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Brain Cholinesterase Activity in Birds After a City-Wide Aerial Application of Malathion

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This bird monitoring project was undertaken as part of a multidisciplinary surveillance program in response to concern about non-target effects of aerial application of malathion over the City of Winnipeg (Manitoba, Canada) in July 1983. The spraying was ordered by the Minister of Health as an emergency measure to control mosquito carriers of the Western Equine Encephalitis virus. Cythion Ultra Low Volume (95 percent malathion) was applied at a rate of 210 ml/ha over the entire city, areas where the test birds were located were sprayed on 24 July 1983.

The toxic action of malathion and other organophosphate insecticides is attributed to their inhibition of cholinesterase (ChE) - an enzyme active in transmission of nerve impulses. The toxicity of malathion to vertebrates is low, but many cases of bird mortalities from exposure to organophosphates *have* been recorded, and ChE inhibition was found in the poisoned birds (Reece 1982, Hill and Flemming 1982, Grue et al. 1983). The degree of inhibition is directly related to the pesticide dose, and measurement of brain ChE activity is a physiologically meaningful and reasonably simple method of assessing the exposure of birds to anti-ChE compounds (Hill et al. 1971; Findlay et al. 1974; Busby et al. 1981, 1983). In the present study house sparrows (*Passer domesticus*) and feral pigeons (rock doves; *Columba livia*) were used as monitors of the spray effect, because of their abundance and wide distribution in the city as well as their unprotected status. The objective was to assess the ChE activity, rate of mortality, and recovery of brain ChE levels after the spraying.

MATERIALS AND METHODS

Sparrows were collected by mist netting in backyards of three Winnipeg residences, pigeons were caught by hand nets in an abandoned school building. In addition to scheduled sampling, dead birds reported by the public were picked up for testing as well. Members of the Rare Bird Alert group of the Manitoba Naturalists Society were asked to report abnormal bird behavior or mortalities observed following the spraying.

Twenty live sparrows were obtained prior to spraying and kept in a cage supplied with sunflower seed, bird seed mixture, and water

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ad libitum. The cage was kept in an open shed for three days and for the last night before spraying in a closed garage, to protect the birds from ground fogging operations carried out in the area. On the morning aerial spraying commenced, the 12-mm open mesh cage was moved to an open area at Fort Whyte Nature Centre where the spray distribution, droplet size, and application rate of the pesticide were monitored by the Environmental Protection Service of Environment Canada. Although the nominal spray coverage was 210 ml/ha, the actual spray deposition rate on the site where the cage was located was 140 ml/ha over the 3 hours of exposure (T.Youmans, Environment Canada, *personal communication*). The cage was left *in situ* during, and for three hours after the pass of the spray aircraft. Food and water supply was not protected from spray, but was replaced afterward.

The sparrows from the cage were sacrificed at 6, 12, and 26-27 hours after exposure to the spray. Additional sparrows were collected 1 and 2 weeks later, from the same backyards. Pigeon collection took place 4 days before and 2, 9, and 15 days after the spraying. A total of 41 sparrows and 39 pigeons were collected by provincial personnel, and eight birds of five other species were submitted by concerned citizens. The brains were excised and kept frozen in individual vials until analysis.

Brains were homogenized with a Polytron homogenizer and centrifuged at 21000 x G to prepare a solution/suspension which was assayed by the colorimetric method of Ellman et al. (1961), using acetylthiocholine as the substrate, at 25°C. Results were expressed as milliunits of enzyme activity per mg of protein (mU/mg). Analytical precision of this method is about +-15%.

RESULTS AND DISCUSSION

The caged sparrows showed neither ill effects nor change in behavior during or after the spraying. Mean values of their brain ChE activity are shown in Table 1. The post-spray specimens' ChE levels, though lower, were not significantly suppressed compared to the pre-spray and 15- to 16-day post-spray samples (about 6 and 12 percent, respectively).

There was no significant change in the ChE activity in any of the pigeon brain samples; less than 10 percent difference between sampling period means was recorded (Table 2).

Four of the samples collected by private citizens were already decomposed when received, which prevented analysis and indicated that the birds died before aerial spraying commenced. ChE activity for the remaining four was as follows (mU/mg): American robin (*Turdus migratorius*) 106.0; Red-winged blackbird (*Agelaius phoeniceus*) 107.0; Purple martin (nestling) (*Progne subis*) 48.7. All were collected 1 day post-spraying. Another purple martin nestling, 5 days post-spraying, was assayed at 104.0. Pre-spray controls for these species were not collected, so that direct pre- vs. post-spray comparison was not possible; in general, the values

Table 1. Brain ChE activity in sparrows

Sampling time[1]	n	ChE activity mU/mg mean (range)	ChE activity suppression, % of: pre-spray	post-spray 15-16 days
Pre-spray (W)	10	34.5 (111-164)	0	6.7
Post-spray				
6 hours (C)	5	28.6 (117-137)	14.4	10.8
12 hours (C)	5	126.6 (101-148)	5.9	12.2
26-27 hours (C)	9	127.0 (113-143)	5.6	11.9
8 days (W)	6	130.2 (126-134)	3.4	9.7
15-16 days (W)	6	144.2 (128-153)	-7.2	0

[1]W = birds from the wild; C = caged birds

of ChE activity were similar to values observed in the pigeon samples, a little lower than those of the sparrow samples. Assuming that the ChE activity of the second purple martin sample was normal (cause of death was unknown), the ChE of the purple martin which died the first day after spraying would have been at a lethally low level. No reports of abnormal behavior or mortalities of birds were received from the Rare Bird Alert members.

According to Ludke et al. (1975), inhibition of brain ChE activity by 20 percent is indicative of exposure to pesticides, and >=50 percent inhibition is sufficient for diagnosing death from anti-ChE poisoning. House sparrows which died from malathion poisoning in experimental studies (Mehrotra et al. 1967; cited by Ludke et al. 1975) had brain ChE suppressed by about 80 percent (range 36 to "near 100" percent). Our results did not approach the lower range; they are similar to the findings of Hill et al. (1971) who reported maximum ChE inhibition of 18 percent in brains of sparrows after an aerial application of malathion at the same rate as in this study.

In evaluating our results, it is not possible to accept without reservation that the pre-spray ChE activity values correspond to normal levels. The birds had been previously exposed several times to pesticides during ground fogging operations (Table 3). In addition, increasing mosquito numbers prompted the City to resume ground fogging on August 8. That, and a second round of aerial spraying (on August 14), precluded continued sampling intended to assess the level at which the ChE activity would stabilize in the post-spray period.

The more or less constant level of ChE activity in pigeons may have been due either to their different habits (e.g., roosting inside the building), or to the confounding effect of the ground fogging, or both. Absence of conspicuous bird mortalities during or following the spraying suggests that the pesticide dose received by the birds from both ground and aerial spraying operations was not excessively high. This applies to both sparrows and pigeons, and probably may be extrapolated to other birds of similar size and habits.

Table 2. Brain ChE activity in pigeons

Sampling Time	n	ChE Activity mU/mg; mean (range)
Pre-spray	9	102.5 (79-128)
Post-spray:		
25-26 hrs.	10	107.0 (89-126)
9 days	10	105.3 (89-136)
15 days	10	111.0 (87-137)

The present study was set up on an emergency basis; although this afforded the advantage of a 'real-life' situation, it also left unanswered a number of important questions. How are insectivorous birds affected by the sudden disappearance of their food supply? As even granivorous songbirds feed their young with insects, are there more severe effects on nestlings, either from the pesticide or from the decreased food supply? Results of Grue and Shipley (1984) suggested that young nestling songbirds may be twice as sensitive as adults. What are the effects on the less visible bird species (e.g., the canopy-dwelling warblers), the less common ones or those which may die in places not easily accessible, where they would not be readily found? Grue et al. (1982) found significantly reduced parental care in birds exposed to sublethal levels of organophosphates. Are there other sublethal effects, e.g., does exposure to the pesticide make the birds more vulnerable to predation? Such questions can be answered only through carefully designed, rigorously controlled experiments.

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Table 3. Times of bird collections and pesticide applications

Date	Birds collected and analyzed	Pesticide applied[1] at collection site of sparrows	pigeons
07/14			Propoxur (Baygon)
07/17		methoxychlor	
07/19	pigeons		
07/20	sparrows		
07/21	sparrows		
07/23		malathion	
07/24	sparrows(C)[2]	AERIAL SPRAYING OF MALATHION	
07/25	sparrows(C), pigeons		
08/01	sparrows		
08/02	pigeons		
08/08	sparrows, pigeons		malathion
08/09	sparrows	malathion	
08/14		AERIAL SPRAYING OF MALATHION	

[1] Ground fogging unless otherwise stated (rates variable). Baygon and malathion are ChE inhibitors, methoxychlor is not.

[2] C = caged specimens; other birds were caught from the wild.

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