

Chemical and Biochemical Evaluation of Swainson's Hawk Mortalities in Argentina

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Several isolated incidents of Swainson's hawk (*Buteo swainsoni*) mortality were reported in the Pampas regions of Argentina during the austral summer of 1996. Eighteen sites containing 5,093 dead hawks were identified. Four sites, I, II, III, and IV, containing 387, 103, 3024, and 595 hawk mortalities, respectively, were investigated. Landowner and pesticide applicator interviews indicated use of the organophosphate pesticides (OP) monocrotophos at sites I and IV and dimethoate at site II. The agent used at site III was unknown due to conflicting reports. However, brain and plasma cholinesterase activity were sufficiently depressed in the hawks found at site III to indicate exposure to an OP. Brain and plasma acetylcholinesterase (AChE) taken from several dead and incapacitated birds were depressed 90% and 50%. Dimethoate, methamidiphos, methyl parathion, and chlorpyrifos were suspected, but only monocrotophos was found in several gastrointestinal (GI) tracts. No other parent OPs were detected from any of the sites. Dimethylphosphoroate, the primary metabolite of a dimethylphosphate insecticide, was detected in GI tracts from sites I and IV. The absence of dialkylthiophosphates precluded the use of any phosphorothioate or phosphorodithioate insecticide. Based upon these findings, monocrotophos was responsible for the mortalities at sites I, III, and IV, and dimethoate for site II.

The Swainson's hawk, (*Buteo swainsoni*), is a neotropical migrant, breeding in the western portions of the United States, Canada, and Mexico and overwintering in the pampas regions of Argentina. The grassland and desert habitats of its breeding grounds provide a variety of prey items including insects, small mammals, reptiles and birds (1). However, the pampas of Argentina, which are primarily agricultural, provide an overwhelming abundance of insect prey. Swainson's hawks seasonally shift their diets to take advantage of the plentiful insect populations (2).

Swainson's hawks are gregarious, gathering in large flocks prior to their annual southward migration. Flocks containing several hundred thousand birds have been reported passing over their southern migration routes (1). They remain in these large groups throughout the wintering period. Common agricultural practices such as cultivating, harvesting, or pesticide application, tend to disturb resident insects Swainson's hawks take advantage by descending in large numbers upon the available prey (1). If insecticides are being applied, or have recently been so, the hawks are subject to direct exposure from application mists, dermal absorption through feet and legs, or oral exposure via their insect prey. The propensity of Swainson's hawks to aggregate in large flocks makes them susceptible to large-scale mortality on the wintering grounds when exposed to insecticides in this manner. Woodbridge and colleagues in 1995, and Goldstein and colleagues in 1996 and 1997 documented that such incidents had occurred in the pampas of Argentina (2-6)

Organophosphate insecticides have largely replaced the once common organochlorine pesticides in the pampas region. Chlorinated organic pesticides and their metabolites tend to be persistent in the environment. For instance, the half-life of p,p'-DDT and its metabolites ranges from 2 to 15 years depending on the environment (7). Their persistence, high affinity for lipophilic tissue, and resistance to metabolism, causes these compounds to biomagnify in top predators. Negative population impacts have been demonstrated in sensitive species (8). Organophosphate pesticides (OPs) have the advantage that they do not persist in the environment and therefore do not bioaccumulate. However, they have the disadvantage that they traditionally exhibit much higher acute toxicities to nontarget species. Five OPs were used in the vicinity of the four hawk mortality sites, monocrotophos (O,O-dimethyl(E)-1-methyl-2-(methylcarbamoyl)vinylphosphate), methamidiphos (O,S-dimethyl phosphoramidothioate), dimethoate (O,O-dimethyl-S-methylcarbamoylmethyl phosphorodithioate), methyl parathion (O,O-dimethyl-O-(4-nitrophenyl) phosphorothioate), and chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) (Figure 1).

The breakdown of OPs into alkylphosphate metabolites is characterized by a specific pattern. Dialkyldithiophosphoroate insecticides can be metabolized into a dialkyldithiophosphate, dialkylthiophosphate, or dialkylphosphate (Figure 2a) In a similar manner, dialkylthiophosphoroate insecticides can be metabolized into a dialkylthiophosphate or dialkylphosphate (Figure 2b) Dialkylphosphoroate

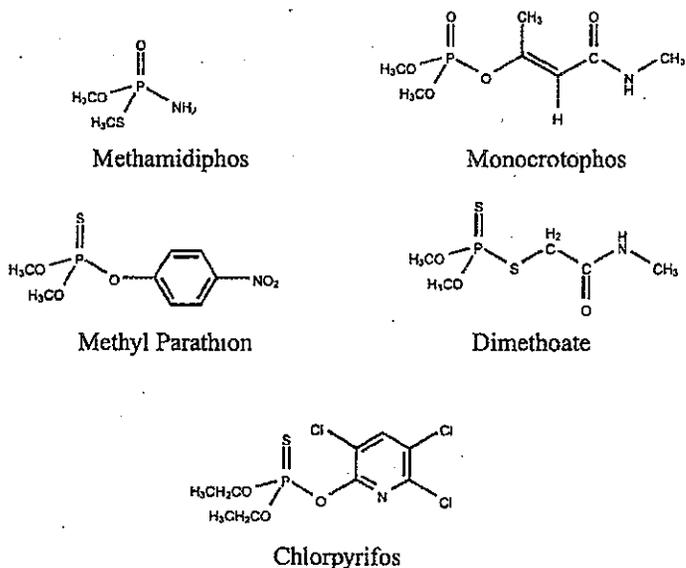


Figure 1. Organophosphorous insecticides suspected in Swainson's hawk mortality sites I, II, III, and IV based on landowner and farmer surveys.

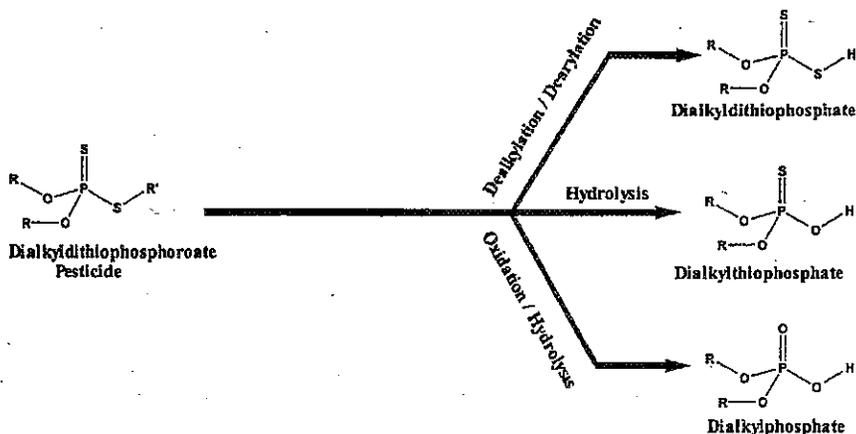


Figure 2a. Metabolic pathway of a dialkyldithiophosphate ester and its respective alkylphosphate metabolites.

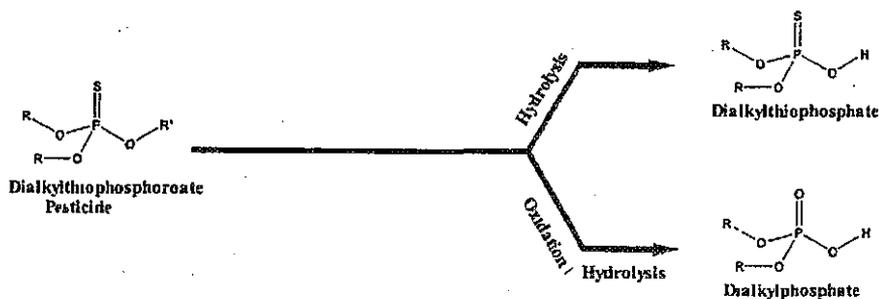


Figure 2b. Metabolic pathway of a dialkylthiophosphate ester and its respective alkylphosphate metabolites

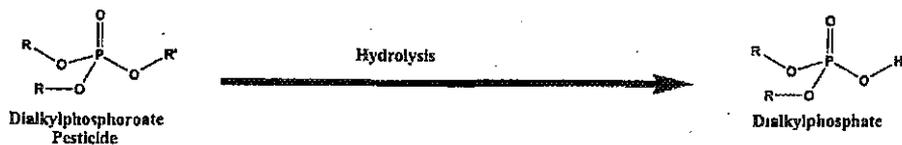


Figure 2c. Metabolic pathway of a dialkylphosphate ester and its respective alkylphosphate metabolites

insecticides can only be broken down into dialkylphosphates (Figure 2c). Putting the current list of pesticides into perspective, dimethoate is a dimethyldithiophosphorothioate, methamidophos and methyl parathion are dimethylthiophosphorothioates, chlorpyrifos is a diethylthiophosphorothioate, and finally, monocrotophos is a dimethylphosphate, lacking thiol groups altogether. If the hawks are exposed to dimethoate, the metabolic products would include dimethyldithiophosphate (DMDTP), dimethylthiophosphate (DMTP), and dimethylphosphate (DMP). Note that the OP degradation does not occur so rapidly that all of the parent compound would be converted to DMP over the course of this study. If the hawks had ingested dimethoate, DMDTP or DMTP would be present. The presence of DMTP or DMP in the absence of DMDTP would indicate the use of methamidophos, methyl parathion or monocrotophos. The presence of DMP and the absence of DMDTP or DMTP in the GI samples would indicate the use of monocrotophos. The presence of any diethylphosphate metabolites would implicate the use of chlorpyrifos.

In a forensic study of this type, it is imperative to link the causative agent with a physiological response. Organophosphate pesticides are anticholinesterase agents. They bind to the serine active sites of acetylcholinesterase, rendering the enzyme nonfunctional. The role of acetylcholinesterase is to promote the hydrolysis of the neurotransmitter, acetylcholine, into its acetyl and choline constituents. In the absence of acetylcholinesterase, acetylcholine builds up within the neural synapse causing stimulatory paralysis. Death ultimately results from depression of the respiratory centers of the brain (9).

The nature of the anticholinesterase agent bound to the serine active site determines the extent to which the agent can be removed and the acetylcholinesterase reactivated. Carbamates, another type of anticholinesterase pesticide, bind to the serine active sites but can be removed from the active site by simple dilution and incubation (10). Cholinesterases inhibited by diethyl OPs may be reactivated with 2-PAM (2-pyridinealdehyde oxime methochloride) (11). Esters containing diisopropyl and dimethyl alkyl groups age quickly, making them unresponsive to reactivation.

Materials and Methods

Eighteen Swainson's hawk mortality incidents, accounting for approximately 5,000 hawks, were found during the 1995-96 austral (southern hemisphere) summer within the pampas region of Argentina (5). A forensic evaluation of sites I through IV was initiated by investigators to determine the cause of the kills. Local residents indicated that the deaths were usually associated with the application of organophosphorous insecticides in hawk foraging areas. The investigation was based on 1) landowner and field worker surveys regarding the pesticide application prior to the hawks dying; 2) analytical determination of parent pesticides and their metabolites in hawk tissues; and 3) biochemical analysis of brain and plasma

cholinesterase activity to link a physiological effect with possible pesticide exposure (5).

Surveys

Landowners and field workers within close proximity of the mortality sites were interviewed. They informed investigators of the timing and nature of pesticide applications. Despite the fact that several reports were deficient or contradictory, the list of probable agents was narrowed down to the five organophosphorous pesticides previously discussed (5).

Analytical

Parent OP and metabolite analyses were performed on hawk gastrointestinal contents, specifically, from the proventriculus. Feather and footwash rinses were collected from affected hawks for OP analysis. Residue determination was initially performed by gas chromatograph (GC) using a flame photometric detector (FPD). Analyte confirmation was performed by dual column chromatography or mass spectrometry (MS).

Sample splits were sent to Novartis Crop Protection, Basel, Switzerland, for monocrotophos analysis more detailed than our laboratory was able to provide (5). The specifics are outlined in the discussion.

Sample Extraction and Cleanup

GI tract. Two gram samples were extracted with 10 ml of 9:1 acetone:hexane using a blender (VITIS Co., Gardiner, NY, USA). The samples were homogenized for 1 minute, after which the eluant was poured through a 5 g granular anhydrous sodium sulfate column into a flat bottom flask. The extraction was repeated a second time. Following the second elution, the sodium sulfate column was rinsed with 25 ml of 9:1 acetone:hexane. The extract was reduced to 2 ml by vacuum rotary evaporation.

The concentrate and rinses (3 x 2 ml 9:1 acetone:hexane) were transferred to the head of a 5 g silica gel column previously conditioned with 9:1 acetone:hexane. The samples were eluted with 35 ml of 9:1 acetone:hexane into a flat bottom flask and again reduced to 2 ml by vacuum rotary evaporation. The concentrate and rinses were brought to a known volume of 5 ml and eluted through a gel permeation chromatography column. Fractions containing the OPs were collected in flat bottom flasks, reduced to 1 ml under vacuum rotary evaporation, and combined with rinses for a final volume of 2 ml. The extracts were loaded into 2 ml autosampler vials for GC analysis.

Alkylphosphate metabolites The original technique for alkyl phosphate analysis is outlined in Weisskopf and Seiber (12). However, several modifications were incorporated to account for differences in sample matrix.

Three ml of deionized water were combined with 0.5 g of the proventriculus contents in polystyrene centrifuge tubes. The homogenate was adjusted to a pH of 4.0 with 1 M sodium hydroxide or 7% acetic acid and vortexed for 20 seconds every 5 minutes for 45 minutes. The slurry was saturated with ammonium sulfate (1.4 g;

Mallinckrodt Analytical Reagent), vortexed for 30 seconds, and then centrifuged for 7 minutes at 2500 rpm.

The supernatant containing the alkylphosphate metabolites was placed onto a 1 g cyclohexyl column preconditioned with 6 ml of 9:1 acetone:hexane, 1:4 methanol:acetone, deionized water, and ammonium sulfate saturated deionized water each. Elution through the column was accomplished using a vacuum manifold. The column was rinsed with 2 ml of 1:9 acetone:hexane and aspirated for 5 minutes to remove residual water.

The alkyl phosphate metabolites were eluted off the cyclohexyl columns with 3.5 ml 1:4 methanol:acetone. The eluant was brought to a known volume of 2 ml and mixed with 0.6 g powdered anhydrous sodium sulfate. The solutions were removed from the sodium sulfate and transferred to 2 ml GC autosampler vials. Immediately prior to running on the GC, 15 μ l of the derivatizing agent, tetrabutyl ammonium hydroxide (TBAH) (1 M in methanol), was added to each vial.

Feathers. Feather samples consisting of 3 to 4 coverts from each of the backs of both wings, the back and the breast, were placed in Erlenmeyer flasks along with 35 ml of 2:1 acetone:hexane. The sample was placed on an orbital shaker and extracted for 30 minutes. The extracted solvent and two 10-ml rinses of 2:1 acetone:hexane were eluted through a 2-g column of granular anhydrous sodium sulfate into a flat bottom flask. The column was rinsed with an additional 10 ml of 2:1 acetone:hexane. The eluant and rinses were reduced to 2 ml using a vacuum rotary evaporator. The extract was filtered through 0.45 μ m PTFE Acrodisc filters into 2 ml autosampler vials for GC analysis.

Footwashes. Ethanol footwashes were reduced to 2 ml, filtered as above, and injected directly onto the GC as described below.

Instrumental Analysis

Quantitation was performed using a Hewlett Packard Model 5890 II gas chromatograph equipped with an autosampler and flame photometric detector (GC/FPD) in the phosphorous mode. When matrix interference was minimal, dual column confirmation was provided by fused silica capillary columns coated with DB-1701 or DB-210 stationary phases (J & W Scientific, Rancho Cordova, CA, USA). The GCs were calibrated with a four point external curve for monocrotophos, methamidiphos, dimethoate, and methyl parathion.

Pesticide recovery was determined by spiking clean crickets with a mixture containing dimethoate, methyl parathion, methamidiphos, and monocrotophos. Crickets were used as a surrogate for the grasshoppers commonly found in the hawk GI tract. The crickets were spiked at two concentrations, 0.1 ppm and 2 ppm. Recoveries ranged from 86% to 123% for the 0.1 ppm spikes and 76% to 121% for the 2 ppm crickets (Table I).

The method level of detection (MLOD) for hawk digesta from site III was 0.037 μ g/g. The digesta from sites I and IV were thicker and contained greater amounts of matrix interference. The MLOD at these sites was correspondingly higher at 0.2 μ g/g.

Table I. Percent Recovery of Organophosphate Pesticides from Spiked Crickets.

	<i>Percent Spike Recoveries</i>	
	<i>0.1 PPM</i>	<i>2.0 PPM</i>
Dimethoate	86.7 ± 8.5 ^a	79.5 ± 6.9 ^a
Methyl Parathion	108 ± 10	76.9 ± 6.1
Methamidiphos	123 ± 6.6	121 ± 16
Monocrotophos	121 ± 6.9	87.6 ± 9.6

^a mean ± standard error

When matrix interference was high, GC-mass spectrometry (GC/MS) was used to confirm GC/FPD detections. GC/MS was performed on a Hewlett Packard Model 5988A quadrupole mass spectrometer operated in the electron impact mode. Full spectrum scans were run for most samples. In samples from areas suspected of monocrotophos contamination, three characteristic ions ($m/z = 127, 192, \text{ and } 223$) were monitored to improve detection limits.

Biochemical Analysis

Sample Preparation

Blood samples were taken from moribund birds euthanized at the field sites as well as birds active enough to be released after capture. The blood was drawn with heparinized needles from the humoral or femoral veins. Blood samples were centrifuged and the plasma removed. The plasma was stored frozen until laboratory analysis, which occurred within two weeks of collection. The samples were thawed and diluted ten-fold for use in cholinesterase (ChE) and reactivation analysis.

Brain tissue was removed from the skulls and diluted 1:4 (weight:volume) with 0.05 Tris buffer. The samples were homogenized for 30 seconds in a tissue homogenizer (VirTis Co., Gardiner, NY, USA). The resulting homogenate was diluted 20-fold, for total dilution of 100-fold, prior to ChE analysis.

Cholinesterase Activity Determination

Cholinesterase (ChE) activity was determined by the Ellman assay (13). This assay measures the hydrolysis of acetylthiocholine (AThCh), a thio ester analogue of acetylcholine. Hydrolyzed thiocholine attacks DTNB (5,5'-dithiobis(2-nitrobenzoic acid)), present in the assay medium. Hydrolyzed DTNB releases a chromophore, TNB (5-thio-nitrobenzoic acid), which can be measured spectrophotometrically. The assay was modified according Hunt and Hooper (10) for use on a UVmax (Molecular Devices Corp., Palo Alto, CA, USA) 96-well plate reader set in kinetic mode at a wavelength of 405 nm with a run time of 2 minutes read at 8 second intervals.

Brain cholinesterase (ChE), predominately acetylcholinesterase (AChE), was measured directly. Plasma AChE activity was measured through the use of Iso-OMPA (tetraisopropyl-pyrophosphoramidate, $1 \times 10^{-4}M$ FC), a butylcholinesterase (BChE) inhibitor. Iso-OMPA was added immediately prior to the 5 minute preincubation. The remaining activity following Iso-OMPA treatment was AChE. BChE activity was computed as the difference between total ChE and AChE activities.

Reactivation Analysis

Dilution and 2-PAM reactivation assessments were performed to test for the presence of carbamate and OP inhibited cholinesterase, respectively. The dilution

reaction involved incubating split sample aliquots at 4°C and 37°C for up to 3 hours. Samples with 37°C incubated activity elevated more than 20% over the 4°C activity were considered to contain carbamate inhibited cholinesterase (10). The 2-PAM reactivation technique included the addition of 2-PAM to one of the incubated aliquots. Samples that showed an increase of 5% or greater in the presence of 2-PAM are considered to contain OP inhibited cholinesterase, most likely of diethyl nature (11).

Results

Site I

Landowner and worker surveys indicated that monocrotophos had recently been applied at or adjacent the Swainson's hawk feeding grounds prior to their deaths (5).

Eleven GI tracts were collected from this site; none contained detectable levels of parent OPs. However, GI samples sent to Novartis Crop Protection Laboratories, Basel, Switzerland, for confirmation, contained detectable concentrations of monocrotophos (0.1 ± 0.03 µg/g) in 6 of the 11 samples. Two of three GI samples analyzed for dialkylphosphate metabolites contained detectable levels of DMP.

Birds from sites I, II, and IV were not found until 9 to 10 days postmortem. Cholinesterase activity at the time of death can be accurately diagnosed up to approximately 4 days after death (14). After this period the data become questionable and were not included in this forensic evaluation.

Site II

The surveys indicated that dimethoate had been applied to the fields where the hawks were found. There were no discrepancies among the reports (5). The carcasses were dehydrated and scavenged preventing the collection of adequate samples for either analytical or biochemical analysis.

Site III

The surveys were confused and often contradictory with respect to pesticide application. The pesticide used at site was initially assumed to be unknown (4, 5). Nine GI tracts were collected from dead birds. Four of these contained detectable levels of monocrotophos (0.089 ± 0.017 µg/g). Two samples collected from euthanized survivors did not contain detectable levels of parent OPs. Only one sample was selected for alkyl phosphate analysis from this site, and it did not contain detectable levels of any metabolites. Novartis confirmation determinations reported detectable levels of monocrotophos (0.13 ± 0.03 µg/g) in 6 of the 11 samples.

Brain cholinesterase activity for 9 dead birds and 2 that were incapacitated and euthanized were depressed $95.4 \pm 1\%$ and 70% , respectively. None of the samples showed any sign of reactivation with dilution and only 2 birds, one dead and one euthanized, were minimally reactivated when incubated with 2-PAM.

Plasma samples were taken from 8 birds, 2 euthanized at the site, and 6 which were later released. ChE was inhibited $60.1 \pm 12.5\%$ in the released birds and 49% in those euthanized. None of the samples reactivated with simple dilution. However, plasma from one of the released hawks showed reactivation when incubated with 2-PAM.

Site IV

The landowners and field workers indicated that a monocrotophos application preceded the arrival of the hawks at this site (4, 5).

No detectable levels of the parent OPs were found in the ten GI samples collected. However Novartis laboratories detected monocrotophos ($0.07 \pm 0.01 \mu\text{g/g}$) in 7 of 9 samples. DMP was detected in 2 of 4 samples analyzed for dialkyl phosphate metabolites (Table II).

Feather and Footwashes

Feathers were collected from 26 birds across the four study sites. A single bird from site III had a detectable level of monocrotophos ($\sim 1 \mu\text{g/g}$).

One footwash, performed on a single bird from site I, tested negative for OP analysis.

Discussion

Site I

Three hundred eighty seven hawks were found at site I. Chemical analysis of 11 proventriculus samples did not indicate the presence of any parent OP (5). Monocrotophos and methamidiphos were applied as oxons ($P=O$). In this activated form, the pesticides are able to interact with the serine esterases. The acidic environment of the proventriculi and the additional serine esterases, released by digesting grasshoppers, enhanced the degradation of these pesticides. Therefore, the probability of these reactive OPs remaining in high concentrations several days postmortem is extremely low. Methyl parathion, dimethoate and chlorpyrifos were applied in the inactive $P=S$ form. These pesticides have to be bioactivated into oxons before they actively bind esterases. Additionally, the inactive states are hydrolyzed at much lower rates than the activated forms. Therefore, if the hawks had consumed grasshoppers treated with methyl parathion, dimethoate, or chlorpyrifos, the pesticides are more stable and would have persisted well beyond the time between death and GI sample analysis.

Table II. Swainson's hawk mortality incidents during January and February of 1996, in the pampas region of Argentina.
(Table modified from Goldstein et al. (5))

Site	# Dead	Survey ^a	ChE % Inhibition						GI Contents MCP µg/g		AP ⁸	Indicated ^h Pesticide
			Brain		Plasma		TIEHH	Novartis ^f				
			Dead	Euthanized	Euthanized	Released						
I	387	MCP	NA	NA	NA	NA	0/11 ^e	0.1±0.03 ^b 0.05-0.19 ^c N=6/11 ^e	NA	2/3	MCP	
II	103	Dimethoate	NA	NA	NA	NA	NA	NA	NA	NA	Dimethoate (?)	
III	3,024	MCP	95.4±1 ^b 89.4-98.3 ^c N=9 ^d	72.7 71.8-73.6 N=2	48.6 38.1-59.2 N=2	60.1±12.5 24.3-97.4 N=6	0.09±0.02 0.05-0.13 N=4/11	0.13±0.03 0.06-0.25 N=6/11	0/1	MCP		
IV	595	MCP	NA	NA	NA	NA	0/10	0.07±0.01 0.05-0.12 N=8/10	2/4	MCP		

NA- Not Available, MCP - monocrotophos, ChE - cholinesterase, TIEHH - The Institute of Environmental and Human Health,

^apesticide implicated by landowner/worker survey, ^bmean±SE, ^crange, ^d# of samples analyzed, ^e# of samples containing monocrotophos/total # of samples analyzed, ^fData from R. Tribolet, Novartis Crop Protection, # of samples containing detectable levels of DMP/total # of samples analyzed, ^hpesticide implicated in hawk mortalities

Despite the fact that our laboratory was unable to detect monocrotophos in the GI tracts, Novartis Crop Protection Laboratories consistently found monocrotophos residues in their samples. Novartis employed a method developed to analyze solely for monocrotophos. Their extraction and cleanup methods catered towards the specific solubility of the analyte. Our method was geared towards the extraction and cleanup of several OPs that spanned a broad range of solubilities. As such, we extracted many more non-pesticide compounds from the samples, increasing the baseline noise and our level of detection above the low concentrations of monocrotophos found in many of the samples.

The presence of DMP in the absence of thiol containing metabolites in the GI samples at this site can only occur with the use of monocrotophos as discussed in the introduction. Therefore, based upon our analytical and biochemical data, landowner surveys, and Novartis findings, it is most probable that the Swainson's hawks were killed by lethal concentrations of monocrotophos at site I.

Site II

One hundred three hawks were found at site II (5). The carcasses were desiccated and scavenged precluding the collection of samples. However, interviews with field workers indicated that dimethoate had been applied to the area just prior to the hawks foraging. The surveys were not contradictory in this regard. Therefore, in the absence of corroborating analytical and biochemical data, dimethoate was designated the likely agent in hawk mortalities at site II.

Site III

By far the most widespread mortality occurred at site III (5). Over 3,000 hawks were found dead or moribund. The presence of detectable monocrotophos in the GI tract indicated recent exposure. The absence of methamidiphos, methyl parathion, or dimethoate in the GI tracts supports the premise that monocrotophos was the only OP responsible for the mortalities at this site.

The extreme depression of brain ChE activity in the birds sampled dead (>90% inhibition) and live (>70% inhibition) from this site indicates that an anticholinesterase agent was responsible for the hawk mortalities. Plasma ChE activity was also significantly depressed compared to normal activities. However, the inhibition was much less than that observed in the brain. This may be attributed to the relatively rapid turnover of ChE in the plasma compared to the brain. Perhaps the plasma ChE was recovering following exposure to the anticholinesterase agent.

The lack of reactivation of ChE by dilution and incubation indicates that the ChE was not inhibited by a carbamate. The infrequent reactivation in the presence of 2-PAM indicates inhibition by an OP, in particular a dimethyl type OP, which ages quickly. Our findings and those of Novartis indicate that the Swainson's hawk at this site experienced a lethal exposure of monocrotophos.

Site IV

Nearly 600 hundred birds were found dead after foraging in an alfalfa field recently treated with monocrotophos. There was no discrepancy among the surveys with regard to the pesticide applied (5).

DMP was found in two of four samples checked for metabolites. The presence of DMP in the absence of DMTP and DMDTP implies the use of monocrotophos. Novartis Crop Protection also found monocrotophos in 7 of 9 GI samples analyzed. Chemical analysis of the GI tract in our labs did not detect any pesticide residues. However, due to the highly reactive nature of monocrotophos and the length of time between exposure and analysis, this result is expected. Our findings, in conjunction with those of Novartis, indicate that the hawks were exposed to monocrotophos.

Conclusions

Based upon our analysis of four Swainson's hawk mortality incidents in the pampas regions of Argentina during the austral summer of 1996, it is evident that current analytical and biochemical techniques can be used to forensically evaluate the impact of agricultural chemicals on wildlife (5). We were able to ascertain the presence of monocrotophos or a primary metabolite in fields where each of the kills occurred. The absence of four additional insecticides was demonstrated through analysis for parent OPs, primary hydrolytic metabolites, and ChE reactivation when appropriate samples were available. This study demonstrates the importance of incorporating multiple disciplines in environmental studies.

Acknowledgments

The authors would like to thank A. Lanusse and S. Salva for their endless hospitality; veterinarians J. Garat and C. Peregalli for their time and help in the field collections; M. Zaccagnini, S. Canavelli, and J. Panigatti of INTA for their support in Argentina; J. Rogers and C. Cline for their assistance in the lab, M. Fuller for satellite coordinates to locate flocks of hawks; B. Woodridge and M. Bechard for their foresight, motivation, and extensive knowledge of Swainson's hawk ecology; and R. Tribolet who performed the monocrotophos analyses at Novartis Crop Protection, Basel, Switzerland.

This study was made possible by support from the National Wildlife Federation, Ecorisk Incorporated, USDA Forest Service, Rachel Kolokoff, Archbold Tropical Research Center, National Fish and Wildlife Foundation, Novartis Crop Protection, and the USFWS Office of International Affairs.

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