 10-3M.

The responses of plasma CHE enzymes of the domestic Chicken, Red-tailed Hawk and Prairie Falcon to different substrates (AThCh and BThCh) and substrate concentrations and the presence of iso-OMPA are compared in Figure 2. Chicken plasma had total CHE values which were comparable using either substrate. This activity was extensively inhibited by 10-4M iso-OMPA, indicating a preponderance of BCHE. Red-tailed Hawk plasma had a mixture of enzymes. Hydrolysis of AThCh was nearly twice that of BThCh. Iso-OMPA inhibited part of the AThCh and all of the BThCh activity. Prairie Falcon plasma contained mostly ACHE with little or no BCHE activity. There was little hydrolysis of BThCh and iso-OMPA inhibition of AThCh hydrolysis was minimal. Numerically, ACHE activities of chicken, Red-tailed Hawk and Prairie Falcon plasmas were 15, 57 and 97 percent respectively of the total CHE activities (using AThCh).

The significance of these findings lies in the fact that studies on blood CHE levels in wildlife have often assumed (based upon clinical studies of mammals) that the CHE in the plasma of wild birds is BCHE and have used BThCh as the substrate of choice for plasma CHE measurements. As shown in this experiment, ACHE has little affinity for BThCh. The more ACHE in the sample, the greater the amount of the plasma CHEs that go unmeasured. In order to monitor total CHE and ACHE, AThCh was used to determine both of their activities.

Table 1 lists the relative activities of total CHE and specific ACHE found in samples of birds examined during the research. Relative activities of ACHE ranged from approximately 12 percent in the Burrowing Owl to more than 95 percent in the Prairie Falcon. Studies on geese, duck, cockatiel and other species are in progress.

Multiple Molecular Forms in Plasma

Both ACHE and BCHE enzyme activities are due to multiple molecular forms; our laboratory and others have studied in detail the ACHE multiple molecular forms of chicken and quail tissues; Massoulie and Bon, 1982, review the work of others on the forms found in mammals. There have been fewer studies of the multiple forms of plasma cholinesterases.

Work in progress on the multiple forms of plasma CHEs adds to the evidence that there are both ACHE and BCHE enzymes in the plasma of birds. Diagrams of sucrose gradient centrifugations of plasma from California Condor, Burrowing Owl, Red-tailed Hawk, and Prairie Falcon are shown in Figure 3. The results show that the extent of migrations of the peak sedimentations of the ACHE and BCHE enzymes did not correspond. In all cases there was a single form of ACHE for each species (even in the Prairie Falcon where ACHE was the sole enzyme present) and there were always several peaks of BCHE activity.

Blood and Brain Enzyme Reactivations

Experiments on the reactivation of OP-poisoned blood and brain enzymes by the oxime 2-PAM are in progress. Tables 2 and 3 show data from an experiment in which live Chukar Partridges were treated with parathion and the CHEs in their blood examined. Birds were treated with 0, 2 or 5 mg/kg of parathion by oral gavage; blood was withdrawn by venipuncture at 0, 6, 24 and 48 hours and samples were examined in triplicate for CHE activity with and without 2-PAM treatment. ACHE activity made up approximately 10 percent of the total CHE activity (Table 2). (The rest was due to BChE activity.) Both enzyme activities were depressed six hours after parathion treatment and much of the activity returned within 24 hours. Total CHE, but not specific ACHE, decreased somewhat with repeated sampling. The rapid recovery of ACHE activity in the plasma, presumably due to synthesis of new enzyme, represents a major difference between plasma CHEs of birds and those found in the blood of other species.

Aliquots of the plasma samples were incubated with and without 2-PAM and assayed to reveal the presence of reactivatable enzyme (Table 3). The reactivation ratio of untreated samples (bottom of table) was much lower in variability than the averages of the activities themselves, illustrating the sensitivity of the technique. Statistical tests showed that OP inhibitions could be detected up to 48 hours after treatment, even though the activities themselves had returned to 78 to 89 percent of their initial values.

Examination of whole blood from rats poisoned with parathion indicated that similar results can be obtained with mammals (data not shown).

CHEs of Condor Blood

From April 1986 through April 1987 the last five wild California Condors, ACs (Adult Condors) 6, 8, 2, 5 and 9 were brought into captivity. Following their capture, blood samples were obtained from all but AC 8 and 2. Additionally, ACs 2 and 5 were captured and released during the period prior to their final capture. Blood samples from both birds were obtained from these captures. Blood samples also were collected from three captive condors at the San Diego Wild Animal Park; two hand-raised juveniles (Hol hol and Paxa) and a wild bird (UN 1) which had been captured approximately two years previously.

Total CHE and ACHE activities are shown in Table 4 for these birds. There was little variability in total CHE and ACHE activities of the wild condors and ACHE accounted for slightly less than 50 percent of the total CHE activity. One bird, AC 5 was sampled twice during the study. ACHE activity from the February 1987 sampling was only 65 percent of the value obtained from the May 1986 sampling, while the BCHE activity (total CHE - ACHE) rose 22 percent. As neither of the samples contained reactivatable CHE, normal individual variation and the presence of aged OP-inhibited CHEs are two possible explanations for the differences.

Attempts to reactivate wild condor blood CHEs using 2-PAM were carried out within several hours of the arrival of each sample (Table 5). None of the samples showed any sign of reactivation. As a days time generally elapsed after their capture and prior to the bleeding of these birds, it is possible that changes in enzyme levels may have occured during this time. As seen in the Chukar experiment, plasma CHE activities increased and reactivatability decreased with time from exposure. It is important to stress that the time interval between capture and blood sampling be minimized, since once the animal is removed from the source of OP exposure (i.e., its habitat), blood cholinesterase levels quickly return to normal.

Wild and Captive birds at UCD

In addition to the condor studies discussed above, we have been working with Dr. Joanne Paul-Murphy of the UC Davis Veterinary Medicine and Teaching Hospital helping to identify wildlife poisoned with OPs. Birds admitted to the clinic that were difficult to diagnose were tested for CHE depression and reactivatibility. To date, three Red-tailed Hawks were identified as suffering from OP poisoning, and the diagnoses were confirmed by positive responses of the birds to atropine therapy.

CHE levels of raptors, kept at the UC Davis Raptor Center are being examined. Data from these birds were included in Table 1. Some of these birds will continue to be bled over the course of a year to investigate possible seasonal fluctuations in blood CHE levels.

Raptors in Almond Orchards

The Sacramento and San Joaquin Valleys of California contain over 400,000 acres of almond orchards, accounting for the majority of the commercial almond production in North America. In order to control pests such as the peach twig borer, San Jose scale and various mite species, the orchards are sprayed during the winter dormant season with a mixture of oil and OP insecticides (Van Steenwyk et al., 1983). Parathion, diazinon and methidathion are commonly used OPs. (Figure 4) The insecticides are applied by ground air blast spray rigs or by aerial methods. The dormant season lasts from late December through the month of January.

Reports of Red-tailed Hawk losses in the vicinity of almond production areas during the dormant season have been made to the Pesticide Investigations Unit of the California Department of Fish and Game (Ed Littrell, personal communication).

To determine if the Red-tailed Hawk losses were related to OP exposure, we focused an investigation on hawks found in and around an area of extensive almond orchards. Northern Butte county in the Sacramento Valley of California has 40,000 acres of almond orchards as well as large numbers of both resident and migratory hawks during the winter dormant season. We trapped hawks alive, banded them with U.S. Fish and Wildlife Service bands, took blood and fecal samples and evaluated them for the presence of OP inhibited CHEs and OP metabolites.

In order to trap in areas of recent OP application, records were obtained from the Butte County Agricultural Commissioner's office indicating where spraying was under way. These records reflected only the use of restricted agricultural chemicals, i.e., parathion and methidathion. As diazinon's use was not restricted, records of its application were not available.

Hawks were trapped opportunistically within and adjacent to almond orchards using Bal Chatri type traps. These traps, weighted down with two horseshoes, are hexagonal wire cages with monofilament nooses attached to the top. Two live mice inside the trap attract the hawk; it approaches, tries to grab the mice through the cage, and becomes snared by the foot. The weight of the trap keeps the bird from escaping.

Blood from a Red-shouldered Hawk and a Rough-legged Hawk and blood and fecal samples from eight Red-tailed Hawks were collected from a group of captive birds kept at the Raptor Center at UC Davis. These birds served as controls for blood CHE levels and for OP spiking and recovery trials in the fecal analysis.

Trapped birds were evaluated for normal U.S. Fish and Wildlife Service banding parameters including age, wing length, tail length, weight, flesh on the keel and crop size. Once the

bird was banded, blood was collected from the femoral/saphenous vein of the leg. The bird was then either released or placed in a holding cage lined with plastic to collect a fecal sample.

Live trapping resulted in the capture and sampling of blood of 12 Red-tailed Hawks, one Red-shouldered Hawk and one Rough-legged Hawk. Fecal samples were obtained from eight of these birds. The trapping locations were distributed throughout the almond orchards of the area (Figure 5).

In addition to the birds caught in the almond orchards, plasma CHE levels and their reactivatability were obtained for other hawks brought for rehabilitation to the Bidwell Nature Center in Chico, CA.

Table 6 shows the levels of CHEs in the blood of wild-caught and captive Red-tailed Hawks during the period of the almond orchard study.

Plasma levels of total CHE and ACHE were determined for each bird. Wide variations were seen in both total CHE and ACHE in the group of 12 Red-tailed Hawks. Values for total CHE ranged from 0.025 to 0.796 units/ml and those for ACHE were between 0.008 and 0.238 units/ml. By comparison, captive hawks from the UC Davis Raptor Center had higher mean and much less variable total CHE and ACHE activities. Total CHE ranged from 0.566 to 1.009 units/ml and ACHE ranged from 0.194 to 0.433 units/ml. Nevertheless, ACHE values averaged 38 percent of the total, similar to that of the wild birds that averaged 35 percent.

The differences in the average CHE levels in the wild Red-tailed Hawks as compared to the captive birds were due to the low levels of plasma enzyme in a number of the birds, several of which were reactivatable with 2-PAM. Total ACHE of six of the 12 Red-tailed Hawks were less than two standard deviations (59%) of the average levels of total CHE of the captive hawks. Seven of the birds had specific plasma ACHE levels less than 2 standard deviations (48%) of the plasma ACHE levels of the captive hawks. All told, eight of the 12 Red-tailed Hawks were low in either one or both CHE activities.

Reactivation of OP-inhibited total CHE was successful in four of the 14 birds (Table 7). Of these 4 hawks, 3 showed reactivation of ACHE activity. All birds with reactivatable cholinesterases were Red-tailed Hawks with initial values more than 2 standard deviations below the mean for the Red-tailed Hawks in this study.

Normal plasma enzyme levels for Red-shouldered and Rough-legged Hawks are not well represented in our baseline data at this time (Table 1). Compared with the single control values for each species, notable differences were seen in the Red-shoulder ACHE (25% of control) and the Rough-legged total CHE and ACHE (60% and 47% of controls, respectively). Lack of

reactivatability of the samples makes their evaluation difficult.

Most of the hawks caught by the Bal Chatri trap behaved normally, displaying no overt signs of toxicity. However, one of the Red-tailed Hawks never attempted to escape after it was snared, acted depressed and could not stand or raise the feathers on the back of its head. This bird (# 10 in Table 7) was taken to the Bidwell Nature Center in Chico, CA. where it was successfully treated for OP poisoning with atropine therapy. Evaluation of blood CHEs revealed 97% depressions of both total CHE and ACHE. Both enzymes were reactivatable to over four times their initial values.

Additional evidence that hawks in Butte County were being exposed to OPs was obtained from the examination of blood and brain samples of five birds brought to the Bidwell Nature Center during the period of the almond orchard study (Table 8). Plasma enzyme levels of these birds were depressed compared to those of the UCD captive birds (means of total CHE and ACHE were 0.158 and 0.015 units/ml plasma, respectively). Brain ACHE levels were approximately one third the 14-15 units expected for normal birds (unpublished data). In addition, total CHE and ACHE activities from plasma of two of the birds were reactivatable with 2-PAM.

During the course of the investigation, a Bald Eagle, a member of an endangered species, was observed traveling through the almond orchard area. This observation and the tentative data on Rough-legged and Red-shouldered Hawks suggest that further investigations should not be confined to Red-tailed Hawks.

Initial results of alkyl phosphate analysis of fecal urate samples of the hawks show evidence for the presence of dimethyl and diethyl substituted thiophosphates as well as dimethyl substituted dithiophosphate. Ms. Carol Weiskopf and Dr. James Seiber are continuing to work on applying the gas chromatographic technique they have used for identification of alkyl phosphates in the urine of farmworkers to fecal urate samples of birds to enable us to identify the OPs to which birds have been exposed by a non-invasive means.

The results of the orchard monitoring project have been presented to Ed Littrell of the Pesticide Investigations Unit of the California Department of Fish and Game, the Agricultural Commissioner of Butte County (Mr. Joe Bandy) and, on his invitation, to a meeting of all the Commissioners.

CONCLUSIONS AND RECOMMENDATIONS

The work of the past year leads to the following specific conclusions:

ACHE activity is found in the plasma of many birds either in combination with BCHE (as in the owls, vultures, eagles and hawks) or as the exclusive enzyme (the falcons). The source of these enzymes is unknown. We are currently beginning a project to study their occurrence in brain, muscle, liver, and kidney.

Determination of plasma CHEs is a suitable method of establishing whether a bird has been recently exposed to an OP, especially when the determinations are coupled with oxime reactivations. It has been shown (Ludke et al., 1975, Fleming, 1981, Westlake et al., 1981) that plasma CHE levels rapidly recover (compared to the slow replacement of brain activity) enabling one to estimate when the exposure may have occurred with increased accuracy. Moreover, the measurements do not require sacrificing the animal; samples are often needed from endangered species or from small local populations that should not be reduced further in numbers.

Oxime reactivation methods should prove practical in analyzing situations where CHE depression measurements do not unequivocally indicate that exposure to an OP has occurred. For example, Kucera, 1987 (and earlier Hill et al., 1971) found that brain CHE activity of sparrows after aerial spraying of malathion was depressed by less than 15-20 percent of normal (as defined by brain levels determined before spraying began); the depressions were not great enough to conclude that the birds were adversely exposed to the OP. However, their conclusions might have been different if the CHE assays had been performed on plasma enzymes and were accompanied by oxime reactivation tests.

OP exposures to wildlife are a particular problem in almond orchards. The blood monitoring study shows that raptors in and around the orchards have been exposed to OPs during the dormant spraying season. The results of documented exposures ranged from depression of blood CHEs with no apparent symptoms to decapacitating toxicity requiring rehabilitation and even death in two Red-tailed Hawks. Work continues on the analysis of fecal samples for OP residues to identify the OP or combination of OPs responsible.

Recommendations

Rapid, accurate methods of assessing exposure to OPs are needed for wildlife. We recommend that efforts be taken to further develop standard techniques for determining both plasma and brain enzymes when applicable, together with oxime reactivations that can be used both in the field and in the clinic. In addition to testing for pesticide exposure, the goal

of a wildlife monitoring program is to use methods which minimize impacts on the study population. The use of blood and fecal sampling can provide the minimal impact option necessary when working with rare and endangered species. The automated techniques applied here enable one to process many samples simultaneously and are suitable to clinics and small laboratories.

The monitoring program initiated in Butte County, has identified a serious problem with OP intoxication of a Red-tailed Hawk population during the dormant spraying season. Further investigation of the effects of dormant sprayed OPs is needed to evaluate the specific hazards they pose to wildlife. Work continues on the analysis of fecal residues to determine which OP(s) are responsible. Prey base analysis and evaluation of dermal absorption or feather adsorption followed by preening should be undertaken to determine the mode of exposure. In addition to the prey base, other selected wildlife in the study area should be monitored for exposure to OPs. Finally, as Butte county represents less than ten percent of the almond acreage (Tippett et al., 1986) and OP (parathion and methidathion) use for dormant spraying (State of California Pesticide Use Report, 1985), other almond areas should be monitored for OP impacts. Kern and Merced counties are responsible for nearly 40% of the state's almond production and of the state's parathion and methidathion use on that crop. Reports of wildlife losses from these areas (Ed Littrell, pers. comm.) should be investigated.

ACKNOWLEDGEMENTS

The project was assisted by the collaborations of wildlife biologist Ed Littrell (California Department of Fish and Game), trappers Philip Dietrich of Wildlife Information Network, Chico, CA and Lynda Spiegel, Terry Schulz and the many volunteers from the Raptor Center of the UCD School of Veterinary Medicine, Marilyn Gammette of the Bidwell Nature Center, Chico, CA, Dr. Patricia Zenone, Mark Robertson and Brian Walton of the Predatory Bird Research Group at the University of California at Santa Cruz and the offices of the Butte County Agricultural Commissioner in Chico and Oroville, CA.

EXTRAMURAL FUNDING

In addition to funding from the CDFG Environmental License Plate Fund, portions of this work were supported on:

University of California Toxic Substance Research and Teaching Grant to study "Reactivation Techniques and the Detection of Exposure to Organophosphate Insecticides"

USDA Western Regional Project: W-169 "Minimizing Occupational exposure to Pesticides".

NIH Grant ES-00202 "Toxicants and Avian Cell Growth and Metabolism"

RESEARCH PRODUCTIVITY

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Table 1. Total CHE and ACHE values from the plasma of selected captive wild birds. Activity expressed as umoles AThCh hydrolyzed/min/ml plasma. Bracketed numbers are the range of values included in the mean. California Condor values are from wild caught birds.

Species	n	Total CHE mean	s.d	ACHE mean	s.d.	% ACHE
Northern Harrier	7	1.062 [0.969-1.256]	0.012	0.310 [0.202-0.418]	0.079	29
Cooper's Hawk	2	1.328 [1.309-1.347]	-----	0.578 [0.541-0.613]	-----	44
Red-tailed Hawk	8	0.790 [0.566-1.009]	0.162	0.302 [0.194-0.433]	0.078	38
Red-shouldered hawk	1	1.551	-----	0.377	-----	24
Rough-legged Hawk	1	1.196	-----	0.462	-----	39
American Kestrel	1	2.112	-----	2.101	-----	100
Prairie Falcon	3	1.285 [1.133-1.435]	0.152	1.267 [1.118-1.423]	0.153	99
Peregrine Falcon	3	1.621 [1.026-2.489]	0.769	1.454 [0.875-2.345]	0.783	90
Golden Eagle	3	0.617 [0.562-0.717]	0.085	0.153 [0.117-0.174]	0.032	25
Bald Eagle	3	0.413 [0.337-0.469]	0.069	0.125 [0.068-0.127]	0.056	30
Turkey Vulture	5	0.739 [0.543-0.987]	0.160	0.384 [0.219-0.648]	0.170	52
California Condor	5	0.600 [0.494-0.706]	0.069	0.286 [0.228-0.356]	0.055	48
Barn Owl	4	3.279 [2.845-3.613]	0.355	0.690 [0.628-0.843]	0.103	21
Burrowing Owl	4	2.207 [1.722-2.819]	0.462	0.265 [0.189-0.368]	0.085	12

Table 2. Inhibition of total CHE and ACHE of Chukar Partridge plasma after exposure to parathion. Values are means ± s.d. of triplicate runs. * Statistically different from controls, ($P < 0.05$). 0 time values: Total CHE 0.920 ± 0.263, ACHE 0.079 ± 0.024 umoles AThCh hydrolyzed/min/ml.

		Percent 0 Time Activity	
Time	Treatment	Total CHE	ACHE
6 Hours	Controls	94.3 ± 0.7	105.2 ± 7.1
	2 mg/kg	41.3 ± 8.0 *	61.7 ± 15.5 *
	5 mg/kg	20.1 ± 12.7 *	37.2 ± 17.1 *
24 Hours	Controls	83.3 ± 3.7	103.6 ± 17.5
	2 mg/kg	64.1 ± 8.7 *	80.3 ± 13.4
	5 mg/kg	57.7 ± 12.9 *	75.3 ± 26.4
48 Hours	Controls	77.0 ± 7.3	92.5 ± 5.2
	2 mg/kg	61.4 ± 7.5 *	77.9 ± 4.9 *
	5 mg/kg	66.4 ± 10.2	89.0 ± 31.6

Table 3. Reactivation of total CHE and ACHE of Chukar Partridge plasma after exposure to parathion. Values are means of reactivation ratios (umoles AlhCh hydrolysed/min/ml plasma with:without 2-PAM) Standard deviations omitted for brevity. * Statistically different from controls, $P < 0.05$. Three birds in each group. Total CHE ratio at 0 time = 0.996 ± 0.014 (n=9). ACHE ratio at 0 time = 0.981 ± 0.055 (n=9).

		Reactivation Ratio	
Time	Treatment	Total CHE	ACHE
0 Hours	Controls	0.989	1.017
	2 mg/kg	1.005	0.988
	5 mg/kg	0.995	0.941
6 Hours	Controls	1.007	0.902
	2 mg/kg	1.331 *	1.406 *
	5 mg/kg	2.262 *	2.014 *
24 Hours	Controls	0.979	0.890
	2 mg/kg	1.021 *	1.083 *
	5 mg/kg	1.057	1.190 *
48 Hours	Controls	0.996	0.928
	2 mg/kg	1.000	1.008 *
	5 mg/kg	1.003	1.073 *

Table 4. CHEs of the California Condor. All values in umoles AThCh hydrolyzed/min/ml plasma.

Source	Date	Bird #	Age	Sex	Total CHE	ACHE	% ACHE
Wild Condors	21 Apr 86	AC 6	adult	M	0.587	0.254	43.3
	21 Apr 86	AC 2	adult	M	0.706	0.345	48.9
	7 May 86	AC 5	adult	M	0.628	0.358	57.0
	27 Feb 87	AC 5	adult	M	0.585	0.228	39.0
	19 Apr 87	AC 9	adult	M	0.494	0.243	49.2
				mean	0.600	0.286	47.5
				s.d.	0.069	0.055	6.1
Captive Condors		Hol hol	juvenile	M	1.171	0.619	52.9
		Paxa	juvenile	M	0.572	0.210	36.7
		UN-1	adult	F	0.981	0.368	37.5

Table 5. Reactivation analysis of wild California Condor plasma CHEs. All values in umoles AThCh hydrolyzed/min/ml plasma.

Bird #	Total CHE				ACHE			
	Pre 2-PAM		Post 2-PAM		Pre 2-PAM		Post 2-PAM	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
AC 6	0.582	0.011	0.562	0.009	0.254	-----	0.212	0.002
AC 2	0.706	-----	0.612	0.001	0.345	0.004	0.280	0.002
AC 5	0.620	0.013	0.564	0.004	0.358	0.001	0.306	0.002
AC 5	0.554	0.006	0.513	0.005	0.235	0.011	0.196	0.005
AC 9	0.494	0.018	0.418	-----	0.243	0.009	0.200	0.004

Table 6. Red-tailed Hawk Cholinesterase Values, January/February 1987. Control birds are from the UCD Raptor Center. All values in umoles acetylthiocholine hydrolyzed/min/ml plasma.

		Total CHE		ACHE	
	n	Mean	S.D.	Mean	S.D.
Control	8	0.790	0.162	0.302	0.078
(range)		(0.566-1.009)		(0.194-0.433)	
Wild-caught	12	0.427	0.231	0.130	0.071
(range)		(0.025-0.796)		(0.008-0.238)	

Table 7. 2-PAM reactivation of total CHE and ACHE from the plasma of live-trapped hawks.

Bird no.	Date	Species	Total CHE		ACHE	
			Pre 2-PAM	Post 2-PAM	Pre 2-PAM	Post 2-PAM
1	1/05	RT a	0.599 ± 0.002 b	0.587 ± 0.004	0.238 ± 0.011	0.212 ± 0.011
2	1/17	RS	1.391 ± 0.039	1.342 ± 0.016	0.092 ± 0.004	0.083 ± 0.002
3	1/18	RT	0.633 ± 0.021	0.616 ± 0.004	0.189 ± 0.002	0.173 ± 0.004
4	1/25	RT	0.515 ± 0.002	0.482 ± 0.004	0.119 ± 0.005	0.112 ± 0.004
5	1/25	RT	0.201 ± 0.007	0.264 ± 0.006 c	0.053 ± 0.002	0.062 ± 0.004 c
6	1/25	RT	0.634 ± 0.004	0.636 ± 0.004	0.194 ± 0.004	0.180 ± 0.001
7	1/31	RT	0.191 ± 0.006	0.193 ± 0.004	0.071 ± 0.002	0.064 ± 0.009
8	1/31	RT	0.796 ± 0.033	0.745 ± 0.004	0.119 ± 0.011	0.116 ± 0.008
9	1/31	RT	0.567 ± 0.018	0.547 ± 0.005	0.179 ± 0.007	0.189 ± 0.002
10	2/01	RT	0.025 ± 0.026	0.106 ± 0.004 c	0.008 ± 0.005	0.037 ± 0.004 c
11	2/07	RL	0.725 ± 0.027	0.714 ± 0.009	0.216 ± 0.002	0.202 ± 0.004
12	2/08	RT	0.316 ± 0.002	0.322 ± 0.001 c	0.138 ± 0.001	0.140 ± 0.002
13	2/08	RT	0.267 ± 0.006	0.315 ± 0.002 c	0.058 ± 0.004	0.078 ± 0.004 c
14	2/08	RT	0.383 ± 0.001	0.390 ± 0.008	0.196 ± 0.006	0.187 ± 0.004

a. RT = Red-tailed Hawk, RS = Red-shoulder Hawk, RL= Rough-legged Hawk
b. units = umoles AThCh hydrolyzed/min/ml plasma
Each value is the mean ± s.d. of triplicate assays.
c. Value is significantly different from pre 2-PAM value, $p < 0.01$.

Table 8. CHE values from Red-tailed Hawks brought to Bidwell Nature Center. Activity in umoles AThCh hydrolyzed/min/ml. Values are the mean ± s.d. of triplicate assays. * = greater than Pre 2-PAM value, P < 0.01.

Enzyme Source	Bird	Date	Total CHE		ACHE	
			Pre 2-PAM	Post 2-PAM	Pre 2-PAM	Post 2-PAM
Blood	RT A	01/18/87	0.222 ± 0.018	0.239 ± 0.007	-------	-------
	RT B	02/06/87	0.201 ± 0.001	0.219 ± 0.005*	0.019 ± 0.001	0.030 ± 0.001*
	RT C	02/06/87	0.138 ± 0.005	0.128 ± 0.001	0.027 ± 0.002	0.025 ± 0.001
	RT D	02/08/87	0.071 ± 0.005	0.179 ± 0.009*	0.000 ± 0.000	0.040 ± 0.007*
Brain	RT D	02/11/87	5.392 ± 0.121	5.334 ± 0.180	5.462 ± 0.147	5.106 ± 0.089
	RT E	02/11/87	4.935 ± 0.092	4.605 ± 0.146	5.076 ± 0.183	4.610 ± 0.110

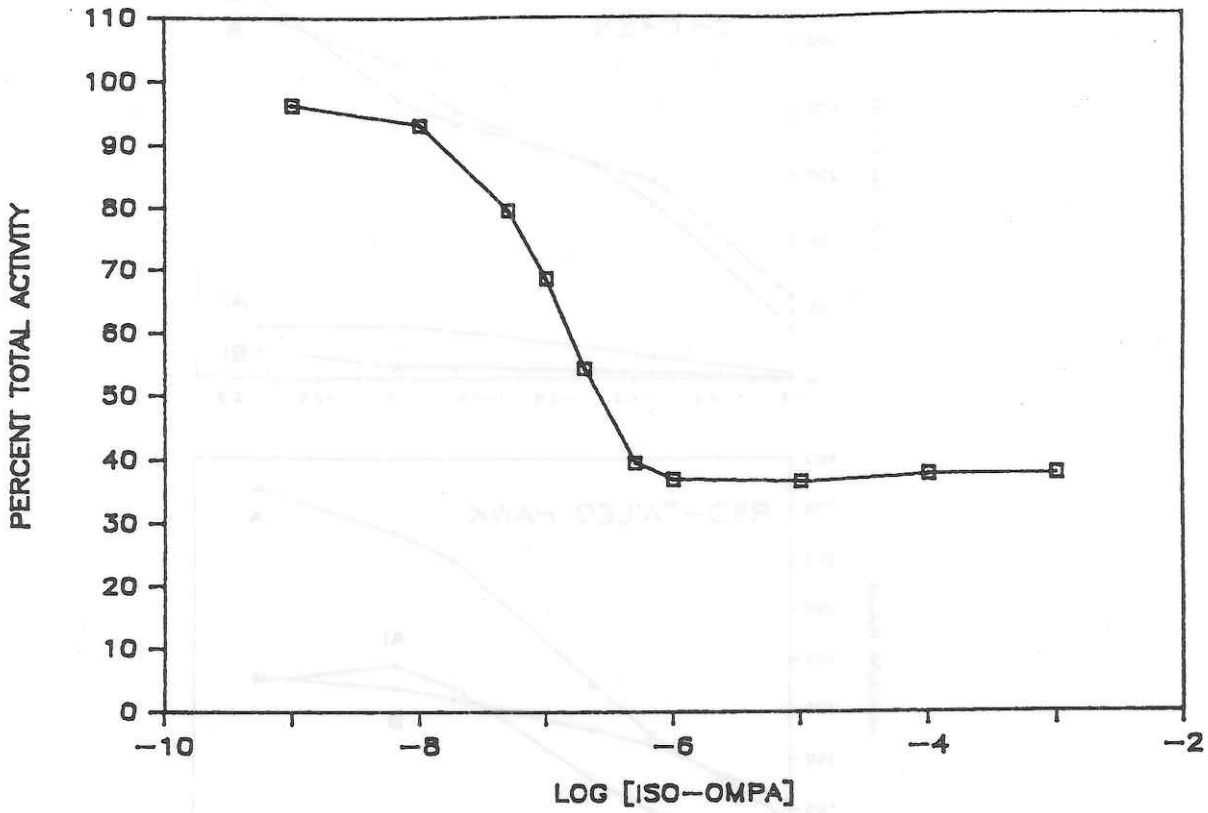


Figure 1. Effect of increasing concentrations of iso-OMPA, an inhibitor of non-specific CHEs, on the plasma CHE of a California Condor. Pre-incubation with iso-OMPA was for five minutes prior to the addition of the substrate, AThCh.

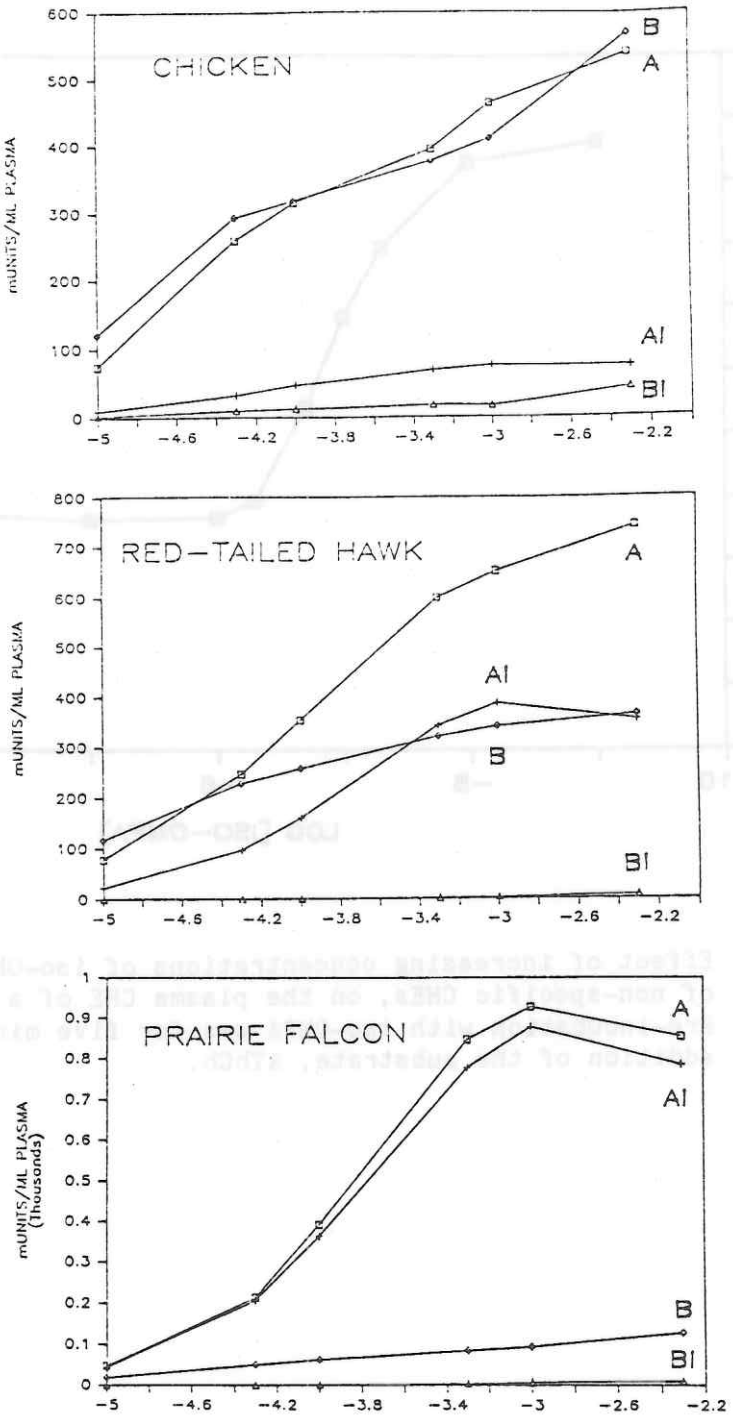


Figure 2. Substrate and substrate concentration effects on the total CHE and ACHE from plasma of the Domestic Chicken, Red-tailed Hawk and the Prairie Falcon. Substrates: A = AThCh, B = BThCh AI = AThCh + iso-OMPA, BI = BThCh + iso-OMPA. Samples with iso-ompa are ACHE, those without are total CHE. Iso-OMPA concentration was 10-5M except for chicken which was 10-4M.

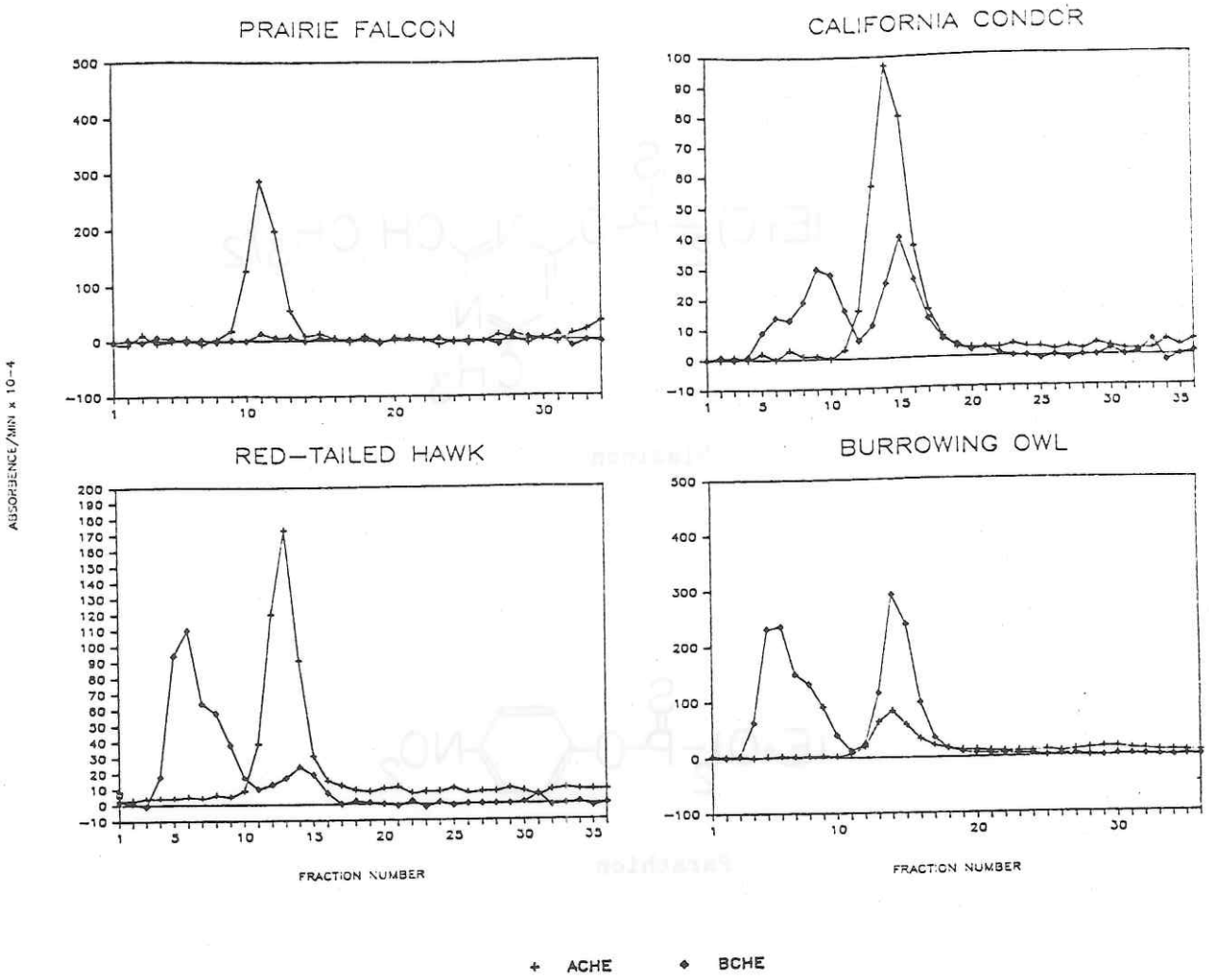
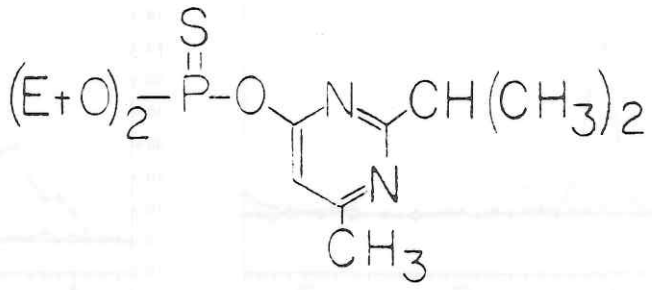
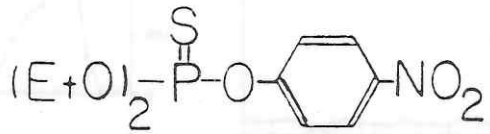


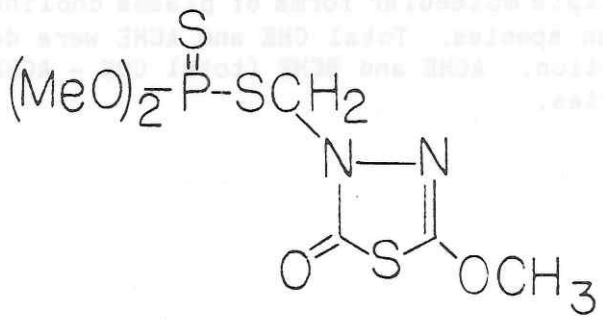
Figure 3. Multiple molecular forms of plasma cholinesterases from several avian species. Total CHE and ACHE were determined for each fraction. ACHE and BCHE (total CHE - ACHE) are shown for each species.



Diazinon



Parathion



Methidathion

Figure 4. Molecular structures for diazinon, parathion and methidathion.

Figure 5. Trapping locations of hawks from dormant spraying study in the northern Sacramento Valley of California (see insert for location). Numbers are bird numbers from Table 7 of this report. Numbers with two circles are birds with OP-inhibited cholinesterase enzymes.