Exposure of the Eastern Screech-owl to selected contaminants in apple orchards of southern Quebec

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ABSTRACT

This study examined the exposure of the Eastern Screech-owl (*Otus asio*) to contaminants in apple orchards of southern Quebec. Using a worst-case scenario approach, secondary exposure to three organophosphorus insecticides, (phosmet, azinphosmethyl and phosalone), two anticoagulant rodenticides, (chlorophacinone and diphacinone), and residues of previously applied organochlorines, particularly DDT and metabolites, was assessed. Exposure to PCBs and trace metals was also considered. Small mammal species preyed upon by Screech-owls were captured in orchards for residue analysis on a continual basis for persistent compounds or after insecticide and rodenticide applications. Beginning in the winter of 2000, 98 nest boxes were constructed and installed in woods inhabited by Screech-owls, adjacent to orchards. These boxes were then repeatedly inspected for pellets and prey remains. Estimated exposure of Screech-owls 0-60 hr post-application was 0.641 mg/kg for phosmet and azinphosmethyl and 0.401 mg/kg for phosalone. Exposure to phosmet at this level may warrant concern. The acute poison zinc phosphide is now the primary means of small mammal control in the study area and the possibility of exposure to anticoagulant rodenticides is diminishing. Observed DDE residues were most elevated in the short-tailed shrew (*Blarina brevicauda*) and ranged from <1.00 to 26.29 μg/g (wet wt) in whole-body pools. A Screech-owl egg found in a nest box between two orchards may have been thinned by as much as 19.8%, of concern because thinning maintained at 15.0 – 20.0% has been linked to population decline. Only background levels of PCBs and trace metals were detected. Finally, over 950 Screech-owl case files were also obtained from one Canadian and seven United States wildlife rehabilitation facilities and analyzed for evidence that pesticide exposure was an underlying or contributing cause of admissions.

RÉSUMÉ

Cette étude a examiné l'exposition aux contaminants du Petit Duc maculé (*Otus asio*) dans les vergers de pommes du sud du Québec. Employant une analyse du pire cas possible, l'étude a évalué l'exposition secondaire au phosmet, à l'azinphos-méthyl, et au phosalone, trois insecticides organophosphorés, au chlorophacinone et au diphacinone, deux rongicides anticoagulants, et aux résidus d'organochlorés répandus avant, surtout au DDT et à ses métabolites. L'exposition secondaire aux résidus de BPC et aux traces de métaux, parmi d'autres contaminants, a également été considérée. Les espèces de petits mammifères consommés par le hibou ont été capturées dans les vergers pour analyse de résidus de contaminants persistants ou d'organophosphorés et d'anticoagulants. À partir de l'hiver en 2000, 98 nichoirs ont été construits et installés dans des boisés dans lesquels des hiboux habitaient et qui étaient situés aux alentours des vergers. Les nichoirs ont ensuite été vérifiés à multiples reprises afin d'y recueillir des boulettes de régurgitation et des restes de proies. L'exposition estimée du Petit Duc était de 0,641 mg/kg au phosmet et à l'azinphos-méthyl et de 0,410 mg/kg au phosalone, ce qui pourrait être inquiétante au niveau du phosmet. Le phosphure de zinc est maintenant le moyen principal de contrôle des petits mammifères et la possibilité d'exposition aux anticoagulants a donc grandement diminué. Les niveaux les plus élevés de DDE été ceux de la grande musaraigne (*Blarina brevicauda*), entre <1,00 à 26,29 μg/g (poids frais). L'écaille d'un œuf de Petit Duc retrouvé dans un nichoir entre deux vergers aurait pu être aminci de 19,8 %. On constate une baisse dans la population lorsqu'un amincissement de la coquille de l'œuf se maintient entre15,0 et 20,0% pour une période prolongée. De faibles niveaux de BPCs et de métaux ont été détectés. Plus de 950 dossiers de Petits Ducs ont été obtenus d'un centre canadien de réhabilitation de la faune et de sept centres américains afin de déterminer si l'exposition aux pesticides aurait pu, de façon directe ou indirecte, avoir contribuée à l'admission du hibou.

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PREFACE

Every year, several insecticide applications and generally one rodenticide application are made in apple orchards to maintain the integrity of apple trees and fruit. Previously, application of organochlorine insecticides, particularly DDT, was also intensive in apple orchards. The Eastern Screech-owl (*Otus asio*) is known to inhabit forests that border apple orchards and to hunt in orchards. In contrast to many of the raptors studied in agricultural systems, the Screech-owl is non-migratory and may therefore be exposed to currently used insecticides and rodenticides and to residues of previously applied pesticides.

The overall aim of this study was to assess the secondary exposure of the Screech-owl to the organophosphorus insecticides, anticoagulant rodenticides and organochlorines currently and previously applied in apple orchards of southern Quebec. A secondary objective was to evaluate the owl's suitability as a monitor of organophosphorus exposure, and of local organochlorine residue persistence.

This study comprised a field and a clinical component. Screech-owl exposure to pesticides and the owl's potential role as a monitor of exposure were pursued in a clinical setting. To this end, case files from a number of wildlife rehabilitation facilities were scrutinized for evidence that exposure played a direct or underlying role in Screech-owl admissions to these facilities.

A further goal was to present the data obtained during this study in a format applicable and relevant not only to the Screech-owl but to other species that might also risk secondary exposure to the pesticides in question. Finally, this is one of the first studies entirely focused on the Eastern Screech-owl in Canada or in Quebec. Moreover, the study was conducted in the northernmost portion of its North American distribution. This provided a unique opportunity to gather baseline information on the species' distribution, basic natural history, and behaviour.

CONTRIBUTIONS OF AUTHORS

As permitted by the Faculty of Graduate Studies and Research, this thesis includes the texts of two manuscripts to be submitted to peer-reviewed scientific journals for publication. The first paper, presented in chapter two, will be submitted to "Archives of Environmental Toxicology and Chemistry" and the second paper, presented in chapter three, has been submitted to "Journal of Wildlife Rehabilitation".

Data collection, data analysis and manuscript preparation were completed independently by the primary author. Pierre Mineau and David Bird appear as co-authors on the chapter two and chapter three manuscripts for their supervisory guidance and provision of research facilities. Pierre Mineau contributed towards the conception of the research project and interpretation of data. Guy Fitzgerald appears as a co-author on the chapter three manuscript for providing the facilities of the Université de Montréal's Clinique des oiseaux de proie during the implementation of a pilot raptor tissue sampling program, for providing Screech-owl case file data for analysis, and for his invaluable input into the analysis of these data.

CHAPTER ONE

Literature and Methods Review

1.1. INTRODUCTION

The apple is Quebec's second most pesticide-intensive crop, marginally below tobacco (S. Tellier, pers. comm.). While orchards only occupy approximately 9,000 ha (Chouinard and Charbonneau 1999), the application rate is estimated at 27.4 kg active ingredient (a.i.) per hectare (ha). (Bélanger 1995). In comparison, corn occupies approximately 350,000 ha, but has an estimated application rate of 3.0 kg a.i./ha (Giroux 1998). Between 11 and 15 pesticide applications are made each year to protect the integrity of apple trees and apples. During a typical growing season, insecticides are applied three to four times and rodenticides are applied once. The remaining applications are of acaricides, herbicides and fungicides, with the latter predominating (Giroux 1998).

1.2. CHOLINESTERASE-INHIBITING INSECTICIDES

From early spring until mid- to late- summer, organophosphorus and carbamate insecticides are applied (Giroux 1998). Both compounds bind to, and in the process inhibit, the enzyme cholinesterase (Vyas *et al.* 1998), responsible for halting neural impulses once they have been transmitted within the body. When cholinesterase is inhibited the nervous system becomes overstimulated (Porter 1993). Uncontrolled impulses overwhelm the respiratory system and suffocation frequently results (Grue *et al.* 1991). The carbamate-cholinesterase bond is reversible because it is relatively unstable. This instability allows for the reactivation or decarbamylation of the inhibited cholinesterase. The organophosphorus-cholinesterase bond is extremely stable (Vyas *et al.* 1998), though the enzyme can be regenerated through *de novo* synthesis. Organophosphorus insecticides are used far more than carbamates in apple orchards and as a result, this literature review focuses on them.

The exposure of raptors to organophosphorus insecticides in agricultural settings and, specifically in orchards, has been studied and documented extensively (Balcomb 1983; Henny *et al.* 1985; 1987, Hooper *et al.* 1989; Newton *et al.* 1990; Wilson *et al.* 1991; Elliott *et al.* 1996; 1997; Mineau *et al.* 1999). These studies have focused on primary exposure. Secondary exposure risk has been considered minimal (Shore and Douben 1994) because in the wild, these compounds degrade rapidly (Edwards 1966; Blus 1996; Buck *et al.* 1996) and have a low associated bioaccumulation potential (Shore and Douben 1994). However, consumption of birds and small mammals exposed to or poisoned by insecticides has been implicated in avian mortality. A severe example is given by Mendelssohn and Paz (1977).

Raptors may be drawn to agricultural areas soon after applications because numerous, highly conspicuous and readily captured prey are available there (Benke and Murphy 1974; Zinkl *et al.* 1977). Prey exposed to organophosphorus insecticides may attract a predator's attention by displaying erratic and uncoordinated movements. They may also be less agile or debilitated and therefore unable to evade capture (Benke and Murphy 1974; Zinkl *et al.* 1977). This predisposes raptors to adopt a diet of contaminated prey (Stehn 1976). Mammals are not as sensitive as insects, birds or fish (Grue *et al.* 1991). Wang *et al.* (1999) found that grey-tailed voles (*Microtus canicaudus*) did not alter their daily movements in response the application of azinphosmethyl, nor did they attempt to avoid sprayed areas. While small mammal prey may not exhibit conspicuous behaviours following exposure, they remain mobile and available to predators. Prior to capture and consumption, they may be present during further spray events.

1.3. ANTICOAGULANT RODENTICIDES AND ACUTE POISONS

Of the small mammals present in Quebec orchards, the meadow vole (*Microtus pennsylvanicus*) is the primary target of population control measures (Chouinard and Charbonneau 1999). Over a year, these very prolific breeders may have as many as 13 litters with 1 to 11 young per litter

(Hamilton 1937). In late autumn, anticoagulant rodenticide pellets are placed at the base of apple trees to minimize the autumnal and hivernal girdling and destruction of root systems by voles. Such damage may jeopardize the survival of the trees or compromise the yield, quality or size of the fruit (Byers 1984). Anticoagulants inhibit vitamin K, which plays a role in the production of blood clotting factors in the liver. Organisms exposed to anticoagulants are predisposed to fatal hemorrhaging (Stone *et al.*1999). However, for anticoagulants to be effective, target species must consume a sufficiently large dose or a series of consistent small doses at the appropriate concentration of active ingredient (Marsh *et al.* 1977; Askham and Poché 1992). Several days or even weeks may elapse between consumption of bait and mortality (Mendenhall and Pank 1980; Merson *et al.* 1984). The increasingly lethargic and eventually moribund state that precedes death (Prier and Derse 1962) facilitates capture of exposed individuals.

While prolonged periods of dry weather promote bait longevity (Fellows *et al.* 1988), toxicant can be leached from bait during inclement weather (Marsh *et al.* 1977). Studies have assessed the efficiency of anticoagulant rodenticides and compared them to acute poisons such as zinc phosphide (Hegdal and Blaskiewicz 1984; Merson *et al.* 1984). This product is effective within 24 hours of ingestion (Byers *et al.* 1982). Upon contact with stomach acid, it is converted to phosphine gas, fatally impeding respiratory functions (Matschke *et al.* 1982). The potential for exposure of non-target wildlife to zinc phosphide has also been investigated (Janda and Bosseova 1970, Shivanandappa *et al.* 1979, Krishnakumari *et al.* 1980; Matschke *et al.* 1982, Fellows *et al.* 1988). Though few studies have assessed potential for secondary exposure to raptors, research conducted thus far indicates little risk either to predators or to scavengers (Bell and Dimmick 1975, Byers 1984; Sterner and Mauldin 1995). It appears that poisoned small mammals retire to underground burrows when they begin to experience symptoms (Bell and Dimmick 1975). Studies have also addressed various forms of vole control,

for example by groundcover management (Anthony and Fisher 1977; Sullivan and Hogue 1987; Sullivan *et al.* 1987; Merwin *et al.* 1999).

1.4. ORGANOCHLORINES AND PESTICIDES CONTAINING HEAVY METALS

Beginning in the late 1940s and continuing into the 1970s, organochlorines were applied in apple orchards. Applications of DDT were particularly intensive (Stringer *et al.* 1974; Blus *et al.* 1987; Blus *et al.* 1989). Use of pesticides such as lead arsenate added residues of heavy metals to orchard soils (Elfving *et al.* 1979; Scanlon *et al.* 1983). Application of DDT in the United States was banned in 1972 (DeWeese *et al.* 1986). In Canada, orchard applications ceased in the mid-1970s (Harris *et al.* 2000), though DDT was not actually banned outright until 1986 (Hebert *et al.* 1994). Despite the length of time that has passed since these applications, residues of organochlorines, in particular of DDT and metabolites, and metals such as lead and arsenic remain more elevated in orchard soils and in orchard biota than in those from other agricultural crops (Miles 1968; Harris and Sans 1969;1971; Frank *et al.* 1976a, b; Blus *et al.* 1987). Harris *et al.* (2000) suggested that old orchard habitats in northern North America are among the environments most contaminated with DDT and metabolites.

1.4.1. DDT and metabolites

Cooke and Stringer (1982) estimated a half-life of almost 58 years for DDT and metabolites, when bound to soil particles. In the soil, the main breakdown product of technical DDT is p,p'-DDE (Stringer *et al.* 1974). Like all organochlorines, DDE is lipophilic and has a propensity to bioaccumulate (Robinson 1970). Concentrations may increase by a factor of 10 with each successive trophic level (Dimond and Sherburne 1969). Due to their position atop the food chain, raptors are especially susceptible to exposure at concentrated levels (Peakall 1970; Frank and Braun 1990). DDE is highly

persistent in avian tissues (Walker 1966) and is the most prevalent organochlorine found in avian carcasses (Sundlof *et al.* 1986).

DDE has a lengthy half-life in the body, about 229 days in Common Grackles (*Quiscalus quiscula*) (Stickel *et al.* 1984). Though a small proportion of absorbed DDE may be excreted during egg-laying (Friend and Trainer 1974; DeWeese *et al.* 1986) or via uropygial secretions, the fraction of the body burden eliminated over time by these mechanisms is likely to be small (Sundlof *et al.* 1986). Johnston (1978) suggested that raptors actually eliminate smaller quantities of pesticide via the uropygial gland than do other birds.

DDE inhibits carbonic anhydrase (Bitman *et al.* 1970), an enzyme that aids in supplying calcium to the shell as it forms in the oviduct. Inhibition slows or halts delivery of calcium and the eggshell is correspondingly thinned (Peakall 1970). However, a reduction in shell thickness does not necessarily result in a decline in reproductive success (Fleming *et al.* 1982; Wiemeyer *et al.* 1989). While a linear relationship exists between eggshell thinning and DDE residues in eggs (Blus *et al.* 1972), a certain threshold of exposure must be exceeded for eggs to actually break (Newton 1988). This level varies by species (Blus *et al.* 1972).

DDE exposure has been indirectly linked to elevated mortality of embryos and fledglings (Enderson and Berger 1970; Price 1977). Exposed parents have been observed consuming broken eggs or newly hatched young (Fyfe *et al.* 1969; Porter and Wiemeyer 1969). Snyder *et al.* (1973) also linked DDE exposure to observed nest desertion and refusal of food proffered by a mate.

Though organochlorines continue to be implicated in wildlife mortality (Fleming *et al.* 1983; Beyer and Krynitsky 1989), relatively few studies, particularly field studies, have addressed the exposure of owls to organochlorines (Blus 1996).

1.4.2. Possible sources of DDE

Aside from residues attributed to previous use, it has been suggested that recent legal use of dicofol may account at least in part for currently observed levels of DDE (Blus *et al.* 1987). DDE can be metabolically derived from one or more of the components of first-generation Kelthane ® (Hunt *et al.* 1986), the principal commercial dicofol product (Clark *et al.* 1990). The old formulation contained p,p'-DDT, o,p'-DDT, Cl-DDT and p,p'-DDE as impurities (Black *et al.* 1971; Rothman 1980; Clark *et al.* 1990). Cl-DDT readily undergoes photochemical dechlorination, which provides a further source of environmental DDE (Brown *et al.* 1986, Risebrough *et al.* 1986). It was required that dicofol products marketed after May 1986 contain no more than 2.5% DDT and related compounds. After 1988, the maximum permitted content was lowered to 0.1%.

Elliott *et al.* (1994) suggested that the small amount of DDT and DDE impurities in dicofol would not rival residues remaining from direct DDT applications made in the past. Interestingly, though, the new Kelthane ® formulation has also been found to decrease shell thickness and shell weight both in Eastern Screech-owls (*Otus asio*) and American Kestrels (*Falco sparverius*) (Wiemeyer *et al.*1989). Currently, Kelthane ® is used sparingly as an acaricide in orchards of southern Quebec, though the old formulation was relied on quite heavily for several years in the early 1990s (S. Bienvenue, pers. comm.).

1.4.3. Heavy metals

Heavy metals are persistent compounds. Exposure to certain metals (such as mercury) has been associated with incidents of severe nephro- and neurotoxicity in avian species (Dieter and Ludke 1975). Uptake of metals does not always increase in proportion to availability, nor do levels necessarily increase across trophic levels (Sharma and Shupe 1977; Beyer 1986; Ma 1987; Ismail and Roberts 1992; Sheffield *et al.* 2001). For example,

zinc uptake is physiologically restricted when present at excessively high environmental levels. Increased uptake of lead and arsenate is balanced by increased rate of excretion (Sharma and Shupe 1977).

1.4.4. Sentinel prey species for assessing secondary exposure of predators to contaminants in orchards

Short-tailed shrews (*Blarina brevicauda*) have a propensity to bioaccumulate p,p'-DDE residues to a far greater extent than many other small mammal species found in the same habitat (Harvey 1967; Elfving *et al.* 1979; Talmage and Walton 1991). Earthworms feature prominently in the diet of these insectivores (Bailey *et al.* 1974) and species such as *Lumbricus terrestris* and *L. rubellus* are known to carry elevated levels of DDE (Harris *et al.* 2000). While earthworm (and by association, shrew) concentrations of DDE depend on soil concentrations, they do not necessarily decrease in proportion to them (Davis and Harrison 1966; Beyer and Krynitsky 1989). Shrews exhibit the highest levels of metals relative to other small mammal species (Elfving *et al.* 1979; Talmage and Walton 1991).

Several studies have reported or discussed organochlorine (particularly DDE) body burdens in migratory songbirds, of concern due to continued use of DDT on wintering grounds (Enderson *et al.* 1982; DeWeese *et al.* 1986; Mora 1997). However, of the birds that occupy orchards and are consumed by Screech-owls, the American Robin (*Turdus migratorius*) is most heavily contaminated with DDE because it consumes earthworms in orchards (Harris *et al.* 2000; Gill *et al.* 2003).

1.5. THE MULTIPLE PESTICIDE EXPOSURE SCENARIO

Studies addressing pesticide exposure of raptors in agricultural areas (or orchards) have almost exclusively focused on one class of compound. It is likely that the multiple pesticide exposure scenario has not been considered because most of the raptors studied, such as the Red-tailed Hawk (*Buteo jamaicensis*), are migratory and occupy large territories. Migratory species

may be absent during some application periods, such as in the autumn when rodenticides are applied (Radvanyi *et al.* 1988). Similarly, a treated area might comprise only a small proportion of the hunting grounds in a large territory (Buck *et al.* 1996). However, non-migratory species that occupy comparatively small territories and live near one or several treated area(s) might be expected to make greater use of them.

1.6. THE EASTERN SCREECH-OWL

In contrast to many of the species previously studied in relation to pesticide exposure, the Eastern Screech-owl is non-migratory (Gehlbach 1994a). Once established in an area, it remains there unless ousted by a competitor or predator, or if their habitat is destroyed (Godfrey 1986). Screech-owls have been observed nesting in the thin strips of forest that separate agricultural cropland (Klaas and Swineford 1976). It has long been known that Screech-owls live in or in the vicinity of orchards (Bent 1938; Smith and Gilbert 1984; Penak 1986; Hegdal and Colvin 1988; Belthoff *et al.* 1993). Parents have been observed delivering food to a row of hungry owlets, all perched on apple branches, in assembly line fashion (P.Wery, pers. comm.).

Screech-owls are opportunistic hunters (Errington 1932; Marti and Hogue 1979; Abbruzzese and Ritchison 1997) and generalist feeders (VanCamp and Henny 1975). Orchards host a number of species preyed upon by Screech-owls: small mammals and birds (Stewart 1969), insects, earthworms and reptiles (Gehlbach 1994a), fish (VanCamp and Henny 1975) and frogs (Sherman 1911, pers. obs.). Exposure to organophosphorus insecticides, anticoagulant rodenticides, organochlorines and metals has also been documented in these species (Benke and Murphy 1974; Kuhr *et al.* 1974; Jett 1986; Hegdal and Colvin 1988; Talmage and Walton 1991; Cobb *et al.* 2000).

1.6.1. Determinants of Screech-owl prey selection

Consumption of species varies with time of year. Prey selection depends on whether the organism is active or conspicuous (Kaufman 1974), the ease with which it can be captured and its abundance (Johnsgard 1988; Abbruzzese and Ritchison 1997). Several studies have highlighted a preponderance of meadow voles in the Screech-owl's diet throughout the year, and many have suggested they are preferentially selected over all other potential prey (Sherman 1911; Wilson 1938; Marti and Hogue 1979). However, avian prey consistently occupies the largest proportion of biomass in the owl's diet (Ritchison and Cavanagh 1992). In the spring, Screech-owls increase their consumption of birds in response to the spring migration influx (VanCamp and Henny 1975).

1.6.2. Previous studies on the Screech-owl

A considerable number of studies have focused on the Eastern Screech-owl, detailing aspects of its physiology (Henny and VanCamp 1979), natural history and ecology (VanCamp and Henny 1975; Gehlbach 1994a, b), selection and use of various habitats (Smith and Gilbert 1984; Belthoff and Ritchison 1990; Belthoff *et al.* 1993) and hunting behaviour and prey preferences (Kaufman 1974; Phelan 1977; Marti and Hogue 1979; Ritchison and Cavanagh 1992). The vast majority of these studies, and the most comprehensive, have been carried out in the United States. By contrast, only a few studies have considered the Eastern Screech-owl in any context in Canada (e.g. James and Martin 1950; Godfrey 1986; Penak 1986) and even fewer have examined the Quebec population (Gauthier and Aubrey 1996).

A number of laboratory studies have examined contaminant exposure in the species (e.g. McLane and Hall 1972; McLane and Hughes 1980; Fleming *et al.* 1982; Serafin 1984; Wiemeyer *et al.* 1989; Wiemeyer and Sparling 1991; Wiemeyer and Hoffman 1996; Vyas *et al.* 1998). These studies were primarily carried out at or in conjunction with the Patuxent

Wildlife Center in Maryland. McLane and Hall (1972) administered dietary DDE to Screech-owls over one breeding study and estimated an average 13.0% decline in eggshell thinning. However, VanCamp and Henny (1975) reported that some of these owls were obtained from their study area, once considered one of the most agriculturally productive in the State of Ohio. As a result, it is unlikely that the eggs of dosed owls were measured against true control values in the McLane and Hall study (1972). If, instead, the average eggshell thickness of dosed owls is measured against that of archival eggs collected by Klaas and Swineford (1976) in Ohio prior to widespread DDE use, a thinning value of 22.2% is obtained. Population decline has been observed when eggshell thinning was maintained between 15.0 and 20.0% over an extended period of time (Anderson and Hickey 1972). No raptor species in North America has been able to sustain a self-perpetuating population with 18.0% eggshell thinning (Lincer 1975).

Wiemeyer and Sparling (1991) dosed Screech-owls, American Kestrels and Northern Bobwhites (*Colinus virginianus*) with one carbamate (carbofuran) and three organophosphorus insecticides (EPN, fenthion, monocrotophos). Interestingly, Screech-owls were 68 times more sensitive to EPN than were kestrels (Wiemeyer and Sparling 1991). Vyas *et al.* (1998) compared the sensitivity of Screech-owls and American Kestrels to a single dietary exposure of an organophosphorus (fenthion) and a carbamate (carbofuran) insecticide. Screech-owls absorbed significantly more than Mallard Ducks (*Anas platyrhynchos*) and Black-crowned Night-herons (*Nycticorax nycticorax*) (Serafin 1984). In these studies, Screech-owls appeared to be equally or less sensitive than American Kestrels.

Species with long digestive tracts usually absorb food more efficiently than species with shorter tracts (Barton and Houston 1994). Generalist raptors have longer digestive tracts and higher digestive efficiencies than specialists (Barton and Houston 1994). There is evidence that Falconiformes and Strigiformes are more sensitive to cholinesterase-inhibitors (Mineau *et al.* 2001). Given interspecies variations in contaminant uptake (Chhabra 1979;

Serafin 1984) and the wide range of sensitivity manifested, caution is required when extrapolating sensitivity from one species to another (Wiemeyer and Sparling 1991).

While laboratory studies address potential risks, field studies assess actual risk (Norton *et al.* 1992). Very few studies (e.g. VanCamp and Henny 1975; Klaas and Swineford 1976) have assessed the Screech-owl's exposure to any pesticide in a field setting. Instead, studies have focused almost exclusively on exposure to anticoagulant rodenticides (e.g. Hegdal and Colvin 1988). Several studies have documented exposure to rodenticides, organochlorines or organophosphorus insecticides in Screech-owl carcasses opportunistically collected for analysis (Johnston 1978; Havera and Duzan 1986; Stone and Okoniewski 1987; Frank and Braun 1990; Okoniewski and Novesky 1993; Mineau *et al.* 1999; Stone *et al.* 1999; 2003).

1.7. MONITORING PESTICIDE EXPOSURE IN RAPTORS

Raptor exposure to an organophosphorus or carbamate insecticide may be assessed in a blood or brain sample. Because it is best correlated with morbidity and mortality, the level of cholinesterase in the brain is considered a more reliable indicator of exposure. A 20.0% depression in brain cholinesterase is considered indicative of exposure, while a 50.0% depression is associated with lethal intoxication (Mineau and Tucker 2002). Though exposure to anticoagulants can be detected in a blood sample, there are ethical concerns, because sampling creates a potential hemorrhage site. It is possible to assess exposure non-invasively, by residue analysis of pellets (Merson *et al.* 1984; Eadsforth *et al.* 1991; 1996). Specific anticoagulants may also be detected by performing a rodenticide screen on the liver (Stone *et al.* 1999; 2003). Organochlorine analyses are most frequently performed on deceased rather than living raptors. Compounds such as DDE and dieldrin may be detected in various tissues, organs, and whole carcasses as described in Sundlof *et al.* (1996) and Frank and Braun (1990).

1.8. REVIEW OF METHODS

Assessment of pesticide exposure, or of ecological risk, requires an interdisciplinary approach (Kendall and Ackerman 1992). Such is also the case when working with an elusive and secretive species like the Screech-owl. A number of field methods were used to evaluate the species' exposure to insecticides, rodenticides and persistent contaminants in orchards. These are reviewed below.

1.8.1. Censusing

Screech-owls defend territories throughout the year individually or in pairs (VanCamp and Henny 1975). They are most vocal during the mating season (Bent 1938; Norwicki 1974). In mid- to late-winter, a low tremolo-like call or a high-pitched whinny is emitted in response to the vocalizations of (perceived) intruders (Johnsgard 1988). Nocturnal censusing may be conducted between sunset and dusk (Cink 1975). However, censusing should not be conducted in the midst of heavy precipitation nor when winds are in excess of 15 km/hr. Even under conditions considered optimal, Screech-owls can be quite uncooperative and do not always respond to broadcast calls (Norwicki 1974). They are even less likely to respond during inclement weather. The wind also muffles the broadcast calls which diminishes the efficiency of censusing (Smith and McKay 1984; Gehlbach 1994a). Presence of a predator, such as a Great Horned Owl (*Bubo virginianus*) or a Barred Owl (*Strix varia*), may also inhibit response to broadcast call (Fuller and Mosher 1981). For ethical reasons, it is important to ensure that censusing efforts do not alert predatory species to the presence and location of resident Screech-owls.

1.8.2. Installation of nest boxes

Screech-owls readily occupy nest boxes (Gehlbach 1994b), particularly when leaf cover is sparse (VanCamp and Henny 1975). This increases the

number of sites available for caching, roosting and nesting. Correspondingly, the possibility of finding and collecting pellets, prey items and eggs for analysis is also increased. Screech-owls are most easily captured within nest-boxes. However, verification can be very time-consuming. Boxes should be placed on trees with trunk diameters equal to or greater than the nest box width and affixed 3 to 4 m from the ground (Gehlbach 1994b) no more than 30 m from the nearest edge (Hegdal and Colvin 1988). If there is running or standing water at the location, several nest boxes should be installed in the vicinity since Screech-owls consume a variety of aquatic organisms and occasionally partake in a bath (VanCamp and Henny 1975; Gehlbach 1994a). Great care must be taken in site selection to ensure that boxes are not placed where adverse human attention may be drawn to the owls. Gehlbach (1994b) reported loss of Screech-owls and nest boxes to vandalism and human intrusion.

1.8.3. Prey inventory and pellet content interpretation

Pellets are soaked in water for several minutes to allow separation of the bones. The identity and number of small mammals is primarily determined by an examination of the mandibles (Yom-Tov and Wool 1997) with the aid of a reference collection of known species. The usefulness of pellet analysis to identify and quantify prey consumption by owls has been strongly emphasized (Errington 1930, 1932; Wilson 1938; Pearson and Pearson 1947; Maser and Brodie 1966; Clark and Wise 1974).

Pellet contents must be interpreted with caution (Glading *et al.* 1943). The number of prey items estimated from pellet remains may be less than that actually consumed (Raczynski and Ruprecht 1974). For example, Screech-owls are highly insectivorous (Allen 1924), however pellet analysis generally underrates the importance of insects in the diet (Turner and Dimmick 1981). On occasion, Screech-owls consume earthworms (Bosakowski and Smith 1992), which may contain important residues of p,p'-DDE (Bailey *et al.* 1974). However, earthworms (and other soft-bodied prey

such as caterpillars) are not detectable in pellets (Brown 1989). It is therefore impossible to use pellet analysis to estimate the potential contribution of earthworms to the overall DDE exposure of Screech-owls.

Finally, a larger number of pellets is required for a generalist feeder than for a specialist (Marti 1987). Long-term pellet collection for analysis is also preferable because an owl's diet will vary from one year to another particularly with changes in habitat and prey availability (Glue 1970).

1.8.4. Biomass calculations

The amount of biomass contributed by each type of prey is a more representative measure of importance in the diet than frequency (Ritchison and Cavanagh 1992). Percent biomass is calculated by multiplying the number of prey individuals by an average, representative weight for the species (Rusch *et al.* 1972). The total of these weights is then summed and the weight contributed by each type of prey is considered as a proportion of the total prey weight (Ritchison and Cavanagh 1992). Given a unit weight of prey consumed by a Screech-owl in an area, and given residue levels of the prey within that area, secondary exposure can be estimated.

1.9. CONCLUSION

Orchards have been and continue to be areas of intensive pesticide use. Several studies have documented exposure of raptors and their prey to cholinesterase-inhibiting insecticides, anticoagulant rodenticides and persistent organochlorine residues in orchards. The majority of these studies have been conducted on migratory birds that occupy large territories. Because a treated area might only comprise a small proportion of the hunting territory, or the bird may be absent during certain pesticide applications, exposure to only one type of pesticide has generally been considered. However, the non-migratory and sedentary Screech-owl occupies a small territory in the vicinity of orchards. Year after year, these owls are present during the entire pesticide application season, consuming a variety of

organisms that may have been exposed to one or several of the pesticides in question. Multiple pesticide exposure is a plausible scenario that warrants further investigation. The case of the Screech-owl in orchards presents an opportunity to do so.

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CHAPTER TWO

Exposure of the Eastern Screech-owl (*Otus asio*) to selected contaminants in orchards of southern Quebec

2.1. INTRODUCTION

A number of pesticides are currently applied in the apple orchards of southern Quebec. Of these, cholinesterase-inhibiting insecticides and anticoagulant rodenticides have received the most scrutiny with regards to raptor exposure. Organophosphorus insecticides are applied to a far greater extent than carbamate insecticides. During an average season, insecticides are applied 3 to 4 times from early spring until late summer. Imidan (phosmet), Guthion (azinphosmethyl) and Zolone (phosalone) are the organophosphorus insecticides recommended for use in orchards of southern Quebec (Ministère de l'Environnement et de la Faune 1998). Sevin (carbaryl), one of the carbamate insecticides recommended for use, is generally applied once in a given season, and only in a portion of the orchard.

The anticoagulant rodenticides Rozol (chlorophacinone) and Ramik (diphacinone) are generally applied in the late autumn and early winter (Askham and Poché 1992). Rodenticide bait can also be applied in the summer if it appears that small mammal populations need to be controlled prior to the winter, but this does not often occur. Anticoagulants are currently being replaced by the acute and rapidly acting poison zinc phosphide.

Starting in the 1940s, organochlorine insecticides such as endrin, dieldrin and especially DDT, were intensively applied (Stringer *et al.* 1974). Records obtained from orchard-owners of southern Quebec indicate that use of these pesticides was largely curtailed in the mid-1970s. Metals in orchard soils also originate from use of pesticides containing heavy metals, such as lead arsenate, and from application of fertilizers, fungicides and trace elements (Frank *et al.* 1976).

Studies addressing exposure of wildlife in agricultural areas and in orchards have focused on raptors such as the Red-tailed Hawk (*Buteo jamaicensis*) and the Great Horned Owl (*Bubo virginianus*) (*e.g.* Wilson *et al.*

1991; Frank and Lutz 1999). Large birds are more easily observed and large carcasses are more likely to be found so that tissues may be analyzed for evidence of residues (Mineau *et al.* 1999; Mineau and Tucker 2002). The majority of the species studied also migrate and occupy large home ranges. This may explain why studies have almost exclusively addressed exposure to one class of compound. Given that currently applied insecticides and rodenticides are applied seasonally, that migratory raptors may be absent for part or all of an application period, and that a treated area may only comprise a small proportion of the hunting grounds, a single pesticide scenario is plausible.

Multiple pesticide exposure is a less scrutinized but equally plausible scenario. The Eastern Screech-owl (*Otus asio*) is non-migratory (VanCamp and Henny 1975) and occupies a small territory relative to other raptors (Belthoff *et al.* 1993). Hegdal and Colvin (1988) estimated an average home range of 132 ha for Screech-owls in orchard habitat, while home ranges in the region of 400 ha have been reported for Great Horned Owls in the vicinity of cropland (Buck *et al.* 1996).

Screech-owls are highly adaptable and occupy a variety of habitats (Gehlbach 1994), including orchards (Smith and Gilbert 1984; Penak 1986; Hegdal and Colvin 1988; Gauthier and Aubry 1996). The Screech-owl's rather secretive nature and diminutive stature may have obscured other traits which make it suitable for a pesticide exposure study (Penak 1986). Screech-owls favour prey that are conspicuous (Metzgar 1967; Kaufman 1974) and that can be captured easily (Abbruzzese and Ritchison 1997). Orchards host a number of species preyed upon by the opportunistic Screech-owl, including small mammals, resident and migratory birds (Stewart 1969), insects, earthworms and reptiles (Gehlbach 1994), fish (VanCamp and Henny 1975) and frogs (Sherman 1911). Exposure of these prey species to organophosphorus insecticides, to anticoagulant rodenticides, and to organochlorines or heavy metals has been documented in treated areas and in orchards (Benke and Murphy 1974, Enderson *et al.* 1982; DeWeese *et al.*

1986; Jett 1986; Mora 1997; Cobb *et al.* 2000; Gill *et al.* 2000; Harris *et al.* 2000). Hegdal and Colvin (1988) radio-tracked Screech-owls in a variety of habitats, including orchards, and postulated that individuals within 1.3 km of a treated area should theoretically be exposed to contaminated prey.

Screech-owls residing near and hunting in orchards (and other agricultural areas) are present during the entire pesticide application season, year after year. Long-term exposure to many compounds may result in additive or synergistic effects (Fimreite *et al.* 1970; Dieter and Ludke 1975). Though the organophosphorus-cholinesterase bond is irreversible (Vyas *et al.* 1998), there is recovery of the enzyme through *de novo* synthesis within the span of a few days or weeks. However, insecticides may also be applied repeatedly over several days or weeks, hampering cholinesterase recovery. The Screech-owl may also be continually exposed to persistent organochlorine and metal residues. Exposure to heavy metals can result in limited cholinesterase inhibition (Dieter and Ludke 1975). Finally, long-term exposure to organochlorines may impair cognitive functions (Frank and Lutz 1999) and predispose raptors to certain types of injuries, such as car strikes (Blus 1996) or collisions with stationary objects (Mineau *et al.*1999).

The natural history and ecology of the Screech-owl has been studied extensively in the United States (*e.g.* VanCamp and Henny 1975; Gehlbach 1994). Very few studies have been carried out in Quebec or in Canada. However, it is known that felling of old trees and lack of suitable nesting sites is a limiting factor for the species within Quebec (Penak 1986; Gauthier and Aubry 1996). Few studies have assessed the species' exposure to pesticides outside a laboratory setting. With a few exceptions (*e.g.* Klaas and Swineford 1976), field studies have focused almost exclusively upon secondary exposure to anticoagulant rodenticides (e.g. Merson *et al.* 1984; Hegdal and Colvin 1988). The possibility of secondary exposure to organophosphorus insecticides has largely been dismissed because of the assumption that these compounds are rapidly metabolized within small mammals and that substantial, measurable residues do not accumulate in body organs (Shore

and Douben 1994; Blus 1996). Few attempts have been made to quantitatively assess residues available to predators in the small mammals on which they prey, during the brief, post-application lifespan of the compounds.

A number of laboratory studies have examined contaminant exposure in Eastern Screech-owls with the objective of assessing species and inter-species sensitivity (McLane and Hall 1972; McLane and Hughes 1980; Fleming *et al.* 1982; Serafin 1984; Wiemeyer *et al.* 1989; Wiemeyer and Hoffman 1996). Organochlorine exposure has also been implicated or diagnostically confirmed in Screech-owl mortality in carcasses collected opportunistically for analysis (Stone and Okoniewski 1988; Frank and Braun 1990; Okoniewski and Noveski 1993; Stone and Stedelin 1999). Exposure to an organophosphorus pesticide has rarely been implicated conclusively in the death of a Screech-owl (Mineau *et al.* 1999).

Our primary objective was to assess the potential for the Eastern Screech-owl to be secondarily exposed to organophosphorus insecticides, anticoagulant rodenticides, organochlorines and residues of other persistent contaminants, from current and previous pesticide applications in apple orchards. Though pesticide exposure may occur through several routes (Tucker and Crabtree 1970), consumption of exposed prey is likely to be an important one (Shirazi *et al.* 1988; Frank and Braun 1990; Wiemeyer and Sparling 1991). To our knowledge, this is also the first study to quantitatively assess whole-carcass small mammal residues of insecticides. A secondary objective was to evaluate the species' suitability as a monitor of exposure to organophosphorus insecticides and of local organochlorine persistence, since sedentary species are generally considered ideal for this purpose (Moore 1966).

Anticoagulant rodenticides are rapidly being replaced by zinc phosphide, an acute poison with minimal potential for secondary exposure, so a third objective was to assess whether remaining uses of anticoagulants might be of concern for the species. Finally, protection of a species is largely dependent on the availability of baseline information (Winger *et al.* 1984).

This study provided a unique opportunity to collect natural history and baseline information on Screech-owls in Quebec.

2.2. FIELD METHODS AND MATERIALS

All fieldwork was carried out in orchards of Saint-Hilaire and Rougemont, in southern Quebec (45°28 073° to 45°32 073°). Rougemont is one of the largest apple-producing domains in the province, whereas Saint-Hilaire is one of the smallest, occupying a total area of 958 and 200 ha, respectively (Ministère de l'Environnement et de la Faune 1998). The very northern fringe of the Eastern Screech-owl's range also coincides with the southern tip of Quebec (Gauthier and Aubry 1996).

Screech-owls have been observed nesting within orchards, in the cavities of standard apple trees (Bent 1938). However, starting in the early 1980s, standard trees were replaced predominantly by the smaller, more productive dwarf varieties (S. Bienvenue, pers. comm.). As a result, resident Screech-owls may have gradually been displaced to the woods that border these orchards or may have vacated the areas altogether. Saint-Hilaire, in particular, is currently under considerable development pressure. From 1996 to 2001, municipal populations in Saint-Hilaire and nearby Otterburn Park increased by almost ten percent. Consultation of aerial photographs taken from 1958 to 2001 shows a clear encroachment on forested lands and a reduction in the forested periphery around Mont-Saint-Hilaire (M-A. Guertin, pers. comm.). The woods bordering orchards presently comprise a large portion of the increasingly limited amount of suitable habitat available to the species within the region.

2.2.1. Censuses

During the winters of 2000-2001 and 2001-2002, woods adjacent to 15 orchards were censused nocturnally for the presence of Screech-owls. From the orchard, facing the adjacent woods, we played a combination of tremolo (bounce) and whinny calls. Presence of an individual or a pair was confirmed

visually with a flashlight or headlamp. Two individuals observed perching on the same branch or in trees several meters apart were considered a pair (P. Wery, pers. comm.). The forest adjacent to a campground near Mont-Saint-Hilaire and a restricted area of the Mont-Saint-Hilaire Biosphere Reserve were also censused as potential control sites. If no response was obtained during the first visit, locations were censused at least once more when logistically possible. In particular, time constraints limited the number of winter visits that could be made to large, snow-bound orchards. Censusing was also conducted in the summer prior to Screech-owl capture attempts. We did not census during heavy precipitation or when winds were in excess of 15 km/hr since Screech-owls, uncooperative even in optimal circumstances, are generally unresponsive during these conditions (Norwicki 1974).

2.2.2. Tabulation of orchard insecticide use

Most orchard-owners keep a log of the pesticides they apply in their orchard every season. When Screech-owls were observed in the vicinity of an orchard during censusing, we asked the orchard-owner if they would provide us with their pesticide use records, dating as far back as available. These records were tabulated and used to draw up a phosmet, azinphosmethyl and phosalone use profile for each orchard.

2.2.3. Nest box installation

Almost 100 nest boxes were constructed and installed throughout the study area. In 2001, 78 nest boxes were installed in woods adjacent to the 11 orchards where owls were located during winter censusing. At one location, 4 previously installed nest boxes were incorporated into the study, bringing the number of boxes in the vicinity of orchards up to 82. We also installed a total of 16 nest boxes at the two control sites: 10 in the forest adjacent to the campground and 6 in an area of the Mont-Saint-Hilaire Biosphere Reserve restricted from public access.

Between spring 2001 and spring 2003, nest boxes were inspected 3 to 4 times annually (depending on accessibility of each site) at different stages in the Screech-owl's life cycle. We divided the calendar year into the territorial season (January-February), the nesting season (March-April), the brooding season (April-June), fledging (June-mid-August) and dispersal (mid-August-December). Inspections were carried out primarily in late May, in August and from mid-November to December. On occasion, boxes were also inspected in early June. Nest boxes were not visited during the territorial season.

2.2.4. Collection of pellets and prey remains

From spring 2001 to spring 2003, pellets and prey remains were collected from nest boxes. Small mammal species were identified by lower mandible (Cahn and Kemp 1930; Raczynski and Ruprecht 1974) using a reference collection of known skulls and mandibles. Avian remains were identified by feather (A. Roth and S. Deshaies, pers. comm.). The percent biomass of small mammal and avian species in the owls' diet was then estimated. The average weight of individual small mammals captured in orchards for pesticide analyses was used in these calculations. Mean avian species weights were obtained from Dunning (1993) and readjusted for the region in consultation with a local wildlife rehabilitator (A. Roth, pers. comm.).

2.2.5. Estimation of biomass consumption and pesticide exposure

The best way to determine pesticide exposure is to take the appropriate samples from the Screech-owls themselves. However, exposure can also be estimated for an owl by considering its biomass requirements, the proportion of prey in its diet, and (whole-body) residue levels in each type of prey. It is essential to account for the toxicokinetics and rate of elimination of the pesticides under consideration (Kendall and Ackerman 1992). This is especially important with regard to organochlorines, which are considerably more persistent than organophosphorus insecticides or anticoagulant rodenticides. Organochlorines are stored almost entirely in adipose tissue

once ingested (Sundlof *et al.* 1986). Campbell and Koplin (1986) examined food and energy balance in a Screech-owl. To adjust for metabolism of lipids, and by association, of organochlorines, we took the original equation of Campbell and Koplin (1986):

$$\textbf{Overall dietary metabolizability coefficient} = \left(1 - \frac{\text{total wt wastes}}{\text{total wt food}}\right) \times 100\%$$

and modified it as follows:

Lipid metabolizability coefficient =

$$\left(1 - \frac{\text{total wt lipid egested in excrement + total wt lipid egested in pellets}}{\text{total wt lipid in food}}\right) \times 100\%$$

then inserted the lipid values provided by the authors, all in units of mg/(kg body wt day):

$$1 - \left(\frac{1{,}306.9 + 69.0}{12{,}712.7}\right) \times 100\%$$

to obtain a lipid metabolizability coefficient of 89.0%.

2.2.6. Small mammal captures

Small mammals were snap-trapped in a total of 12 orchards from June to October 2001 and in June and July 2002 for pesticide analyses. Screech-owls had been observed in 8 of these orchards. Pesticide use records were also obtained for 3 of the 4 additional orchards. Abundant grassy and moist areas within the orchard played a key role in site selection since this makes an attractive habitat for many small mammal species (Hamilton 1940; Getz 1961). After meticulous inspection of each orchard, traps were set at the base of apple trees, and beside brush piles and ditches, where vegetation was plentiful and runways or burrows were noted.

Soil residues of DDE may not be uniformly distributed within an orchard (Weaver *et al.* 1990). With this in mind, an attempt was made to capture individuals at different locations within the orchard to integrate more accurately the overall residue distributions and potential exposure risk to the

Screech-owl. When insecticide or rodenticide applications were monitored, we only set traps within the treated area of the orchard.

All traps were baited with a mixture of peanut butter and cracked corn, and topped with a v-shaped wooden cover to minimize non-target captures. Traps were checked every 12 hr and rebaited at each visit. Captured individuals were identified to species (Burt and Grossenheider 1976; Beaudin and Quintin 1993), sexed when possible and weighed to the nearest gram with a 60 g or 100 g pesola spring scale. Subsequent procedures depended on the class of pesticide (or contaminant) being analyzed in the specimens; they were analyzed for organophosphorus insecticides, for anticoagulants, or for organochlorines, trace metals and other persistent contaminants, as outlined below.

2.2.7. Selection of small mammal species for organophosphorus insecticide and organochlorine analysis

Short-tailed shrews (*Blarina brevicauda*) were the primary target species for the organochlorine/metal analyses because they exhibit high levels of DDE and heavy metals relative to other species (Dimond and Sherburne 1969; Elfving *et al.* 1979; Talmage and Walton 1991). Organophosphorus insecticides are short-lived compounds (Stone 1979; Blus 1996). To obtain an overall idea of exposure in small mammals, any species captured within the interval of interest was taken for analysis. This is realistic given that Screech-owls opportunistically consume the species that are available to them. However, we determined that analyzing residues in a single, representative species would better enable us to detect patterns of persistence and assess the behaviour of the insecticides post-application. Of the species found within orchards, the meadow vole (*Microtus pennsylvanicus*) resides almost entirely within the confines of orchards and is known to comprise a large component of the Screech-owl's diet (Sherman 1911; Wilson 1938; Marti and Hogue 1979; Ritchison and Cavanagh 1992). We therefore concentrated our trapping efforts on this prey species.

2.2.8. Captures following application of organophosphorus insecticides

Small mammal captures

Orchard-owners were asked to provide notification just before or immediately after spraying with phosmet or azinphosmethyl. Snap-traps were then set in the orchard as soon as possible so that initial captures would encompass the first 12 hr post-application. After application, traps were checked at least once every 12 hr, generally spanning 48 to 60 hr post-application. When an individual was found in a trap, a spring-loaded pesola scale was clamped to the animal's tail and the trap was carefully released to minimize contact with the fur. After identification and weighing, the animal was dropped into a chemically cleaned glass jar. The pesola clamp was rinsed off with hexane after contact with each animal.

In 2001, a volume of hexane (10% v/v) equivalent to twice the animal's body weight was measured into a graduated glass cylinder (100 ml or 200 ml depending on the size of the animal) and poured onto the fur in the confines of the jar. The jar was firmly sealed with a teflon-lined lid and the contents swirled for a minute to ensure complete immersion with the hexane, which was added to halt further degradation of organophosphorus residues. Jars were placed in a cooler on blue ice while in the field and later transferred to a -20° C freezer. They were later transported to the Canadian Wildlife Service (CWS) laboratory, in Hull Quebec, in the same cooler on blue ice. In 2002, the field processing procedure was modified so that no hexane was added to the small mammal. All other aspects remained the same.

A separate sample of small mammals was also captured and treated with a spiking solution to ensure that loss in transportation could be accounted for, and recovery could be calculated. In 2001, 6 individuals (3 *Blarina* and 2 *Sorex* sp. and 1 *Zapodidae*) were spiked with 10 ml of solution of known concentration of phosmet (0.021 mg/ml) and azinphosmethyl (0.228 mg/ml). In 2002, the volume and concentration of spiking solution administered was considerably reduced. Nine individuals (2 *Microtus*, 2

Zapodidae, 2 *Peromyscus*, 2 *Blarina* and 1 *Sorex*) were spiked with 0.5 ml of known concentration of phosmet (5.112×10^{-3} mg/ml) or of azinphosmethyl (4.792×10^{-3} mg/ml).

Screech-owl captures

Broadcast whinny and bounce calls and a bal-chatri trap (Smith and Walsh 1981) baited with a white mouse were used to draw Screech-owls into a mist net set up in orchards. Our initial intention was to capture resident owls and fit them with a 4.0-g transmitter (Holohil Inc., PD-4) to monitor their hunting behaviour within the orchards, to determine their whereabouts during and following insecticide applications, and to recapture them and take a blood sample. An attempt was made to capture Screech-owls as soon after applications as possible since the detection interval for exposure to an organophosphorus insecticide through a cholinesterase assay is approximately 48 hours (Mineau and Tucker 2002). All captured adults and young were banded (under permit no. 10309 AM).

2.2.9. Small mammal captures following application of anticoagulant rodenticides

Orchard owners were asked to provide notification as soon as possible before or after autumn application of anticoagulant rodenticides. Snap traps were set primarily beside the entrance to burrows at the base of apple trees. Captures spanned 14 d with 35 traps/night. After weighing and identification, specimens were dropped into a chemically cleaned glass jar and stored in a cooler on blue ice until transferred from the field to a –20°C freezer. They were later transported to the CWS in Hull for storage.

2.2.10. Organochlorine, trace metal and persistent contaminant analysis

Once captured individuals were identified and weighed, they were placed in a chemically cleaned glass jar and sealed with an aluminum foil-lined teflon lid. In the field, jars were stored in a cooler on blue ice until

transported to a –20°C freezer. Samples were later transported in a cooler on dry ice to the CWS laboratory.

In 2003, a Screech-owl egg was recovered from an orchard nest box (under permit no. 2003-02-27-103-16-SF). This egg was processed, stored and transported in the same manner as described above for the small mammal specimens.

2.3. LABORATORY METHODS AND MATERIALS

Like many owl species, Screech-owls generally consume their prey whole. Contaminants can be present at different concentrations in different tissues once absorbed (Fox and Lock 1978). For these two reasons, analysis of whole-body homogenate should provide the most appropriate and representative measure of exposure likely to be incurred by a Screech-owl.

2.3.1. Analysis of organophosphorus insecticides in small mammal specimens

In 2001 and 2002, specimens were analyzed for residues of phosmet or azinphosmethyl at the CWS laboratory in Hull. Since there were technical difficulties associated with the recovery of the hexane-immersed small mammals in 2001, only 2002 procedures, where samples were left dry prior to analyses, are reported. Specimens were homogenized individually and then weighed into a Sorvall Omni-Mixer container. An amount of anhydrous Na_2SO_4 1.5 times the specimen mass was added to the homogenate and mixed with a stainless steel spatula. The mixture was left to stand for approximately 15 min. This duration was not sufficient for the sample to desiccate. To this, 100 ml dichloromethane (DCM) was added and mixed for 10 min with the Sorvall Omni-Mixer (at speed setting ~ 2). The liquid was then decanted through a funnel plugged with glass wool and topped with Na_2SO_4 that had been wetted with DCM. The filtrate was collected in a 500-ml flat-bottomed evaporator flask. The specimen mixture was extracted twice more,

each time with 100 ml DCM but mixed for 5 min. The Na_2SO_4 in the funnel was washed with approximately 30 ml of DCM.

The three extracts were combined and evaporated with a rotary evaporator and made up to a concentration of 1.0 ml/g with 1:1 hexane:DCM. Using a 10 ml luer-lock tip syringe filter, between 2 and 3 ml of the extracted liquid was filtered through a CHROMOSPEC 0.45 μm PTFE filter into a gel permeation chromatography (GPC) glass tube. Sufficient hexane:DCM (1:1) was added to make up to 10 ml. Lipids and biogenic materials were removed by GPC. The collected solvent fraction was evaporated using a rotary evaporator to a small volume and made up to a final, suitable volume (1 ml/g, 1 ml/2g or 1 ml/3g), depending on the amount of sample taken, using iso-octane. Residues were obtained via analysis by gas chromatography (Agilent 6890N Network GC System, Palo Alto, California)/mass selective detector (Agilent 5973 Network MSD, Palo Alto, California).

2.3.2. Assessment of organophosphorus insecticide exposure in Screech-owls admitted to a local raptor rehabilitation facility

Capture of wild Screech-owls within the post-application detection interval is not always easily accomplished, or successful. However, Screech-owls exposed to organophosphorus insecticides (and to other pesticides) may be found debilitated or injured and admitted to a rehabilitation facility. In 2000 and 2001, a pilot sampling program was implemented at the University of Montreal's Clinique des oiseaux de proie (COP), faculty of veterinary medicine, in Saint-Hyacinthe, Quebec. This facility receives raptors from the entire province of Quebec and some Screech-owls are admitted from rural and agricultural areas. To assess the potential role that insecticide exposure may have played in admission, levels of serum cholinesterase (butirylcholinesterase or BChE) and brain cholinesterase (acetylcholinesterase or AChE) were analyzed. A 0.75 to 1.0 ml blood sample was taken from the femoral vein or brachial vein of Screech-owls as soon as possible after admission. Poor body condition (*e.g.* emaciation and

dehydration) at arrival delayed sampling because of the difficulty in obtaining a sufficient volume of blood for analysis and the possibility that sampling might compromise the owl's survival.

Blood was placed in a heparinized tube, shaken and then transferred to a Whatman filter paper via a capillary tube (Trudeau *et al.* 1995). The paper was placed in a Tupperware container and kept elevated above desiccant silicon dioxide (SiO_2) for 48 hrs. The filter paper was then enveloped in a paper towel and placed in a small Ziploc© bag containing a tablespoon of desiccant, placed in a padded envelope and mailed to the CWS in Hull. Deceased or euthanized Screech-owls were decapitated or the brain was excised and samples were placed in a Whirl-pak bag. Samples were analyzed at the CWS biomarker laboratory according to the method of Trudeau *et al.* 1995. Precinorm U, a control serum from Boehringer Mannheim (Laval, Quebec), was analyzed with each series of samples for quality control purposes. With the exception of reactivated samples, analyses were carried out in duplicate. Samples were incubated with and without 2-PAM (2-pralidoxime chloride) to attempt reactivating samples with low enzyme titers. Control brain tissues, from birds killed following exposure to an organophosphorus or carbamate, were reactivated at the same time as the samples.

2.3.3. Analysis of anticoagulant rodenticides in small mammal specimens

Analyses were conducted at the Illinois Animal Disease Laboratory in Centralia, Illinois using a high-performance liquid chromatography (HPLC) screening procedure based on Chalermchaikit *et al.* (1993). After acetone extraction and solid-phase cleanup using Florisil and C-18 Sep Pak cartridges (Waters Corportation, Taunton, Massachusetts) in tandem, identification and quantification of 12 different anticoagulants was achieved via reverse-phase separation using UV and fluorescence detectors (Shimadzu models SPD-M10AVP and RF-10AXL; Shimadzu Scientific Instruments, Inc., Columbia,

Maryland). Additional sensitivity and confirmation were obtained as needed using an ion-paired method according to Hunter (1985). Detection limits for chlorophacinone and diphacinone were 0.05 μg/g (wet wt).

2.3.4. Analyses of organochlorines, trace metals and other persistent contaminants in small mammal specimens and in a Screech-owl egg

Between 2001 and 2002, 24 small mammal pools from 12 orchards were analyzed for organochlorine residues (6.0 g aliquot) at the CWS. With two exceptions, pools were comprised of 3 individuals of the same species, representing one of 7 possible species: *Blarina brevicauda*, *Sorex cinereus*, *Microtus pennsylvanicus*, *Peromyscus maniculatus, P. leucopus*, *Napaeozapus insignis* and *Zapus hudsonius. Zapodidae* and *Peromyscus* sp. pools were later consolidated for statistical analysis. Carcasses were whole-ground in a Robot Coupe food processor (Blixes Bx3). Pools were run against a diluted Herring Gull (*Larus argentatus*) egg reference sample for quality assurance. Samples were first dehydrated with anhydrous sodium sulphate (Na_2SO_4) followed by neutral extraction with a 1:1 solution of dichloromethane (DCM) to hexane. Lipids and biogenic materials were removed by GPC and additional cleanup was accomplished via Florisil column chromatography. Quantitative analysis of organochlorines and PCBs was performed using a gas chromatograph coupled with a mass selective detector operated in selected ion monitoring mode. Each cleaned sample was injected twice, the first to determine organochlorines using 21 standards, the second to determine PCBs using Aroclors 1242/1254/1260, in a 1:1:1 ratio. Twenty persistent compounds were measured: DDT, DDE, DDD (p,p'-compounds), 1,2,4,5- and 1,2,3,4- tetrachlorobenzene(TCB), pentachlorobenzene (QCB), alpha-HCH, beta-HCH, gamma-HCH, hexachlorobenzene (HCB), octachlorostyrene (OCS), heptachlor epoxide (HE), oxy-chlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, dieldrin, mirex and photomirex. Levels of PCB congeners were also examined, (IUPAC

numbers 16, 17, 18, 20, 22, 28, 31, 32, 33, 42, 44, 47, 48, 49, 52, 56, 60, 64, 66, 70, 74, 76, 85, 87, 90, 92, 95, 97, 99, 101, 110, 105, 118, 128, 130, 137, 138, 141, 146, 149,151,153, 156, 157, 158, 170, 171, 172, 174, 176, 177, 178, 179, 180, 183,187, 190, 194, 195, 196, 200, 201, 202, 203, 206, 207, 208). Total PCB levels were measured as the sum of these congeners. For all compounds, detection limits were in the 100 ppt range.

In addition to the above, the 10 whole body pooled homogenates obtained during the 2001 captures were also analyzed for 23 trace metals (Ag, Al, As, Ba, Be, Bo, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Tl, U, V, Zn). The carcasses were homogenized at the CWS laboratory in Hull prior to shipment to Philip Analytical Services in Halifax, Nova Scotia. The specimens were digested in mineral acids and metals were analyzed by ICP-MS (Perkin Elmer Elan 5000 ref. U.S. EPA method no. 200.8). Detection limit ranged from 0.02 to 5.0 mg/kg. Finally, total Hg of specimens was determined at the CWS laboratory in Hull without acid digestion on an AMA-254. Detection limit was 0.040 µg/g (wet wt).

A Screech-owl egg collected from a nest box in a forest between two orchards was analyzed for organochlorines and persistent contaminants but not for trace metals. The contents of the egg were analyzed in the same manner as the small mammal specimens, as described above. The eggshell was air dried then measured at the mid-line to determine the thickness of the shell and the inner membrane. Five measures of shell thickness were taken using a dial gauge micrometer calibrated in 0.001 mm units and the average of these values was calculated (in mm).

2.4. RESULTS

Data obtained during this study are presented in a format applicable and relevant not only to the Screech-owl but to other species that might be at risk of secondary exposure to the compounds in question.

2.4.1. Censuses

Screech-owls were observed in 9 of 15 orchard locations and at both control locations during nocturnal censusing. All owls observed were grey phase except the red-phase owl observed near orchard I. Table 2.1, below, shows results of winter and summer censuses conducted from 2000 to 2002.

2.4.2. Orchard insecticide use profiles

Pesticide use records were obtained for the 14 of the 15 orchards where Screech-owls were observed and small mammals were captured. Table 2.2, shown below, summarizes the approximate area and age, as well as the number of trees within, each orchard. To maintain confidentiality, orchards were identified by a letter. The span of pesticide use records and the number of years covered by these records is also provided. The earliest records, obtained for Orchard I, dated back to 1974. The highest number of yearly phosmet, azinphosmethyl and phosalone applications (max no. recorded applications) were contrasted against the most common number of yearly applications. When pesticide use records were not available, we estimated yearly frequency of application in consultation with orchard-owners. However, these estimates are based on current rates of use rather than on historical use.

Table 2.1
Screech-owl observations during 2000-2002 winter and summer censuses of orchard and control locations in Saint-Hilaire and Rougemont and number of nest boxes installed at each

Location	Winter 2000-2001 Date (d/m/y)	Summer 2001 date (d/m/y)	Winter 2001-2002 date (d/m/y)	Summer 2002 Date (d/m/y)	No. nest boxes installed
A/A1*	15/02/01 **pair**	31/07/01 **2 individuals**	18/02/02 **adult**	04/06/02 **individual**	9
B	17/01/01 **adult**	30/07/01 **individual**	27/01/02 27/02/02 **NR**	08/09/02 **individual**	10
C	20/03/01 **adult**	24/07/01 **individual**	27/01/02 **adult**	06/10/02 06/24/02 01/08/02 **NR**	5
D/F*	18/12/00 **pair**	04/06/01 **individual** 25/07/02 **1 adult, 2 young**	05/01/02 **adult**	06/06/02 **NR** 06/24/02 **individual**	11
G	03/01/01 **adult**	24/07/01 **NR**	28/02/02 **NR**	06/10/02 **NR**	7
I	22/10/01 **NR**	03/06/01 **individual**	04/01/02 **adult**	**NC**	15
K	17/01/01 **adult**	24/07/01 **individual**	05/01/02 **NR** 27/01/02 **adult**	06/06/02 **individual**	9
M	**NC**	**NC**	18/02/02 **adult**	18/07/02 08/08/02 **NR**	7
N	07/12/00 **NR** 18/12/00 **pair**	17/07/01 19/07/01 30/07/01 **NR**	05/01/02 27/02/02 **NR**	08/08/02 **NR**	9
Campground	02/02/01 **adult**	**NC**	26/12/01 **pair**	24/05/02 **adult**[+]	10
MSH Biosphere Reserve	**NC**	07/08/01 **individual**	08/01/02 18/02/02 **NR**	22/06/02 20/09/02 **individual**[++]	6

*Orchards adjacent to one another
[+] observed perching in tree at location, no censusing conducted
[++] heard, no censusing conducted
Individual = adult or juvenile
NR = no response
NC = not censused
MSH = Mont-Saint-Hilaire

Table 2.2
Summary of phosmet, azinphosmethyl and phosalone use in Saint-Hilaire and Rougemont orchards where Screech-owls were observed and/or small mammals were captured 2000-2002

Orchard	Area (ha)	No. trees	Age of orchard (y)	Span of pesticide use records (y)	No. years applied during span of records	Max recorded no. applications	Most common no. seasonal applications
**A	1.5	1,500	~80	Bio	-	-	-
**A1	18	5,000	~75	1991 – 2001 (n = 11)	*5 +11 ±4	*3 +3 ± 6	*1 – 2 +1 ±2 – 3
B	10	5,000	~75	1981 – 2001 (n = 21)	*7 +16 ±5	*, +, ±2	* 1 + 1 ±2
C	2	1,200	12	N/A	N/A	N/A	*1 +3 ±0-1
**D	2	1,000	~10	N/A	N/A	N/A	*1 +1-2 ±1-2
E	7	3,000	> 70	N/A	N/A	N/A	*1-2 +0 ±0
**F	21	3,000	~ 150	1994 – 2001 (n = 8)	*8 +0 ±1	*4 +0 ±1	*2 +0 ±1
G	25	17,000	~ 70	2000 – 2001 (n = 2)	*2 +0 ±2	*5 +1 ±3	*3 +1 ±2 – 3
H	1.2	650	~ 100	1990 – 2001 (n = 12)	*6 +10 ±0	*, +2 ±0	*1 +1 ± 0
I	8	1,000	~75	1974 – 2001¤ (n = 26)	*26 +8 ±9	*7 +2 ±6	*3-4 + 1-2 ±3
J	10	5,000	~75	N/A	N/A	N/A	* 1-2 +0 ±1-2
K	11	~2,500	> 40	1995 – 2001¤ (n = 5)	*0 +1 ±2	*0 +1 ±1	*0 +1 ±1
M	14	8,500	~70	1986 – 2001¤ (n = 15)	*2 +13 ±11	*1 +3 ±5	*1 +1-2 ±2
N	5	900	~70	1977-2001¤ (n = 24)	*1 +11 ±19	*1 +3 ±6	*1 +1 ±1,2,3

*phosmet +azinphosmethyl ±phosalone
**Adjacent to one another = A and A1, D and F
¤ = missing year(s) from records I: 1982,1989 K: 1998, 2000 M: 1992 N: 1982
bio = biological orchard, no organophosphorus insecticides applied
N/A = no records available, number of applications estimated in consultation with orchard-owners

2.4.3. Collection of pellets and prey remains

A total of 164 pellets and prey remains were retrieved from nest boxes between 2001 and 2003: 58 from orchard locations and 106 from the two control locations. Pellets collected from Orchards N and C were excluded from analysis because only 1 and 4 pellets were collected from single nest boxes during the entire inspection period, respectively.

The remaining pellets (n = 39) and prey remains (n = 14) were collected from two adjacent orchards in Rougemont (Table 2.3a) and in Mont-Saint-Hilaire (Table 2.3b). Both pairs of orchards were separated by less than 1 km. The Mont-Saint-Hilaire orchards were connected by a series of forest patches. A continuous forest patch joined the Rougemont orchards. Pellets and prey remains were pooled for each pair of orchards and used to estimate the percent biomass of small mammal and avian species.

For comparison, Tables 2.4a and 2.4b list the pellet content and prey remains found at the two control sites. Interestingly, the highest small mammal biomass proportion was observed in pellets found at the Mont-Saint-Hilaire Biosphere Reserve. Voles comprised the highest proportion of small mammal biomass consumed at all sites. We made the assumption that voles found in pellets at orchard locations were *Microtus* rather than *Clethryonomis*. Appendices 2.1a to 2.1d detail when pellets and prey remains were found in relation to the Screech-owl's annual cycle.

Table 2.3a
Analysis of Screech-owl pellets (n = 8) and prey remains (n = 3) collected from two Rougemont orchard sites (B and K) 2001-2003

Species	Average weight (g)	Approximate biomass contribution (g)	% Biomass contribution
Short-tailed Shrew *B. brevicauda* (n = 2)	20.11	44.0	9.0
UID Vole *M. pennsylvanicus* or *Clethrionomys gapperi* (n = 5)	32.50	163.0	33.4
American Robin *Turdus migratorius* (n = 1)	80.0	80.0	16.4
Hairy Woodpecker *Picoides villosus* (n = 1)	66.0	66.0	13.5
Mourning Dove *Zenaida macroura* (n = 1)	135.0	135.0	27.7

UID: unidentified
Total biomass = 488 grams
Total small mammal biomass contribution: 42.4%
Total avian biomass contribution: 57.6%

Table 2.3b
Analysis of Screech-owl pellets (n = 31) and prey remains (n = 11) collected from two Saint-Hilaire orchard sites (D and F) 2001-2003

Species	Average weight (g)	Approximate biomass contribution g)	% Biomass contribution
UID Vole *M. pennsylvanicus* or *C. gapperi* (n = 6)	32.5	195.0	16.9
***Sorex* sp.** (n = 5)	4.9	25.0	2.2
Peromyscus* sp.** or ***Zapodidae (n = 1)	18.0	18.0	1.6
UID Rodents* (n = 2)	25.3	50.5	4.4
Eastern Phoebe *Sayornis phoebe* (n = 1)	20.0	20.0	1.7
Mourning Dove *Zenaida macroura* (n = 4)	135.0	540.0	46.7
UID Thrush *Turdidae* (n = 2)	47.0	94.0	8.1
Blue Jay *Cyanocitta cristata* (n = 1)	90.0	90.0	7.8
Black-capped Chickadee *Parus atricapillus* (n = 1)	12.0	12.0	1.0
Brown-headed Cowbird *Molothrus ater* (n = 1)	43.0	43.0	3.7
Brown Thrasher *Toxostoma rufum* (n = 1)	68.0	68.0	5.9

UID: unidentified
Total biomass: 1,156.0 g
Total small mammal biomass contribution: 25.0%
Total avian biomass contribution: 75.0%
*the average of known rodent species weights (*Microtus* or *Clethrionomys* and *Peromyscus* or *Zapodidae)*

Table 2.4a
Analysis of Screech-owl pellets (n = 42) and prey remains (n = 20) collected from Saint-Hilaire campground control site 2001-2003

Species	Average weight (g)	Approximate biomass contribution (g)	% Biomass contribution
UID Vole *M. pennsylvanicus* or *C. gapperi* (n = 19)	32.5	617.5	20.0
Peromyscus* sp.** or ***Zapodidae (n = 5)	18.0	90.0	2.9
Short-tailed Shrew *B. brevicauda* (n = 2)	20.1	40.2	1.3
Mourning Dove *Zenaida macroura* (n = 6)	135.0	810.0	26.2
Rock Dove *Columbia livia* (n = 3)	300.0	900.0	29.1
Cedar Waxwing *Bombycilla cedrorum* (n = 2)	40.0	80.0	2.6
American Goldfinch *Carduellis tristis* (n = 1)	14.0	14.0	0.5
American Robin *Turdus migratorius* (n = 1)	80.0	80.0	2.6
Blue Jay *Cyanocitta cristata* (n = 1)	90.0	90.0	2.9
Common Grackle *Quiscalus quiscula* (n = 1)	112.0	112.0	3.6
Dark-eyed Junco *Junco hyemalis* (n = 1)	20.0	20.0	0.6
Hairy Woodpecker *Picoides villosus* (n = 1)	66.0	66.0	2.1
UID Flycatcher *Tyrannidae* (n = 1)	35.0	35.0	1.1
Northern Flicker *Colaptus auratus* (n = 1)	120.0	120.0	3.9
Green Frog *Rana clamitans* (n = 1)	18.0	18.0	0.6

UID: unidentified **Note:** scales of unidentified fish also found
Total biomass: 3,092.7 g Total small mammal biomass contribution: 24.2 %
Total avian biomass contribution: 75.2%

Table 2.4b
Analysis of Screech-owl pellets (n = 45) and prey remains (n = 4) collected from the Mont-Saint-Hilaire Biosphere Reserve control site 2001-2003

Species	Average weight (g)	Approximate biomass contribution	% Biomass contribution
UID Vole* *M. pennsylvanicus* or *C. gapperi* (n = 46)	32.5	1,495.0	69.5
Peromyscus* sp.** or ***Zapodidae (n = 11)	18.0	198.0	9.2
Short-tailed Shrew *Blarina brevicauda* (n = 3)	20.1	60.3	2.8
***Sorex* sp.** (n = 1)	4.9	4.9	0.2
Blue Jay *Cyanocitta cristata* (n = 1)	90.0	90.0	4.2
European Starling *Sturnus vulgaris* (n = 1)	78.0	78.0	3.6
Mourning Dove *Zenaida macroura* (n = 1)	135.0	135.0	6.3
Northern Saw-whet Owl *Aegolius acadicus* (n = 1)	91.0	91.0	4.2

UID = unidentified
*given habitat adjacent to nest boxes, assume *C. gapperi*
Total biomass: 2,152.2 g
Total small mammal biomass contribution: 81.7%
Total avian biomass contribution: 18.3%

2.4.4. Analysis of organophosphorus insecticides in small mammal specimens

In 2001, we monitored 1 azinphosmethyl and 3 phosmet applications. Recovery rates from spiked samples were below or slightly above 1.0%, indicating serious problems with the collection procedure. In 2002, we were notified of 1 phosmet and 1 azinphosmethyl application but only captured 1 *Blarina* during the latter. Average recoveries from spiked samples using the new procedures were 81.84% for phosmet and 86.98% for azinphosmethyl. Given the extremely poor recovery values for 2001, we have chosen to only report on the 2002 phosmet capture. In the Discussion, these values are considered in relation to exposure of Screech-owls to either insecticide, and to phosalone, since it is also applied in the study area, based on a 'Residue per unit dose' (RUD) concept (Hoerger and Kenaga 1972).

In June of 2002, 8 *Microtus* were captured between 8 and 52 hr after a 2 kg phosmet (50% w.p.) /ha application by a tractor-pulled air blast sprayer (Table 2.5). Residues were plotted against time first in ng/g and then in $ng/g^{0.67}$. In the second instance, body weight was raised to the power of 0.67 because this factor relates surface area to body weight (Mineau *et al.* 1996). When residues were simply plotted as ng/g, the relationship was not as good ($r^2 = 0.69$ versus $r^2 = 0.88$, respectively) (Figures 2.1 and 2.2).

Table 2.5
Whole-body residues of phosmet in *M. pennsylvanicus* (MV) 0-52 hours post-application (ng/animal)

Sample	Time post-spray (hr)	Weight (g)	Residues (ng/g)	Residues ($ng/g^{0.67}$)
MV1	8	46	1020	3608
MV2	24	20	680	1836
MV3	28	11	1337	2984
MV4	51	39	247	823
MV5	51	39	133	445
MV7	51	20	133	445
MV6	52	39	310	837

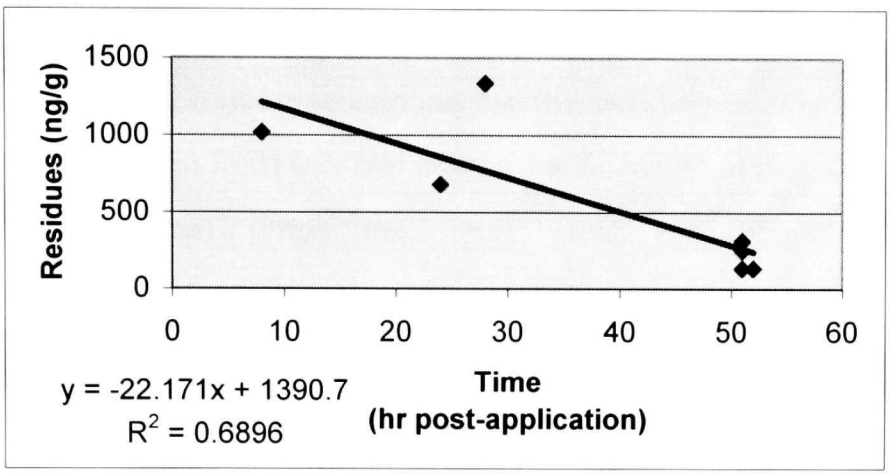

Figure 2.1
Phosmet residues in *M. pennsylvanicus* 0-52 hr post-application
Residues (ng/g)

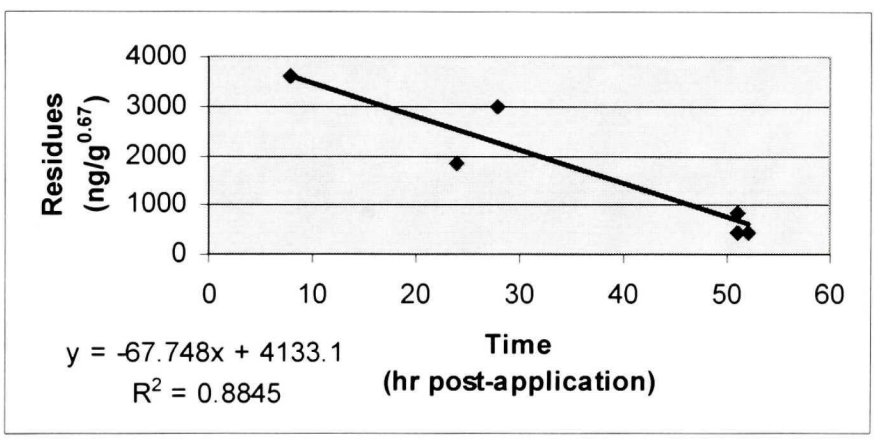

Figure 2.2
Phosmet residues in *M. pennsylvanicus* 0-52 hr post-application
Residues in ng/g$^{0.67}$

2.4.5. Screech-owl captures

Four adults and 5 juveniles were captured and banded between 2001 and 2002 (Table 2.6). In 2002, 2 juveniles were also observed at Orchard A/A1 and an adult and 2 juveniles were observed at Orchard B, but all evaded capture. Our attempts at radio-tracking were well-intentioned but unsuccessful; the adults fitted with transmitters shed them within 2 days.

Despite numerous attempts, we were unable to capture Screech-owls in the 0-48 hr interval post-insecticide application. Only one of the 9 owls was bled and this owl was captured approximately 72 hr post-application. This

individual was captured in a forest patch adjacent to Orchard I, where *Microtus* were captured post-phosmet application (reported in Table 2.5). Phosmet was applied in this orchard during the day of July 19, 2001 and the adult Screech-owl was captured in the evening of July 22, 2001. The blood sample taken from this owl showed a BChE level of 1304 μmol/min/L blood (1294 and 1313 μmol/min/L).

Table 2.6
Screech-owls captured and banded in orchard and control locations of Saint-Hilaire and Rougemont 2001-2002

Location	Capture date (d/m/y)	Captured individual
Campground	13/07/02	1 adult* 3 juveniles
Orchard A/A1	09/08/02	1 adult* 1 juvenile
Orchard D/F	29/08/02	1 juvenile
Orchard I	22/07/01	1 adult
Orchard K	31/07/02	1 adult

*fitted with radio-transmitter
N/A= unsuccessful

2.4.6. Cholinesterase levels in Screech-owls admitted to a local raptor rehabilitation facility

From 2000 to 2001, blood samples were taken from 18 Screech-owls at the Clinique des oiseaux de proie. Four of these owls had been in captivity for at least one year and so were used as references (1158-2127 μmol/min/L blood). Owls no. 2025 and 2828 were sampled twice and the average of the two samplings is also included in Table 2.7.

For the 14 'non-reference' owls, BChE levels ranged from 1217 to 3947 μmol/min/L (Table 2.8). Individuals admitted from agricultural areas beginning in late April until late July were of particular interest, since this encompasses the typical period of insecticide applications. Four of the14 were admitted during the timeframe of interest, unfortunately, they were all sampled too late (78 to 90 days after admission). Acetylcholinesterase levels in the brains of 4 individuals ranged from 21.0 to 28.0 μmol/min/g (Table 2.9). Owl 2228 was released in May of 2000 at the edge of an apple orchard and

found dead in the woods adjacent to this orchard several days later. The owl had a level of 26.0 μmol/min/g.

Table 2.7
Mean plasma butirylcholinesterase (BChE) activity (μmol/min/L) in captive Screech-owls sampled at the Clinique des oiseaux de proie (COP) 2000-2001

Sample	Age	In captivity since	Date Sampled	BChE level	
2025a 2025b	AHY	07/12/97	02/05/01 23/05/01	1158 1348	1253
2465	AHY	23/11/99	22/07/01	1249	
2828a 2828b	AHY	Unknown*, at least 1 yr	02/05/01 23/05/01	1515 1844	1680
2228	ASY	11/12/98	17/05/00	2127	

*Transferred from the Owl Foundation in Vineland, Ontario
AHY: after hatching year
ASY: after second year

Table 2.8
Mean plasma (BChE) activity (μmol/min/L) in Screech-owls admitted to the Clinique des oiseaux de proie (COP) 2000-2001

Sample	Age	Date Admitted (d/m/y)	Date sampled (d/m/y)	BChE level	No. days admission to sampling	Location
2487	AHY	05/01/00	16/05/00	1300	131	St-Valérien
2500	AHY	27/01/00	17/05/00	1714	110	St-Damase
2502	AHY	27/01/00	18/05/00	2249	111	Hudson
2504	AHY	02/02/00	18/05/00	2461	105	Montreal
2511	SY	15/02/00	16/05/00	1479	90	St-Basile
2542	UNK	08/05/00	10/08/00	1521	94	St-Valérien
2545 *	UNK	14/05/00	13/10/00	1252	149	Montreal
2557	HY	08/06/00	04/09/00	1535	88	Montreal
2587	HY	20/06/00	06/09/00	2040	78	Hudson
2669 *	UNK	09/09/00	12/09/00	1217	3	Hudson
2754	AHY	14/12/00	18/12/00	1884	4	St-Robert
2785	HY	01/02/01	08/02/01	1290	7	Hemmingford
2788a	HY	03/02/01	03/02/01	2712	0	Laval
2788b			01/03/01	2326	26	
2794a	HY	17/02/01	17/02/01	3947	0	St-Ours
2794b			01/03/01	2383	12	

*Owl died, brain also analyzed
AHY: after hatching year
ASY: after second year
HY: hatching year
SY: second year
UNK: unknown

Table 2.9
Mean brain (AChE) activity (μmole/min/g) in Screech-owls admitted to the Clinique des oiseaux de proie (COP) 2000-2001

Sample	Age	Time of admission (d/m/y)	Time of death (d/m/y)	AChe level
1641-2228*	ASY	++	~26/05/00	26.0
1641-2545	UNK	14/05/00	13/10/00	21.0
1641-2931*	HY	25/07/01	26/07/01	23.5
1641-2669*	UNK	09/09/00	12/09/00	28.1

*Blood sample also taken
~Released 17/05/00, found dead 26/05/00
++ No admission, owl found dead in woods adjacent to an orchard
ASY: after second year
HY: hatching year
UNK: unknown

2.4.7. Analysis of anticoagulant rodenticides in small mammal specimens

Only two orchards could be located for captures post-anticoagulant application. Though numerous runways, burrows at the base of apple trees and plentiful, luxuriant vegetation were noted in both orchards, small mammals were only captured in one. Chlorophacinone residues were detected in only 2 of the 16 individuals captured, 0.3 and 0.1 μg/g, respectively. No other anticoagulants were detected in any of the samples.

2.4.8. Analyses of organochlorines, trace metals and other persistent contaminants in a Screech-owl egg and in small mammal specimens

Where appropriate, percent moisture in samples has been reported so that residue values can be converted to dry wt and compared with data collected during other studies. Trace metal values are reported on a wet and dry wt basis.

DDT and metabolites

In 2003, a Screech-owl egg was collected from a nest box in the forest connecting Orchards B and K. The egg contained 2.61 μg/g p,p'-DDE (fresh wt, corrected for recovery). Percent moisture, percent lipid and percent recovery were 80.77, 5.56 and 82.20, respectively. With the exception of p,p'-DDE, all residues of persistent contaminants were negligible.

Small mammals captured in 12 orchards were analyzed for DDT and metabolites. Full results as well as percent moisture, percent recovery and percent lipid are reported on both a species and an orchard-by-orchard basis (Table 2.10). Results were corrected for recovery. The p,p'- DDT and p,p'-DDE residue levels were log-transformed to satisfy assumptions of normality and heterogeneity of variance. Associated standard deviation for the geometric mean and observed residue range in the field is reported. Highest levels were always observed in *Blarina*.

Residue levels of DDD are generally not reported, often because levels are below detection limits (Mora 1997). However in our samples, 22 of the 24 pools contained detectable residues of p,p'-DDD ranging from the detection limit to 1.15 μg/g. Samples of *Blarina* exhibited a mean p,p'-DDT residue level of 0.90 μg/g with an observed range of 0.08 – 10.43 μg/g (Table 2.11). Mean p,p'-DDE residue level was 6.20 μg/g with an observed range of 0.94 to 26.29 μg/g (Table 2.12).

Trace metals

Only small mammal pools from the 2001 captures were analyzed for trace metals because levels observed in these samples were not sufficiently elevated to be of further concern. Eleven of the 23 trace metals analyzed were detected at low levels. Full values are summarized on a wet and dry wt basis in Appendix 2.2a. Traces of Hg were found in 4 of 10 samples and ranged from 0.07 – 0.30 μg/g (wet wt). Traces were only detected in shrew pools. Results are summarized in Appendix 2.2b on a dry wt basis.

Other persistent contaminants

Sixty-seven PCB congeners were analyzed in 2001 and 2002 samples and 35 were detected at low levels. Of these, five were most frequently detected: IUPAC # 118, 138, 153 and 187. These results, and the sum of PCB congeners are summarized in Appendix 2.3 on a μg/kg (wet wt) basis. Low levels of TCB, QCB, HCB, trans-nonachlor, trans-chlordane and dieldrin were detected in some samples and are also summarized in Appendix 2.3 With the exception of dieldrin, results were not corrected for recovery.

Table 2.10
Residues of DDT and metabolites in small mammal pools (n = 24) from orchards (n = 11) of Saint-Hilaire and Rougemont 2001 and 2002 (µg/g wet wt)

Orchard	Species	p,p'-DDE	p,p'-DDT	p,p'-DDD	% recovery	% moisture	% lipid
+A	STS	7.20	0.94	0.47	85.95	72.87	3.07
+A1	STS	4.38	0.47	0.06	85.17	72.62	2.83
	JM**	0.03	0.00	0.00	85.73	68.96	4.77
+B	MV	0.03	0.00	0.00	91.77	75.72	2.18
	Per	0.00	0.00	0.00	85.95	76.12	4.20
	JM	0.02	0.00	0.00	78.03	70.91	3.74
+D	MV	0.02	0.02	0.00	87.56	76.21	4.62
D/F*	Per	0.02	0.00	0.00	92.02	70.48	4.42
E	STS	26.29	10.43	1.15	77.43	68.93	3.63
	MV	0.29	0.02	0.00	90.26	74.15	2.3
	Per	0.02	0.00	0.00	83.22	73.96	3.07
+F	MS	0.15	0.01	0.03	94.98	73.86	3.80
	MV	0.06	0.00	0.00	87.31	74.62	2.86
+G	STS	4.35	0.48	0.09	82.44	68.69	4.15
H	STS	5.14	0.53	0.17	94.85	70.46	2.37
	MV	1.12	0.05	0.01	91.75	76.08	4.63
+I	MV	0.08	0.00	0.00	81.60	75.34	3.28
J	STS	9.11	3.57	0.13	84.36	70.61	2.80
+K	STS	13.72	1.37	0.31	91.72	71.16	4.44
	MV	0.20	0.00	0.00	110.83	73.95	3.2
	Per	0.07	0.01	0.00	83.35	74.44	0.52
L	STS	0.94	0.08	0.02	93.19	70.39	3.35
	MV**	0.12	0.01	0.00	90.83	74.79	3.21
	JM	0.01	0.00	0.00	83.86	69.99	3.72

*D/F: captured between two orchards
**Pool of 2 individuals only
+Screech-owl observed in vicinity during censusing
STS: Short-tailed Shrew (*B. brevicauda*) (n = 8 pools)
MS: Masked Shrew (*Sorex cinereus*) (n = 1 pool)
MV: Meadow Vole (*M. pennsylvanicus*) (n = 8 pools)
Per: *Peromyscus* sp. (n = 4 pools)
JM: *Zapodidae* (n = 3 pools)

Table 2.11
P,p'-DDT residue ranges in small mammal pools from orchards of Saint-Hilaire and Rougemont 2001 and 2002 (µg/g wet wt)

Species and No. pools	Geometric mean	Log-transformed value and standard deviation	Residue range observed in the field
B. brevicauda (n = 8)	0.9044	-0.0436 ± 0.63867	0.0783 – 10.4266
S. cinereus (n = 1)	0.0097	N/A	N/A
M. pennsylvanicus (n = 8)	0.0162	-1.7912 ± 1.3586	0.0000 – 0.0461
Peromyscus sp. (n = 4)	0.0010	-3.0019 ± 0.8303	0.0001 – 0.0072
Zapodidae (n = 3)	0.0022	-2.6528 ± 0.2338	0.0013 – 0.0038

Table 2.12
P,p'-DDE residue ranges in small mammals pools from orchards of Saint-Hilaire and Rougemont 2001 and 2002 (pools in µg/g wet wt)

Species and No. pools	Geometric mean	Log-transformed value and standard deviation	Residue range observed in the field
B. brevicauda (n = 8)	6.1957	0.7921 ± 0.4258	0.9396 – 26.2914
S. cinereus (n = 1)	0.1516	N/A	N/A
M. pennsylvanicus (n = 8)	0.1636	-0.7863 ± 0.8248	0.0168 - 1.1221
Peromyscus sp. (n = 4)	0.0167	-1.7779 ± 0.5983	0.0026 - 0.0726
Zapodidae (n = 3)	0.0188	-1.7249 ± 0.2203	05 - 0.0260

2.5. DISCUSSION

2.5.1. Organophosphorus insecticides

Potential phosmet, azinphosmethyl and phosalone exposure

We were only able to capture one Screech-owl in the vicinity of an orchard shortly after an insecticide application (approximately 72 hr post-application). As a result, we determined the risk of exposure through an assessment process. Assuming that the vole skulls identified in Screech-owl pellets from orchard locations were in fact those of *Microtus*, the species represents the majority of the small mammal biomass consumed (Tables 2.3a and 2.3b). Studies have also demonstrated the preponderance of *Microtus* in the small mammal component of the Screech-owl's diet (Sherman 1911;

Wilson 1938; Ritchison and Cavanagh 1992), and it has even been suggested that the species is preferred over other potential prey (Marti and Hogue 1979). Juveniles tend to be more active and conspicuous than adults, a trait favoured by the opportunistic Screech-owl (Metzgar 1967). When captive Screech-owls were offered both large and small prey, they always selected the latter (Marti and Hogue 1979). We therefore reasoned that assessing the risk of insecticide exposure to the Screech-owl, based on the species' consumption of juvenile *Microtus*, would be most appropriate.

Using the residues observed in the 8 *Microtus* captured post-phosmet application, our intention was to construct a worst-case scenario where a Screech-owl hunts for voles in an orchard shortly after an application is made, and over the interval over which residues persist in these prey. We first plotted residues in ng/g against hr post-application and obtained an r^2 value of 0.69 (Figure 2.3). Given the short lifespan of organophosphorus insecticides, the majority of residues observed prior to their dissipation in the environment are likely to be those on the surface of the vole. Small-bodied individuals have a large surface to volume ratio, and therefore a larger surface on which insecticide can be deposited, relative to larger-bodied individuals. In this instance, the Screech-owl's apparent predilection for small prey may predispose it to adopt a diet of more contaminated individuals. With these factors in mind, we plotted residues in $ng/g^{0.67}$, because this relates body weight to surface area (Mineau *et al.* 1996), and obtained a better fit (r^2=0.88) (Figure 2.4).

We retained this regression and its associated equation for our risk assessment because it enabled us to model Screech-owl exposure from the consumption of small-bodied individuals (a true worst-case exposure scenario), in this case juveniles, and to account for the relationship between body weight and surface area. From the equation $y = -67.748x + 4133.1$, where y = residues ($ng/g^{0.67}$) and x = time (hr post-application), we determined that residues in *Microtus* should have dissipated (y = 0) approximately 61 hr post-application.

Screech-owls are nocturnal, which implies that they hunt sometime within the (approximately) 12 hours of darkness in a 24 hour day. Within 60 hours, then, an owl would have a maximum of three hunting periods, and thus three intervals, each theoretically spanning 12 hr, when exposure could take place. Two exposure scenarios spanning 0 – 60 hr post-application, were drawn up. In the evening application scenario, the Screech-owl is exposed during the first 12-hr interval post-application, shortly after an insecticide is applied. Twelve hours pass, during which the owl is at rest. Exposure occurs again during the 36- and 60-hr post-application interval. This worst-case scenario might occur if an insecticide was applied at dusk, in the late evening, or in the early morning. In fact, these are considered optimal application times since wind speed is frequently higher during the day (J. Boucher, pers. comm). Nocturnal applications are also recommended when bees are kept in the orchards to pollinate the apple blossoms (Ministère de l'Environnement et de la Faune *et al.* 1996). This precaution is particularly important with respect to phosmet because it is highly toxic to honeybees (*Apis mellifera*) (http://www.epa.gov/pesticides/op/phosmet).

In the morning application scenario, the owl is at rest during the first 12-hr post-application interval. Exposure occurs 12 hours after an insecticide has been applied and then from 36 to 48 hr post-application. As a result of the delay between insecticide application and the owl's active period, the morning application represents the best-case scenario.

Assuming that a female Screech-owl weighs 180 g and a male weighs 150 g (A. Roth, pers. comm.), then an 'average' Screech-owl weighs165 g. We estimated the daily food requirement of a wild Screech-owl from the equation and factors provided by Nagy (1997):

From the equation: $y = ax^b$
where y = daily food requirement (g/d) (dry wt)
x = body mass (g), in this case 165 g
a = 0.648
b = 0.651
daily food requirement for a 165 g Screech-owl = 17.995 g/d

Since all residues in our small mammal samples were reported on a wet wt basis, the estimated daily food requirement was also converted to wet wt. However, percent moisture values were not available for the specimens captured post-phosmet application. Instead the average of the percent moisture values (75.11%, n = 8) for the *Microtus* pools (Table 2.10) was used, providing a readjusted value of 54 g. The average weight of the 3 smallest juveniles captured post-phosmet application was 18 g (Table 2.5), so we reasoned that 3-18 g voles could be consumed to meet the estimated 54 g daily food requirement.

We estimated the residue level available to a Screech-owl in an 18 g individual at each exposure interval of interest, then multiplied this value by 3. In keeping with our worst-case scenario, exposure was calculated based on residues available at the 12, 24, 36, 48 or 60-hr mark rather than over each 12-hr interval of activity. We also assumed that the product was not metabolized over the entire 60-hr interval. For example, for the first 12 hours of the evening application scenario:

$y = -67.748 * 12 + 4133.1$

$y = 3{,}320 \text{ ng/g}^{0.67} \text{ vole}$

$$\frac{3{,}320 \text{ ng}}{\text{g}^{0.67} \text{ vole}} * 18 \text{ g}^{0.67}\text{vole}$$

$23{,}025 \text{ ng} * 3 = 69{,}075 \text{ ng}$

The formulations of phosmet, azinphosmethyl and phosalone recommended for use in orchards of the study area all contain the same percentage of active ingredient (50% w.p.). Both phosmet and azinphosmethyl are applied at an average rate of 2.0 kg a.i. /ha. From the 'Residue per Unit Dose' (RUD) principle proposed by Hoerger and Kenaga (1972), this suggests that the exposure values calculated for phosmet are also applicable to azinphosmethyl. Phosalone, however, is applied at an average rate of 1.25 kg a.i./ha. Total exposure to phosalone was calculated by multiplying the value obtained for phosmet and azinphosmethyl by 0.625, the ratio of the two application rates. Finally, total exposure was converted

from ng insecticide to mg insecticide per 0.165 kg Screech-owl. This conversion was carried out in order to compare our exposure values with LD_{50}s (expressed in mg/kg) for each insecticide. Scenario 1, our worst-case scenario, provided the most elevated exposure values for all three insecticides.

Table 2.13
Phosmet, azinphosmethyl and phosalone exposure (in ng) for a 165 g Screech-owl that consumes 3- 20g *Microtus* in alternating 12 hr intervals 0-60 hr post-application

Intervals (hr post-application)	**Scenario 1 Evening Application (worst-case)**	**Scenario 2 Morning Application (best-case)**
0 –12	23,025 x 3 = 69,075	**N/A**
12 – 24	**N/A**	17,387 x 3 = 52,161
24 – 36	11,749 x 3 =35,247	**N/A**
36 – 48	**N/A**	6,111 x 3 =18,333
48-60	473 x 3 =1,419	**N/A**
Total exposure 0-48 hr (ng)	*105,741 +66,088	*70,494 +44,059
Total Screech-owl exposure 0-48 hr (mg/kg)	***0.641** **+0.401**	***0.427** **+0.267**

N/A = owl at rest, not hunting
*Phosmet and azinphosmethyl
+Phosalone

What do the calculated exposure values mean for the Screech-owl?

Studies have assessed the sensitivity of Screech-owls to several organophosphorus insecticides (Wiemeyer and Sparling 1991; Vyas *et al.* 1998), but neither phosmet, azinphosmethyl, nor phosalone was among them. Consequently, no Screech-owl LD_{50}s for the three insecticides are available with which to compare and assess our projected exposure values. Indeed, information pertaining to the toxicity of a pesticide to the species of interest is rarely available (Mineau *et al.* 1996). Instead, sensitivity is generally extrapolated on the basis of available acute toxicity measurements from other species. However, frequently tested species such as the Bobwhite (*Colinus virginianus*) and the Mallard Duck (*Anas platyrhynchos*) may exhibit sensitivities markedly different from those species most likely to be exposed

to the insecticide when it is applied in the environment (Wiemeyer and Sparling 1991; Mineau *et al.* 1996).

Small-bodied birds are often more sensitive to pesticides, yet the values for the largest and least sensitive species are frequently used in the final determination of pesticide toxicity (Mineau *et al.* 2001). For example, values of avian sensitivity to phosmet were available for five species (Table 2.14, below), however the U.S. EPA's assessment of the toxicity of phosmet was based only on the sensitivity of the Mallard, the largest and least sensitive species in the dataset. While the phosmet LD_{50} reported for the Mallard was 1830 mg/kg (from Hudson *et al.* 1984), the LD_{50} for the most sensitive species, the Red-winged Blackbird (*Agelaius phoeniceus*), was 18 mg/kg (from Shafer *et al.* 1983). This latter value is conspicuously absent from the EPA's assessment of phosmet. Not surprisingly, the assessment also describes phosmet as being moderately to practically nontoxic to avian species (http://www.epa.gov/pesticides/op/phosmet).

Given that the largest and smallest test species exhibited such a large discrepancy in acute sensitivity, it would certainly have been prudent to exercise more caution when assessing the avian toxicity of phosmet. Figure 2.3 shows that the slope of the phosmet LD_{50} regression line is heavily influenced by these two values. The steepness of the slope is largely dictated by the fact that the dataset is dominated by sensitivity values at both extremes of the potential sensitivity spectrum. The positive slope indicates that phosmet is more toxic to small-bodied birds than to large-bodied birds.

While body weight accounts for a large component of the variation observed in LD_{50}s, and for observed sensitivities, it is not the only factor responsible, and the relationship is not linear. Mineau *et al.* (2001) used a distribution-based approach that incorporated scaling factors to account for sensitivity of various test species to a number of pesticides for which acute toxicity data were available. Their resultant HD_5 or Hazardous Dose 5% value represents the lower 5% tail of the distribution of avian LD_{50} values, calculated with a 50% probability of overestimation. The HD_5 therefore

depends not only on the extent of interspecific variation in the existing dataset but also on how the toxicity of a specific pesticide appears to scale to bodyweight (Mineau *et al.* 1996).

Hazardous dose values can also be estimated using the DOS program ETX, developed by Aldenberg (and Slob) (1993) and the program HCREGFIT developed by Collins (in Mineau *et al.* 2001). However, while Aldenberg (and Slob) (1993) drew from procedures similar to those used by Mineau *et al.* (2001), they did not integrate body weight scaling as a factor. HCREGFIT, which is modified from Aldenberg (and Slob) (1993), does scale for body weight. This program first runs a regression of sensitivity (ln LD_{50}) against (ln) weight and estimates the residual variance. Following this step, the program runs a simulation, using from 10,000 to 40,000 iterations (we used 40,000 iterations) to estimate the Aldenburg-Slob factor. This factor is then applied to extrapolate a hazardous dose specific to the species weight of interest. Though body weight is not the sole factor responsible for species sensitivity to an insecticide, it does account for a potentially large amount of inter-species variation. Estimating the residual variance provides a means of removing some of this variation from the dataset.

Referring to the acute avian sensitivity values in Tables 2.14 and 2.15, we used ETX and HCREGFIT to assess sensitivity to phosmet and azinphosmethyl at the lower bounds of the distribution and to compare hazardous doses estimated with and without body weight scaling. For our phosmet assessment, we also conducted our analyses excluding the Red-winged Blackbird sensitivity value (Table 2.16).

Only one LD_{50} was available for phosalone, so we were unable to assess sensitivity to the insecticide in the same manner. However, Luttik and Aldenberg (1997) developed safety factors that could be used to estimate hazardous doses for birds and mammals when only small sample sizes of LD_{50}s ($n = 1 - 4$) were available. An assessment based on a small number of LD_{50}s will likely underestimate the risk and Luttik and Aldenberg (1997) recommend using the safety factor from the 95.0% confidence limit (32.9) for

a more conservative estimation approach. The sole phosalone LD_{50}, reported for the Mallard is >2150 mg/kg (Tomlin 1994). Applying the safety factor, we estimated a hazardous dose of 65.34 mg/kg.

Table 2.14
Acute avian sensitivity reference values (LD_{50}) for phosmet (in mg/kg)

Species	Weight (g)	Geometric mean LD50 (mg/kg)	Source
Ring-necked pheasant (*Phasianus coichicus*)	1135	243.4	Hudson *et al.* 1984
Mallard Duck (*Anas platyrhynchos*)	1082	1945	www.epa.gov/opprrd1/op/phosmet www.epa.gov/pesticides/op/phosmet Hudson *et al.* 1984
Red-legged Partridge (*Alectoris rufa*)	450	435.8	Grolleau and Cartier 1986
Gray Partridge (*Perdrix perdrix*)	370	438.20	
Red-winged Blackbird (*Agelaius phoeniceus*)	53	17.80	Shafer *et al.* 1983

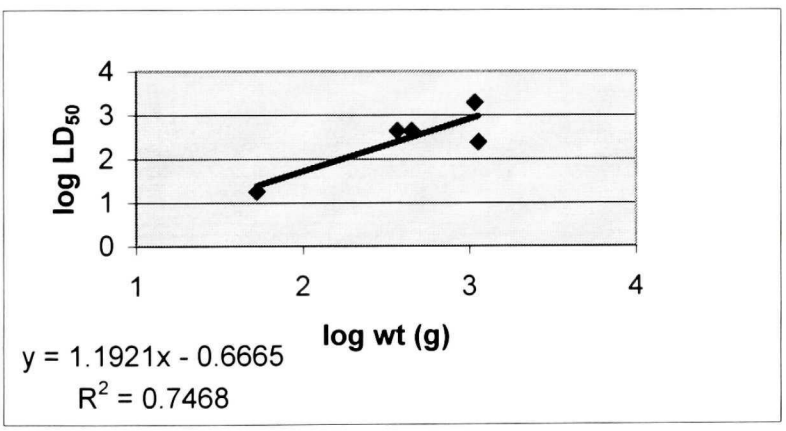

Figure 2.3
Log LD_{50} phosmet sensitivity reference values against log wt (g) test species

Table 2.15
Acute avian sensitivity reference values (LD_{50}) for azinphosmethyl (in mg/kg)

Species	Weight (g)	Geometric mean LD50 (mg/kg)	Source
Ring-necked Pheasant (*Phasianus coichicus*)	1135	75	Hudson *et al.* 1984
Mallard Duck (*Anas platyrhynchos*)	1080	136	
Chukar (*Alectoris chukar*)	578	84.2	www.epa.gov/opprrd1/op/azinphos Hudson *et al.* 1984
Bobwhite Quail (*Colinus virginianus*)	200	44.8	
Japanese Quail (*Coturnix japonica*)	175	10	Grun *et al.* 1995
European Starling (*Sturnus vulgaris*)	82	27	DeCino 1963 Shafer *et al.* 1983
Red-winged Blackbird (*Agelaius phoeniceus*)	53	8.45	

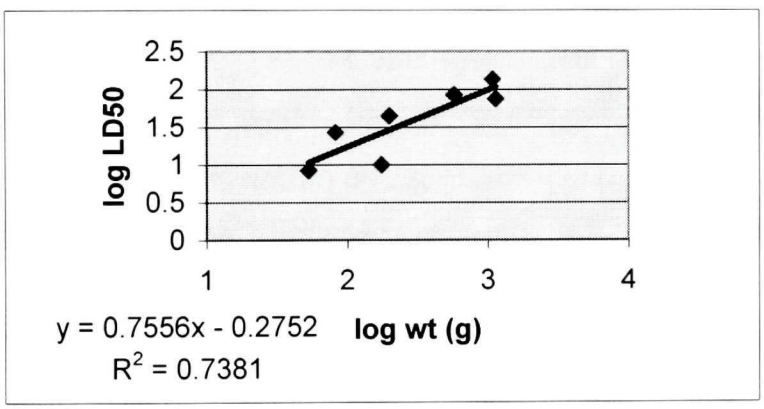

Figure 2.4
Log LD_{50} azinphosmethyl sensitivity reference values against log wt (g) test species

Table 2.16
Estimated hazardous dose values for phosmet and azinphosmethyl (in mg/kg)

Insecticide	Program used	50% confidence	95% confidence
Phosmet	ETX[a]	11.6 99.7*	0.13 4.19*
	HCREGFIT[b]	14.24	5.59
Azinphosmethyl	ETX[a]	5.43	0.78
	HCREGFIT[b]	8.49	3.93

*analysis run excluding Red-winged Blackbird data
a: from Aldenberg and Slob 1993
b: from Collins 1998 (in Mineau *et al.* 2001)

Our worst-case (evening application scenario) exposure levels were 0.641 mg/kg for phosmet and azinphosmethyl and 0.401 mg/kg for phosalone 0-60 hr following an insecticide application (Table 2.13). These levels exceed the hazardous dose estimated for phosmet with 95.0% confidence, and retaining the Red-winged Blackbird data. If one wishes to be extremely cautious in assessing the toxicity of phosmet to a Screech-owl, exposure 0-60 hr post-application may be of particular concern.

The total calculated exposure to phosalone is well below the estimated hazardous dose (0.401 mg/kg versus 65.34 mg/kg, respectively). However, the margin between total calculated exposure to azinphosmethyl and the hazardous dose, estimated with 95.0% confidence (0.641 mg/kg versus 0.78 mg/kg), is considerably narrower. We emphasize that incidents of mortality are still expected at this estimated level of exposure. Hazardous doses are modified LD_{50}s, and LD_{50}s represent the single acute oral dose that elicits 50.0% mortality within a test group. So as the estimated exposure approaches the hazardous dose, estimated mortality approaches the 50.0% mark.

It must also be noted that our assessment only addressed mortality as a result of exposure. Chronic effects such as debilitation and reproductive impairment may also be expected as a result of exposure to insecticides.

Our analysis was confined to a single application scenario where residues available in prey steadily decreased until complete dissipation. In reality, between 3 to 4 insecticide applications are made every season in apple orchards. Consultation of pesticide use records for orchards of southern Quebec revealed that as many as 6 and 7 seasonal applications of phosalone and phosmet have been made, respectively (Table 2.2). One year, phosalone was applied consecutively for 5 days in Orchard A1. However, record entries indicate that consecutive applications of phosmet, azinphosmethyl or phosalone usually span 2-3 days at most.

A Screech-owl's territory, approximately 1.5 km^2 in size (P. Wery, pers. comm.), may be comprised of several orchards, and these may be sprayed

concurrently. Multiple applications of one or several pesticides may result in repeated dosing (Tiebout and Brugger 1995), prolong the effects of exposure, and enhance sensitivity during subsequent exposure (Grue *et al.* 1988). Azinphosmethyl is almost always applied between April and May, during the week following petal fall and up to a month afterwards. It is also applied infrequently near the middle of the summer. Phosmet and phosalone are applied during the remainder of the season, between June and July, coinciding with the fledging and dispersal period. We only assessed the risk of exposure to adult Screech-owls. However, during fledging and, particularly, during dispersal, juveniles may be even more predisposed to consume contaminated prey than adults. Juveniles practice their hunting skills at this time and would most likely prey on the most conspicuous and easily captured prey.

Orchards are sometimes mowed at the same time as insecticides are applied (pers. observ). This can greatly limit small mammal activity within the orchard during and following the spray event. Initial insecticide applications are carried out before small mammal activity begins in earnest. However numerous resident and migratory songbirds are present in the orchards in April and May. The spring is a period of peak avian prey consumption for Screech-owls (VanCamp and Henny 1975). Whole-carcass phosmet, azinphosmethyl and phosalone residues of selected avian prey species would enable us to assemble a more complete scenario of the Screech-owl's seasonal insecticide intake. However, since the owls pluck feathers prior to consumption, and exposure is primarily a surface phenomenon, further exposure to either insecticide from avian prey may not warrant concern.

Screech-owls also consume aquatic organisms (VanCamp and Henny 1975). The majority of orchards in southern Quebec contain a network of ditches, streams, ponds and reservoirs. Giroux (1998) found traces of phosmet, azinphosmethyl and phosalone among others, in streams of certain Rougemont orchards and suggested that aquatic inhabitants are exposed as

a result. Consumption of aquatic organisms may provide a further source of insecticide exposure to the species, albeit minimal.

Cholinesterase levels in Screech-owls admitted to a local rehabilitation facility and in a wild owl

None of the serum or brain cholinesterase levels from birds sampled were indicative of inhibition associated with exposure to a cholinesterase inhibitor. Owls no. 2788 and 2794, admitted to the Clinique des oiseaux de proie in February, were sampled twice, at admission and then 26 and 12 d post-admission (Table 2.8). A 14.0 and 40.0% depression from the initial cholinesterase sampling level, respectively, was observed. A 14.0% change is well within the variation seen in control birds (Table 2.7). We have no explanation for the levels observed in owl no. 2794. An attempt was made to plot mean BChE levels versus time since admission, however no obvious trend was detected.

A Screech-owl was captured in woods adjacent to an orchard in the late evening of July 22, 2001 and had a level of 1304 μmol/min/L. Phosmet was applied in this orchard on July 19. While not the lowest, this level is certainly on the lower end of the observed BChE range. It is noteworthy that our worst-case scenario is based on residue levels in *Microtus* that were captured in this orchard.

Not all birds exposed to cholinesterase inhibitors become intoxicated, nor do all intoxicated birds die (Blus 1996). Most birds exposed to lethal levels, and therefore of interest in terms of sampling, though, die in the wild (Porter 1993). On May 26, 2001, an adult Screech-owl was found dead in the forest adjacent to an apple orchard. The owl, rehabilitated at the Clinique des oiseaux de proie, was released at the edge of the orchard on May 17, 2001. An analysis of its brain did not reveal an AChe level indicative of inhibition.

The owl was found several kilometers from the orchard, in habitat considered more suitable for Barred Owls (*Strix varia*), which were observed in these same woods in August 2001. The deceased may have been chased

deeper into the woods by a resident Screech-owl or by a predator. Prior to release, this owl had been in captivity for almost two years. It is also possible that this owl had lost some of its hunting prowess or that it was simply unable to adjust to the stresses of its new environment.

While none of Screech-owls sampled exhibited cholinesterase levels indicative of inhibition, observed levels may be attributed to a number of factors that mask exposure, such as length of time in the wild prior to capture. Difficulties in assessing exposure in a clinical setting are discussed in the next chapter.

2.5.2. Anticoagulant rodenticides

Chlorophacinone levels in our two samples were 0.1 and 0.3 μg/g, respectively. In a laboratory setting, Askham and Poché (1992) staged a worst-case scenario by feeding 5 Red-tailed Hawks and one Great Horned Owl a diet exclusively composed of chlorophacinone-killed montane voles (*M. montanus*) for 6 consecutive days. All dosed birds survived and none manifested the signs of discomfort or bleeding associated with anticoagulant exposure (Radvanyi *et al.* 1988). Askham and Poché (1992) suggested that chlorophacinone was metabolized in, or excreted by, the voles and surmised that the small amounts of chlorophacinone retained by voles at death would not cause injury or death to raptors. Average whole body residues of voles fed to the raptors were 3.2 μg/g, well above the levels in our two samples.

2.5.3. Organochlorines, trace metals and other persistent contaminants

DDE levels and eggshell thinning in Screech-owl eggs

A Screech-owl egg collected in 2003 from a nest box in the forest between Orchards B and K contained 2.61 μg/g p,p'-DDE. The average of 5 eggshell thickness measurements for this egg was 0.195 mm (0.189 – 0.243 mm). McLane and Hall (1972) reported that Screech-owls administered 2.8 μg/g (wet wt) dietary DDE per day, prior to the breeding season, laid eggs with shells thinned by an average of 13.0%. The average eggshell thickness

from the dosed group was 0.189 mm, while that for the control group was 0.218 mm. However, many of the owls in the McLane and Hall study were actually wild-caught, obtained in 1967 from the area in northern Ohio where VanCamp and Henny (1975) conducted their 30-year study on Screech-owls. This area was once considered to be one of the most productive agricultural regions in the State.

Eggs collected from the same area in 1973 were approximately 8.0% thicker than those from the McLane and Hall study control group (VanCamp and Henny 1975). This suggests that owls in the control (and dosed group) had been exposed to DDE prior to the study. Consequently, the reported DDE intake and subsequent eggshell thinning were probably underestimated because the eggs from the dosed group were not measured against a true control.

To correct for this, we substituted the average eggshell thickness reported by McLane and Hall (1972) for the control group with the average thickness of eggs obtained from nests in Pennsylvania and Ohio prior to 1943 (Table 2.17), and before widespread use of organochlorines (Stringer *et al.* 1974). Dividing the average shell thickness of the Screech-owl egg from our study by the new control value, we estimated that our egg could have been thinned by as much as 19.8%. Dividing the average shell thickness from the McLane and Hall dosed group by the new control value, we calculated an eggshell thinning value of 22.2% (Table 2.18).

Table 2.17
Mean thickness (mm) of Screech-owl eggs (n = 49) collected from Pennsylvania and Ohio prior to 1943

Source	No. eggs measured	Mean thickness (mm)	Range (mm)
Pennsylvania	37	0.241	0.197 – 0.277
Ohio	12	0.244	0.230 – 0.287

From Klaas and Swineford (1976)

Table 2.18
Mean levels of p,p'-DDE and average percent shell thickness in Screech-owl eggs collected from agricultural areas in Quebec, Ohio and Oregon

Source of egg(s) No. samples	Arithmetic mean level p,p'-DDE (μg/g)	Range level p,p'-DDE (μg/g)	Average eggshell thickness (mm)	Range eggshell thickness (mm)	% eggshell thinning (mm)
Orchards B and K (n = 1)	N/A 2.61	N/A	N/A 0.195	N/A	19.8[+]
Klaas and Swineford 1976 (n = 35)	1.29	0.33 - 2.8	0.234* 0.243**	0.157 – 0.270	0.0 - 4.0[+]
Henny *et al.* 1984 (n = 7)	1.65	<0.10 –3.94	0.212	0.189 – 0.243	7.4[++]

*eggs collected during incubation
** addled eggs
[+](average) eggshell thickness divided by 0.243 mm, pre-1943 mean from Ohio and Pennsylvania
[++]average eggshell thickness divided by 0.229 mm, pre-1947 mean from Oregon and Washington

Assuming that the eggs collected from Ohio and Pennsylvania prior to 1943 serve as a more appropriate control, both the dosed birds and the owl from our study area laid eggs thinned by approximately 20.0%. Population declines have been observed in species when eggshell thinning was maintained at 15.0 to 20.0% for an extended period of time (Anderson and Hickey 1972).

While the calculated level of eggshell thinning observed in the egg from our study warrants concern, this concern is based on a sample size of one. As a result, we constructed a risk assessment based on DDE levels in prey captured by owls in orchards of the study area, and on the proportion of these prey consumed from pellets and prey remains retrieved from nest boxes. Orchards B and K were among the orchards for which both residue and prey data was available. Having an actual measure of exposure in a Screech-owl from these orchards, in the form of the egg, provided a valuable reference to evaluate the assumptions and validity of the risk assessment, and to propose improvements to the process as needed. We also reviewed studies assessing eggshell thickness of Screech-owls in agricultural areas because these were based on a much larger number of eggs and enabled us to evaluate the range of eggshell thicknesses observed.

Reference values and assumptions for calculating DDE exposure

McLane and Hall dosed owls from late September, 1970 through to the 1971 breeding season. The number of days during which dosing was conducted is not specified in the study. However, we reasoned that treatment would have ceased once eggs were laid and that this should have taken place by the end of April. From this, we estimated that dosage (2.8 μg/g DDE/d) was conducted over 220 d (September 21, 1970 to April 30, 1971) and that over this period owls were administered approximately 616 μg/g. Assuming that a female Screech-owl weighs 180 g and a male weighs 150 g (A. Roth, pers. comm.), then an 'average' owl, weighing 165 g, would have been administered 101,640 μg dietary DDE. Applying our lipid metabolism coefficient of 89.0%, we determined that 90,460 μg of this would have been metabolized.

Pellets and prey remains were obtained from 2 pairs of adjacent orchards, one in Saint-Hilaire and one in Rougemont. A forest patch with a maximum width of 30 m separated the orchards D and F in Saint-Hilaire. A patch of woods less than a kilometer in length connects Rougemont orchards B and K. Given the small distances between them as well as their overall layout, each pair of orchards was considered the territory of one individual or pair (P. Wery, pers. comm.) and pellets and prey remains were pooled.

Using our previous estimate of daily food requirement, we determined that a wild Screech-owl, weighing 165 g, would require approximately 54 g of biomass per day or 11,800 g over 220 d. Assessing exposure over 220 d enabled us to directly compare our results with those from the McLane and Hall study (1972). We also calculated total exposure over an entire year as a worst-case scenario. Biomass intake was estimated from pellets and prey remains recovered in nest boxes in the woods linking the two pairs of orchards (Tables 2.3a and 2.3b). We considered that all vole skulls identified in pellets from orchard locations were those of *M. pennsylvanicus* rather than *C. gapperi*.

In orchards of the Okanagan Valley, British Columbia, resident birds contained more elevated levels of DDE than neotropical migrants (Harris *et al.* 2000) and American Robins contained the most elevated levels of all (Gill *et al.* 2003). Consequently, the consumption of robins was factored into the risk assessment. We made the assumption that the two unidentified *Turdidae* found at Orchards D and F were robins. No whole-body DDE levels for robins from orchards of Saint-Hilaire or Rougemont were available to us. However, Elliott *et al.* (1994) estimated that the ratio between DDE in an egg and in whole body burden is 1:5. The average of mean DDE levels in robin eggs (n = 7 mean DDE levels) from orchards in the Okanagan Valley is 41.57 µg/g (Gill *et al.* 2003), which equates to a whole-body burden of 83.14 µg/g.

Table 2.19
Calculated exposure of Screech-owls from Saint-Hilaire and Rougemont orchards to DDE (220d)

Orchards	Species	Percent Biomass+	Biomass proportion (g)	DDE residues (μg/g)++	DDE contributed (μg)	Total DDE intake (μg)*
B and K	*Blarina*	9.0	1,062	13.72	14,571	156,253 – 156,849
	Microtus	33.4	3,941	0.03 (B) 0.20 (K)	118 – 788	
	T. migratorius	16.4	1,935	(N/A) 83.14	160,876	₸261,019-262,016
D and F	*Sorex*	2.2	260	0.15	39	70,813 – 70,884
	Microtus	16.9	1994	0.02 (D) 0.06 (F)	40-120	
	Per/Zapodidae	1.6	189	0.02 (D)	3.8	₸118,266 – 118,385
	T. migratorius	8.1	956	(N/A) 83.14	79,482	

+ From Tables 2.3a and 2.3b
++ From Table 2.10
₸ worst-case scenario
*after 89.0% metabolism coefficient applied
N/A = 41.57μg/g x 2, estimated from 0.5:1 egg to carcass DDE ratio

The total DDE intake calculated for Orchards D and F over 220 d was approximately 19,600 μg below that estimated by McLane and Hall (1972). By contrast, the total calculated for Orchards B and K for this same timeframe exceeded it by approximately 66,000μg (Table 2.19). The Screech-owl egg found near B and K was thinned by 19.8%, while owls from the McLane and Hall dosed group laid eggs thinned by 22.2%. These discrepancies might exist because our risk assessment overestimated DDE intake or because the McLane and Hall study underestimated DDE exposure. We also note that it would be most appropriate to measure our Screech-owl egg against archival eggs from the region, or from Quebec, however to our knowledge, none are available.

Our estimates of biomass proportion are based on 8 pellets and 3 avian prey items, collected over only two years. Given the admittedly small sample size and the relatively brief interval over which these items were collected, the estimated biomass consumption may not be entirely

representative. This is most important with regards to the calculated proportion of robins consumed, because of the elevated estimated body burden in these prey. It is also possible that our risk assessment greatly overestimated the DDE body burden of robins. Long-term collection of pellets and prey, and whole-body DDE residue analysis of robins from B and K, D and F, and other orchards where Screech-owls have been observed, would provide more accurate values with which to construct a risk assessment. Finally, Screech-owls in the dosed group of the Mclane and Hall study may have been exposed to DDE prior to the study. As a result, the estimated DDE intake, based on the administration of 2.8 μg/g dietary DDE for 220 d, may not be entirely representative of total intake. Total DDE intake calculated for a wild Screech-owl in the vicinity of Orchards B and K was well above the McLane and Hall reference value. However, the average eggshell thickness for dosed owls was less than that of our wild owl from B and K. In our worst-case scenario, total yearly intake at D and F was well above the reference value as well.

Klaas and Swineford (1976) collected eggs from northwestern Ohio, where (Eastern) Screech-owls were nesting in narrow strips of riparian habitat beside an extensive network of corn, wheat and soybean fields. Estimated eggshell thinning was quite low, though eggs thinned by as much as 35.4% were observed at the lower end of the range (Table 2.18). Overall, Klaas and Swineford (1976) concluded that the level of DDE observed in the eggs did not have any adverse effect on reproductive success. Between 1979 and 1981, Henny *et al.* (1984) collected a single Western Screech-owl (*Otus kennicottii*) egg from 7 nests adjacent to fields in Umatilla and Morrow counties, Oregon. Wheat is the primary crop in this heavily agricultural area. Eggshells were thinned by an average of 7.4% (Table 2.18).

While the DDE level in, and the average shell thickness of, the egg collected from our study area falls within the range observed by Klaas and Swineford (1976) and Henny *et al.* (1984), it is on the low end of both ranges (Table 2.18). Screech-owls that consume small mammals and robins in

orchards of Saint-Hilaire and Rougemont are exposed to DDE. Secondary exposure of Screech-owls in the study region may be sufficient to affect reproductive success in the long-term. However, given the range of eggshell thicknesses observed pre- and post-organochlorine use (Tables 2.17 and 2.18), it would be prudent to obtain additional Screech-owl eggs from other orchards to more fully assess the extent of eggshell thinning throughout the study area.

Secondary exposure to DDE may also have more direct repercussions on the stability of the Screech-owl population. Chronic exposure to organochlorines can diminish motor skills and delay cognitive development, which may predispose to predation or accidents such as car strikes (Blus 1996) or lead to disease and starvation (Frank and Lutz 1999). Extended periods of weight loss may be fatal to birds carrying a potentially lethal DDE body burden (Porter and Wiemeyer 1972). Local rehabilitation facilities receive many Screech-owls every year, possibly debilitated or injured as a result of exposure to a contaminant. Owls admitted from the vicinity of southern Quebec orchards, or from agricultural areas, could be sampled for DDE exposure.

Trace metals

Observed trace metal levels were below or comparable to reference site values reported by Talmage and Walton (1991) and by Sheffield *et al.* (2001). We note that these authors did not report on Al, Ba or Sr. Of the trace metals analyzed, Se exposure has been studied with regards to effects on Screech-owl reproductivity. Wiemeyer and Hoffman 1996 concluded that consumption of prey with body burdens in excess of 4.4 μg/g wet wt (or 10 μg/g dry wt) Se could adversely affect hatching success and cause developmental malformations in young. Observed selenium levels in our small mammal samples ranged from below detection level to 0.70 μg/g wet wt (Appendix 2.2).

Other persistent contaminants

Thirty-five of 67 PCBs were detected at levels close to detection limits. Exposure to PCBs may affect reproduction, reduce resistance to infectious disease and cause pathological changes in the liver (Stone and Okoniewski 1988) but these effects are unlikely at the exposure levels observed. McLane and Hughes (1980) administered 3.0 μg/g (wet wt) dietary Aroclor 1248 to captive Screech-owls on a daily basis 8 weeks prior to egg-laying. The level administered, considerably more elevated than was observed in our samples, did not appear to adversely affect reproductive success. June beetles (*Phyllophaga* sp.) in the stomach of a poisoned Screech-owl contained 0.8 μg/g chlordane-related compounds (Okoniewski and Novesky 1993). Only one of our samples contained t-Chlordane (0.5 μg/kg), well below this value (Appendix 2.3).

2.6. CONCLUSION

Screech-owls are non-migratory and sedentary. Eastern Screech-owls in the vicinity of southern Quebec apple orchards are present during the entire pesticide application season, year after year. Secondary exposure to the organophosphorus insecticides and anticoagulant rodenticides currently applied, and to residues of previously used organochlorines, through consumption of small mammal prey, is possible.

We have shown that insecticide levels in small mammal prey can be analyzed on a whole-carcass basis and have demonstrated how these levels may be used to extrapolate exposure to the Screech-owl. The exposure level calculated for phosalone appears to be well below that of concern. If one wished to be conservative, incidents of mortality might be expected from the calculated exposure to azinphosmethyl. The calculated exposure level for phosmet may also warrant concern. This is based on an assessment of the full extent of LD_{50} values and contrasts with the EPA view that phosmet is non-toxic. The EPA view is based on the sensitivity of the Mallard, the least

sensitive of 4 other species for which toxicity data are available. If anything, our calculated insecticide exposure for the Screech-owl shows the weakness in placing too much emphasis on a paper risk assessment, derived from values selected at the upper end of possible inter-species sensitivity, when a more intensive field study may be needed.

Such a field study might prove challenging, however, because of the small number of owls at each location, as well as the difficulty of finding nests and capturing individuals. Though their occupation of orchards and dietary preferences render them suitable for assessment of exposure, Screech-owls may not be ideal study candidates, nor ideal monitors. They are often difficult to capture, and this poses a great deal of uncertainty as to whether data can be obtained within the detection interval post-insecticide application. Though the whereabouts of a Screech-owl can be ascertained by radio-tracking, capture within the post-application interval remains uncertain. Finally, the owl's small body size and secretive nature may hamper the search for and recovery of carcasses.

Observed anticoagulant residues were low and zinc phosphide is now almost exclusively relied upon for vole population control. We conclude that opportunities for Screech-owls to be exposed secondarily to anticoagulants in orchards of the study area are minimal. We note that in most orchards, zinc phosphide bait was observed on the ground, readily available to a variety of non-target organisms. Granivorous songbirds are more likely to be at risk of exposure.

Of the organochlorines analyzed in small mammal prey, levels of p,p'-DDE were most elevated. Observed residue levels were highest in *Blarina* samples. Estimated whole-body levels for *T. migratorius* were higher still. There can be no doubt that Screech-owls hunting in orchards of southern Quebec are secondarily exposed to DDE. A Screech-owl egg, collected from a nest box in a forest joining two orchards, may have been thinned by as much as 19.8%. Population declines have been observed when eggshell

thinning was maintained at 15.0 to 20.0% over an extended period of time (Wiemeyer *et al.* 1989).

The Screech-owl may be a suitable monitor of local organochlorine persistence in a field setting. Harris *et al.* (2000) maintained that, given the intensity of previous organochlorine applications, old orchard habitats probably represented some of the most contaminated environments in North America. Indeed, DDE levels observed in migrant songbirds are considerably less elevated than those observed in American Robins feeding in orchards.

Screech-owl exposure could be assessed non-invasively by collecting addled eggs from nest boxes after the young have fledged. Eggs could be collected as part of a long-term study, which would provide an opportunity to monitor eggshell thinning over time and better assess the stability of the population. Reference measurements could be taken from the eggs of Screech-owls captive-bred owls at local rehabilitation facilities. Pellets and prey remains could also be collected over a longer period of time to ensure a more representative estimate of biomass proportion of robins in the owl's diet. It would also be appropriate to analyze whole-carcass levels of DDE in robins from orchards of the study area so that the exposure risk to Screech-owls would be based on values specific to the area.

It might also be appropriate to monitor Screech-owl exposure to organochlorines, particularly DDE, and to assess local persistence of these compounds in a clinical setting. Chronic exposure to organochlorines may affect cognitive responses and behaviour and predispose Screech-owls to injuries such as car strikes. Owls admitted to local rehabilitation from the vicinity of orchards could be sampled to address the role that exposure to DDE might have played in admission, and to address effects on the population of southern Quebec. Levels of metals, PCBs and the other contaminants analyzed do not appear sufficiently elevated to cause concern over a short or long-term duration.

Habitat loss poses a distinct threat to the Screech-owl population in southern Quebec; the presence of owls depends on the availability of suitable

habitat in the area. In Saint-Hilaire especially, forested regions are rapidly succumbing to development pressure (M.A- Guertin, pers. comm.). Screech-owls require sufficient food resources, cavities and shelter, not always available in housing developments. Orchards are areas of intensive pesticide use. However, from the perspective of a Screech-owl amid dwindling habitat, orchards satisfy all the essential requirements. Thus, the presence of orchards may help to ensure the presence of a Screech-owl population in southern Quebec. However, the stability of that population remains in question.

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Appendix 2.1a

Timing of pellet and prey remains recovery from nest boxes of Orchards B and K in relation to Screech-owl annual cycle

Life stage spanned	Date spanned by inspections	Small mammal prey consumed	Small mammal biomass (g)	Avian prey consumed	Avian biomass (g)
Fledging-Dispersal	June 7 to August 21, 2001	**N/A**	**N/A**	**Mourning Dove** *Z. macroura*	**135.0**
Dispersal-Nesting	December 17, 2002 To April 17, 2003	**UID Vole**	**32.5**	**Hairy Woodpecker** *P. villosus* **American Robin** *T. migratorius*	**146.0**
Dispersal-Brooding	November 17 to May 27	**Short-tailed shrew** *B. brevicauda* **UID Vole**	**52.6**	**N/A**	**N/A**
Dispersal	August 15 to December 17	**Short-tailed shrew** *B. brevicauda* **UID Vole** (n = 3)	**117.6**	**N/A**	**N/A**

N/A = not found during inspection period
UID Vole = *M. pennsylvanicus* or *C. gapperi*
n = 1 unless otherwise indicated

Appendix 2.1b

Timing of pellet and prey remains recovery from nest boxes of Orchards D and F in relation to Screech-owl annual cycle

Life stage spanned	Date spanned by inspections	Small mammal prey consumed	Small mammal biomass (g)	Avian prey consumed	Avian biomass (g)
Dispersal – Nesting	December 17, 2002 to April 17, 2003	**UID Vole** **Peromyscus sp. or *Zapodidae***	**50.5**	**Blue Jay** *Cyanocitta cristata* **Mourning Dove** *Zenaida macroura*	**225.0**
Dispersal – Brooding	December 20, 2001 to May 23, 2002	**Sorex** sp. **UID Vole** (n = 3)	**102.4**	**Black-capped Chickadee** *Parus atricapillus* **Eastern Phoebe** *Sayornis phoebe*	**32.0**
Dispersal – Fledging	December 3, 2000 to June 6, 2001	**UID Vole**	**32.5**	**Mourning Dove** *Z. macroura*	**135.0**
Fledging – Dispersal	June 7, 2001 to August 22, 2001	**Sorex sp.** (n = 4) **UID Vole** (n = 2)	**84.6**	**Brown-headed Cowbird** *Molothrus ater* **Brown Thrasher** *Toxostoma rufum* **Mourning Dove** *Z. macroura* **UID Thrush** *Turdidae* (n = 2)	**340.0**

n=1 unless otherwise indicated

Appendix 2.1c

Timing of pellet and prey remains recovery from campground control site in relation to Screech-owl annual cycle

Life stage spanned	Date spanned by inspections	Small mammal prey consumed	Small mammal biomass (g)	Avian prey consumed	Avian biomass (g)
Dispersal-Nesting +	December 17, 2002 to April 12, 2003	**Short-tailed shrew** *Blarina brevicauda* **UID Vole** (n =9)	313.0	**N/A**	**N/A**
Breeding-Dispersal	February 19 to June 27, 2001	**N/A**	**N/A**	**Blue Jay** *Cyanocitta cristata* **Cedar Waxwing** *Bombycilla cedrorum* **Common Grackle** *Quiscalus quiscula* **Hairy Woodpecker** *Picoides villosus* **Mourning Dove** *Z. macroura* (n =4) **Rock Dove** *Columbia livia* (n =3)	1,748.0
Brooding-Dispersal	May 23 to June 15, 2002	**N/A**	**N/A**	**American Goldfinch** *Carduellis tristis*	14.0
Fledging – Dispersal	June 27 to August 24, 2001	**N/A**	**N/A**	**Mourning Dove** *Z. macroura*	135.0
Dispersal ++	August 15 To December 17*	**Short-tailed shrew** *B. brevicauda*	20.1	**Northern Flicker** *Colaptus auratus*	120.0
Dispersal-Brooding	December 2, 2001 to May 24, 2002	**Peromyscus sp. or *Zapodidae*** **UID Vole**	50.5	**Cedar Waxwing** *B. cedrorum* **UID Flycatcher** *Tyrannidae*	75.0

N/A = not found during inspection period
n = 1 unless otherwise indicated
UID Vole = *M. pennsylvanicus* or *C. gapperi*, but given habitat, assume C. gapperi
+ unidentified fish scales and bones also found, ++cached frog also found

Appendix 2.1d

Timing of pellet and prey remains recovery from Mont-Saint-Hilaire Biosphere Reserve control site in relation to Screech-owl annual cycle

Life stage spanned	Months spanned	Small mammal prey consumed	Small mammal biomass (g)	Avian prey	Avian biomass (g)
Dispersal	August 15 To December 18, 2002	**Peromyscus sp. or *Zapodidae*** (n = 4) **UID Vole** (n = 9)	364.5	**N/A**	**N/A**
Dispersal-Nesting	December 18, 2002 To April 17, 2003	**Peromyscus sp. or *Zapodidae*** (n = 6) **Short-tailed shrew** *Blarina brevicauda* **Sorex sp.** **UID Vole** (n = 32)	1,173	**Blue Jay** *Cyanocitta cristata* **European Starling** *Sturnus vulgaris* **Mourning Dove** *Zenaida macroura* **Northern Saw-whet Owl** *Aegolius acadicus*	393
Dispersal-Brooding	November 1, 2001 to May 28, 2002	**Peromyscus sp. or *Zapodidae*** **Short-tailed shrew** *B. brevicauda* **UID Vole** (n = 5)	201	**N/A**	**N/A**

N/A = not found during inspection period
n = 1 unless otherwise indicated
UID Vole = *M. pennsylvanicus* or *C. gapperi*

Appendix 2.2a

Detectable trace metal ranges in small mammal pools from orchards of Saint-Hilaire and Rougemont 2001 (in mg/kg, wet wt)

Trace Element	Detection Limit (wet wt)	Range (wet wt)	Range (dry wt)
Strontium (Sr)	1.50	4.70-16.00	17.98 – 65.90
Aluminum (Al)	2.50	6.40 - 96.0	24.48 – 332.87
Barium (Ba)	1.50	2.00 – 7.90	7.35 – 32.54
Cadmium (Cd)	0.08	< 0.08 – 0.36	BDL – 1.38
Chromium (Cr)	0.50	< 0.50 – 0.80	BDL – 3.07
Copper (Cu)	0.50	1.80 – 4.70	7.09 – 17.98
Iron (Fe)	5.00	65.00 – 240.00	256.11 – 766.53
Lead (Pb)	0.18	< 0.18 – 1.40	BDL – 4.85
Manganese (Mn)	0.50	2.10 – 11.00	8.27 – 35.13
Selenium (Se)	0.50	<0.50 – 0.70	BDL – 2.30
Zinc (Zn)	0.50	23.00 – 33.00	83.22 – 122.42

BDL: below detection limit

Appendix 2.2b

Detectable traces of Hg in small mammals pools from orchards of Saint-Hilaire and Rougemont 2001 (µg/g dry wt)

Orchard	Species	[Hg]	% Moisture
F	MV	BDL	71.0
	MS	0.0494	67.1
B	MV	BDL	73.6
	Per	BDL	74.0
K	MV	BDL	72.7
	STS	0.209	70.2
	Per	BDL	65.8
H	MV	BDL	70.8
	STS	0.188	69.2
G	STS	0.187	65.5

BDL: below detection limit
MV: *M. pennsylvanicus*
MS: *Sorex* sp.
Per: *Peromyscus* sp.
STS: *B. brevicauda*

Appendix 2.3

PCBs and persistent contaminants detected in small mammal pools from orchards in Saint-Hilaire and Rougemont 2001 (in µg/kg wet weight)

Contaminant	STS	MS	MV	Per	JM
PCB # 118 (n = 12)	0.2 – 1.1 (n = 7)	ND	0.1 - 0.2 (n = 2)	0.2 – 0.3 (n = 2)	0.1 (n = 1)
PCB # 138 (n = 23)	0.5 – 1.8 (n = 8)	0.6 (n = 1)	0.1 – 0.6 (n = 8)	0.2 – 0.5 (n = 4)	0.1 (n = 2)
PCB # 153 (n = 23)	0.6 – 2.3 (n = 8)	0.8 (n = 1)	0.1 – 0.5 (n = 8)	1.1– 0.7 (n = 4)	0.1 – 0.2 (n = 2)
PCB # 180 (n = 22)	0.1 – 1.2 (n = 7)	0.7 (n = 1)	0.1 – 1.3 (n = 8)	0.1– 0.4 (n = 4)	0.1 – 0.4 (n = 3)
PCB # 187 (n = 10)	0.1 – 0.7 (n = 7)	ND	0.3 (n = 1)	0.2 – 0.3 (n = 2)	ND
Sum PCB (n = 24)	1.8 – 14.9 (n = 8)	2.0 (n = 1)	0.1 – 14.5 (n = 8)	0.6 – 2.2 (n = 4)	0.4 – 0.8 (n = 3)
TCB (n = 12)	0.1 (n = 4)	ND	0.1 (n = 3)	0.1 (n = 2)	0.1 (n = 3)
QCB (n = 3)	0.1 (n = 1)	ND	0.2 (n = 1)	ND	0.2 (n = 1)
HCB (n = 21)	0.1 - 0.2 (n = 7)	0.1 (n = 1)	0.1 - 0.3 (n = 8)	0.2 (n = 3)	0.1 - 0.2 (n = 2)
t-Nonachlor (n = 10)	0.4 – 1.2 (n = 8)	0.3 (n = 1)	ND	0.3 (n = 1)	ND
t-Chlordane (n = 1)	0.5 (n = 1)	ND	ND	ND	ND
Dieldrin (n = 10)	5.1 – 110.1 (n = 6)	2.2 (n = 1)	1.4 – 1.8 (n = 2)	ND	1.8 (n = 1)

(n= no. samples detected in)
ND: Not Detected
TCB: 1,2,4,5-Tetrachlorobenzene
QCB: Pentachlorobenzene
HCB: Hexachlorobenzene
STS: Short-tailed Shrew (*B. brevicauda*)
MS: Masked Shrew (*S. cinereus*)
MV: Meadow Vole (*M. pennsylvanicus*)
Per: *Peromyscus* sp.
JM: *Zapodidae*

CONNECTING STATEMENT

Chapter two established that apple orchards are areas of intensive pesticide usage. Numerous studies documenting topical or secondary exposure of raptors to organophosphorus insecticides, anticoagulant rodenticides and organochlorines (and heavy metals to a much lesser extent) in orchards were discussed. The mode of action of these compounds and mechanisms of secondary exposure were also described. The lack of studies conducted on non-migratory, sedentary raptors was highlighted and the reality of the multiple pesticide exposure scenario was argued using the example of the Screech-owl. Residue levels of each class of compound were measured in the owls' small mammal prey base of orchards of southern Quebec. Risk of secondary exposure and ensuing adverse effects were evaluated by comparing the preponderance of each small mammal species within the owls' diet to the residue levels observed in each species.

Chapter three offers a broader perspective on the potential exposure of the Screech-owl and its suitability as a monitor of exposure. Case files of Screech-owls admitted to various rehabilitation facilities were analyzed for evidence showing that exposure to one of the three classes of pesticide contributed directly or indirectly to admissions. The agricultural status of the location from which the owl originated was also of interest. The breadth of supporting information used in the analyses, such as pesticide use inventories and population census, and biases inherent within this type of data are discussed. Factors that can enhance or impede the availability of toxicological information used to diagnostically confirm or eliminate pesticide exposure as a cause of injury or admission are also reviewed.

CHAPTER THREE

An evaluation of Eastern Screech-owl (*Otus asio*) admissions to rehabilitation facilities: does pesticide exposure play a role?

3.1. INTRODUCTION

This chapter addresses potential exposure of Eastern Screech-owls (*Otus asio*) to pesticides used in agricultural areas from a clinical perspective. An implicit objective was to assess whether the Screech-owl can serve as a good monitor of pesticide exposure at rehabilitation facilities that receive this species. Over 950 Screech-owl case files spanning 1998 to 2001 admissions were obtained from eight rehabilitation facilities. The question that directed the analysis of these case files was whether pesticide exposure was an underlying or contributing cause of admissions. Analysis is restricted to insecticide exposure because this is the only class of pesticides for which relevant information was available. The generalized agricultural and agricultural pesticide information used in the analyses may have resulted in an ambiguous interpretation of results. The feasibility of increasing sampling efforts at rehabilitation facilities to more conclusively identify or eliminate pesticide exposure as a contributing factor in Screech-owl admissions was considered. Finally, factors that could impede tissue sampling at rehabilitation facilities were briefly addressed.

3.2. WHY IS PESTICIDE EXPOSURE OF INTEREST?

Birds of prey, including Screech-owls, are known to occupy agricultural areas. Numerous studies have documented their exposure to a variety of pesticides in agricultural areas and have outlined subsequent adverse effects (Henny et al. 1987, Hegdal and Colvin 1988, Blus et al. 1989, Mineau et al. 1999). There is anecdotal evidence (reviewed in Mineau et al. 1999) that exposure to at least one class of pesticides (i.e. cholinesterase-inhibiting insecticides) may predispose birds to mishaps such as hitting stationary objects and entanglement in fences. As their name implies, cholinesterase-

inhibiting insecticides, henceforth referred to simply as insecticides, depress the level of the cholinesterase enzymes within the body. Acetylcholinesterase, one form of cholinesterase, is necessary for timely breakdown of the neuro-messenger acetylcholine. When acetylcholinesterase levels become depressed, acetylcholine accumulates and the nervous system becomes overstimulated, often shutting down completely (Mineau and Tucker 2002a,b). Fatalities are usually a result of respiratory failure (Porter 1993).

Insecticides are used in numerous agricultural settings, including orchards. Exposure may be primary (flying through a spray, or, more likely, perching on contaminated surfaces) or secondary (consuming contaminated prey). A second group of pesticides of concern are rodenticides. These are applied in orchards to reduce tree trunk damage by voles and other small mammals. Anticoagulant rodenticides are of particular interest because secondary exposure can arise through ingestion of target or non-target species. Such exposure results in hemorrhaging, which can be fatal. Though theoretically no longer in use, organochlorines that persist in the environment have been linked to eggshell thinning and avian mortality (McLane and Hall 1972, Cooke et al. 1982, Okoniewski and Novesky 1993). Historical use of DDT was especially intensive in orchards and present DDE levels are high in earthworms and in the wildlife that consume them (Dimond and Sherburne 1969, Bailey et al. 1974, Blus et al. 1987).

In contrast to many species that frequent agricultural areas, the Screech-owl is non-migratory and sedentary. Thus, owls that inhabit agricultural areas are present during the entire application season and may therefore be exposed to a variety of pesticides on a continual basis. The Screech-owl consumes birds, small mammals, reptiles and fish (Gehlbach 1994), all potentially exposed organisms. Once exposed, organisms often adopt conspicuous behaviours or are lethargic (Benke and Murphy 1974, Stehn 1976), which may promote or facilitate their capture by the opportunistic Screech-owl. To assess exposure, individuals can be captured in the field after an insecticide application and a blood sample taken.

However, there is a short detection interval (about 48 hours) and Screech-owls are not always readily captured. Tissue samples can also be taken when owls are admitted to rehabilitation facilities and the brain can be removed from deceased owls for analysis. Difficulties associated with these procedures are discussed in the last section.

3.3. OBTAINING, PROCESSING AND INTERPRETING THE CASE FILES

Case files are generally consulted to strengthen or supplement diagnosis once exposure has been confirmed. Several studies have examined case files in this manner (Porter 1993, Mineau et al. 1999). In 2000, a call was put out on an ornithological listserv describing the project and asking for Screech-owl case files spanning 1998 to 2001 admissions. Seven U.S. and one Canadian facility collaborated, and during a two-year period over 950 case files were obtained. These files detailed the owls' age, date of admission, location, circumstance and outcome, as well as results from any analyses conducted. This information was adjusted to reflect the date of initial receipt from the wild and to standardize ages, circumstances and outcomes across facilities. Permanently captive owls admitted for a physical examination were excluded from the data.

Unadjusted Screech-owl admission numbers were used to estimate total volume of birds of prey and proportion of Screech-owls admitted to a given facility. This is because it was not possible to adjust the other bird of prey records in a similar manner. If information was missing from the files, the primary and associated facilities were contacted for clarification. The question that directed our scrutiny of the case files was whether pesticide exposure could have been a direct or underlying cause of Screech-owl admissions. We were also interested in investigating factors inherent in the owls' life cycle and in land use and demographic patterns that could influence admissions.

The number of Screech-owls admitted relative to the total number of birds of prey was examined for all facilities. Variables examined in the case files were age, date of admission (time during life cycle), circumstance and

outcome. Information on agriculture and agricultural pesticide use on a county basis was obtained from the Census of Agriculture website (www.nass.usda.gov). Information was available for insecticides only and the most recent data that could be obtained were for 1997. Theoretically, persistent organochlorines are no longer in use and rodenticide use is of minimal economic importance, hence they were not covered by the survey. Population density and size of county were obtained from the U.S. Census Bureau website (www.census.gov); the most recent data were from 2000. Efforts to obtain information on the density of roadways by county are ongoing. The independent variables examined were area of county admitted from, distance from county center to facility, county human population density, proportion of owls admitted from county, county proportion of land in agriculture and in orchards, and county proportion of land in crop or orchard sprayed with insecticides. The dependent variable was the number of Screech-owls admitted per county, corrected for county area.

3.4. RESULTS AND DISCUSSION

3.4.1. Proportion of Screech-owl to bird of prey admissions

The proportion of Screech-owls admitted relative to the total number of birds of prey varies considerably between facilities but shows little fluctuation within a facility from year to year. Table 3.1 lists the total proportion of Screech-owl to total bird of prey admissions by size of facility, for 1998 to 2001. Total admissions of Screech-owls and birds of prey are given in Appendix 1.

Table 3.2 shows total admissions and proportions by year. The Raptor Center (TRC) at the University of Minnesota in St. Paul and the Clinique des oiseaux de proie (COP) in St.-Hyacinthe, Quebec, Canada, are among the largest facilities but have the smallest proportion of Screech-owls admitted. Both are located on the outermost fringes of the Screech-owl's northern limit. The COP serves the entire province of Quebec (594,857 mi^2, or 1,540,680 km^2 an enormous area relative to other facilities), and receives about 25

species of birds of prey every year. TRC receives a similar caseload (e.g. 31 species in 2001). By contrast, the River Raisin Raptor Center (RR), in Manchester, Michigan, is the smallest facility and has one of the highest admission proportions probably because it admits and raises numerous orphans. This facility sees approximately 7-10 species in a given year.

Table 3.1a
Total admission proportion of Screech-owls (EASO) to birds of prey (BOP) (1998-2001)

Facility	Total no. BOP	Total no. EASO	Total admission Proportion (%)
CRC	2539	332	13
TRC*	2287	57	3
WCtrVA	1152	248	22
COP	1076	45	4
Tri-State	839	105	13
Tufts	561	61	11
SWFI	480	136	28
RR	134	36	27

*data available from 1998 to 2000 only, but this is actually the largest facility

Table 3.1b
Proportion of Screech-owl (EASO) to birds of prey (BOP) admitted by year (1998-2001)

Facility	EASO 1998	EASO/ BOP 1998 (%)	EASO 1999	EASO/ BOP 1999 (%)	EASO 2000	EASO/ BOP 2000 (%)	EASO 2001	EASO/ BOP 2001 (%)
CRC	77	13	67	12	92	14	96	13
TRC	25	4	15	2	17	2	15	2
WCtrVA	65	27	59	19	65	23	59	18
COP	9	4	9	4	18	6	9	3
Tri-State	18	11	33	17	22	10	32	13
Tufts	12	12	18	13	17	11	14	8
SWFI	29	30	33	27	44	30	30	26
RR	8	33	5	14	7	22	12	29

CRC: Carolina Raptor Center (North and South Carolina)
TRC: The Raptor Center (Minnesota)
WCtrVA: The Wildlife Center of Virginia (Virginia)
COP: Clinique des oiseaux de proie (Quebec)
Tri-State: Tri-State Bird Rescue and Research, Inc. (Delaware, Pennsylvania, Maryland)
Tufts: Tufts University Wildlife Clinic (Massachusetts)
SWFI: Conservancy Wildlife Center of Southwest Florida (Florida)
RR: River Raisin Raptor Center (Michigan)

Young can account for a high number of admissions to a facility, as discussed in the following sections. Some facilities are not equipped or mandated to raise young. Often, young are diverted to smaller, supporting facilities (such as RR) thus do not show up in the larger facilities' records. Thus, differences in admission proportions may arise from a combination of factors including facility location relative to the Screech-owl's range, area served by the facility, relative abundance of other species and mandate or specialization of facility. Land use and demographics may also influence admission proportions as discussed in a later section.

3.4.2. Admission circumstances

Some circumstances are age-related and seasonal. For example, "fell from nest" is associated with nestlings and fledglings. In colder climates, owls may seek refuge in chimneys in late autumn and winter. However, car strikes occur throughout the year. It was estimated that car strikes (and road-related circumstances) form the majority of admission circumstances followed by age-related circumstances. This is the case, as shown in Table 3.3.

Table 3.2
Proportion (%) of known* Screech-owl admission circumstances (1998- 2001)**

Facility	Rd	HBC	PA	FFN/ Orp	HD	Other	Chimney/ Confinement
CRC	28	39	1	20	6	3	2
WCtrVA	37	30	5	12	2	11	4
COP	37	10	7	0	3	33	10
Tri-State	31	20	4	19	19	4	4
Tufts	44	31	2	4	10	2	6
SWFI	19	22	12	8	14	22	3
RR	6	10	3	74	3	3	0

*Owls found on the ground or under unknown circumstances were omitted from this analysis and proportions were adjusted accordingly.
**TRC excluded due to largely unavailable data

Rd: found on middle or side of road
HBC: hit by vehicle or flew into vehicle
PA: predator attack (e.g. cat) or mobbing (e.g. by crows)
FFN/Orp: fell from nest or orphaned, nestling or juvenile brought in to be raised
HD: habitat destruction, tree cut down or blown down during storm
Chimney/confinement: found in house or garage
Other: see Appendix 2 for detailed list

Facility specialization must be considered in this context to avoid the erroneous conclusion that some circumstances are more problematic or frequent in certain areas. For example, River Raisin received the highest proportion of young that were orphaned or fell from the nest, but one of the facility's roles is to raise young. This is reflected in Figure 3.1, which shows age proportion of admissions by facility.

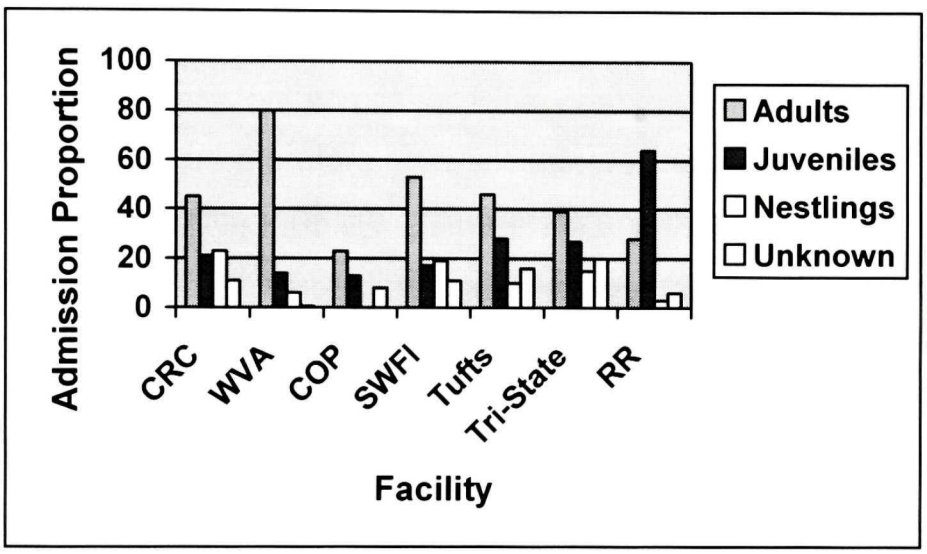

Figure 3.1
Total age proportion of Screech-owls admitted by facility (1998 – 2001)

CRC: Carolina Raptor Center (North and South Carolina)
WVA: The Wildlife Center of Virginia (Virginia)
COP: Clinique des oiseaux de proie (Quebec)
SWFI: Conservancy Wildlife Center of Southwest Florida (Florida)
Tufts: Tufts University Wildlife Clinic (Massachusetts)
Tri-State: Tri-State Bird Rescue and Research, Inc. (Delaware, Pennsylvania, Maryland)
RR: River Raisin Raptor Center (Michigan)

Differences in proportions are also influenced by the way facilities assign admission circumstances. Occasionally, circumstances are not known or are rather ambiguous. Without concrete knowledge of what occurred prior to admission, more conservative facilities may record circumstances as unknown. Other facilities might assign a circumstance based on experience, find location (*e.g.* side of road or beneath windowsill) and injuries observed. We chose to be conservative and grouped birds recorded as unknown or as found on the ground. Owls found on the side or in the middle of the road were

grouped as road-related. Though ambiguous, we would like to draw attention to this category. Some birds exposed to insecticides develop paralysis while perching and fall to the ground (Shlosberg 1976). Very few owls (2-3 of the 950) were actually seen falling from a tree by the finder before they were picked up from the ground.

3.4.3. Life cycle stage and activity level

We assigned five life cycle stages corresponding to timing of admissions and to changes in mobility and activity of individuals. These stages, consistent across facilities, enabled us to standardize admission times and are therefore more meaningful than calendar dates. However, we emphasize that they are approximate and there is overlap between stages. We considered the *territorial* stage to be from January to February. During this period adults are restricted to a defined territory. Though time is spent defending the territory and hunting, movement from the territory is minimal. The *nesting* stage was defined as March and April. This is when the nest site is selected and eggs are laid and incubated. At this time the male is the sole food provisioner and he remains in proximity of the nest. *Brooding* spans April to June. Most of the young are still in the nest at this point, but there is no longer room for the female. Thus, both parents now actively feed and protect the young. The *fledging* stage spans June to mid-August. Young leave the nest and begin to fly. At this time both the young and adults are active and mobile. *Dispersal* spans mid-August to December. At their parents' prompting, young drift from the natal territory in search of suitable habitat. Their increased mobility may also expose them to more hazards, but it is difficult to distinguish adults and juveniles from one another at this time.

Figure 3.2 shows total number of admissions by age and life stage. The number of admissions for adults, young of the year and birds of undetermined age does indeed peak during the fledging and dispersal stages. There are more opportunities for mishap and for discovery by humans, and young inept fliers may be easier to capture. Pesticide application times often

correspond with fledging and dispersal, confounding our efforts to identify exposure as an underlying or contributing cause of admission. An increase in the number of individuals during pesticide application periods does not imply a causal relationship between pesticides and admissions.

3.4.4. Outcomes

Despite being admitted with extensive injuries (generally car strikes and road-related), Screech-owls are hardy and exhibit a high recovery rate. A successful release outcome is also likely for the many orphaned or kidnapped (but otherwise healthy) young admitted to most facilities every year (see Figure 3.1). Release is the majority outcome, as shown in Table 3.3. Regardless of geographical location or facility size (with the exception of RR), there is little fluctuation in the proportion of released and deceased individuals. River Raisin shows very high release proportions, but again this is expected because the majority of Screech-owl admissions are orphans *intended* for release.

Table 3.3
Proportion (%) of total Screech-owl (EASO) outcomes (1998-2001)

Facility	Released EASOs	Deceased EASOs (DOA, DI, EU)	Non-releasable Pending Unknown
CRC	63	29	8
TRC*	61	30	9
WCtrVA	53	43	4
COP	45	48	7
Tri-State	63	30	7
Tufts	43	38	19
SWFI	60	40	N/A
RR	81	11	8

*Data available for 1998 to 2000 only

CRC: Carolina Raptor Center (North and South Carolina)
TRC: The Raptor Center (Minnesota)
WCtrVA: The Wildlife Center of Virginia (Virginia)
COP: Clinique des oiseaux de proie (Quebec)
Tri-State: Tri-State Bird Rescue and Research, Inc. (Delaware, Pennsylvania, Maryland)
Tufts: Tufts University Wildlife Clinic (Massachusetts)
SWFI: Conservancy Wildlife Center of Southwest Florida (Florida)
RR: River Raisin Raptor Center (Michigan)

The COP and Tufts showed the lowest release proportions. Both are veterinary teaching institutions and lower proportions may be partly attributable to handling more severe cases. In fact, both facilities have among the highest proportions of road-related and car strike admissions. However, it is more probable that the lower release proportion is related to the low to nonexistent proportion of young admitted (see Table 3.3 and Figure 3.2). When young are transferred or diverted to supporting facilities as soon as possible, potentially releasable individuals do not appear in the analysis. Release proportions decrease accordingly.

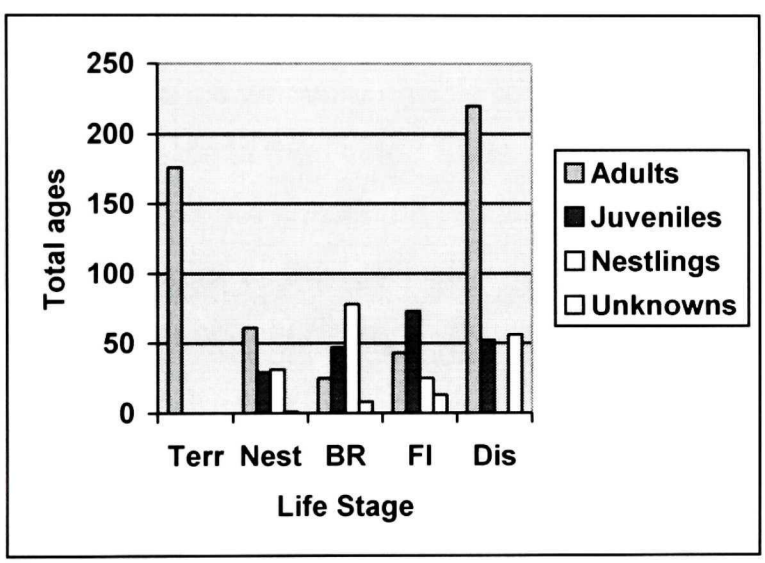

Figure 3.2
Total Screech-owl age and life stage at admission across facilities
Terr: territorial
Nest: nesting
BR: brooding
FI: fledging
Dis: dispersal

3.5. ANALYSIS OF LAND USE PATTERNS AND DEMOGRAPHICS OF COUNTIES SCREECH-OWLS WERE ADMITTED FROM

In an attempt to explain among-county differences in the number of owls admitted, we examined several independent variables in a multiple regression analysis. The number of Screech-owls admitted from counties and adjusted for county area (no./1000 mi^2 or 2,590 km^2) served as our dependent variable. We were unable to find sufficient owl density information

in Christmas Bird Counts (CBC) or Breeding Bird Surveys (BBS) to correct admission numbers for owl density. Independent variables were facility size (as measured by the total number of raptors admitted from 1998 to 2001), distance from county center to facility (since we might expect rehabilitation facilities to more actively serve nearby counties), human population density (as a reflection of both probability of discovery and road traffic), proportion of the county area in cropland, the proportion in orchards, and the proportion of the combined area of cropland and orchards, which is sprayed with insecticides. The proportion of land sprayed is a measure of total insecticide use without regard to toxicity or risk of exposure. For these analyses, we excluded data from TRC because it was largely incomplete, and from the COP because we were unable to obtain Canadian data strictly comparable to that available in the United States.

Table 3.4 shows the results of our analyses. The analysis was first carried out for all the facilities (spanning 130 counties). In this analysis, distance of county center to facility and human population density were found to be very highly significant ($p = 0.0004$ and $p = 0.001$, respectively). No other factor added to our understanding of admission numbers by county in this analysis. Facility size did not explain admissions on a county by county level. This may be because we excluded from the analysis two of the largest facilities, serving the largest areas, and on the outermost limits of the Screech-owl's range. However, facilities in the analysis varied considerably in size. The two significant variables only accounted for about 19% of the total variance in admission numbers, indicating that other unexplained variables (or chance) were most important. We plan to repeat the analyses once road density information is obtained.

Next, individual analyses were carried out for each of the two largest facilities. For the CRC (62 counties served), distance to facility and proportion of county area in cropland were significant ($p = 0.036$ and $p = 0.046$, respectively), whereas human population density did not quite reach significance ($p = 0.096$). For the WCtrVa (44 counties served), distance to

facility center was found to be highly significant (p = 0.008) while population density just attained significance (p = 0.047). All analyses were repeated with known adults only, but results were similar and are not reported here. However, because of the difficulty in distinguishing age groups in the autumn we may have under- or overestimated the number of adults in our analysis.

Table 3.4
Results of multiple regression analysis

Analysis	Distance county center to facility	Human population density	Proportion of county land in crop
All Counties (n = 130)	p = 0.0004	p = 0.001	NSS
CRC (n = 62)	p = 0.036	p = 0.096	P = 0.046
WCtrVA (n = 44)	p = 0.008	p = 0.047	NSS

CRC: Carolina Rehabilitation Center
WCtrVA: Wildlife Center of Virginia
NSS: Not statistically significant

These results suggest that the likelihood of receiving an owl from a county decreases with distance from a facility, which was expected. Facilities also seem more likely to receive owls from counties with higher population densities. This may be because injured owls are more likely to be reported where human population density is higher. Alternatively, more injuries may be sustained as a result of increased human activities (*e.g.* more road traffic) so road density may be a better predictor of admission numbers. After proximity to facility, the proportion of land in crop was a significant factor in explaining admissions to the Carolina facility. However, this may reflect suitable habitat rather than a causal relationship between agricultural use of land and need for rehabilitation. At this scale of analysis, we found no visible effect of pesticide use as measured by the proportion of cropland or orchard sprayed with all insecticides combined.

It is possible that insecticide use does not predispose Screech-owls to circumstances necessitating rehabilitation. Alternatively, the number of reported cases might not reflect the number of actual cases. Owls exposed to insecticides or other pesticides may perish in fields or other remote locations and not be reported to rehabilitation facilities. Analyses of this scale are biased towards cases involving human contact, especially car strikes (Glue 1971). Finally, even though there is evidence that many insecticides currently registered cause mortality and, presumably, sub-lethal intoxication in birds (Mineau and Tucker 2002a,b), products vary in their toxicity and use patterns. This may be completely obscured in an analysis (such as this one) that only looks at total insecticide use.

The two primary causes of admission were road- and age-related. In both these categories, birds are highly visible (Weir 1971) and readily captured. It has been suggested that pesticide exposure predisposes birds of prey to incidents such as car strikes (Blus 1996). Indeed, cholinesterase-inhibiting insecticides have been shown to cause visual and motor impairment (Porter 1993). However, street and road embankments are edges and Screech-owls favour edge habitat (Gehlbach 1994). Many birds of prey also hunt by light. Roads and roadsides may simply be perceived by owls as suitable habitat (Glue 1971). An owl seeing its prey in a vehicle's headlights may ignore the approaching vehicle. We may therefore not need to invoke sublethal intoxication and compromised reflexes to explain the high number of car strikes sustained by Screech-owls. Without concrete toxicological evidence, we cannot link any of the examined cases to pesticide exposure. Examining these case files has provided us with a greater understanding of factors involved in Screech-owl admissions and leads to other questions. Without proper biochemical or toxicological screening, we do not think that the extent of reported Screech-owl mortality or debilitation can be a suitable indicator of exposure to insecticide, nor possibly to other pesticides.

3.6. SAMPLING PROTOCOLS AT REHABILITATION FACILITIES: A PARTNERSHIP AND LEARNING OPPORTUNITY

To assess whether pesticide exposure is a contributing factor in admissions, monitoring efforts could be increased at rehabilitation facilities. A trial tissue sampling protocol was conducted at the COP. In this section we discuss complications that arose during the implementation of this protocol. We contrast toxicological considerations with concerns espoused by rehabilitation facilities. Here, we focus on Screech-owls, but our discussion applies to other species as well.

3.6.1. Administration and organization

The general aspects of screening individuals for pesticide exposure were recently discussed in two back-to-back issues of the Journal of Wildlife Rehabilitation (Mineau and Tucker 2002a,b). The expectations and contributions of the rehabilitation facility and agency initiating the sampling must be clearly established at the onset. Prior to implementing a protocol, facility personnel may not be familiar with the associated sampling, preparation, storage and shipping methods. Training may be required. Appropriate collection equipment must be purchased and accurate records of samples taken need to be kept. Several agencies or researchers may simultaneously express interest in the same species or tissues and these tissues need to be allocated appropriately. All these steps require time, money and personnel, admittedly scarce resources in the rehabilitation milieu. When a facility is understaffed, the time required to implement a monitoring program may be perceived to compete with more fundamental forms of care, or even with bestowing additional attention to admittees.

3.6.2. Condition at admission and detection

A cholinesterase assay can be run with a single drop of blood – about 20 μl. However, analyzing for specific cholinesterase-inhibitors or for pesticides which are not cholinesterase-inhibitors is likely to require a larger

volume of blood (Mineau and Tucker 2002a). A maximal volume corresponding to approximately 1% of the total body weight can be drawn (for instance, 1 ml for a bird of 100 g). A rough rule of thumb for detecting exposure through a cholinesterase assay is about 48 hours. An owl brought to a rehabilitation facility could have been in the wild for several days prior to admission. Furthermore, it may be 2 to 4 days before it is physically possible, or in the owl's best interest, to draw blood. At the time of admission, an efficient initial examination is favoured so the owl can be stabilized as soon as possible. Blood sampling lengthens this examination and provides another source of stress. Screech-owls have small veins which makes sampling more difficult and can increase handling time. If the owl is emaciated, dehydrated or in shock, the veins will constrict and drawing blood is difficult without compromising the bird's well-being.

3.7. CONCLUSION

Over 950 case files from 8 facilities, spanning admissions from 1998 to 2001 were examined. Facilities serving the largest areas, and located on the fringes of the owls' geographic range, showed the lowest admitted proportion of Screech-owls relative to other birds of prey. Road- and car-related circumstances followed by age-related circumstances formed the highest proportion of admissions. Birds found on the ground are of interest because insecticide exposure may induce paralysis; a very small number of owls were observed falling from trees onto the ground before admission to a facility. Admissions were also examined in the context of life stage and activity level. The number of adult and especially juvenile admissions peaked during fledging and dispersal. The time of fledging frequently coincides with time of pesticide application, but an increase in individuals at this time does not imply a correlation with exposure. Rather, there are many easily discovered and readily captured young. Releases formed the majority of outcomes. Facilities that took in and raised healthy young had correspondingly higher release proportions than other facilities. A multiple regression analysis revealed that

distance of county (center) to facility and human population density were very highly significant. A separate analysis was also conducted for the two largest facilities. In the case of the CRC, distance from county center to facility and proportion of county in cropland was found to be significant. However, for the WCtrVA, only distance was found to be significant.

At this scale of analysis, we were unable to assign pesticide exposure as a contributing or underlying cause of Screech-owl admissions. One way to reduce ambiguity is to increase sampling efforts at rehabilitation facilities. Logistical and physiological or ethical factors beyond the control of regulatory agencies may threaten a potential partnership. Facilities often operate under tremendous financial constraints. Of necessity, sampling efforts may be accorded a lower priority. There is also a clear difference in the mandate of a rehabilitation facility and in that of an agency intent on tracking the impacts of pesticides released in the environment. To the facility, admittees are perceived as patients and individuals but to agencies, they are samples from a population. Ultimately, both parties seek to identify problems and solutions that will benefit individuals and populations. By demonstrating the difficulty in reaching conclusions about pesticide exposure, and acknowledging some of the complications that arise during sampling, we hope to encourage dialogue between rehabilitation facilities, regulatory agencies and wildlife researchers.

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Appendix 3.1

Total Admissions Screech-owls (EASO) and Birds of Prey (BOP) 1998-2001

Facility	EASO 1998	BOP 1998	EASO 1999	BOP 1999	EASO 2000	BOP 2000	EASO 2001	BOP 2001
CRC	77	577	67	568	92	676	96	718
WCtrVA	65	245	59	304	65	281	59	322
SWFI	29	98	33	122	44	145	30	115
TRC	25	709	15	739	17	839	15	666
Tri-State	18	162	33	196	22	225	32	256
Tufts	12	97	18	144	17	151	14	169
COP	9	206	9	254	18	292	9	324
RR	8	24	5	36	7	32	12	42

Appendix 3.2

Circumstances categorized as 'Other'

Bleached

Caught in net

Caught in trap

Disease

Electrocuted

Fell from wall during blast

Fence/barbed wire

Golf course

Hayfield

Oiled

Poolside/waterside

Trapped in tree

Window collision

(Found on) Windshield of stationary vehicle, in parking lot

****Fell from tree:** adult seen falling from tree by finder. Considered noteworthy because

birds exposed to insecticides may develop paralysis and fall from trees while perching.

SUMMARY OF THESIS

This study had three objectives. The overall objective was to assess the potential exposure of the Eastern Screech-owl to organophosphorus insecticides, to anticoagulant rodenticides, to organochlorine pesticides, and to other persistent contaminants, specifically in apple orchards of southern Quebec and, more generally, in agricultural settings. The second objective was to evaluate the species' suitability as a monitor of exposure to organophosphorus insecticides and of local organochlorine persistence. The final, underlying objective, was to gather baseline information on Screech-owls in Quebec, since this was the first study to focus on the species within the province.

Chapter one provided a comprehensive literature review of past and present pesticide regimes in orchards, of the mode of action of selected pesticides, documented exposure of raptors in orchards and in other agricultural settings, and the manner in which exposure is detected. Field methods required for exposure studies, and for studies involving elusive species such as the Screech-owl, were also discussed. Emphasis was placed on the fact that previous studies have focused primarily on exposure of migratory raptors to a single pesticide or contaminant. The Screech-owl was cited as an example of a raptor that is a year-long resident of orchards and agricultural areas to demonstrate that exposure to multiple contaminants is a possible and likely scenario.

However, the Screech-owl does not simply serve as a case study. Of all the raptor species that occupy orchards at one period of the year or another, the Screech-owl may be especially susceptible to long-term exposure to the insecticides and rodenticides currently applied and to persistent residues of organochlorines, particularly DDE, remaining from previous use. Chapter two focused on the potential for Screech-owls to be secondarily exposed to three organophosphorus insecticides, (phosmet, azinphosmethyl and phosalone), to two anticoagulant rodenticides, (diphacinone and chlorophacinone), and to residues of organochlorines,

especially DDE, as a result of consuming small mammal prey in apple orchards of southern Quebec. Referring to the proportion of small mammal species consumed by Screech-owls, and to residue levels observed in these prey, risk assessments were drawn up.

We concluded that there is a minimal risk of exposure to anticoagulant rodenticides, based primarily on the fact that the acute poison zinc phosphide is now being used for small mammal control by most orchard-owners. Capture efforts were largely unsuccessful and, as a result, analyses were conducted on a small number of specimens (n = 16). Studies carried out thus far indicate that predators are not likely to be secondarily exposed to zinc phosphide. However, bait is often left on bare ground and this certainly poses a risk to granivorous songbirds.

Residue levels in meadow voles captured after a phosmet application were used to determine secondary exposure of the Screech-owl to organophosphorus insecticides. The risk assessment and worst-case scenario spanned 0-60 hr, encompassing the period immediately after the application to the period when residues would no longer be detected in voles. Total calculated exposure was 0.641 mg/kg for phosmet and azinphosmethyl and 0.401 mg/kg for phosalone. This exposure level is well below the hazardous dose estimated for phosalone and does not appear to be of concern. Though the exposure level is below the hazardous dose estimated for azinphosmethyl, incidents of mortality may still be expected. If one wished to be conservative and cautious, the exposure level calculated for phosmet might warrant concern. It is important to note that our risk assessment only addressed direct mortality as a result of insecticide exposure. Chronic effects, such as debilitation and decrease in reproductive success, are also possible.

Screech-owls may not be appropriate monitors of insecticide exposure, especially because they can be difficult to capture. To effectively assess insecticide exposure, it is essential that the species of interest be captured within the narrow detection interval. Screech-owls are also small and secretive, which hampers the search for and recovery of carcasses.

Our risk assessment of insecticide exposure was based on residue levels in 8 *Microtus*, an admittedly small sample size. Capturing small mammals post-insecticide application proved challenging, especially in 2002. Unfortunately, data from 2001, the first year of captures, was lost due to poor recovery rates and problems with the storage and analysis of the small mammal specimens. However, as a result of the 2001 and 2002 captures, we were able to test and validate a method of whole-body insecticide analysis in small mammals. Though other studies have assessed insecticide exposure in small mammals, this was the first to assess exposure on the basis of whole-body residue levels. This method of analysis may also be used to assess secondary insecticide exposure of other avian and mammal predators.

Short-tailed shrews captured in some of the orchards contained elevated levels of DDE. Even higher levels of DDE may be observed in American Robins. Consumption of robins was therefore incorporated into the risk assessment and worst-case scenario. We calculated a DDE intake reference value based on a laboratory study in which Screech-owls were administered dietary DDE on a daily basis over one breeding season. The authors of the study reported 13.0% eggshell thinning as a result of this exposure. However, owls from the control (and dosed) group were not all captive-bred; some were obtained from an area that was once intensively managed for agricultural purposes. This suggested that the eggshell thickness of dosed owls was not evaluated against a true control. To correct for this, the average eggshell thickness of the dosed group was measured against the average thickness of archival eggs obtained from the area prior to widespread application of organochlorines. From this, we estimated that eggs in the laboratory study might actually have been thinned by as much as 22.2%.

Small mammal residue data as well as pellet and prey remains were both obtained from two pairs of adjacent orchards, one pair in St-Hilaire (Orchards D and F) and the other in Rougemont (Orchards B and K). In the best-case scenario, total exposure at D and F was elevated, but below the

reference DDE intake value from the laboratory study. In the worst-case scenario, total exposure at this pair of orchards was well above the DDE intake value. In both scenarios, exposure at Orchards B and K was well above the value. A Screech-owl egg found in a nest box in the forest between Orchards B and K may have been thinned by as much as 19.8%. Population declines have been observed when 15.0 to 20.0% eggshell thinning has persisted over time.

Screech-owls may be suitable indicators of the persistence of local organochlorines, especially DDE, because they are likely to have been exposed to local sources. Orchards represent some of the most DDE-contaminated environments in North America. Resident American Robins feeding in orchards contain very elevated levels of DDE and are consumed by Screech-owls.

Residues of organophosphorus insecticides are available to Screech-owls in small mammal prey, and, possibly, in avian prey, among others. Secondary exposure in orchards is possible and may warrant concern. Whole-carcass residue analyses of avian prey would enable us to assess their contribution of organophosphorus insecticides. Screech-owls are secondarily exposed to potentially elevated levels of DDE in orchards, which may adversely affect the population. American Robins are undoubtedly their most contaminated prey, though this is based on estimates from data obtained in British Columbia orchards. Our estimate of robin consumption was also based on a small sample size of pellets and prey remains.

The level of eggshell thinning observed in a Screech-owl egg obtained from a southern Quebec orchard location may warrant concern. Addled eggs should be collected from nest boxes installed near orchards as part of a long-term study. This would provide a larger sample size, from several orchards rather than one location, and would enable us to monitor eggshell thickness over time. This would also enable us to collect additional pellets and prey items and obtain a better idea of the proportion of American Robins in the diet. Whole-carcass analysis of DDE residues in robins from orchards of the

study area should also be conducted. Finally, eggs should be obtained from Screech-owls captive bred at local rehabilitation facilities so that the shell thickness of eggs laid by owls in the vicinity of southern Quebec orchards can be measured against control values from the region or the province.

Habitat loss may pose a more immediate threat to the Screech-owls of the region. In conjunction with pesticide exposure, habitat loss may ultimately threaten the continued presence of the species in the region. In particular, Saint-Hilaire has undergone considerable development pressure since the 1950s and encroachment into previously forested land is evident from aerial photographs. Although Screech-owls risk pesticide exposure by living near and hunting in orchards, these areas do provide critical, and now dwindling habitat.

Chapter three addressed the broader objective of assessing exposure of Screech-owls in agricultural areas. This chapter discussed pesticide exposure assessment in a clinical setting. Over 950 case files of Screech-owls were analyzed for evidence that pesticide exposure played an underlying or contributing role in admissions. Based on the data available to us, this does not seem to be the case. We do not imply by this that pesticide exposure does not serve as an underlying or contributing cause of Screech-owl admission. Rather, we were limited in our ability to address this question directly. We were, however, able to determine that factors such as distance from facility and type of circumstance (such as car strikes) appear to most heavily influence admissions. Biases associated with the analysis of this type of data, and factors that would impede sampling and obscure exposure, were also discussed.