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Effects of Azinphos-Methyl on American Robins Breeding in Fruit Orchards

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The Okanagan Valley of British Columbia is an important fruit growing region accounting for 21% of Canadian fruit production (Statistics Canada 1997a, Statistics Canada 1997b). Pests such as coddling moth (*Cydia pomonella*) are controlled mainly through spraying with the organophosphate, azinphos-methyl (Guthion™). Azinphos-methyl is extremely toxic (Class I, Hill et al. 1975) with an LD50 value of 8.5 mg/kg in Red-winged blackbirds (*Agelaius phoeniceus*) (Smith 1987). Studies have shown that cholinesterase (ChE) inhibition, a useful measure of physiological stress caused by organophosphate insecticides, exceeding 20% is indicative of exposure, while inhibition greater than 50% is sufficient for attributing the death of a bird to the insecticide (Ludke et al. 1975; Busby et al. 1981). Mortality in birds following exposure to azinphos-methyl (35% W.P.) has been recorded in Washington apple orchards (Stinson and Bromley 1991).

The purpose of this project was to evaluate the exposure effects of azinphos-methyl on breeding birds in Okanagan orchards using the American robin (*Turdus migratorius*) as an indicator species. Their nestlings may be exposed to azinphos-methyl because the nests are open and nestlings are present during spray events. The objectives of our study were:

1) To determine if robins were exposed to azinphos-methyl by measuring plasma ChE activities in nestlings before and after spray events, measuring brain ChE activities in nestlings found dead during the study period and measuring azinphos-methyl residues deposited in model nests placed in trees for spray events.
2) To determine if exposure to azinphos-methyl affected reproduction of robins in orchards.

MATERIALS AND METHODS

Apple orchards in the Okanagan Valley (the Penticton and Naramata areas in 1993 and Kelowna in 1994) were surveyed systematically tree by tree for American robin nests between May and August. One hundred thirty-three nests were located in orchards treated with azinphos-methyl (here on referred to as "sprayed" orchards), 24 nests were found in orchards where orchardists sprayed but had not done so prior to nest production, 32 were located in orchards not treated with any organophosphates (here on referred to as "control" orchards), and five nests were located in non-orchard habitat adjacent to an orchard (here on referred to as "periphery" nests).

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Azinphos-methyl was applied two to four times per season at 1.4 kg Guthion™ 50% W.P. per hectare by tractor-pulled air blast sprayers. In our study, birds sampled from sprayed orchards were exposed to only one azinphos-methyl spray event. In both control and sprayed orchards, other chemicals such as the fungicides, captan and manzoceb were used, but not during the period when the nests were active and therefore, they should not affect the results of our study.

Blood samples (150 µl) were collected in the field from the brachial vein using heparinized capillary tubes (Fisherbrand™) and placed on ice. Within two hours of collection, blood samples were centrifuged (Canlab-Medifuge™) at 10,000 g for five minutes and the plasma was then stored in cryovials in liquid nitrogen until analysis. Heads of birds found dead in the orchards were frozen at -20°C as soon as possible. Frozen plasma and heads were sent to the National Wildlife Research Centre (NWRC) in Hull, Quebec for determination of plasma and brain ChE activity levels. Cholinesterase activity in plasma and brain was determined based on Hill and Flemming's (1982) modification of the method of Ellman et al. (1961). Quality assurance procedures included duplicate analysis of a standard commercial serum control and preparation of control charts. Samples were kept frozen until analysis and activity was assayed colorimetrically at 406 nm at 30°C using acetylthiocholine iodide (Sigma Chemical Company, St. Louis, MO) as the enzyme substrate. Enzyme activity was expressed as micromole of substrate hydrolyzed per minute per liter (plasma) or gram (brain).

Age of nestlings from which plasma samples were collected ranged from two to 12 days old. Plasma samples were collected from 44 nestlings from control orchards. Two to five days following the first blood sampling, 27 of the 44 were bled a second time. A third sample was obtained from 14 of these robins, two to five days following the second sampling. Another 14 samples were collected from nestlings in sprayed orchards, prior to any organophosphate application. All groups showed no significant difference; therefore these 99 samples were combined to provide an age profile of ChE activities of nestlings not exposed to azinphos-methyl.

We collected plasma samples from 44 nestlings from 21 nests exposed to azinphos-methyl. Time post-exposure and age of nestling when sampled were recorded. The 17 post-exposure times were classified into five groups for analysis: up to one day, one to four days, five to ten days, 11 to 20 days and 21 to 26 days. For comparison of age susceptibility to azinphos-methyl effects, the ages of the nestlings were classified into four groups: up to five days, six to seven days, eight to nine days and ten to 12 days post-hatch. Both groupings were done because sample size for some post-exposure time or age was small or there was no sample.

Twenty-eight nestling robins were found dead in orchards in 1994. Although the exact time of death was unknown, 12 were found prior to spraying and 16 after spraying. The probable cause of mortality of any nestling found dead was documented. The carcasses were collected and stored in a cooler with ice during transportation from the field to the lab and brain tissues were analyzed for cholinesterase activity levels.

We attempted to assess exposure of nestlings to azinphos-methyl by measuring the presence or absence of pesticide deposited during one spray event onto model nests placed in trees surrounding a nearby active robins' nest. Pesticide deposition was measured in a total of 20 nests (five model nests around four active nests), over three spray periods. The model nests were made of a 250 ml glass bowl, which was lined with Whatman™ glass microfibre filter paper (diameter of 15 cm) supported by a 500 ml plastic container, which was secured to the tree using wire. Inside each model nest were three large glass beads (diameter of 24.32 mm) covered with Whatman™ glass microfibre filter paper (diameter of 9 cm). The model nests were placed three to eight feet above the base of the tree immediately prior to spraying, and were removed 60 minutes after spraying to allow the pesticide particles to settle while minimizing degradation and/or volatilization of the active ingredient. Five field spikes (10 ppm azinphos-methyl) and one blank of model nests were sent in for quality assurance of handling, shipping and analysis. Samples of the pesticide mix from the spray tanks from each of the three spray periods were collected and analyzed for azinphos-methyl. The National Wildlife Research Centre in Hull, Quebec, conducted analysis of azinphos-methyl based on the methodology used by Corneliussen et al. (1984). Due to accidental spillage, only qualitative data were obtained.

Productivity was surveyed in 150 nests (112 in sprayed orchards, 17 in orchards where orchardists sprayed but after nest production, 16 in control orchards and five in non-orchard habitat adjacent to an orchard). These nests did not include those used for blood sampling. Nests were visited as often as required to determine outcome. Each nest was monitored for clutch size, hatching success and fledging success. Incubation started the day after the last egg was laid. The incubation period was estimated to be 12 days and nestlings were assumed to have fledged at 14 days after hatching. Nests were considered successful if at least one offspring fledged. Nests whose young disappeared before day ten were considered unsuccessful under the assumption that the young were depredated or prematurely fledged. The Mayfield method was used to calculate nesting success (Mayfield 1975).

To provide an age profile of ChE activities of nestlings not exposed to azinphos-methyl, samples from control orchards were combined with those from sprayed orchards prior to spraying. To compare the level of plasma ChE inhibition among different age groups of nestlings exposed to the spraying and time post exposure to the spray, least squared analysis of covariance (ANCOVA) was used with time post exposure or age as a covariate, respectively. Plasma and brain ChE activities in exposed nestling robins compared time post exposure using orthogonal contrasts. These statistical analyses were conducted with the help of JMP software program (SAS Institute Inc 1995). To determine if a statistical difference was present in the variables used to determine reproductive success in sprayed versus control orchards, the χ^2 test was applied.

RESULTS AND DISCUSSION

Routes of exposure important in the uptake of organophosphorus pesticides in birds, in addition to ingestion of contaminated food, may include dermal absorption, ingestion through preening and inhalation (Driver et al. 1991, Wilson et al. 1991).

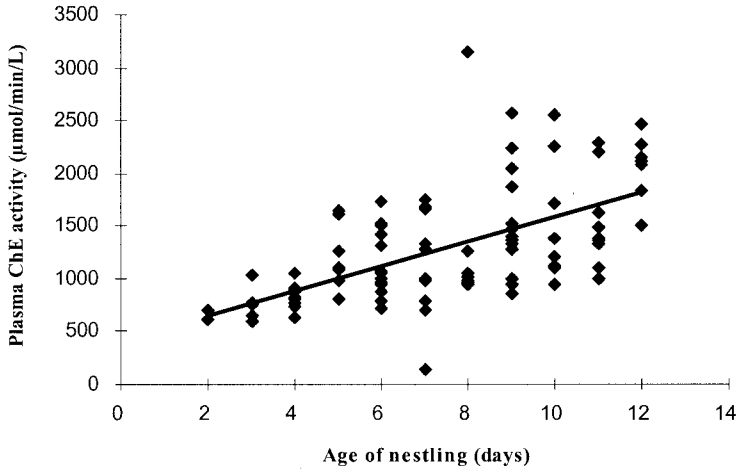

Figure 1. Basal plasma cholinesterase (ChE) activity in nestling American robins not exposed to azinphos-methyl (including nestlings from control orchards and those from sprayed orchards prior to organophosphate application) compared to age of nestling (n=99). ChE activity is seen to increase linearly with age [ChE activity = 405 + 118 (age), r=0.6, P<0.0001].

Nestling exposure to azinphos-methyl was confirmed by the presence of residues in all 20 model nests sampled (data not shown) and the presence of azinphos-methyl in the pesticide mix from the spray tanks.

There was no significant difference in the mean plasma ChE activity in nestlings from the control orchards (first, second and third bleed) and the pre-sprayed sites (sites in which orchardist sprayed azinphos-methyl but had not sprayed at time sample was collected from nestling), therefore, they were pooled to provide an age profile of plasma ChE activities of nestlings not exposed to azinphos-methyl (Figure 1). Plasma ChE activity regressed significantly (r = 0.6, P<0.0001) on age in control nestlings (n= 99). Basal plasma ChE activity increased linearly (P<0.0001) with age [ChE activity = 405 + 118 (age)] (Figure 1). After standardizing for age variations (least squared means from ANCOVA), plasma ChE levels of nestlings sampled between one and four days post exposure were significantly lower (orthogonal contrast, P< 0.04) than those sampled before the spraying (Figure 2A). There was 29.4% and 34.5% inhibition of plasma ChE activity during the first day and between two and four days post exposure, respectively. Plasma ChE level of nestlings sampled between five and 26 days post exposure was not significantly different from pre-spray samples. There was no significant difference between samples collected in 1993 and 1994. Samples collected in May, June and July were also not statistically different. In a separate analysis, we found no significant difference in the level of plasma ChE inhibition among the different age groups of nestlings exposed to the spraying, after standardizing for time post exposure (ANCOVA).

Brain ChE activity of control nestlings increased significantly (P<0.0001) with age. After standardizing for age variations (ANCOVA), brain ChE activity in nestlings sampled during one to four days post exposure was found to be significantly lower

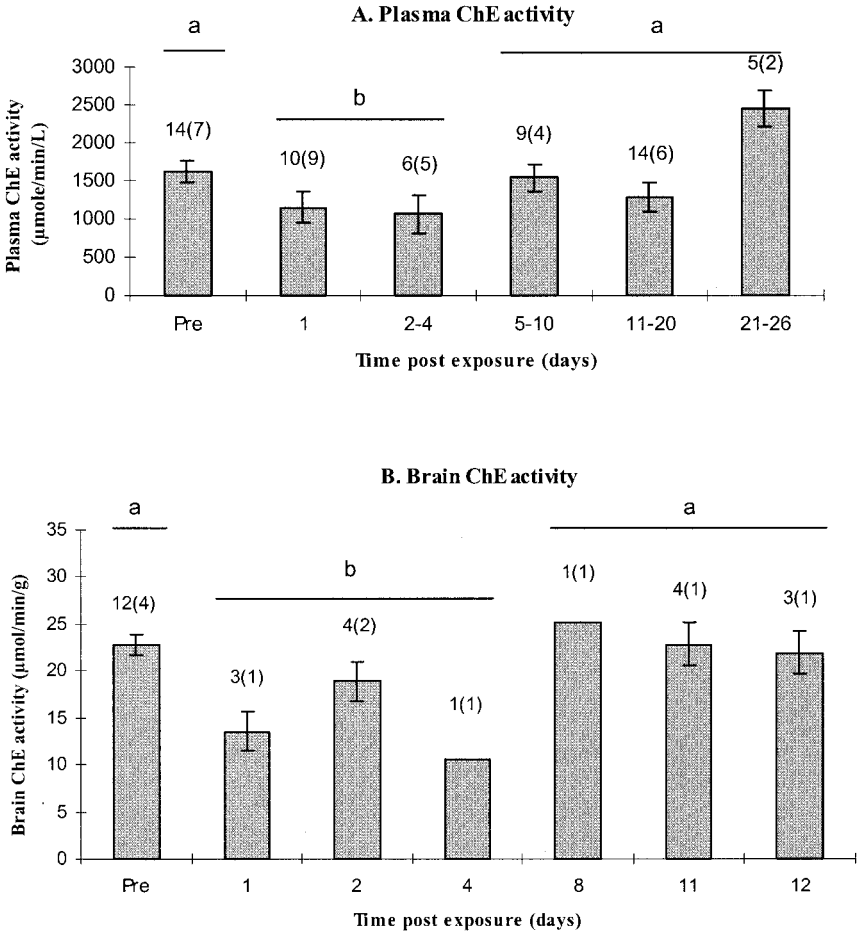

Figure 2. Mean plasma (A) and brain (B) cholinesterase (ChE) activity in nestling American robins collected before (plasma n=14; brain n=12) and after (plasma n=44; brain n=28) azinphos-methyl application. Results shown are means ± SE. Bars with "a" present are seen to be statistically different than those with "b" present (orthogonal contrast, plasma P=0.0106 and brain P=0.0004). Values above each bar represent number of individuals sampled with numbers in parenthesis representing number of nests sampled. Nestlings from same nest may have been bleed at more than one time period.

(orthogonal contrast, $P < 0.001$) than those sampled before spraying (Figure 2B). At one, two and four days post-exposure, nestlings showed 40.8%, 17.4% and 53.8% ChE inhibition, respectively. Four days post-exposure, the nestling sampled showed brain ChE inhibition greater than the critical level of 50% inhibition, therefore, it is likely that individual died from acute exposure to azinphos-methyl (Ludke et al. 1975; Busby et al. 1981). Three nestlings found dead one day following exposure showed brain ChE inhibition greater than the 20% threshold indicative of anticholinesterase exposure, however, we cannot conclude it was the primary cause of death. Those sampled from eight to 12 days post exposure were not significantly

Table 1. A comparison of nest productivity parameters of American robins exposed to azinphos-methyl during either the incubation or nestling stage with those from control orchards.

	Sprayed	n	Control	n
Incubation stage				
Clutch size[1]	3.42	38	3.15	13
Unsuccessful nests[2]	38	98	11	29
Nests w/ clutch reduction [3]	10*	26	3	23
Hatching rate [4]	90.0%	38	87.9%	13
Nestling stage				
Brood size[5]	3.08	38	2.77	13
Unsuccessful broods[6]	22	59	6	17
Nests w/ brood reduction[7]	3	38	1	13
Fledging rate[8]	57.9%	38	61.5%	13

[1] number of eggs laid per clutch.
[2] proportion of nests surveyed that were abandoned or totally depredated during incubation.
[3] proportion of nests surveyed that showed loss of eggs (not whole clutch) during incubation (* χ^2 test, $P<0.05$).
[4] % eggs hatched per clutch.
[5] number of young hatched per nest.
[6] proportion of nests with young surveyed that were abandoned or totally depredated before young fledged.
[7] proportion of nests surveyed with reduction in number of young without losing whole brood.
[8] % young fledged per brood.

different from those sampled before spraying. Six nestling appeared to have been predated. The other 18 showed no apparent injuries and showed less than 20% ChE inhibition. The cause of death of these nestlings was unknown.

One day following the spraying, plasma and brain ChE levels in nestling robins were significantly inhibited and maximum inhibition for both plasma (34.5%, n=6) and brain (53.8%, n=1) occurred four days post-exposure, after which time they returned to normal levels. The time lag in maximum ChE inhibition to the organophosphate acephate occurred six days after exposure in Chipping sparrows (*Spizella passerina*) (Zinkl et al. 1980). Graham and DesGranges (1993) found that plasma cholinesterase of adult robins returned to normal activity levels two days following exposure to azinphos-methyl. As nestlings from our study took longer to recover, it may indicate that nestling robins may be more sensitive to azinphos-methyl exposure than adults. This sensitivity in nestlings to organophosphates has also been noted in European starlings (*Sturnus vulgaris*) exposed to dicrotophos (Grue and Shipley 1984) and Tree swallows (*Tachycineta bicolor*) and Eastern bluebirds (*Sialia sialis*) exposed to azinphos-methyl, phosmet, diazinon and phosalone (Burgess et al. 1999).

Measurement of ChE activity has been successfully employed to assess the extent of exposure to organophosphate and carbamate insecticide spraying on birds (Zinkl et al. 1980; Busby et al. 1981), however, this alone may not indicate harmful levels of exposure (Graham and DesGranges 1993). Using the Mayfield method, the probability that an egg present at the start of incubation would produce a fledgling was 35.1% in control orchards and 28.3 % in sprayed orchards, a difference not significant by the χ^2 test. A comparison of nests from sprayed orchards with nests from control orchards (Table 1) found that the proportion of nests with unhatched

eggs was significantly higher in exposed orchards (χ^2 test, $P< 0.05$) suggesting that eggs may be more susceptible to azinphos-methyl exposure than nestlings. All other parameters showed no significant difference. American robins exposed to organophosphates (including azinphos-methyl), carbamates and organochlorine pesticides in apple orchards in Pennsylvania showed no difference in clutch size or the number of fledglings produced compared to robins nesting in organic orchards, however, hatching success of eggs was significantly lower in conventional than in organic orchards in one of two years of their study (Fluetsch and Sparling 1994). Thrush species, such as American robins and Eastern bluebirds, may not be suitable indicators to assess the effects of anticholinesterase pesticides on birds breeding in orchards. Tree swallows appeared to be more sensitive than Eastern bluebirds to the effects of organophosphates, carbamates and organochlorine pesticides on reproduction (Bishop et al. 2000). This suggests that thrush species may be more tolerant and resistant to organophosphate exposure.

In conclusion, robin nestlings were exposed to azinphos-methyl as plasma and brain ChE levels in nestlings were inhibited and presence of residues in model nests was observed. Some nestlings were acutely exposed to the pesticide although most experienced sub-lethal exposure. Although there were no noticeable effects on the reproductive success to the fledging stage of nestlings, orchards sprayed with azinphos-methyl had a significantly higher number of unhatched eggs than control orchards.

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