INVESTIGATIONS OF ORGANOCHLORINE INSECTICIDES, POLYCHLORINATED BIPHENYLS AND MERCURY IN GREAT HORNED OWLS IN MANITOBA

By

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A practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of Master of Natural Resources Management.

ABSTRACT

This study determined the concentrations of selected biocides in Manitoba Great Horned Owls and investigated analytical techniques applicable to a variety of biological tissues.

Polychlorinated biphenyls (PCBs), p,p'-DDE, heptachlor epoxide/oxychlordane, dieldrin, and mercury were all present at levels below which adverse effects are noted in the literature. Levels of these chemicals were generally comparable to those found in owls and other raptors utilizing similar habitats and food sources. Reproduction by some members of the population was probably affected adversely by p,p'-DDE during the period when DDT use was common. Egg PCB, p,p'-DDE, heptachlor epoxide/oxychlordane, and dieldrin did not decline significantly between 1967/68 and the early/mid 1980s. Contaminant levels were not sex or age related.

Results of this study suggest that compounds such as heptachlor epoxide, oxychlordane, and dieldrin might be de-emphasized when non-migratory species are analyzed. There is little information about the levels of coplanar PCBs, dioxins, toxaphenes and carbamates present in birds resident in, or migratory to Manitoba. These should be determined in a future study. More information is required about the effects of long-term storage on residues present in tissues.

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INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Environmental contamination by organochlorine insecticides, polychlorinated biphenyls (PCBs), and mercury has contributed to population declines in predatory and fish-eating birds (DeSmet 1987, Heinz et al. 1979, Cooke 1973, Blus et al. 1972). Organochlorine insecticides, PCBs and mercury are concentrated to potentially harmful levels in upper trophic level organisms by their lipid solubility and persistence (Shaw and Connell 1986. Gardner et al. 1978, Fyfe et al. 1976). Raptors and fish-eating birds can be exposed to significant concentrations of these ubiquitous environmental contaminants by virtue of their position at or near the top of their respective food chains (DeWeese et al. 1986, Bröö and Odsjo 1984, Delbeke et al. 1984).

A number of Manitoba raptors such as the Swainson's Hawk, (systematic names of birds mentioned are listed in the appendix) Cooper's Hawk, Merlin and Burrowing Owl, and fish-eating birds including the American White Pelican, Red-necked Grebe, Double-crested Cormorant, and Osprey, have experienced population decline in some or all of their North

American range. The Committee on the Status of Endangered Species in Canada (COSEWIC) reports (De Smet 1984, De Smet 1982, Penak 1981, Wedgewood 1978) indicate that environmental contaminants are a factor limiting some of these populations.

There is a paucity of information on the chemical body burden carried by Manitoba's migratory and non-migratory raptors and fish-eating birds. Whether birds are being exposed to toxic chemicals in Manitoba, or primarily on their wintering grounds is unclear. Information about residue levels in Manitoba birds is needed if those populations currently suffering decline are to be properly managed, and if the status of less threatened species is to be ensured. The Manitoba Department of Natural Resources was interested in ensuring that techniques which could be used to analyze a wide variety of biological samples were available. Great Horned Owl (Bubo virginianus Gmelin) carcasses were analyzed to assess available methods and to determine the levels in this terrestrial predator.

1.2 OBJECTIVES

The primary objective of this study was to determine organochlorine insecticide, PCB, and mercury residue levels in Manitoba's Great Horned Owls.

This study also

1. determined whether owl organochlorine and mercury levels are correlated with maturity and/or sex,
2. determined whether organochlorine insecticide and PCB levels have changed between the late-1960s and the mid-1980s,
3. indicated whether the organochlorine insecticide, PCB, and mercury residues borne by Great Horned Owls could be affecting them via morbidity and/or mortality and
4. acted as a pilot study for the Department of Natural Resources' province-wide toxic chemical monitoring program by investigating techniques which could be used to analyze a wide variety of biological tissues.

1.3 DEFINITION OF TERMS

1. Organochlorine insecticide(s) may include DDT (and its metabolites DDD and DDE), dieldrin, endrin, heptachlor epoxide, oxychlordane, and cis- and trans-chlordane.
2. "Total chlordanes" includes heptachlor epoxide and oxychlordane.
3. The "DDT era" refers to the time period before DDT was banned for most uses in Canada and the U.S. while the "post-DDT" period refers to the period since the ban was instituted. These terms are being used to distinguish between eggs collected during the late 1960s and those collected during the 1980s.

4. The term "biocides" is used in a general sense and includes organochlorine insecticides, PCBs, and Hg.
5. The term "addled" eggs refers to eggs which were nonviable and were collected after failing to hatch.

1.4 LIMITATIONS

This study was primarily concerned with determining the concentrations of selected biocides present in Great Horned Owls. Analytical difficulties made it problematic to look for organochlorines other than PCBs, p,p'-DDE, dieldrin, and total chlordanes. These difficulties made interpretation of chromatograms tenuous if samples contained levels of dieldrin and/or total chlordanes near the detection limit. More difficulties were experienced when dieldrin levels were low. Caution should therefore be used when interpreting results near the detection limit.

The samples analyzed during this study primarily included road-kills and addled eggs, i.e.,

1. samples collected non-systematicaly and
2. samples which may have undergone decay before being collected and were subsequently stored for varying but considerable lengths of time.

These factors highlight the need for caution when extrapolating from this study to the population as a whole. This study is also limited by the paucity of literature on

effects of residues on Great Horned Owls and by the inconsistent way in which levels and effects of residues present in other species are reported. Interpreting the implications of residues present in Great Horned Owls is therefore difficult.

1.5 SUMMARY

Some biocides have caused a significant population decline in numerous raptorial and fish-eating bird species (Elliott et al. 1988, Newton and Bogan 1978, Fyfe et al. 1976, Hickey and Anderson 1967). The Manitoba Department of Natural Resources and the World Wildlife Fund Canada were concerned that these contaminants might be contributing to the population decline being experienced by some Manitoba raptors and fish-eating birds, though residue levels borne by many of these birds are not known.

The Great Horned Owl is relatively abundant throughout the province. As a top level predator it is expected to reflect residue levels in its food chain. Concentrations of selected biocides borne by Manitoba's Great Horned Owls were therefore determined. Effects of maturity and sex on Great Horned Owl biocide burdens were sought as was evidence of a decline in organochlorine insecticides and PCBs between the late 1960s and the mid-1980s. Potential impacts of Great Horned Owl biocide burdens were inferred from the literature.

Chapter II

LITERATURE REVIEW

The literature dealing with effects of environmental contaminants on selected raptors and fish-eating birds is extensive. Field and laboratory studies have provided insight into concentrations of organochlorine insecticides, PCBs, and mercury which detrimentally affect some raptor and fish-eating species (Peakall 1986, Peakall 1985, Cooke 1973). Advantages and disadvantages of analytical procedures have been discussed in the literature (Tessari et al. 1980, Hutzinger et al. 1974).

Organochlorine insecticides, PCBs, and mercury are widespread, persistent environmental contaminants. These contaminants are bioconcentrated in food chains by virtue of their chemical stability and lipid solubility (Rudd et al. 1981, Woodwell et al. 1971). Accumulation in raptors and fish-eating birds results from their occupation of upper trophic levels in ecosystems. Bioaccumulation of PCBs varies depending on the degree of chlorination and steric characteristics of individual congeners. Most PCB's have higher bioaccumulation factors and are generally more persistent than DDT and its related compounds (Shaw and Connell 1986).

The causal role of toxic chemicals in population declines of Peregrine Falcons and Merlins (Fyfe et al. 1976), Bald Eagles (Kaiser et al. 1980), Ospreys (Wiemeyer et al. 1980), and Brown Pelicans (Blus et al. 1979), has been established. COSEWIC reports identify biocides as limiting factors for Red-necked Grebes (De Smet 1982), Burrowing Owls (Wedgewood 1978), Cooper's Hawks (Penak 1981), and Merlins (De Smet 1984).

2.1 IMPACTS OF CHLORINATED INSECTICIDES ON BIRDS

The mechanisms by which some environmental contaminants affect bird populations are identified in the literature. DDT, DDD, DDE, and to a lesser extent, other organochlorine insecticides, affect bird populations primarily by impairing reproduction (Porter and Wiemeyer 1969, Hickey and Anderson 1967). Though the susceptibility of different species to organochlorines varies, Peakall (1970) identified characteristic chronic reproductive effects of organochlorines as the "pesticide raptor syndrome". It includes

1. delayed breeding,
2. reduction in clutch size,
3. diminished re-nesting if the first clutch is not viable,
4. thin eggshells and resultant egg breakage,

5. increased embryonic mortality, and
6. complete failure to lay eggs.

DDT and its metabolites cause the pesticide raptor syndrome by inducing a bird's hepatic mixed function oxidase (MFO) system and inhibiting carbonic anhydrase activity in the female shell gland. MFOs, which are induced by and metabolize steroid hormones as well as xenobiotics, reduced circulating estradiol (Peakall 1967) and estrogen and progesterone (Peakall 1970) levels in birds dosed with p,p'-DDT or p,p'-DDE. Resultant depressed sex hormone levels delayed breeding and diminished renesting efforts while inhibition of carbonic anhydrase reduced eggshell thickness and increased the incidence of egg breakage (Bitman et al. 1970, Peakall 1970).

Peregrine Falcons experienced reproductive failure at egg levels of 15-20 ppm p,p'-DDE while reproduction in Prairie Falcons and Merlins was affected at levels as low as 2.5 and 6.0 ppm, respectively (Fyfe et al. 1976). Prairie Falcons experienced reproductive failure when p,p'-DDE concentrations reached 12.5 ppm wet weight in eggs. Egg p,p'-DDE levels above 3.5 ppm decreased Bald Eagle productivity to a level below that required for population stability (Wiemeyer et al. 1984) while Osprey's experienced significant (10%) shell thinning at egg levels of 2 ppm p,p'-DDE (Wiemeyer et al. 1988). Cooper's Hawk reproduction was unhindered in eggs containing mean p,p'-DDE levels of

3.2 ppm but egg damage and altered feeding behavior ocurred when eggs were contaminated with 6-8 ppm (Pattee et al. 1985). American Kestrels exposed to a diet containing 2.8 ppm p,p'-DDE (wet weight) for 1 year produced thin-shelled eggs contaminated with an average of 32 ppm p,p'-DDE (Wiemeyer and Porter 1970). Eastern Screech Owls fed 2.8 ppm p,p'-DDE for 6 months experienced greater eggshell thinning than that noted in American Kestrels (McLane and Hall 1972). Mendenhall et al. (1983) suggested that Barn Owls were among the most sensitive species examined as 4 months of exposure to dietary levels of 2.83 ppm p,p'-DDE caused significant eggshell thinning and impaired reproduction.

Dieldrin, which causes little eggshell thinning in Barn Owls, primarily affects bird populations through adult mortality (Mendenhall et al. 1983). Findholt (1984) and Newton and Bogan (1978) failed to find significant correlations between dieldrin and low reproductive success (in American Kestrels and Snowy Egrets). Dieldrin induced MFOs and thereby stimulated testosterone and progesterone metabolism (Peakall 1967) though it has not been implicated in behavior-based breeding failure. Henney et al. (1982) suggested that egg concentrations above 1.3 ppm may have affected reproduction in Peregrine Falcons by reducing embryo viability. The breeding success of Golden Eagles has been related to dieldrin burdens (Lockie et al. 1969).

These eggs, which contained relatively low levels of p,p'-DDE (0.99 ppm), contained mean dieldrin levels of 1.34 ppm. Dieldrin concentrations of 1 ppm were judged the threshold above which breeding success was impaired.

Though endrin is not generally implicated as a cause of the reproductive failure experienced by some birds, Eastern Screech Owl embryonic mortality occured when egg endrin concentrations exceeded 0.3 ppm (Fleming et al. 1982). Blus et al. (1979) found a significant correlation between egg endrin levels of 0.5 ppm and low reproductive success in Brown Pelicans. They proposed endrin contamination as the primary cause of a major decline of Louisiana Brown Pelicans. Heptachlor has, at concentrations in eggs above 1.5 ppm, impaired productivity in American Kestrels (Henny et al. 1983), though neither it nor other chlordanes or toxaphenes are associated with widespread population impacts.

2.2 EFFECTS OF PCB RESIDUES

The literature is not clear as to the overall impacts of PCBs on raptor and fish-eating bird populations. Researchers have unsuccessfully sought a correlation between PCB levels in eggs and impaired reproduction (Findholt 1984, Fyfe et al. 1976) though any effects may have been masked by the presence and therefore the effects of p,p'-DDE on reproductive success. A significant correlation between egg

addling and egg PCB concentrations was noted in Kestrels (Newton and Bogan 1978) though these researchers later concluded that the PCB levels found were not likely to have had the effect noted earlier (Newton et al. 1986). McLane and Hughes (1980) detected no effect on Eastern Screech Owl reproduction when adults were fed 3 ppm Aroclor 1248 over 2 breeding seasons, a level of exposure which produced whole body burdens of 12.8 ppm and egg levels of 7.12 ppm wet weight. No eggshell thinning was detected in Ring Doves injected with PCBs immediately before laying eggs (Peakall 1971). Based on a review of acute and chronic feeding studies, Peakall (1986) concluded that PCBs are not sufficiently toxic to pose an acute hazard to birds.

PCBs elicit a number of subtle physiological and behavioral changes in birds. Peakall (1985) noted that behavioral responses are not as sensitive an indicator of PCB or chlorinated insecticide exposure than physiological changes. Mourning Doves fed a diet containing 10 ppm Aroclor 1254 spent increased amounts of time engaged in less intense courtship behavior than did controls (Tori and Peterle 1983). They also exhibited depressed circulating and peak progesterone levels, inhibited follicle development, and delayed ovulation (Koval et al. 1987). Heinz et al. (1980) depressed brain dopamine and norepinephrine production by feeding Ring Doves 10 ppm Aroclor 1254 for 8 weeks. They and MacArthur et al. (1983)

speculated that PCBs could adversely affect the reproductive success of wild populations by temporaly altering breeding and increasing its energetic cost, thereby affecting the fitness and/or number of offspring. Bird et al. (1983) reduced American Kestrel sperm concentrations 22-27% by feeding them a diet contaminated with 33 ppm Aroclor 1254. They suggested that this reduction, when combined with altered breeding behavior, might reduce the reproductive fitness of a highly contaminated population.

Like DDT and its metabolites, PCBs induce MFOs. MFO induction (and therefore toxicity) tends to increase with increasing chlorine content but is also dependent on a congener's stereochemistry. In domestic chickens, 5 ppm of Aroclors 1254 and 1268 induced MFOs though the same concentration of lower chlorine content Aroclors did not (Cecil et al. 1978). Safe et al. (1985) and Tanabe (1988) indicated that co-planar PCBs, i.e., those which sterically resemble 2,3,7,8-tetrachlorodibenzodioxin (TCDD), are the most potent MFO inducers and most toxic of the PCB congeners. Toxicity decreases as congeners become less planar.

Tanabe et al. (1987) suggested that co-planar PCBs pose a greater hazard to people and wildlife than do dioxins or furans since they tend to persist at higher residual concentrations and therefore usually compose a major portion of a sample's TCDD toxic equivalency. An animal's

susceptibility to co-planar PCBs is dependent on its liver MFO activity. Birds generally have lower MFO enzyme activities than do mammals and are therefore likely to be more susceptible to PCBs (Tanabe 1988).

Kubiak et al. (1989) attributed much of the reproductive impairment experienced by a Lake Michigan Forster's Tern colony contaminated by dioxins (including TCDD), PCBs, and other organochlorine insecticides to MFO inducing PCBs. PCB congeners 118, and 156 contributed approximately 0.3% and 0.2% of the total 2,3,7,8-TCDD equivalents in Forster's Tern eggs while 128, 138, and 158 contributed insignificant amounts. Congener 105, a potent MFO inducer contributed between 2.5 and 3% of the PCB total in Green Bay Forster's Tern eggs but contributed up to 30% of the total 2,3,7,8-TCDD equivalents. Congener 126 (0.03% of the total PCB content) contributed 70% or more of the 2,3,7,8-TCDD equivalents to these Forster's Tern eggs. Total PCBs were 4-5 times higher (20 ppm) while 2,3,7,8-TCDD equivalents were 11 fold higher in Green Bay Forster's Tern eggs than in the less contaminated inland population located at Lake Poygan, Wisconsin. Unlike the Green Bay population, reproduction in the Lake Poygan population was unimpaired.

2.3 EFFECTS OF MERCURY RESIDUES

Mercury was not associated with eggshell thinning (Peakall and Lincer 1972) though as little as 0.5 ug/g was teratogenic to Mallard embryos (Scheuhammer 1987). Fimreite et al. (1970) reported Ring-necked Pheasant hen's and a Peregrine Falcon's liver mercury levels to be 5-9 times greater than those in their eggs while Barrett et al. (1985) found egg mercury concentrations to be between 10 and 20% of those in the female's liver. Background liver mercury concentrations in raptors are considered to be below 1 ppm (Stanley and Elliot 1975, Fimreite et al. 1970).

Red-tailed Hawks fed prey containing 7-10 ppm of methylmercury (MeHg) for an extended period accumulated toxic levels of 17-20 ppm in their livers. American Kestrels were less susceptible, accumulating liver concentrations of 50 ppm before exhibiting significant toxic symptoms (Fimreite and Karstad 1971). Dale et al. (1974) indicated that mercury is unlikely to have toxic effects on pelagic seabirds if liver concentrations are below 10 ug/g. Scheuhammer (1987) advised that liver selenium levels be considered when assessing potential MeHg toxicity. Birds exposed to normally toxic levels of MeHg showed no adverse effects if dietary levels of selenium were sufficiently high.

Reproduction can be impaired at dietary mercury levels easily tolerated by adults. Black Duck hatching success was impaired when adults were fed a diet containing 3 ppm MeHg for two 28 week periods during consecutive breeding seasons (Finley and Stendell 1978). Adult female Black Duck livers contained an average of 23 ppm mercury after treatment although adults exhibited no detrimental effects. Heinz (1974) exposed adult Mallards to 2-3 ppm MeHg for 21 weeks. Though adults were unaffected by liver levels of 11.1 ppm, reproductive impairment reduced the number of one week old ducklings by 50-60% over controls. Mallard reproduction was affected at egg levels of 0.79 to 0.86 ppm (Heinz 1979). Twelve weeks on a diet containing 2-3 ppm MeHg had no detrimental effect on Ring-necked Pheasant adults (Fimreite 1971). This level of exposure, which produced mercury concentrations of 2 ppm in adult livers and 0.5-1.5 ppm in eggs, decreased egg hatchability significantly by increasing embryonic mortality and the number of unfertilized eggs.

While either total (inorganic and organic) mercury and organic (MeHg) can be determined when mercury contamination is monitored, MeHg is both much more toxic and more readily bioaccumulated (Scheuhammer 1987, Stickel et al. 1977, Berglund and Berlin 1969). The difference in bioaccumulation between inorganic and MeHg were demonstrated in a salt marsh containing sediment contaminated with 0.06 to 1.7 ppm inorganic mercury but no detectable levels of

MeHg. A variety of fish species carried muscle mercury levels between 0.3 and 2.4 ppm of which close to 100% was methylated (Gardner et al. 1978).

Though the literature contains a good compilation of the chlorinated hydrocarbon and mercury concentrations which affect a variety of species, there are no toxicity data for Great Horned Owls. If the susceptibility of both Eastern Screech and Barn Owls to p,p'-DDE is indicative of that of owls generally, Great Horned Owls may be very susceptible to p,p'-DDE.

2.4 LEVELS OF ORGANOCHLORINES IN OWLS AND RAPTORS

The literature contains a good compilation of organochlorines and mercury levels in different birds. Bald Eagle eggs collected in Minnesota (1978) and Wisconsin (1979) contained geometric mean p,p'-DDE levels of 2.5 and 2.2 ppm respectively. DDE levels in eagle eggs collected in eastern U.S. states were generally 4-5 fold higher than those from Minnesota and Wisconsin (Wiemeyer et al. 1984). The eggs of a variety of Manitoba raptors carried p,p'-DDE levels ranging from 0.04 to 1.34 ppm (DeSmet 1988).

Geometric mean total PCB levels in Common Loon, Red-necked Grebe, Ring-billed Gull, and Herring Gull were 4.66, 4.18, 1.33, and 16.76 ppm respectively. PCBs were not detected (detection limit < 0.8 ppm) in the American

Kestrel, Merlin, Swainson's Hawk, and Great Grey Owl eggs tested.

Alaska Peregrine Falcon p,p'-DDE concentrations varied from 28.9 ppm in muscle to 103 ppm in liver (Lincer et al. 1970). Kaiser et al. (1980) reported p,p'-DDE residues in Bald Eagle carcasses as 4.0 ppm while PCBs were present at 5.5 ppm. Seidensticker and Reynolds (1971), found a combined DDT, DDD, and p,p'-DDE concentration of 9.19 ppm in a sample of Great Horned Owl muscle taken by biopsy. Dieldrin and heptachlor epoxide were present at 0.15 and 0.19 ppm, respectively. Havera and Duzan (1986) determined contaminant residue loads in Great Horned Owls and other raptors collected between 1974 and 1981. Mean Great Horned Owl liver and breast muscle p,p'-DDE, dieldrin, and PCB residue levels exceeded those in Screech and Barn Owls, and Red-tailed, Rough-legged, and Cooper's hawks.

Fimreite et al. (1970) found variation in liver mercury levels between different Alberta raptors. Short-eared Owl livers contained 6.8 (±) 3.3 ppm. Burrowing Owl livers were contaminated with 3.7 ppm of mercury. A Prairie Falcon and an American Kestrel had liver levels of 1.2 and 0.75 ppm respectively. Great Horned Owl liver mercury concentrations ranged from 0.076 ppm (Saskatchewan) to 1.97 ppm (Alberta).

2.5 EFFECTS OF CONTAMINANT COMBINATIONS

Though contamination by a number of chemicals is common, there has been little research concerned with the effect of a combination of toxic chemicals. MacArthur et al. (1983) exposed Ring Doves to a combination of Aroclor 1254 (0.297 or 8 ppm), p,p'-DDE (0.897 or 2.8 ppm), mirex (0.095 or 1.67 ppm), and photomirex (0.32 or 4.61 ppm) to imitate the low and high level residue mixtures present in Lake Ontario Herring Gull eggs. These mixtures produced dose-related changes in courtship behavior, parental care, and fledgling success by depressing circulating levels of estrogens and androgens. The behavioral abnormalities and decreased nesting success were similar to those reported in Lake Ontario Herring Gull populations (Fox et al. 1978). Mendenhall et al. (1983) investigated the reproductive effects of two years on a diet containing 2.83 ppm p,p'-DDE and/or 0.58 ppm dieldrin on Barn Owls. Though dieldrin caused slight eggshell thinning, which was additive to that caused by p,p'-DDE, the reproductive effect of both was not different from that attributed to p,p'-DDE alone.

2.6 FACTORS AFFECTING ACCUMULATION OR RETENTION OF RESIDUES

2.6.1 EFFECT OF ONE CONTAMINANT ON THE RETENTION OF ANOTHER

The influence of the presence of one contaminant on the retention of another compound has been studied. While the

presence of p,p'-DDE and MeHg did not affect the accumulation of either compound in Mallards (Heinz 1987), DDT increased PCB retention in Robins (Södergren and Ulfstrand 1972). American Kestrels excreted increased amounts of mirex if fed a diet containing both mirex and Aroclor 1254 rather than just mirex (Bird et al. 1983).

2.6.2 EFFECTS OF TROPHIC LEVEL

Fyfe et al. (1976) attributed the Prairie Falcon's toxic chemical burden, higher than that of the Merlin, in part to falcon's feeding at a higher trophic level. Niethammer et al. (1986) reported a similar relationship between three species of herons feeding at different trophic levels and their tendency to accumulate contaminants. Lindberg et al. (1985) found higher levels of p,p'-DDE and mercury in Peregrine Falcons from northern Sweden than those present in southern birds. This within-species variation was attributed to prey availability since northern falcons primarily ate aquatic birds while southern falcons preyed predominately on terrestrial species.

Braune (1987) investigated mercury levels in nine marine bird species and found that those feeding on fish and benthic organisms carried higher mercury loads than did those feeding on lower invertebrates. Delbeke et al. (1984) determined mercury levels in thirty species of raptors and aquatic birds collected in Belgium between 1971 and 1981.

Raptor liver mercury levels increased as diets changed from insects to mammals to birds. Bröö and Odsjo (1981) attributed a significantly higher level of mercury contamination in coastal Swedish Eagle Owls, relative to inland owls, to the coastal owls' more contaminated food chain.

2.6.3 EFFECTS OF NUTRITIONAL CONDITION

Several researchers have investigated the effect of body fat content on residue concentration. Frank et al. (1983) analyzed 174 Common Loons for mercury and organochlorine residues. Breast muscles of visibly emaciated adults had 4 1/2 fold less extractable lipid while total DDTs and PCBs were concentrated 5-7 and 5 fold respectively. Liver fat levels were unchanged but total (inorganic and organic) liver mercury increased by a factor of 3. Anderson et al. (1984) examined the seasonal dynamics of organochlorines in Cackling Geese. Increases in body residue concentrations were attributed to cyclical decreases in overall body fat content. The insecticides were most concentrated when energy stores (fats) were mobilized. This is most common during periods of stress such as

1. winter, when cold temperatures and/or reduced food supplies could induce lipid catabolism,
2. during migration (food intake may not offset the energetic cost of migration), and

3. as the breeding season progresses since adults are required to feed both themselves and their offspring and would catabolize body fat reserves as their food intake declined (Mora et al. 1987, Anderson et al. 1984).

Body lipid reserves are reduced during these parts of the year.

Few researchers indicate a specimen's body fat content. This makes interpretation of the impacts of residues reported in the literature difficult since

1. body organochlorine burdens are diluted or concentrated by seasonal or stress-caused changes in body fat levels (Heinz et al. 1979) and
2. birds with considerable lipid reserves tolerate higher dietary levels of organochlorines than thin birds do (Ecobichon and Saschenbrecker 1969, Stickel et al. 1965).

Body (tissue) fat content is usually reported on a percent basis (Mora et al. 1987, Anderson et al. 1984, Frank et al. 1983, Bogan and Newton 1977).

Any stress which causes stored body fat to be mobilized can also cause organochlorines to relocate. Findlay and De Freitas (1971) indicated that organochlorine residues relocate to muscle tissue rather than liver and brain if

pigeons are starved to lose 50-75% of their body fat. Södergren and Ulfstrand (1972) starved Robins to the point where body fat reserves were essentially depleted. They found an increase in muscle, brain, and whole body residue levels at that point. Bogan and Newton (1977) reported that p,p'-DDE relocated to muscle, liver, and brain (increasing by factors of 2, 6, and 13 respectively) when body fat reserves of American Kestrels had all but disappeared.

2.6.4 INFLUENCE OF HABITAT

A raptor's migratory habits influence its body residue levels. Peregrine Falcons breeding in Canada and the United States, and Snowy Egrets breeding in Idaho, accumulated organochlorines on their Central and South American wintering grounds (Findholt 1984, Henney et al. 1982). Great Horned Owls, being non-migratory, should reflect breeding ground residue levels. Those inhabiting relatively uncontaminated locales are therefore often exposed to lower biocide concentrations than migratory birds (Havera and Duzan 1986, Seidensticker and Reynolds 1971).

2.6.5 INFLUENCE OF SEX

Mature (breeding) female raptors normally carry lower levels of PCBs and chlorinated insecticides than males (Newton and Bogan 1978). Mendenhall et al. (1983) indicated that 16 months on a diet containing 2.83 ppm

p,p'-DDE produced female Eastern Screech Owl whole carcass p,p'-DDE levels of 78 ppm while male carcasses contained 112 ppm. As significant quantities of organochlorines are stored in body fat, female birds excrete some of these contaminants when laying eggs (Newton and Bogan 1978).

Though MeHg concentrates primarily in liver, it is also excreted through deposition in eggs. Mercury's biological half-life in Bonaparte's Gulls was affected by egg laying and moulting (Braune and Gaskin 1987). Male and female pre-moult (pre-egg laying) head feather mercury levels were similar. Mercury concentrations in post-moult (post-egg laying) female head feathers were significantly lower than those in males. Furness et al. (1990), who collected feather samples during breeding season, found no sex related differences in Red-billed Gull mercury levels.

2.6.6 EFFECTS OF AGE AND OTHER FACTORS

Niethammer et al. (1986) and Newton et al. (1981) have demonstrated increasing organochlorine concentrations with age, though males accumulated contaminants more rapidly than females. Given species with a similar degree of susceptibility to p,p'-DDE or any other persistent contaminant, those with longer life cycles are more likely to be affected (e.g., large predatory and fish-eating birds) than species with high turnover rates. Furness et al. (1990) found that while mercury levels in Red-billed Gull

chicks were approximately 80% of those in adults there was no age related accumulation in adults ranging from 2 to 15 years old. In contrast, Braune and Gaskin (1987) noted increasing mercury levels between juveniles, second year, and adult Bonaparte's Gulls though they did not distinguish between adults of differing ages.

Deposition in feathers during the annual moult is the primary method by which birds excrete mercury. The amount excreted varies between species and between sexes within species. Female Bonaparte's Gulls distributed 68% of their total mercury body burden to feathers while males excreted 59% via this route (Braune and Gaskin 1987). Black-eared Kites deposited 70% of their body mercury load into feathers during their annual moult (Honda et al. 1986a).

Different abilities to metabolize and excrete contaminants also contribute to differences in toxic chemical body burdens between species (Lemmetyinen et al. 1982). Serafin (1984) discovered significant differences in intestinal absorption of a C-14 labelled hexachlorobiphenyl, dieldrin, and organic mercury. Screech Owls and American Kestrels absorbed double the quantity of the PCB congener as did Black-Crowned Night Herons and Mallards; they also absorbed twice as much dieldrin as Herons and 3-4 times as much as Mallards. Mallards absorbed one-eighth, Herons one-fourth, and Screech Owls one-half as much mercury as Kestrels. These differences in xenobiotic absorption

demonstrate that levels of environmental contamination are not necessarily indicative of the degree to which a certain species is exposed.

2.7 CHOICE OF TISSUE FOR ORGANOCHLORINE DETERMINATION

Tissues chosen for analysis must be representative of whole body contaminant burdens under diverse physiological conditions since residue concentrations and storage locations are affected by body fat content (Hutzinger et al. 1974). Various tissues are analyzed for organochlorine and mercury residues. Total DDT levels in the blood plasma of American Kestrels and other raptors have been correlated with egg and brain DDT levels (Henney and Meeker 1981). Henney and Meeker (1981) and Henney et al. (1982) indicated that live trapping and blood sampling can be particularily useful for monitoring DDT and other organochlorines in discrete populations of endangered species.

Body fat deposits are frequently analyzed for organochlorines (Havera and Duzan 1986, Hutzinger et al. 1974, Dindal et al. 1970). Organochlorines are however, not distributed evenly between fat reserves. Samples of fat from different parts of a carcass can contain significantly different concentrations of organochlorine pesticides and PCBs. Mallards exposed (for 30 d) to a marsh treated with DDT accumulated 12.1 ppm p,p'-DDE in subcutaneous leg fat, 8.2 ppm in post-ventral body fat, and 2.4 ppm in mesenteric

fat (Dindal 1970). Dindal suggested that this be considered before fat samples are taken for analysis. This variation in contamination may be a result of a certain lipid deposit's function. Mesenteric fat may primarily serve a protective function while subcutaneous reserves may function primarily as energy stores. Subcutaneous reserves could therefore be more metabolically active and reflect recent exposure to a contaminant more readily than would mesenteric fat. Mesenteric fat might reflect long term exposure more accurately than do subcutaneous fat reserves.

Body fat reserves can be an unreliable indicator of whole body residue burdens, since body fat levels can change quickly so as to dilute or concentrate organochlorine residues (Heinz et al. 1979, Hutzinger et al. 1974). Body fat deposits may be non-existent if a bird is sampled during winter, towards the end of migration, or during breeding season (Mora et al. 1987, Anderson et al. 1984, Frank et al. 1983, Capen and Leiker 1979). Fat samples might be difficult or impossible to obtain if carcasses are collected at those times (Capen and Leiker 1979). Breast muscle lipid contents are also subject to substantial variability (Frank et al. 1983) though this variation is not nearly as great as that of fat reserves.

Since researchers (Newton and Bogan 1978, Södergren and Ulfstrand 1972, Findlay and De Freitas 1971) have demonstrated that organochlorines relocate during

starvation, samples drawn from whole carcasses should provide the most accurate assessment of overall biocide burdens. Heinz et al. (1979) suggested that whole carcasses are the best samples to use for chlorinated hydrocarbon analysis, though breast muscle is suitable if whole carcass analysis is impractical. Norstrom et al. (1986) determined lipid weight ratios of p,p'-DDE relative to whole body levels in a variety of Herring Gull tissues. Levels in muscle were 80-90% of whole body concentrations while liver, egg, and brain residues (50-70, 40, and 10% of whole body concentrations respectively) were lower. They concluded that the lipid weight p,p'-DDE ratio between breast muscle and whole body lipid pools was nearly at equilibrium indicating that breast muscle more closely approximated whole body levels than did the other tissues analyzed.

2.8 CHOICE OF TISSUE FOR MERCURY ANALYSIS

Tail or flight feathers are commonly analyzed to detect mercury contamination in waterfowl (Hesse et al. 1975). Feathers are chosen since they are easily obtained from hunters. The literature does not indicate whether feathers reflect whole body mercury residues better than other tissues though feathers may accumulate 60-70% of a bird's mercury burden as they grow (Braune and Gaskin 1987, Honda 1986a). Feathers accumulate mercury during the yearly molt (Berg et al. 1966) and reflect mercury levels present in a

bird's bloodstream while each feather is forming. Rose and Parker (1982) cautioned that feathers may adsorb mercury once they have formed. If a bird is exposed to significant airborne deposition, feather mercury levels might be artificially inflated. Bröö and Odsjo (1981) noted that since changing food sources may alter the amounts of mercury being ingested, caution should be used if drawing inferences about morbidity or mortality from feather mercury levels outside the moulting period.

Liver mercury levels are affected by variables which can make interpretation difficult. Liver mercury levels vary depending on a bird's nutritional state. MeHg, which usually makes up more than 90% of a bird's total liver mercury load (Scheuhammer 1987, Westermark et al. 1975), is lipid soluble and would be diluted or concentrated as whole body and/or liver fat reserves undergo accretion or mobilization (Honda et al. 1986b, Osborn 1979). Liver and whole body mercury levels decrease during the moult and increase once moulting is complete. Honda et al. (1986a) found that Black-eared Kite liver mercury levels decrease by two-thirds over the moult. Braune and Gaskin (1987) reported similar changes in Bonaparte's Gull liver mercury levels during that bird's moulting period.

Liver is the tissue most commonly sampled if researchers are investigating the potential toxic effects of mercury on birds under controlled laboratory conditions. A number of

researchers (Wiemeyer et al. 1980, Heinz 1976, Hesse et al. 1975, Heinz 1974, Fimreite 1974, Fimreite and Karstad 1971, and Borg et al. 1970) analyzed liver since it accumulates significant amounts of MeHg and reflects increases in body mercury concentrations resulting from laboratory exposure. Since the liver is always metabolically active, its mercury levels reflect body burdens at the time of a bird's death.

2.9 STABILITY OF CONTAMINANTS UNDERGOING LONGTERM STORAGE

The stability of a compound being stored for long periods of time depends on the compound's chemical stability, the storage conditions, and the type and condition of the matrix it is found in. DDT, DDD, and hexachlorohexane are readily degraded in freeze-dried Herring Gull eggs stored at room temperature while p,p'-DDE, penta-, hexa-, and heptachlorobiphenyls, cis-chlordane, trans-nonachlor, heptachlor epoxide, and dieldrin are stable for a year under like conditions (Norstrom and Won 1985). DDT is also dehydrochlorinated in bird liver (half life at -12 to -15° C-65 days) (Jefferies and Walker 1966) and whole blood (half life at -20° C-28 days) (Ecobichon and Saschenbrecker 1967). The extent of DDT dehydrochlorination in whole blood depended on the amount of hemolysis a sample had undergone. Ecobichon and Saschenbrecker (1967) suggested that DDT could be dehydrochlorinated in tissues containing substantial quantities of reduced porphyrins, coenzymes, and other

metalloproteins. Total DDT and PCB levels in frozen seal blubber were unaffected by two years of storage (Anas 1974) while PCBs, p,p'-DDE, heptachlor epoxide, dieldrin, oxychlordane, mirex, and hexachlorobenzene, present in naturally contaminated Herring Gull egg homogenates, were essentially unaffected by 2-3 years of storage at -18 to -28° C (Norstrom and Won 1985). Norstrom and Won (1985) concluded that low temperature storage was probably a satisfactory method of storing biological materials for years but that additional matricies should be studied.

2.10 METHODS OF ORGANOCHLORINE DETERMINATION

Techniques used for organochlorine analysis vary depending on the contaminants expected and the equipment available. Complete homogenization of samples in sodium sulfate is followed by extraction with hexane using a polytron, Soxhlet or ball-mill apparatus (Cromartie et al. 1975, Hutzinger et al. 1974, Grussendorf et al. 1970). Initial clean up may make use of gel permeation chromatography or liquid-liquid partitioning (Tessari et al. 1980). These techniques can be combined with elution through alumina, and/or florisil, and/or silica gel columns which provide additional clean up and allow samples to be fractionated (Bidleman et al. 1978, Hutzinger et al. 1974, Holden and Marsden 1969). Fractionation is necessary if PCBs are present since they interfere with the

quantification of co-eluting organochlorine insecticides, although PCBs are not completely separated from all other organochlorines. Liver, which accumulates chemicals that could interfere with organochlorine analysis, is more difficult to clean up than other body tissues.

The literature contains a good compilation of organochlorine insecticide, PCB, and mercury concentrations at which some species have experienced population decline. It is not complete concerning the susceptibility of, and levels of toxic chemicals in, the Great Horned Owl and other raptors and fish-eating birds resident in or migratory to Manitoba. It is acknowledged that residue levels can be concentrated or diluted as a bird's lipid stores are metabolized or undergo accretion. It is also apparent that birds with substantial lipid reserves can tolerate a higher organochlorine intake than can low fat birds. However, few studies give any indication of the lipid levels present in the samples analyzed. Road-killed birds are often analyzed but the implications of collecting samples of unknown quality in a non-random manner are not discussed adequately. While the literature indicates that long term, low temperature storage is likely satisfactory, there is insufficient discussion of the effects of long term storage on residue levels present in a carcass. These factors combine to make interpretation of the effects of residue levels found in Great Horned Owls difficult.

Chapter III

METHODS

3.1 CARCASS COLLECTION

Great Horned Owl road-kills were collected by Natural Resource Officers and were turned in by members of the general public. Taxidermists were alerted to submit carcasses from birds brought in for mounting. Several injured birds, which died in captivity, were obtained from veterinarians. Owls were frozen (at -20^{0} C) as soon as possible after discovery.

Addled Great Horned Owl eggs have been collected in Saskatchewan since 1967, by Dr. C.S. Houston, and are stored in the Canadian Wildlife Service's tissue bank. Twelve eggs, 6 from 1967-1968 and the remainder from 1980-1985, were obtained for analysis of PCBs and organochlorine insecticides. The locations where Great Horned Owl carcasses and eggs were obtained are indicated in Figures 1 and 2 respectively.

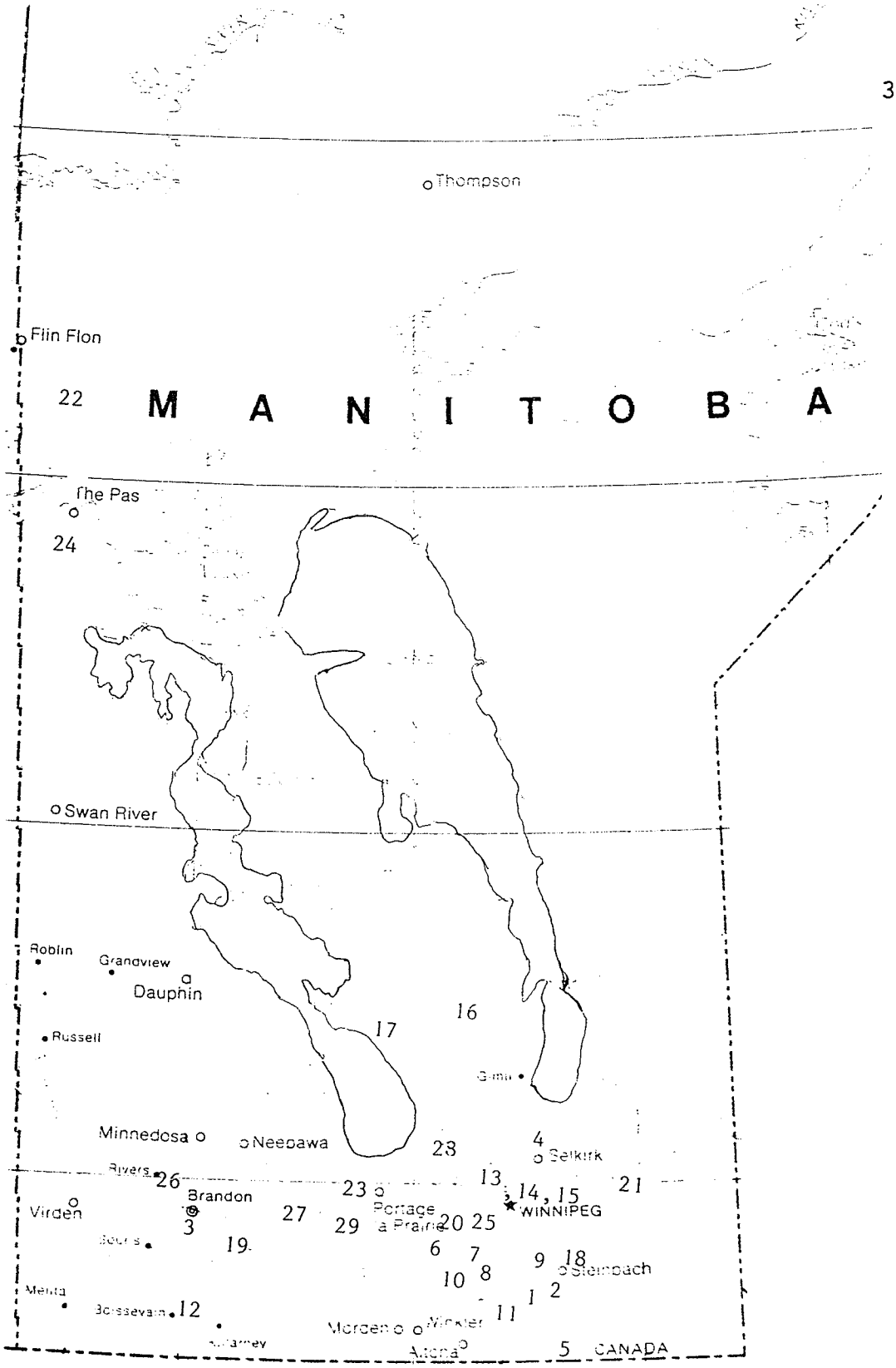

Figure 1. Locations in Manitoba from which Great Horned Owls were obtained. Owls are identified by sample number.

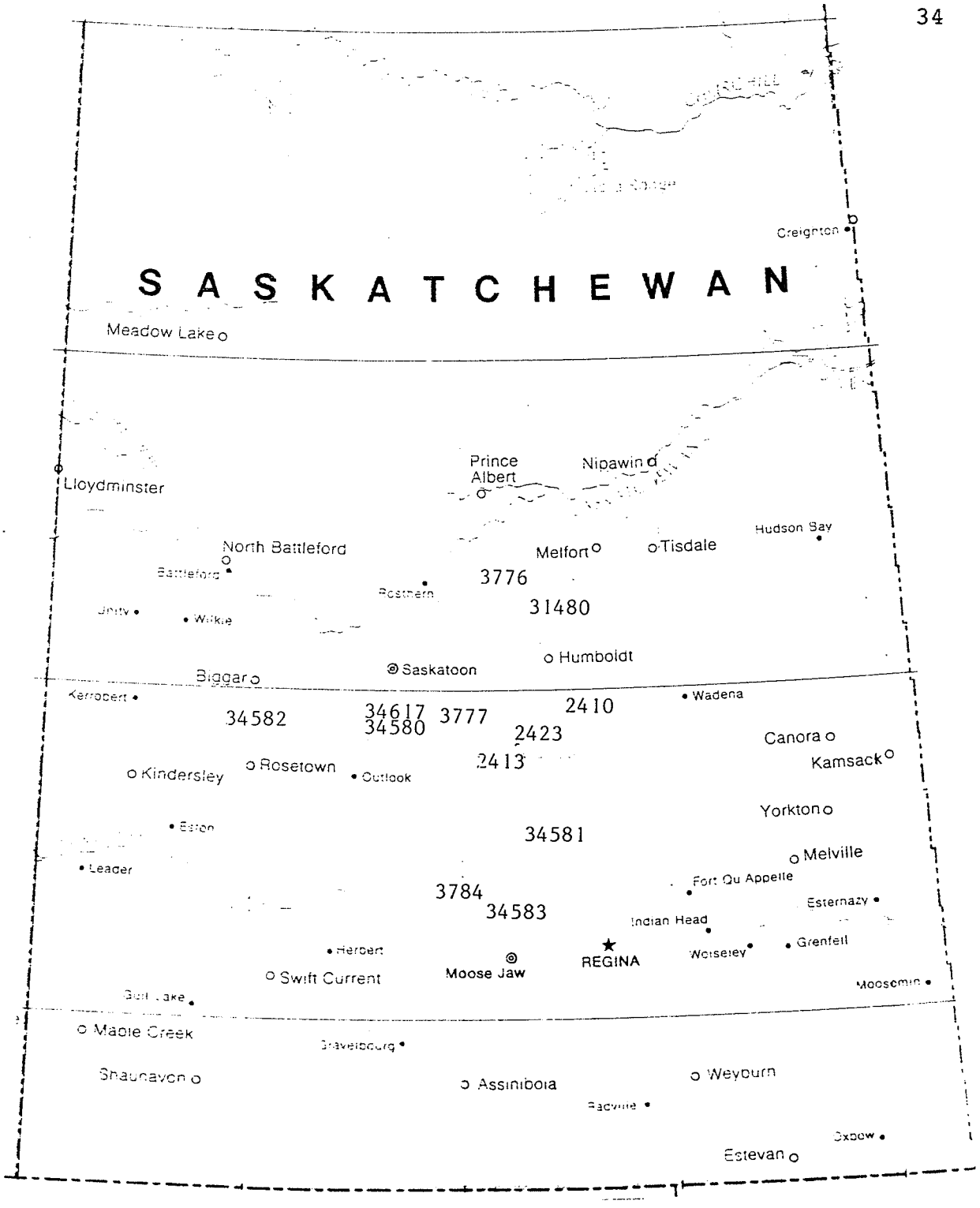

Figure 2. Locations in Saskatchewan from which addled Great Horned Owl eggs were obtained. Eggs are identified by sample number.

3.2 AGEING, SEXING, AND CARCASS CONDITION

Maturity was established by examining each bird's flight feathers. Immature birds' flight feathers are in the same age class and, therefore, show similar wear patterns and a similar degree of fading. Mature birds (those which have moulted once) have flight feathers of different ages which therefore show different wear patterns (R.W. Nero 1987 pers. commun.). Maturity of females and the sex of all owls was confirmed by examination of the sex organs.

The general internal body condition, including the presence and extent of subcutaneous and coronary fat deposits, was noted. Both the time of year a bird was found and the condition of its tissues were used to judge whether a bird had undergone significant decay. The condition of the intestines was the primary factor used to discriminate between birds chosen for analysis and those discarded. It was assumed that intestinal decay would proceed more rapidly and become evident sooner than would the decay of other tissues. Birds chosen for analysis were those judged to have been frozen soon enough after their demise so that significant post-mortem decay and possible alterations of contaminant levels were minimized.

3.3 DEGRADATION STUDY

A degradation study was carried out to determine the extent to which organochlorines may have undergone post-mortem decay between the time an owl died and the time it was frozen. It was assumed that any carcass exposed to room temperatures for 3 days would have undergone noticeable decay and would therefore have been discarded. Muscle samples removed from a carcass (already analyzed for organochlorines) found in December 1987 were placed in vials and stored at 20° C for 1, 2, and 3 days. (It was assumed this specimen was frozen soon after death and had therefore undergone little decay before being picked up.) These samples were stored (at -35° C) for 2 months, cleaned up, and analyzed to determine the extent of organochlorine residue breakdown. The extent of PCB and organchlorine insecticide breakdown was determined by comparing levels present in tissues before and after exposure to room temperature for 1, 2, or 3 days.

3.4 MERCURY ANALYSIS

The Department of Fisheries and Oceans' Freshwater Institute staff analyzed liver samples for total mercury using flameless atomic absorption spectrophotometry (AAS) (Armstrong and Uthe 1971). Liver samples (0.1g) were digested in nitric (1 mL) and sulfuric (4 mL) acids at 58° C for 2 h. $KMnO_4$ (15 mL- 6%) solution was added and samples

were allowed to oxidize overnight after which 30% H_2O_2 was added to clear the solution. The solution was made up to 25 mL and analysed by flameless AAS at a wavelength of 253.7 nanometers.

3.5 ORGANOCHLORINE ANALYSIS

3.5.1 SUBMISSION OF SAMPLES FOR ANALYSIS AT THE TECHNICAL SERVICES LABORATORY

Great Horned Owl samples were initialy submitted to the TSL for organochlorine insecticide and PCB analysis. The samples were analyzed by the standard method used at the TSL for determining organochlorines in fish and other biological samples. Tissue samples (10 g) were ground with anhydrous $NaSO_4$, Soxhlet extracted, and evaporated to a volume suitable for application to a clean up column of 10 g of fully activated florisil. Samples were then adjusted to a volume suitable for injection onto a GC equipped with a electron capture detector (ECD). Difficulties encountered during sample clean up led to contamination of the capillary column equipped GC. Samples were then diluted to varying degrees and injected on a GC equipped with a packed column. This increased the minimum detectable levels so that PCBs and organochlorine insecticides, other than p,p'-DDE, were undetectable in most samples. These results were not considered satisfactory. Since many researchers (Elliott et al. 1988, DeSmet 1987, Wiemeyer et al. 1984, Frank et al. 1983, Grier 1982) had recently detected organochlorines in a

variety of bird species resident in, or migratory to Canada and the northern U.S., it seemed reasonable to believe that organochlorines other than p,p'-DDE should have been detected in the tissues analyzed.

3.5.2 ANALYSIS OF SAMPLES BY THE AUTHOR

The author then decided to evaluate the analytical methods available. Samples were subsequently re-analyzed by him in the Pesticide Research Laboratory (PRL), Department of Soil Science, University of Manitoba.

All solvents were pesticide analysis grade, obtained from Caledon Laboratories Inc. (Georgetown, Ontario). PCB and insecticide standards were obtained from the U.S. Environmental Protection Agency (Research Triangle Park, North Carolina).

Breast muscle samples were homogenized in a blender where possible. Samples (5 g of tissue, 1.7 g of egg) were ground with 5 g of anhydrous sodium sulphate (Fisher-reagent grade heated at 600° C for 6 h) (Fisher Scientific, Winnipeg, Manitoba). The sample was transferred to a ball-mill apparatus for extraction. Hexane (25 mL) was added and the sample was shaken on a wrist action shaker for 45 min. The sample was centrifuged at 3000 rpm for 2 min and the extract transferred to a round bottom flask. One milliliter was removed for determination of extractable lipids.

The sample was then rotoevaporated (33° C) to approximately 2 mL. The extract was transferred to a graduated test tube and its volume adjusted, by evaporation under a stream of dry nitrogen, to 2 mL. A 10 g alumina (Woelm-neutral, activated at 800° C for 4 h; deactivated with 5% HPLC grade water) (ICN Biomedicals Inc., Mississauga, Ontario) clean up column was packed, topped off with 1 cm anhydrous $NaSO_4$, and rinsed with hexane. The sample was transferred to the column and 2 fractions were eluted with 130 mL hexane. Fraction 1 (Figure 3) (18 mL) contained the PCBs and p,p'-DDE while fraction 2 (Figure 4) (112 mL) contained the more polar dieldrin and chlordanes (heptachlor epoxide, oxychlordane, and cis- and trans-chlordane).

The resulting fractions were rotoevaporated to 2 mL, and transferred to graduated test tubes with a 2 mL isooctane and 2-2 mL hexane rinses. The first fraction did not adversely affect the GC and was therefore evaporated to 2 mL (in isooctane) under nitrogen and transferred to a storage vial. The second fraction contained lipids which could not be separated from the sample by polarity based clean up techniques (adsorption or liquid-liquid partitioning), saponification, or freeze-out. Fraction 2, which was initially stored in 2 mL, contaminated the GC injector, column, and detector when injected in an undiluted form. The effect of this contamination was to make the GC unusable

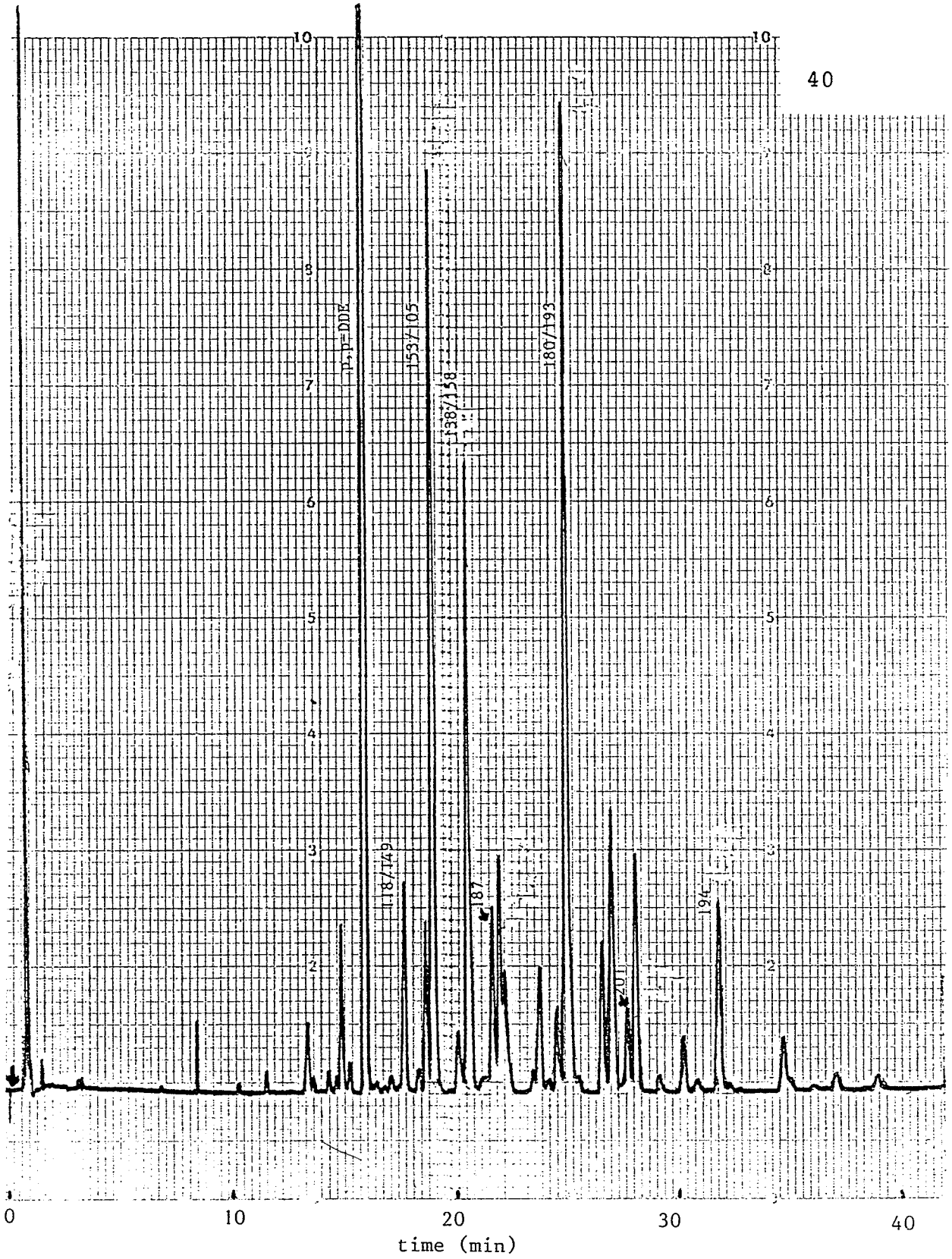

Figure 3. Chromatogram of fraction 1 of a sample. PCB congeners are identified by their IUPAC numbers (Ballschmiter and Zell 1980). Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow.

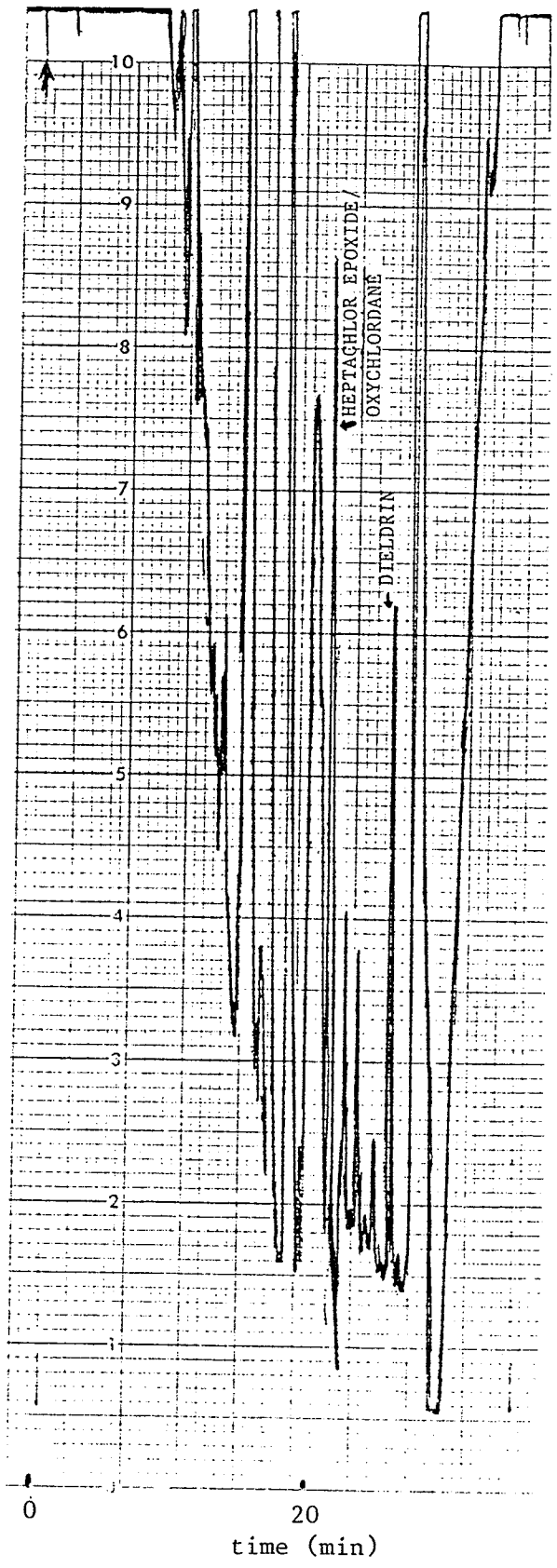

Figure 4. Chromatogram of fraction 2 of a sample. Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow. The chart recorder was set at its maximum sensitivity. The baseline therefore starts at the top of the chart and descends as the run proceeds.

for considerable periods. Second fractions of tissue samples were then diluted by a factor of 8-9, and egg samples by a factor of 9 in order to minimize contamination of the GC. Duplicates of the breast muscle samples were analyzed.

A minimum of 2-1 uL injections were made per extract. The gas chromatograph was a Hewlett Packard 5890 equipped with an electron capture detector (ECD) (Hewlett Packard, Mississauga, Ontario) and a 30 M (0.53 mm i.d.) DB-5 megabore column (Chromatographic Specialties, Brockville, Ontario). An initial temperature of 90° C was held for 2 min, raised to 180° C at 30° C/min, to 200° C at 10° C/min, and thereafter at 2° C/min to a final temperature of 262° C. The splitless injection port was held at 200° C while the detector temperature was 350° C. Helium (9.33 mL/min) was used as the carrier gas while the makeup gas consisted of 5% argon-methane (44.58 mL/min). Detection limits were as follows: PCBs-0.001 ppm, p,p'-DDE-0.05 ppb, and total chlordanes, and dieldrin-0.5 ppb.

Standards of a 1:1 mixture of Aroclors 1254 and 1260 (figure 5) or organochlorine insecticides (Figure 6) were injected every 6 to 8 samples as were solvent blanks. Procedural blanks were also run to monitor for cross contamination between samples and contamination from glassware, sodium sulfate, alumina, and solvents. Recoveries from owl muscle tissue and chicken egg samples

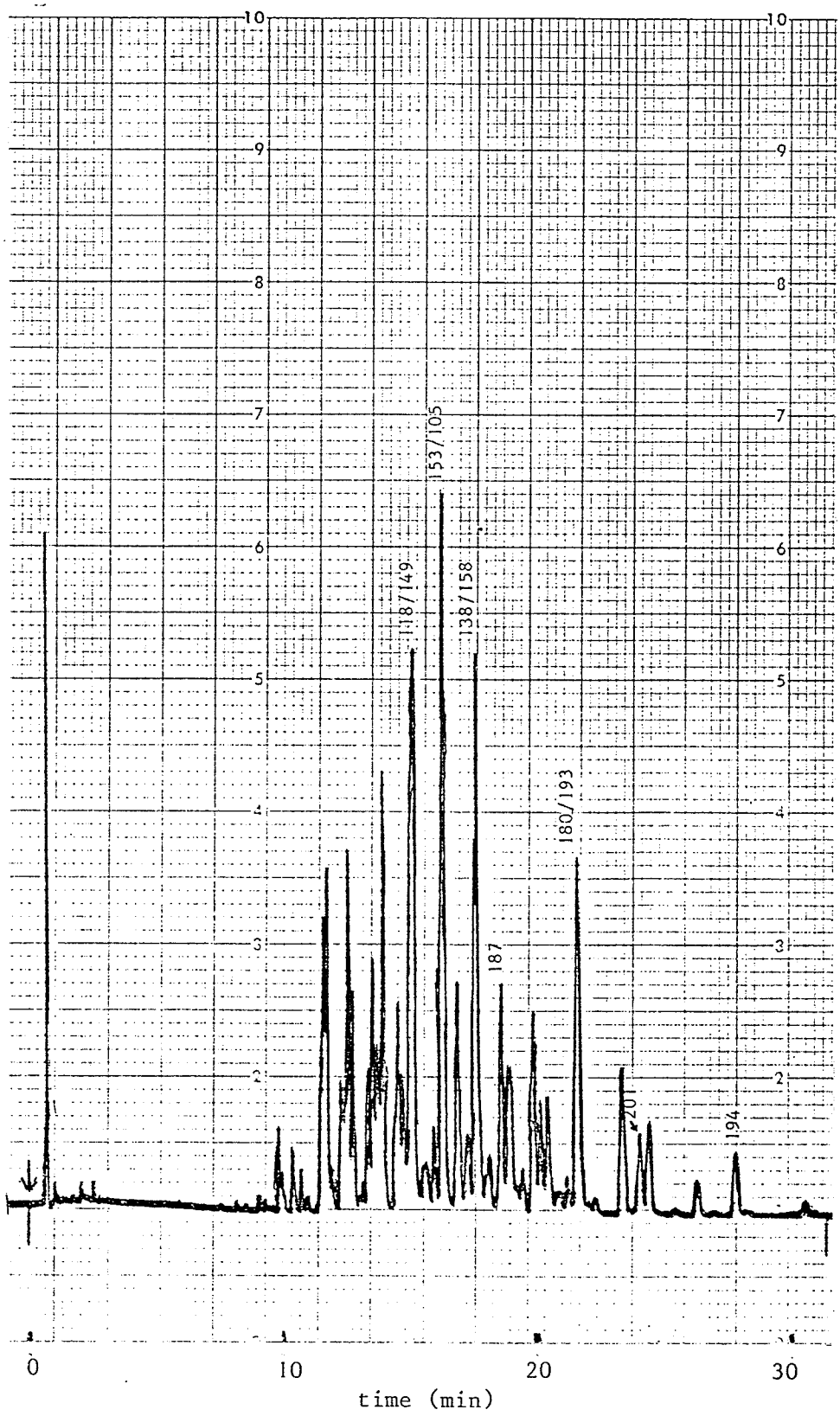

Figure 5. Chromatogram of an Aroclor 1254/1260 (1:1) standard. PCB congeners are identified by their IUPAC numbers (Ballschmiter and Zell 1980). Only those peaks used for identification are identified. Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow.

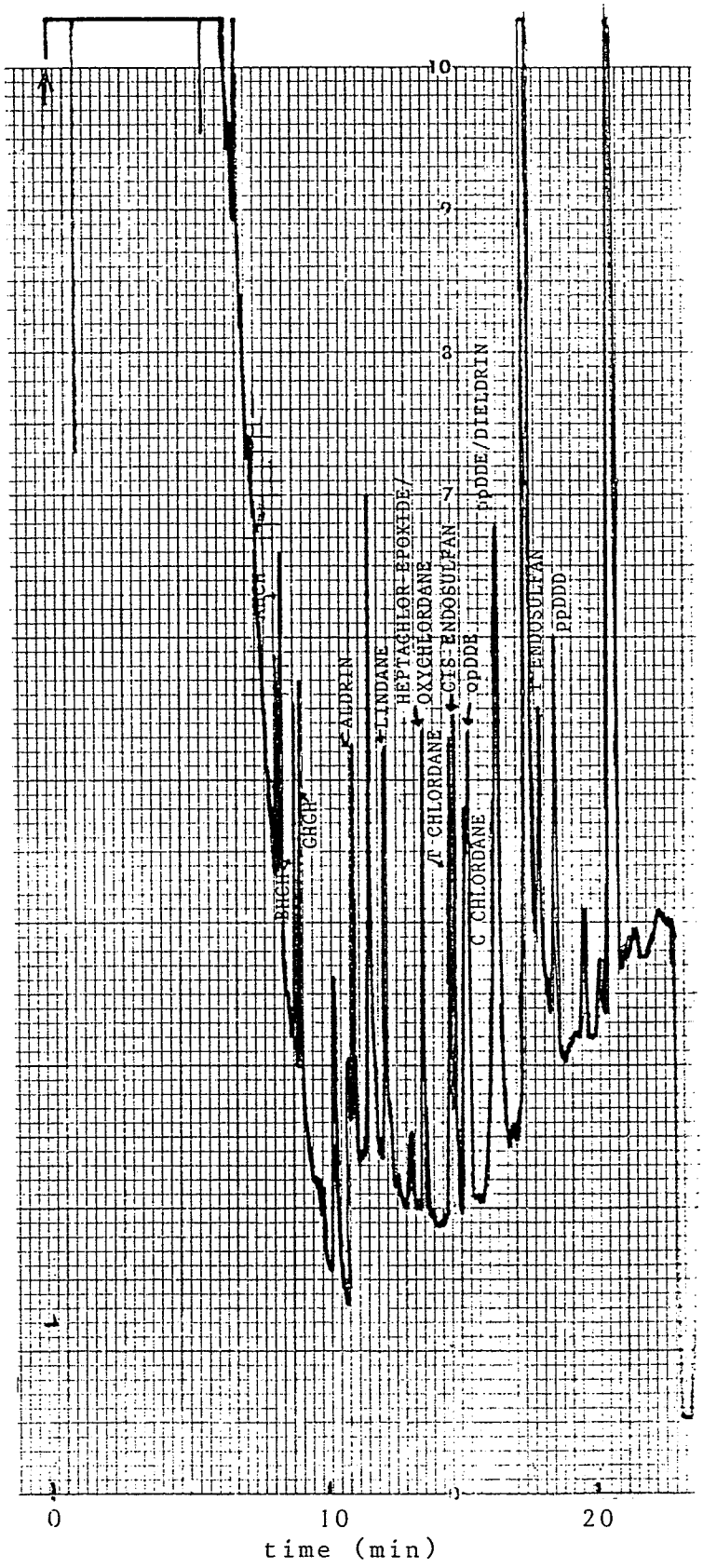

Figure 6. Chromatogram of the organochlorine insecticide standard. Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow. The chart recorder was set at its maximum sensitivity. The baseline therefore starts at the top of the chart and descends as the run proceeds.

(spiked with 0.0015 or 0.2 ppm of an organochlorine insecticide and 0.023 or 0.5 ppm of an Aroclor 1254:1260 (1:1) standard) ranged between 78 and 105% with a mean recovery of 97% for p,p-DDE, 92% for PCBs, 92% for heptachlor epoxide/oxychlordane, and 85% for dieldrin. Organochlorine insecticides and PCBs were quantified by peak heights. PCB quantification was based on a 1:1 standard mixture of Aroclors 1254 and 1260 (Figure 5). Four peaks used for quantification (118/149, 153/105, 138/158 and 180/193) were made up of 2 PCB congeners. PCB congeners are identified by their International Union of Pure and Applied Chemists (IUPAC) numbers as determined by Ballschmiter and Zell (1980). Seven major peaks common to all samples (Figures 3 and 5),

1. 118/149
   (2,3',4,4',5-penta and 2,2',3,4',5',6-hexachlorobiphenyl),
2. 153/105
   (2,2',4,4',5,5'-hexa and 2,3,3',4,4-pentachlorobiphenyl),
3. 138/158
   (2,2'3,4,4',5'-hexa and 2,3,3',4,4',6-hexachlorobiphenyl),
4. 187
   (2,2',3,4',5,5',6-heptachlorobiphenyl),
5. 180/193
   (2,2',3,4,4',5,5'-hepta and
   2,3,3',4',5,5',6-heptachlorobiphenyl),
6. 201
   (2,2',3,3',4',5,5',6-octachlorobiphenyl) and

7. 194

(2,2',3,3',4,4',5,5'-octachlorobiphenyl).

were measured to determine total PCBs. Of these the 118, 153, 138, and 180 congeners usually predominate in wildlife samples (Focardi et al. 1988). The chromatographic pattern was used along with retention times when selecting PCB peaks for quantification.

Organochlorine insecticides and PCBs in 2 samples were confirmed with High Resolution Capillary GC-ECD (HRGC) at the Department of Fisheries and Oceans' Freshwater Institute on a Varian 6000 (equipped with a model 651 data system) (Varian Inc., Georgetown, Ontario) containing a 60m DB-5 column (0.25 um i.d.). The injector was set at 220° C, the detector at 300° C, and the hydrogen carrier and nitrogen makeup gas flow rates were 0.96 and 30 mL/min respectively. The runs were programmed from 100 to 150° C at 15°/min, to 265° at 3°/min, and held at that temperature for 15.34 min. These samples were further cleaned up with gel permeation chromatography and were fractionated into 3 fractions. Fraction 1 (Figure 7) contained PCBs, p,p'-DDE, and some other organochlorines while fraction 2 (Figure 8) contained organochlorine insecticides (including oxychlordane) and toxaphene. Fraction 3 (Figure 9) included heptachlor epoxide and dieldrin. PCBs and p,p'-DDE were also structurally confirmed at the PRL (in 1 egg and 2 tissue samples) on a Hewlett Packard 5890 GC equipped with a

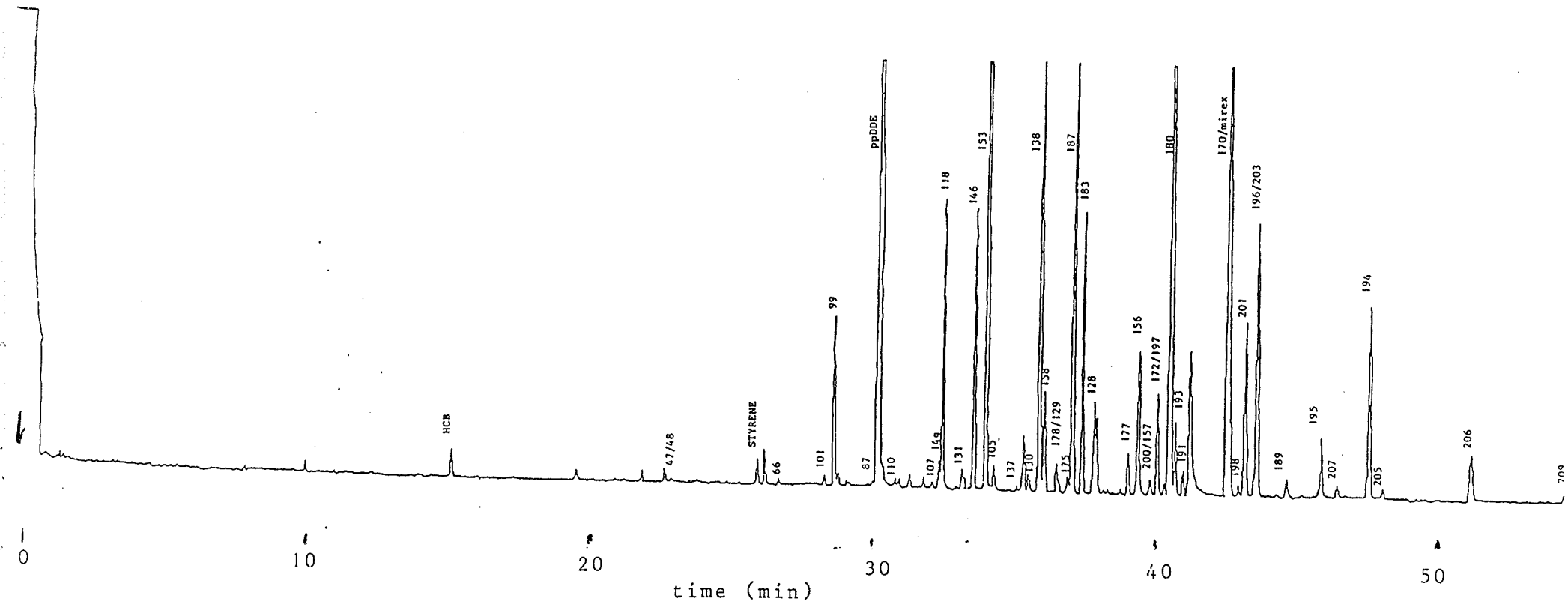

Figure 7. Confirmatory chromatogram of fraction 1 of a sample (analyzed by HRGC). PCB congeners are identified by their IUPAC numbers (Ballschmiter and Zell 1980). Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow.

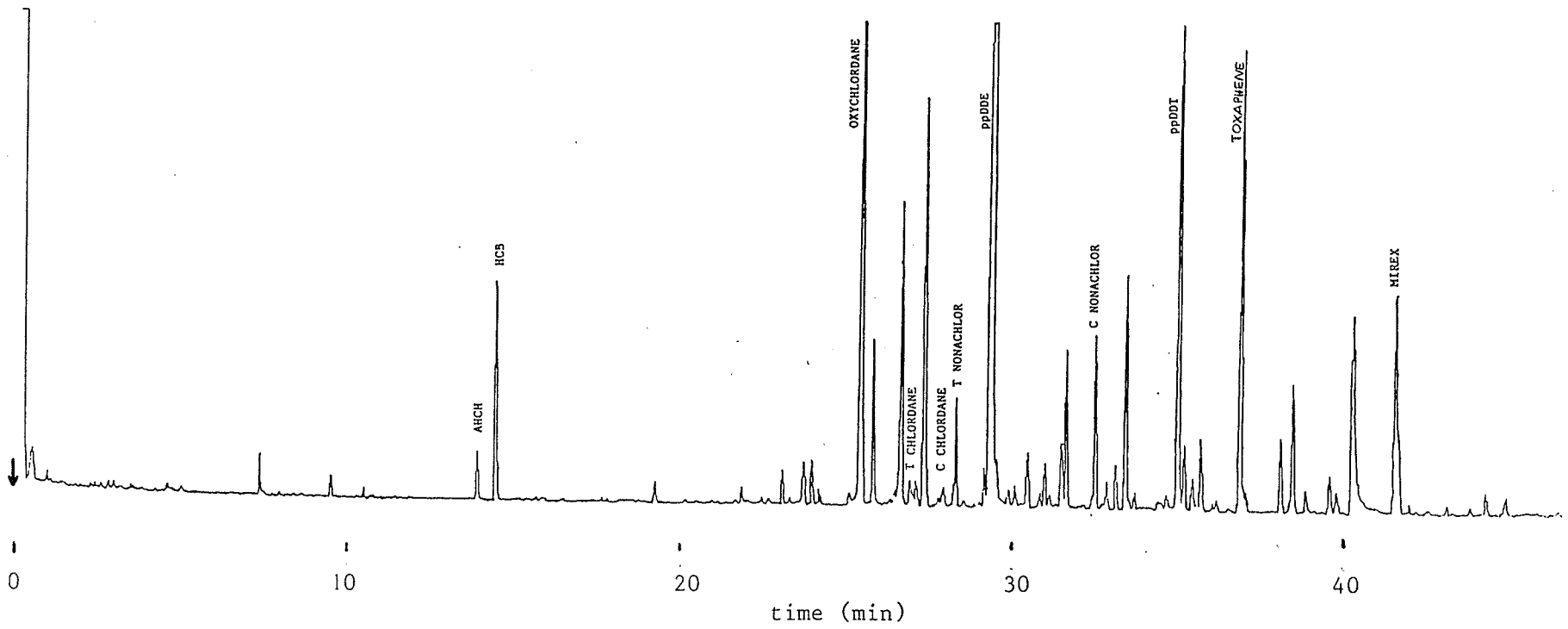

Figure 8. Confirmatory chromatogram of fraction 2 of a sample (analyzed by HRGC). Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow.

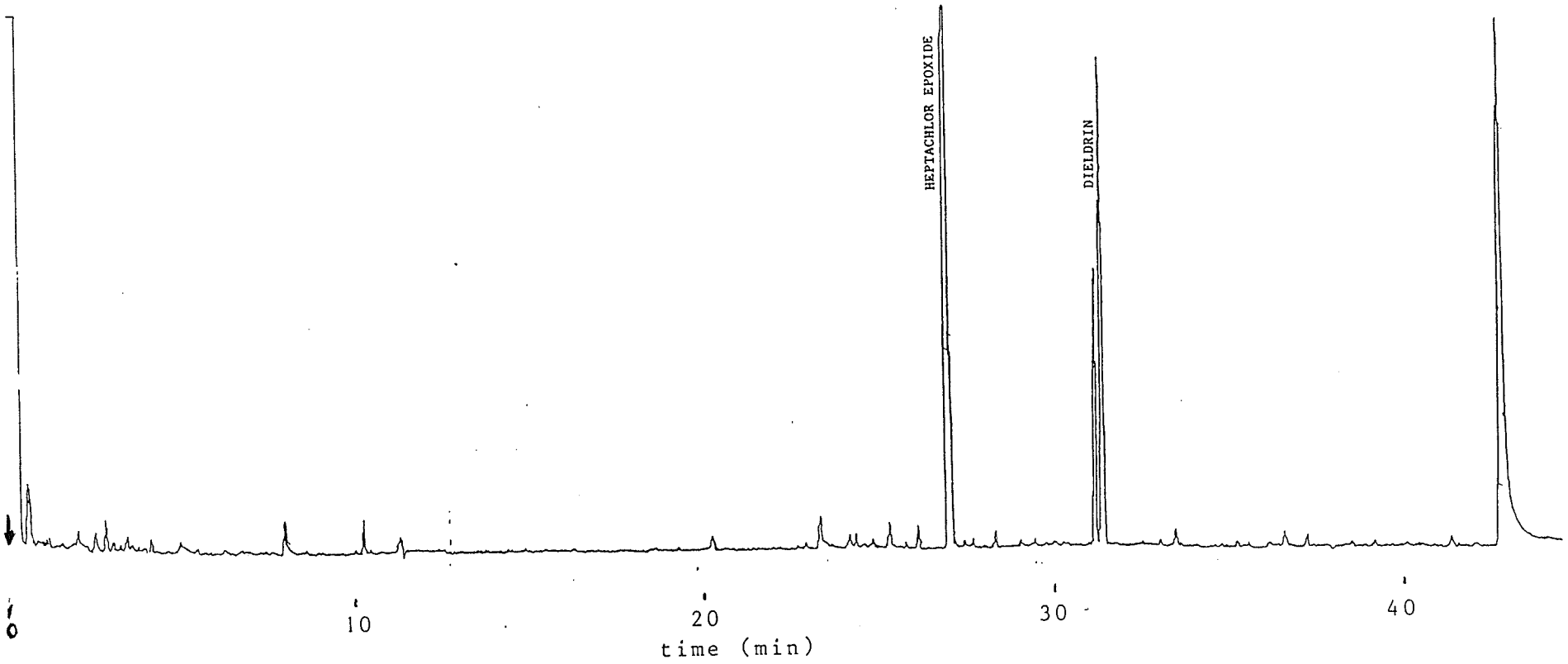

Figure 9. Confirmatory chromatogram of fraction 3 of a sample (analyzed by HRGC). Run times are indicated at 10 minute intervals. The beginning of the run is indicated by the arrow.

Finnigan Ion Trap Detector (model 801) (electron multiplier-1500 Volts) (Finnigan-Mat, San Jose, California). The injector temperature was 200° C. The oven temperature was held at 90° C for 2 min, raised to 180° C at 30°/min, to 200° C at 10°/min, and to a final temperature of 262° C at 2°/min The column was a 30m DB-5 capillary column (0.25 i.d.) with a nitrogen carrier gas flow rate of 1.7 mL/min.

3.6 STATISTICAL METHODS

All statistical tests were carried out using SAS statistical packages available through the University of Manitoba's computer services. Analysis of Variance (ANOVA) was used to test for differences between DDT era and post-DDT egg insecticide and PCB levels. Effects of sex, maturity, and year on muscle organochlorine insecticide, PCB, and Hg levels were tested with the General Linear Models (GLM) procedure for unbalanced designs. Correlations between insecticide, PCB, Hg, and % lipid levels were determined using the Pearson product-moment correlation procedure.

3.7 DETERMINATION OF EFFECTS OF CONTAMINANTS

The literature does not contain information about the susceptibility of Great Horned Owls to biocides. However, the effects of some contaminants on other species are known. The potential effects of contaminants borne by Great Horned Owls were inferred from their effects on other bird species.

Chapter IV

RESULTS

Mean muscle lipid percentages, which ranged from 0.17 to 2.43 (Table 1) indicate that birds varied considerably as to their nutritional state. Body lipid content, as indicated by both muscle lipids and presence or absence of subcutaneous and heart lipid deposits, was not necessarily indicative of an emaciated bird. Most of the low lipid birds appeared to be in good condition despite their low body lipid content. A few birds (numbers 2, 3, 10, and 11) were thin and had atrophied breast muscles.

About one-third of the owls analyzed had no subcutaneous fat deposits while 25% lacked fat deposits around their heart (Table 1). Those without fat deposits around their hearts had low muscle lipid contents. PCB, p,p'-DDE and mercury levels (Table 2) were significantly ($p < 0.01$) related to the presence or absence of visible fat deposits around a bird's heart. This suggests that these birds had lost sufficient amounts of body fat to cause these compounds to be mobilized and concentrated in the muscles and other locations (Frank et al. 1983, Bogan and Newton 1977). None of the birds with high tissue residue contents (Table 2) appear to have lost significant amounts of moisture (Table

Table 1. Tissue moisture (%), % lipid and presence (+) or absence of subcutaneous and heart fat deposits in Great Horned Owls.

SAMPLE NO.	%MOISTURE	%LIPID	SUBCUTANEOUS FAT	HEART FAT
1	83	0.17	-	-
2	75	0.72	+	+
3	75	0.26	-	-
4	70	1.73	+	+
5	76	0.60	+	+
6	74	0.18	-	-
7	69	1.36	+	+
8	68	1.05	+	+
9	73	1.65	+	+
10	74	0.24	-	-
11	72	1.14	+	+
12	68	2.04	+	+
13	75	1.42	+	+
14	73	1.3	+	+
15	71	1.17	+	+
16	72	2.4	+	+
17	72	0.73	-	-
18	74	1.1	+	+
19	76	0.60	+	+
20	74	1.63	+	+
21	72	1.1	+	+
22	78	0.60	-	-
23	75	2.43	+	+
24	73	1.17	+	+
25	73	0.23	-	-
26	75	0.36	-	-
27	84	1.88	-	+
28	74	1.67	-	+
29	74	1.68	+	+
MEAN	74	1.1		

Table 2. Levels of PCBs, p,p'-DDE, total chlordanes, dieldrin, and mercury (ppm wet weight) (means, standard deviations (SD), and geometric means are indicated) in Manitoba Great Horned Owls collected between 1982 and 1988. Their sex (F=female; M=male) and maturity (IMMAT=immature; MAT=mature) are indicated. Numbers followed by a (*) were difficult to quantify because of interferences. Levels below the detection limit were set at half the detection limit when calculating geometric means. (NA=not analyzed)

SAMPLE NO.	YEAR	SEX	MATURITY	p,p-DDE	TOTAL PCBs (ppm wet weight)	TOTAL CHLORDANES	DIELDRIN	MERCURY
1	1982	F	IMMAT	0.43	0.87	0.003	0.004*	0.58
2	1982	F	MAT	0.11	0.74	0.006	0.02	0.28
3	1982	M	IMMAT	0.07	0.28	0.001*	<0.0005	0.58
4	1982	M	MAT	0.14	0.09	0.003	0.001*	0.17
5	1983	F	MAT	0.19	0.15	0.0007	<0.0005	0.43
6	1983	F	MAT	0.02	0.03	0.03	0.02	0.38
7	1983	F	IMMAT	0.03	0.01	0.001	<0.0005	0.38
8	1983	F	MAT	0.03	0.05	0.0007	0.01	0.18
9	1983	F	MAT	0.22	0.35	0.04	0.04	0.63
10	1983	M	IMMAT	1.3	0.30	0.02	0.02	0.26
11	1983	M	MAT	0.07	0.002	0.001*	<0.0005	0.37
12	1984	M	MAT	0.71	0.81	0.005	0.009	0.36
13	1984	M	MAT	0.16	0.09	0.01	0.008	0.15
14	1984	M	MAT	0.46	0.04	0.03	<0.0005	0.27
15	1984	M	MAT	0.07	1.6	0.003	0.002*	0.23
16	1985	F	MAT	0.33	0.09	0.001*	<0.0005	0.25
17	1985	F	MAT	1.3	0.11	0.08	0.06	2.4
18	1985	F	MAT	0.13	0.21	0.003	0.006	0.20
19	1986	M	MAT	0.08	0.24	0.1	0.009	0.30
20	1986	F	MAT	0.29	3.5	0.0007*	0.002*	0.30
21	1986	F	MAT	0.10	0.24	0.002*	<0.0005	0.34
22	1986	F	MAT	2.6	0.14	0.07	0.04	1.68
23	1987	F	MAT	0.37	6.9	0.008	0.008	NA
24	1987	F	MAT	0.08	0.20	0.003	<0.0005	NA
25	1987	F	MAT	1.4	0.25	0.03	0.007	1.67
26	1987	F	IMMAT	0.18	0.12	0.002	0.004*	0.63
27	1987	M	MAT	0.59	0.79	0.007	0.06	0.36
28	1987	M	MAT	0.06	0.12	0.002	0.01	NA
29	1988	F	MAT	0.46	0.79	0.006	0.005	NA
MEAN (SD)				0.41 (0.57)	0.66 (1.4)	0.02 (0.03)	0.01 (0.02)	0.54 (0.55)
GEOMETRIC MEAN				0.20	0.20	0.005	0.005	0.39

1) indicating that moisture loss did not cause residues to be concentrated in the more highly contaminated birds.

PCBs, p,p'-DDE, heptachlor epoxide/oxychlordane, and mercury were detected in 100% of samples analyzed while dieldrin was detected in 72% (Table 2). Mean variations between the 29 duplicate (breast muscle) samples were: p,p'-DDE-7.12%, PCBs-10.53%, heptachlor epoxide/oxychlordane-12.5%, and dieldrin-21.6%. Comparison of individual fraction 2 chromatograms with solvent blanks indicated that interfering peaks were primarily associated with column bleed, injector and/or detector contamination. Coextractives did not normally affect total chlordane quantification. The higher variation between dieldrin levels is due to interfering peaks (coextractives and/or column bleed) which made accurate quantification difficult in some cases. Quantification was more consistent if higher ($\geq$ 0.004 ppm) levels of dieldrin were present in fraction 2 (Figure 4) since the significance of a coeluting peak decreased as the size of the dieldrin peak increased. While cis- and trans-chlordane, p,p'-DDD, p,p'-DDT, o,p-DDE, endrin, and α- and γ-HCH were present in the insecticide standard (Figure 6), they were not detectable in any sample because of interfering peaks or because they were present at concentrations below the minimum detectable level.

The sample and standard chromatograms of fraction 1 (Figures 3 and 5) indicate the peaks used for PCB

quantification. The pattern shown (Figure 3) is typical of the samples analyzed with peaks 153/105, 138/158, and 180/193 predominating. The 7 peaks chosen for quantification comprise approximately 60% of the total PCB content of the samples. High resolution gas chromatography of fraction 1 (Figure 7) indicates that congeners 149, 105, 158, and 193 are minor constituents of peaks 118, 153, 138, and 180. Figure 7 also indicates the presence of mirex (coeluting with 170) and the toxic congener 105. Sample chromatograms (analysed by HRGC) of the second (containing oxychlordane) and third (containing heptachlor epoxide) fractions confirm the presence of oxychlordane, (Figure 8) and heptachlor epoxide and dieldrin (Figure 9). Figure 8 also indicates that a number of other organochlorine residues (α-HCH, hexachlorobenzene, cis- and trans-chlordane, cis- and trans-nonachlor, p,p'-DDT, and toxaphene) are present. These were either not sought or could not be detected during routine analysis because of interfering peaks.

There were no significant trends ($p < 0.05$) in mean owl PCB, p,p'-DDE, total chlordane, dieldrin, or mercury levels between 1982 and 1988 (Figure 10). Sample sizes were small and the variation within a year was often larger than the mean for that year. Trends were therefore very difficult to detect.

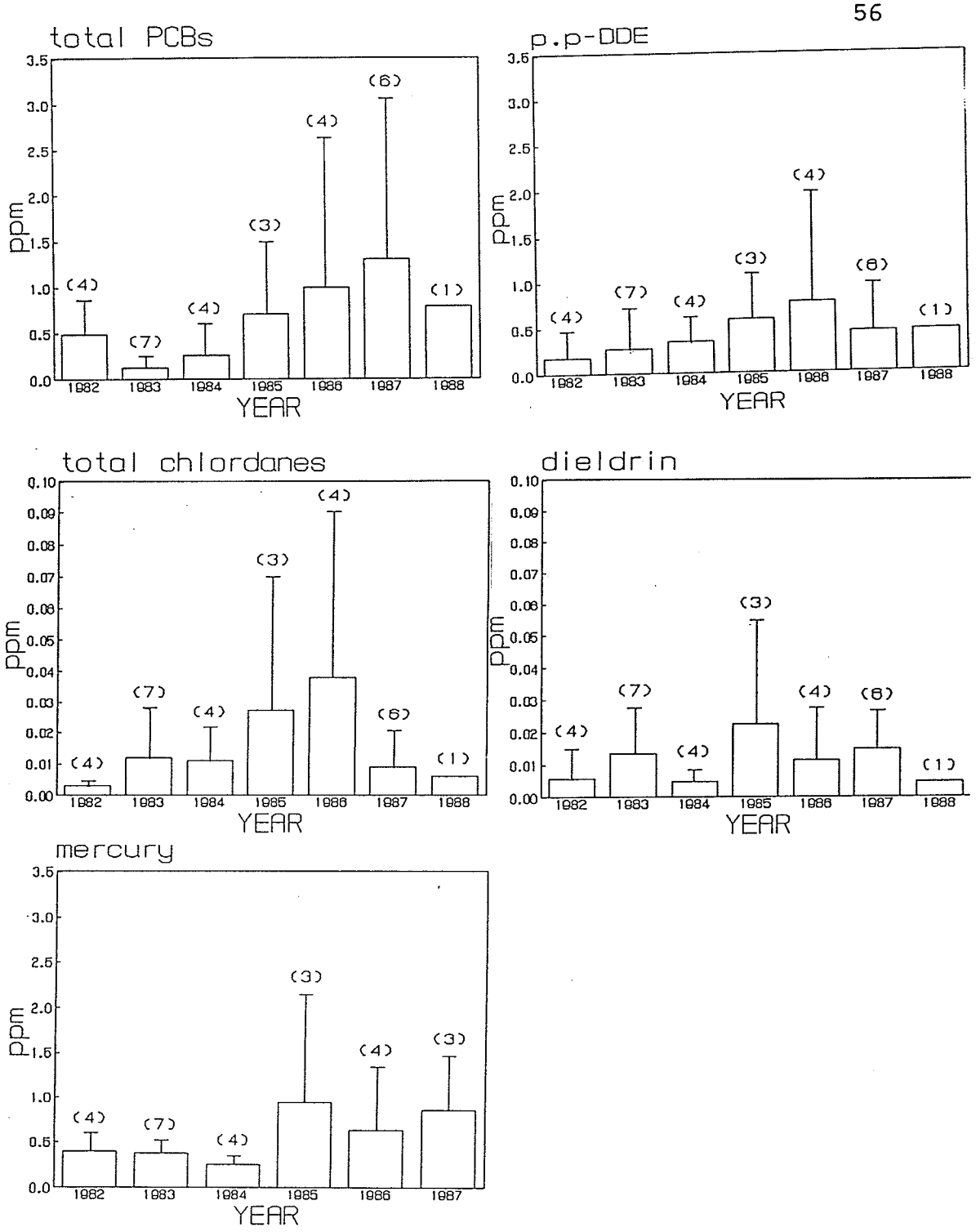

Figure 10. Residue level trends of PCBs, p,p'-DDE, chlordanes, dieldrin, and mercury (mean and SD-ppm) in Great Horned Owls from Manitoba, 1982-1988. Mercury residues were determined from 1982-1987. There were no significant differences ($p < 0.05$) between years. Number of samples analyzed/year indicated above each SD bar.

There were no significant trends ($p < 0.05$) in mean organochlorine levels present in eggs during the pre- and post-DDT periods (Table 3), if the very high levels found in egg 3784 were included when means were calculated. Trends then approached significance for p,p'-DDE ($p < 0.06$), chlordanes ($p < 0.1$), and dieldrin ($p < 0.07$). If means from pre- and post-DDT era eggs were compared and results for egg 3784 were excluded, p,p'-DDE levels have decreased significantly ($p < 0.008$) since the late 1960s. Neither PCBs ($p < 0.28$), chlordanes ($p < 0.14$), or dieldrin ($p < 0.17$) decreased significantly when means were calculated in this way. Exclusion of the levels found in egg 34583 (which contained the highest p,p'-DDE and PCB levels in post-DDT era eggs) in addition to those of egg 3784, when means were calculated, did not affect these results. The small sample sizes and very large variability in residue levels (Table 3) made residue level trends difficult to detect.

There were no differences ($p < 0.05$) between levels of PCBs, p,p'-DDE, chlordanes, dieldrin, and mercury present in male and female owls or immature and mature birds (Table 4). The small sample sizes and considerable variability in contamination made detection of differences difficult.

The degradation study did not reveal any significant breakdown of PCBs, p,p'-DDE, chlordanes, or dieldrin in tissues left at room temperature for up to 72 hours and stored at -35° C for 2 months ($p < 0.05$).

Table 3. Levels of p,p'-DDE, PCBs, total chlordanes, and dieldrin (ppm wet weight) in Great Horned Owl eggs collected in Saskatchewan during 1967/1968 and 1980/1985. Means, standard deviations (SD), and geometric means are indicated for eggs collected during the DDT and the post-DDT time periods. Means between the DDT and post-DDT periods are not significantly different ($p < 0.05$).

SAMPLE NO.	YEAR	p,p-DDE	TOTAL PCBs	TOTAL CHLORDANES	DIELDRIN
			(ppm wet weight)		
2410	1967	3.5	2.1	0.2	0.2
2413	1967	4.5	4.4	0.3	0.1
2423	1967	1.7	0.50	0.09	0.03
3776	1968	1.5	1.3	0.3	0.05
3777	1968	2.2	0.74	0.1	0.02
3784	1968	13	14	0.9	0.4
MEAN		4.4	3.8	0.3	0.1
(SD)		(4.3)	(5.1)	(0.3)	(0.2)
GEOMETRIC MEAN		3.2	2.0	0.2	0.08
31480	1980	0.27	0.69	0.02	0.007
34617	1982	0.04	0.09	0.04	0.005
34580	1984	0.47	0.76	0.08	0.03
34581	1985	0.88	0.96	0.3	0.03
34582	1985	0.68	1.0	0.1	0.1
34583	1985	1.8	2.4	0.05	0.01
MEAN		0.69	0.98	0.1	0.03
(SD)		(0.56)	(0.75)	(0.09)	(0.03)
GEOMETRIC MEAN		0.42	0.69	0.07	0.02

Table 4. Means and standard deviations (SD) of p,p'-DDE, PCB, total chlordane, dieldrin, and mercury concentrations (ppm wet weight) in male and female, and immature and mature owls. Means between sexes and between immature and mature birds are not significantly different ($p < 0.05$).

SEX	NO. OF SAMPLES		p,p-DDE	TOTAL PCBs	TOTAL CHLORDANES	DIELDRIN	MERCURY
			(ppm wet weight)				
MALE	11	MEAN	0.34	0.20	0.02	0.01	0.69
		(SD)	(0.40)	(0.22)	(0.03)	(0.02)	(0.70)
FEMALE	18	MEAN	0.46	0.92	0.02	0.01	0.31
		(SD)	(0.66)	(1.7)	(0.03)	(0.02)	(0.12)
MATURITY							
IMMATURE	5	MEAN	0.41	0.33	0.005	0.005	0.49
		(SD)	(0.53)	(0.32)	(0.006)	(0.008)	(0.16)
MATURE	24	MEAN	0.41	0.71	0.02	0.01	0.55
		(SD)	(0.59)	(1.5)	(0.03)	(0.02)	(0.61)

Levels of the various biocides determined in owls were negatively correlated with tissue lipid content though these correlations were weak and insignificant ($p < 0.05$) (Table 5). Correlations between lipid content, mercury, and PCB levels and between p,p'-DDE and total chlordane levels were positive and approached significance (Table 5). There were positive correlations between the levels of PCBs, p,p'-DDE, total chlordanes, and dieldrin found in eggs, and egg lipid contents (Table 6). There were also positive correlations between the levels of each organochlorine contaminant (e.g., between PCBs and p,p'-DDE, between PCBs and dieldrin etc.) determined in eggs (Table 6).

Table 5. Correlation between %lipid, PCB, p,p'-DDE, total chlordane, dieldrin, and mercury concentrations in Great Horned Owl tissues. The significance (p <) of any relationship is indicated.

	PCBs	p,p-DDE	TOTAL CHLORDANES	DIELDRIN	MERCURY
LIPID	-0.31 (0.11)	-0.22 (0.25)	-0.27 (0.16)	-0.04 (0.85)	-0.35 (0.09)
PCBs		0.69 (0.0001)	0.26 (0.17)	0.18 (0.34)	0.71 (0.0001)
p,p-DDE			0.33 (0.08)	0.48 (0.008)	0.73 (0.0001)
TOTAL CHLORDANES				0.46 (0.01)	0.49 (0.01)
DIELDRIN					0.56 (0.004)

Table 6. Correlation between PCB, p,p'-DDE, total chlordane, dieldrin, and mercury concentrations in Great Horned Owl eggs. The significance (p <) of any relationship is indicated.

	p,p-DDE	TOTAL CHLORDANES	DIELDRIN
PCBs	0.99 (0.0001)	0.93 (0.0001)	0.94 (0.0001)
p,p-DDE		0.93 (0.0001)	0.97 (0.0001)
TOTAL CHLORDANES			0.92 (0.0001)

Chapter V

DISCUSSION

5.1 SIGNIFICANCE OF PCB RESIDUES

The PCB levels present in the Great Horned Owls analyzed are not likely to have any adverse impact on the population. Since the literature contains no information on the susceptibility of Great Horned Owls to PCBs or any of the other chemicals sought, data for other birds are being used to determine the owls' possible susceptibility.

A number of researchers have examined the acute and chronic effects of PCBs on birds. It is quite apparent that PCBs (mean-0.66, maximum-6.9 ppm wet weight) pose no acute threat to the birds analyzed. Seventy days on a diet containing 144 ppm Aroclor 1254 caused no mortality in White Pelicans (Greichus et al. 1975) while 84 days exposure to a dietary dosage of 150 ppm Aroclor 1242 caused no increases in Mallard mortality (Haseltine and Prouty 1980). The exposure levels in the above studies were much higher than those the owls examined in this study would have been exposed to, as dietary levels of 3 ppm Aroclor 1248 only produced whole body levels of 12.8 ppm in Eastern Screech Owls (McLane and Hughes 1980).

It is very unlikely that Great Horned Owls are experiencing reduced reproductive success due to their PCB loads. The highest concentrations found in eggs from the 1960s and 1980s (14 and 2.4 ppm) are far lower than the levels associated with reduced reproduction in Bald Eagles -19 ppm- (Wiemeyer et al. 1984), White-tailed Sea Eagles -40 ppm- (Helander et al. 1982), or Northern Gannnets -30 ppm- (Elliott et al. 1988). These researchers pointed out that high p,p'-DDE levels made a cause and effect relationship difficult to establish.

Eastern Screech Owls, which accumulated adult whole body levels of 12.8 ppm and produced eggs with mean levels of 7.12 ppm after exposure (for 2 breeding seasons) to 3 ppm Aroclor 1248, (McLane and Hughes 1980) reproduced normally. These levels were higher than both the mean and maximum post-DDT egg (0.98 ppm and 2.4 ppm) and tissue (0.66 ppm and 6.9 ppm) levels determined in this study. Dietary levels of 0.5 ppm of Aroclor 1254 or 1260 induced MFOs and thereby promoted the metabolism of estradiol in American Kestrels (Lincer and Peakall 1970). The more contaminated owls analyzed in this study could have been exposed to similar PCB levels and therefore be affected in a similar way. The studies discussed above indicate that Great Horned Owl reproduction is not impaired by this exposure.

Some of the PCB congeners which are present in commercial Aroclor mixtures, and accumulate in birds and other

wildlife, induce MFOs in laboratory animals (birds and mammals) (Kubiak et al. 1989, Tanabe 1988, Safe et al. 1985). Kubiak et al. (1989) attributed much of the reproductive impairment experienced by a Lake Michigan Forster's Tern colony contaminated by dioxins (including TCDD), PCBs, and a variety of organochlorine insecticides, to MFO inducing PCBs. Two pentachlorinated PCBs (congeners 105 and 126) accounted for over 90% of the median TCDD equivalents in the egg samples.

MFO inducing congeners were present in the samples analyzed using HRGC (Figure 7). Congener 105 (0.35% of the samples analyzed by HRGC), which contributed 2.5 to 3% to the total PCB load but 30% of the TCDD equivalents to Green Bay Forster's Tern eggs (Kubiak et al. 1988), is a potent MFO inducer. Other MFO inducing congeners found in samples analyzed by HRGC include 118 (3.7% of the PCB total), 128 (0.65%), 138 (9.81%), 156 (2.71%), 158 (1.01%), and 170 (4.82%). Congeners 118 and 138 are also present in significant quantities in other wildlife and human samples (Kubiak et al. 1989, Focardi et al. 1988, Safe et al. 1985). Since the samples analyzed by HRGC were those most highly contaminated with PCBs, other less contaminated samples were unlikely to contain a higher quantity of MFO inducing PCBs.

As most of these congeners did not make a significant contribution to the 2,3,7,8-TCDD equivalents in Green Bay Forsters' Tern it follows that they were not adversely

impacting the owls in this study. However, knowledge about levels of the toxic coplanar congeners is necessary before the importance of MFO inducing PCBs present in owls or other birds can be assessed properly. Whether highly toxic coplanar congeners such as 126 are present at significant levels in Great Horned Owls is not known since these samples were analyzed for total PCBs rather than for specific congeners. It seems unlikely that the more toxic congeners pose a significant threat to owls given their generally low total PCB loads though preferential bioaccumulation of toxic congeners by terrestrial predators such as owls cannot be ruled out.

PCB muscle levels in Manitoba Great Horned Owls varied from 0.002 to 6.9 ppm (arithmetic mean 0.66 ppm, geometric mean 0.2). These levels are similar to PCB levels reported in birds from relatively unindustrialized parts of North America and Europe. Breast muscle PCB levels present in Great Horned Owls from Florida (0.59 and 0.83 ppm) (Sundlof et al. 1986) and Eagle Owls from Norway (mean-0.5 ppm) (Froslie et al. 1986) were similar to those in Manitoba Owls. Levels in Manitoba Great Horned Owls are lower than PCB residues present in raptors from more contaminated, industrial locales. Great Horned Owls collected in Illinois carried mean levels of 25.29 ppm (Seidensticker and Reynolds 1986) while Long-eared, Tawny, and Barn Owls collected in Belgium between 1972 and 1982 had median muscle PCB levels

of 1.82, 4.31, and 7.6 ppm respectively (Joiris and Delbeke 1985).

The geometric mean PCB level present in the post-DDT era eggs analyzed during this study (0.69 ppm) is below those found in eggs of most of the species of aquatic birds collected in Manitoba during 1986 and 1987 (DeSmet 1988). PCBs were not detected (detection limit < 0.8 ppm) in the American Kestrel, Merlin, Swainson's Hawk, and Great Grey Owl eggs tested. Both DDT era (25.9 ppm lipid weight) and post-DDT era (12.9 ppm lipid weight) owl eggs are substantially less contaminated by PCBs than Red-necked Grebe eggs collected in Turtle Mountain Provincial Park during 1981. These contained mean PCB levels of 194 ppm-lipid weight (DeSmet 1987). Great Horned Owl eggs are also less contaminated than are Bald Eagle eggs from Minnesota and Wisconsin (geometric mean PCB levels were 2.7 and 3.0 ppm respectively) (Wiemeyer et al. 1984). This difference in contamination is probably a reflection of these birds' feeding niches as well as the regions they inhabit.

5.2 SIGNIFICANCE OF DDE RESIDUES

Laboratory and field studies of a number of bird species suggest that the p,p'-DDE residues present in Great Horned Owls and their eggs are not adversly affecting the population. Lethal body levels (35,300-63,100 ppm-lipid weight basis) (Stickel et al. 1984) are between 3 and 4

orders of magnitude higher than the highest level found in this study.

Levels in the eggs collected during the 1980s were not high enough to have skewed the mean by impairing hatching. Mean p,p'-DDE levels in post-DDT era eggs analyzed in this study were 0.69 ppm while the highest recorded was 1.8 ppm (Table 3). These levels are similar to those present in apparently viable Great Horned Owl eggs (0.74 ppm) (Seidensticker and Reynolds 1971) and below those at which Screech Owl productivity is unaffected (1.21 ppm) (Klaas et al. 1976). The geometric mean p,p'-DDE level present in post-DDT eggs (0.42 ppm) is below that at which reproduction is impaired in

1. Cooper's Hawks (6-8 ppm) (Pattee et al. 1985),
2. Bald Eagles (> 3.5 ppm) (Wiemeyer et al. 1984),
3. Brown Pelicans and Prairie Falcons (2.5 ppm) (Blus et al. 1972, Fyfe et al. 1976), and
4. Ospreys (2.0 ppm) (Wiemeyer et al. 1988).

Unless Great Horned Owls are as or more sensitive to p,p'-DDE than the most vulnerable species noted above it is unlikely that the population as a whole is experiencing any reproductive impairment though the most contaminated egg could have been thinned by about 8% assuming a sensitivity similar to that of Ospreys.

If Great Horned Owls are as susceptible as Bald Eagles, Ospreys, and Prairie Falcons, p,p'-DDE probably caused reproductive impairment in the population during the 1960s. DDE levels in addled eggs collected in Saskatchewan during the late 1960s ranged from 1.5 to 13 ppm with a mean of 4.4 ppm (Table 3). It should be noted that this mean may not be representative of the p,p'-DDE levels prevailing in owl eggs from that period since hatching in the eggs containing 3.5, 4.5, and 13 ppm p,p'-DDE may have been impaired by those levels. The actual population mean could have been lower than that indicated by this study.

Geometric mean p,p'-DDE levels in the post-DDT eggs analyzed during this study were substantially lower (0.42 ppm) while those collected during the late 1960s (geometric mean-3.22 ppm) had somewhat higher levels than eagle eggs from Minnesota (2.5 ppm) and Wisconsin (2.2 ppm) (Wiemeyer et al. 1984). DDE levels in post-DDT eggs were 20 or more fold lower than those in eggs laid by eagles inhabiting the more industrial, contaminated eastern U.S. states (Wiemeyer et al. 1984), and Peregrine Falcons nesting in Alaska and Greenland but wintering in Central or South America (Springer et al. 1984).

Geometric mean p,p'-DDE levels in post-DDT eggs (0.42 ppm) were lower than those found in the eggs of most of the aquatic bird species collected in Manitoba during 1986-1987 (DeSmet 1988). Geometric means were lower in the Great Gray

Owl (0.04 ppm), American Kestrel (0.05 ppm), Swainson's Hawk (0.18ppm), and Short-eared Owl (0.31 ppm).

The mean and highest p,p'-DDE levels present in the owl carcasses analyzed in this study (0.41 and 2.6 ppm) are well below those present in Great Horned Owls collected in Illinois (mean-4.48, range-0.04-18.49 ppm) (Havera and Duzan 1986). Great Horned Owls from southern Manitoba carry p,p'-DDE burdens similar to those carried by raptors from other relatively uncontaminated locales. Eagle Owls, Tawny Owls, and Golden Eagles collected in Norway during 1983 carried mean liver p,p'-DDE residue levels of 0.8, 0.2, and 0.4 ppm respectively (Froslie et al. 1986).

5.3 SIGNIFICANCE OF CHLORDANE AND DIELDRIN RESIDUES

It is apparent that neither heptachlor epoxide or oxychlordane adversely affected Great Horned Owl reproduction or adult survival during the DDT or post-DDT era. Mean total chlordane egg levels were 0.3 ppm in the 1960s eggs (maximum-0.9 ppm) and 0.1 ppm (geometric mean 0.07 ppm) in the eggs collected during the 1980's (Table 3). While American Kestrel productivity decreased at egg heptachlor epoxide levels of 1.5 ppm (Henney et al. 1983), Cooper's Hawk reproduction was unaffected at total chlordane levels up to 0.89 ppm (Pattee et al. 1985). Reproduction in Bald Eagles was unimpaired by oxychlordane concentrations of 0.6 ppm (Wiemeyer et al. 1984). The levels present in post-

DDT eggs are comparable to those reported in Bald Eagle eggs collected in Minnesota (0.03 ppm) and Wisconsin (0.08 ppm) during the late 1970s (Wiemeyer et al. 1984). They are also comparable to the heptachlor epoxide levels found in a Ferruginous Hawk egg (0.07 ppm), a Merlin egg (0.05 ppm), and the eggs of aquatic birds collected in Manitoba during 1986 and 1987 (DeSmet 1988).

Total chlordane tissue levels were low (mean 0.02 ppm, range 0.0007-0.1 ppm, Table 2). These levels are well below those present in Great Horned Owls from Illinois (heptachlor epoxide residues-1.11 ppm) (Havera and Duzan 1986). They are also less than levels of heptachlor epoxide (0.41 ppm) and oxychlordane (0.15 ppm in whole blood) which did not affect Ospreys (Wiemeyer et al. 1980).

The mean and maximum dieldrin levels present in the eggs analyzed (DDT era-0.1 and 0.4 ppm; post DDT era-0.03 ppm, Table 3) were well below those which affect reproduction in other species.

Egg dieldrin levels above 1 ppm impaired reproduction in Golden Eagles (Lockie et al. 1969) and Brown Pelicans (Blus et al. 1982). Wiemeyer et al. (1984) suggested that levels above 1 ppm may also affect Bald Eagle reproduction. White-tailed Sea Eagle (Helander et al. 1982) and Sparrowhawk (Newton and Bogan 1978) reproduction was unimpaired by egg dieldrin levels of 0.44 and 1.5 ppm, respectively. Dieldrin

levels of 8.1 ppm produced statistically significant but biologically insignificant shell thinning (i.e., the egg shell thinning produced was of a magnitude which did not appear to affect reproduction) in Barn Owls (Mendenhall et al. 1983).

Brain dieldrin levels of 4-5 ppm are generally associated with adult mortality (Linder et al., 1970 Stickel et al. 1969). Wiemeyer et al. (1980) associated carcass levels of 1.3 ppm with brain levels of 3.8 ppm. The mean tissue dieldrin levels carried by the owls analyzed in this study are well below that level (0.01 ppm) while the highest level found (0.06 ppm) is also very low. Mean and maximum dieldrin levels found in these owls are lower than those found in Great Horned Owls from Florida (0.13 and 0.6 ppm) (Sundlof et al. 1986) and Illinois (1.1 ppm) (Havera and Duzan 1986). Short-eared Owl and Merlin eggs from Manitoba (DeSmet 1988) and Eagle, Tawny, Long-eared, and Hawk owls collected in Norway (Froslie et al. 1986) all carried levels comparable to those found in this study.

5.4 SIGNIFICANCE OF MERCURY RESIDUES

The total mercury (organic and inorganic) levels present in Manitoba owls appear to be below those which cause adverse impacts in birds. Though the mercury levels include both the organic and inorganic mercury present in a bird's liver it appears reasonable to assume that most of the

mercury present is in the more toxic methylated form. Inorganic mercury is poorly absorbed while the absorption of the lipid soluble MeHg approaches 100% (Berglund and Berlin 1969). MeHg's half-life in birds varies between 2-3 months while other organomercurials and inorganic mercury have half-lives of 1-2 weeks (Stickel et al. 1977, Westermark et al. 1974).

Over 90% of the total mercury present in Atlantic Puffin, Fulmar and Manx Shearwater livers consisted of MeHg (Osborn et al. 1979). Most of the mercury in feathers of a variety of Swedish raptors and owls consisted of MeHg (Westermark et al. 1974) while Vermeer et al. (1973) found MeHg constituted 69-99% of the mercury present in breast muscles of a variety of duck species collected in the Canadian Prairies.

White-tailed Sea Eagle reproduction was unaffected by egg mercury levels up to 1.09 ppm (Helander et al. 1982). These levels appear to correspond to adult liver mercury levels of 5 ppm or more (Barrett et al. 1985, Fimreite 1971, Fimreite et al. 1970). The mean liver mercury concentration determined in this study (0.54 ppm) is below the level which affects adult birds. The mean is also below the level which might cause reproductive problems in these owls. The highest liver Hg level found in an owl was 2.4 ppm, a level below that at which Red-tailed Hawk and Mallard adults, and Ring-necked Pheasant reproduction, appeared to be affected (Heinz 1974, Fimreite 1971, Fimreite and Karstad 1971).

Mean liver mercury concentrations in Manitoba Great Horned Owls (0.54 ppm) are well below those found in Short-eared (6.84 ppm) and Burrowing Owls (3.74 ppm) collected in Alberta (where use of mercury treated seed was common up to the early 1970s) during the late 1960s. Great Horned Owls from Saskatchewan were less contaminated (mean liver mercury level-0.076 ppm) during the 1960s than the Manitoba Great Horned Owls are now (Fimreite et al. 1970).

Manitoba Great Horned Owls carry liver mercury loads similar to those carried by Eagle, Tawny, Barn, and other owls from Norway (range from 0.1 to 1.1 ppm) (Froslie et al. 1986) and England (mean-0.4 ppm) (Stanley and Elliot 1975). These and other studies (Wiemeyer et al. 1980, Stanley and Elliot 1975, Fimreite et al. 1970) suggested that owl and raptor liver mercury concentrations below 1 ppm are attributable to background contamination. It appears that only 3 of the 25 owls analyzed for Hg in this study were exposed to mercury levels above background. As might be expected, Manitoba owl mercury residue levels are much lower than those found in Alberta owls during the late 1960s when mercury treated seed was used on up to 75% of Alberta's agricultural lands (Fimreite 1970).

The generally low mercury levels present in owls are expected given that mercury levels in Prairie Falcons and Merlins decreased between the late 1960s and mid-1970s as a result of restrictions on the use of organo-mercurials for

seed dressing (Fyfe et al. 1976). A ban on MeHg seed dressings was followed by decreased mercury levels in Swedish birds within 2-3 years (Westermark et al. 1975).

5.5 SOURCE OF RESIDUES

Mean PCB, p,p'-DDE, heptachlor epoxide/oxychlordane, dieldrin, and mercury levels present in Manitoba Great Horned Owls and the levels of organochlorine contamination found in addled eggs from Saskatchewan indicate that these owl populations are not threatened by these contaminants. The low levels of contamination are indicative of low levels of contamination in the Great Horned Owl's food chain. Being basically non-migratory, Manitoba Great Horned Owls are not directly exposed to the elevated contaminant levels found in parts of Latin America (DeWeese et al. 1986, Henney et al. 1982) or the more industrial parts of the United States (Wiemeyer et al. 1988, Wiemeyer et al. 1984).

Great Horned Owls are opportunistic feeders preying on mammals such as hares, mice, and voles, and a variety of birds (grouse, waterfowl, and other birds). A study carried out in central Alberta, (McInvaille and Keith 1974) found Snowshoe Hares (Lepus Americanus Erexleben) formed the bulk of the Great Horned Owls' prey biomass (81%) when the hares were at or near the peak of their population cycle. The percent biomass composed of mammals ranged from 52-90% while birds made up between 10 and 42% (mean over 6 years-24.8%).

The importance of birds as prey items increased as Snowshoe Hare availability decreased. Houston (1987) found that Great Horned Owl productivity in Saskatchewan was nearly synchronous with Snowshoe Hare numbers.

The relatively low levels of contamination found in this study are not surprising considering this species' reliance on non-migratory mammalian food sources. The more highly contaminated individuals may have had a higher percentage of migratory birds in their diet than did other owls. A number of researchers (Joiris and Delbeke 1985, Lincer et al. 1970) reported higher biocide residue levels in species consuming higher proportions of birds, particularly if these birds migrated to Latin America (DeWeese et al. 1986), than in those which preyed primarily on mammals.

The eggs examined in this study were generally less contaminated with PCBs, p,p'-DDE, and dieldrin than were the eggs of the migratory aquatic birds recently analyzed by the Manitoba Department of Natural Resources (DeSmet 1988). This difference in contamination reflects both the owl's non-migratory nature and its terrestrial, primarily mammalian food chain. Biocides are more readily biomagnified in aquatic food chains than in terrestrial food chains since uptake of contaminants occurs via absorption through the gills as well as from food (Shaw and Connell 1986).

5.6 EFFECT OF MUSCLE LIPID AND MOISTURE LEVELS ON RESIDUE CONCENTRATIONS

The concentrations of PCBs, p,p'-DDE, and/or mercury present in the most highly contaminated birds were in part a reflection of their low muscle and whole body lipid levels. Of these individuals (numbers 10, 17, 22, and 25) only owl #10 was small and immature. All 4 had extractable lipid levels below the mean while owls 10 and 25 had lipid levels about 5 1/2 times less than the average (Table 1). They all lacked subcutaneous fat and had no fat deposits around their hearts (Table 1). A number of researchers (Mora et al. 1987, Anderson et al. 1984, Frank et al. 1983, Capen and Leiker 1979, Bogan and Newton 1977, Söderdgren and Ulfstrand 1972) noted substantial increases in body residue concentration as a bird's body lipid levels decreased. Though liver lipid levels were unchanged despite a mean 4 1/2 fold breast muscle lipid level decrease in emaciated loons, mean liver mercury contents also increased (by a factor of 3) (Frank et al. 1983).

Though individual low body lipid and therefore high contaminant concentrations serve to skew the mean population concentration of contaminants upwards they are also indicative of residue concentrations when individuals are faced with other substantial environmental stresses. These "worst case scenarios" allow speculation as to whether a biocide could affect an individual bird during winter, its

breeding season, or during migration, i.e., those periods when food intake may fall and an individual's physical condition deteriorates (Mora et al. 1987, Anderson et al. 1984). Tissue moisture levels were similar (Table 1) indicating that moisture loss did not artificially increase detected residue levels. Whether the eggs had undergone moisture loss (and therefore biocide concentration) before they were collected is unknown.

5.7 CORRELATIONS BETWEEN CONTAMINANTS

Numerous authors (Elliott et al. 1988, Wiemeyer et al. 1988, DeSmet 1987, Wiemeyer et al. 1984) reported significant correlations between levels of various residues in wildlife samples. Of the contaminants determined in owl breast muscles, PCB, p,p'-DDE, and mercury show significant positive correlations with one another as do dieldrin, total chlordanes, and mercury (Table 5). All the biocides measured were negatively correlated with lipid levels though none of these correlations were significant. PCB, p,p'-DDE, dieldrin, and heptachlor epoxide/oxychlordane concentrations were positively correlated with one another in eggs (Table 6).

5.8 RESIDUE LEVEL TRENDS

There were no significant differences between mean breast muscle contaminant levels in owls found in different years (Figure 10). Though there appeared to be large differences between mean PCB and p,p'-DDE contamination in different years, these apparent differences were often negated by standard deviations which were larger than the mean. The very small sample sizes also served to make differences between years difficult to detect. Since Barrett et al. (1983), failed to find significant decreases in contaminant levels between samples collected from 1972 to 1983 it was not surprising that no evidence of decreasing organochlorine levels was found in these samples, all of which were collected during the 1980s.

Though a slow rate of decrease in levels of organochlorines may be expected over time, mercury levels, which appeared to be at background, could remain unchanged over the long term. Mercury levels carried by Swedish raptors and other birds decreased rapidly (within 2-3 years) after MeHg was banned from use as a seed dressing (Westermark et al. 1974). Levels in inland Swedish Eagle Owls had decreased to those present in feathers collected before mercury came into use as a seed dressing within 5 years of MeHg being banned (Bröö and Odsjo 1981).

While none of the organochlorines determined in the late 1960s and 1980s eggs decreased significantly ($p < 0.05$) if all samples were included when means were calculated, trends in p,p'- DDE ($p < 0.06$), total chlordane ($p < 0.1$), and dieldrin ($p < 0.07$) levels approached significance (Table 3). PCB levels did not decrease from the late 1960s to the 1980s ($p < 0.2$) though the large sample variance may have masked any decrease. As indicated in chapter 4, p,p'-DDE levels underwent a significant decrease ($p < 0.006$) if sample 3784 (Table 3) was treated as an outlier when mean pre- and post-DDT egg residue levels were compared. Neither PCBs, total chlordanes, or dieldrin levels decreased if means were calculated in this way. Substantial variation between samples and the small sample size made it difficult to find trends. Sample sizes would have to be increased (perhaps doubled to 12 from each era) in order to allow more definite conclusions regarding residue level trends to be made.

Decreases between the DDT and post-DDT era levels of p,p'-DDE, PCBs, dieldrin, and other organochlorine contaminants are noted in the literature. While Moksnes and Norheim (1986) noted significant decreases in Herring Gull egg p,p'-DDE, levels the same was not true for PCBs. DDE, PCB, dieldrin, and oxychlordane levels decreased in Northern Gannets between 1968 and 1984 while heptachlor epoxide levels remained unchanged (Elliott et al. 1988). DDE levels

decreased among Bald Eagles nesting in Northern Ontario from 1967 to 1981 though PCB levels stayed about the same (Grier 1982).

The PCB/DDE ratio changed from 0.89 in the DDT era eggs to 1.4 in the post-DDT era eggs reflecting the decrease in p,p'-DDE levels relative to PCBs. Increases in the PCB/DDE ratio were also found in White-tailed Sea Eagle eggs (Helander et al. 1982), Herring Gull eggs (Moksnes and Norheim 1986), and Bald Eagles (Grier 1982). PCB/DDE ratios in Northern Gannets changed from 1.1 in 1968 to 8.52 in 1984 (Elliott et al. 1988). Increases in the PCB/DDE ratio are expected following the Canadian and American ban on DDT use during the early 1970s (Wiemeyer et al. 1980). Though PCB production was restricted during the mid-1970s, environmental PCB levels can only be expected to decrease slowly since substantial sources still exist (Tanabe 1988). PCB/DDE ratios in the aquatic birds eggs collected in Manitoba during 1986 and 1987 (DeSmet 1988) were often higher than those present in the post-DDT owl eggs analyzed during this study. This is probably a reflection of the migratory nature of most of the birds analyzed during that study.

5.9 EFFECTS OF SEX AND MATURITY ON RESIDUE LEVELS

There were no significant differences between the contaminant levels found in males and those present in females. Differences are reported in the literature with females carrying lower levels. Female birds often carry lower levels because they can excrete organochlorines and mercury in the fat they add to eggs (Braune and Gaskin 1987, Mendenhall et al. 1983, Newton and Bogan 1978). Since most of the owls were collected in late fall, winter, and early spring it is possible that levels of contaminants had risen since the previous breeding season to obscure any differences. Great Horned Owls may not be sufficiently contaminated to make a small difference between the sexes (resulting from egg laying) detectable. Three of the 4 owls most contaminated with p,p'-DDE and all 3 of the samples carrying over 1 ppm PCBs and mercury were female and had low levels of body fat. These birds may have skewed the residue levels found in females making any difference undetectable.

The literature reports that organochlorines undergo age dependent accumulation (Niethammer et al. 1986, Newton et al. 1981). Whether mercury also accumulates as adults age is subject to debate but accumulation between juveniles and adults has been demonstrated (Furness et al. 1990, Braune and Gaskin 1987, Honda et al. 1986a). No age dependent accumulation was detected in this study but only 5 of the 29 owls analyzed were juveniles. The sample size in this study

may have been too small to reveal any differences which might exist in the general population. The sample size would have to be increased substantially to determine whether there is any difference in contamination between males and females or between immature and mature birds.

5.10 IMPLICATIONS OF SAMPLING AND LONGTERM STORAGE

The carcasses analyzed in this study were primarily road killed. Though this is not the preferred way of obtaining samples for trace analysis it is common in studies which are monitoring levels present in birds of prey (Sundlof et al. 1986, Delbeke et al. 1984, Frank et al. 1983, Kaiser et al. 1980, Wiemeyer et al. 1980). One cannot assume that road killed birds are representative of the population since they are collected non-systematically. It is difficult to determine the type of habitat occupied and therefore the prey species a bird is likely to have relied on. Different habitats can cause significant intra-species differences in contaminant loads (Joiris and Delbeke 1985, Bröö and Odsjo 1984). These differences occur if one area is significantly more contaminated than another or if food sources of birds resident in different habitats are differentially contaminated.

As samples were collected non-systematically, it is unknown whether a bird had occupied a predominantly forested, agricultural or other habitat. The Great Horned

Owl's principal food source changes from hares in forested regions, to voles and mice in agricultural environments. Predation on migratory waterfowl, which could be a significant source of contaminants, has not been correlated with any particular habitat (McInvaille and Keith 1974). Since the principal food source remains mammalian there should not be a substantial difference in biocide uptake between owls occupying adjacent forested and agricultural regions. Given the mechanisms by which organochlorines and PCBs are dispersed in the environment (primarily via atmospheric transport though some organochlorines and mercury may be residual in a region as a result of past use) (Tanabe 1988, Westermark et al. 1974, Woodwell et al. 1971), there is little likelihood that some regions of southern Manitoba are significantly more contaminated than others. Since road killed birds (which probably nest and feed within 1-2 Km of a road (McInvaille and Keith 1974)) are likely exposed to similar levels of contamination to those nesting further from roads, the owls analyzed in this study should have been representative of organochlorine and mercury contamination in agricultural Manitoba.

As road-kill collection is dependent both on birds being present and on the traffic volume of the road, more populous regions are likely to be overrepresented. This is the case in this study since most carcasses were collected in southern Manitoba (Figure 1). This study may be more

representative of contaminant levels in owls from agricultural Manitoba than in the province as a whole. Differences in contamination could be reasonably expected as one moves further from the sources (agricultural/industrial regions) of organochlorine and mercury contamination. Great Horned Owls inhabiting the northern boreal forest should therefore be less contaminated than those living in southern Manitoba, all other factors being equal.

Birds undergo substantial changes in body composition during the year (Mora et al. 1987, Anderson et al. 1984, Frank et al. 1983, Capen and Leiker 1979). Sample collection should, under ideal circumstances, be representative of seasonal changes in body fat levels. The samples analyzed in this study were primarily collected during fall and winter, as more carcasses were available from that time of year and since it was assumed that carcasses collected during cold months had experienced minimal decay. Since birds collected during winter are probably under cold and nutritional stress their body lipid stores are likely lower than at other times of the year. If this is the case, levels determined during this study may overestimate average yearly residue burdens. Contamination levels determined in this study should reflect residue loads at the most stressful time of year. This information is valuable since one can then estimate the maximum hazard a population is exposed to.

Seasonal changes in the diet of a predator could affect its contaminant uptake. The diet of the Great Horned Owl probably undergoes a seasonal shift since migratory birds are not available during late fall/winter. Whether this shift is significant in terms of the population's exposure to contaminants is unknown. The uncertainty introduced by prey availability could be reduced if samples were available from throughout the year.

The proportion of females killed and analyzed is higher than that of males but this did not affect the results since males and females were similarly contaminated. While 24 of 29 owls analyzed were mature, the results were unaffected by this factor. Given the relatively low residue levels present in these owls it is unlikely that the contaminant load of any bird impaired it sufficiently to cause it to hit a car, or meet its demise in any other manner. Sampling was presumably not skewed towards highly contaminated birds by virtue of their level of contamination.

The degradation study found no evidence of significant PCB or organochlorine insecticide breakdown over 3 days of exposure to 20° C. This did not necessarily mimic conditions under which post-mortem organochlorine breakdown might have occurred since a threshold temperature above 20° C might be necessary to stimulate rapid organochlorine breakdown. However, most of the carcasses (23 of 29) analyzed were picked up between mid-October and mid-March

(cooler months) while 6 were found between April and July. The majority of samples should therefore not have undergone a substantial amount of decay before being frozen.

Despite the apparent stability (under conditions of the degradation study) of the biocides considered during this study, one cannot be certain about the stability of these residues when samples are stored for years. DDT would probably have undergone considerable breakdown while p,p'-DDE (a DDT breakdown product) levels might have increased (Norstrom and Won 1985, Ecobichon and Saschenbrecker 1967). Degradation of PCBs, p,p'-DDE, hexachlorobenzene, mirex, oxychlordane, heptachlor epoxide, and dieldrin should have been minimal (especially in the eggs) (Norstrom and Won 1985).

Though few other substrates have been reported on to date, some degradation might be expected in muscle and other tissues which have substantial blood supplies. The extent of degradation is probably primarily dependent on the amount of hemolysis and general cellular disruption a sample underwent before being frozen. If cellular decay prior to and during storage is sufficient to release significant quantities of reduced coenzymes and other reactive compounds (Ecobichon and Saschenbrecker 1967), organochlorine breakdown might occur. Substrates such as lipid deposits, which are not normally as metabolically active as liver or muscle, might contain fewer compounds capable of catalyzing

post-mortem organochlorine breakdown. Such matrices might be more suitable for analysis if samples are archived for a long period though sampling fat deposits presents other problems (Chapter 2.6.3).

While indicating the need for research concerning the long term stability of organochlorines in substrates such as muscle, Norstrom and Won (1985) concluded that storing biological materials at low temperatures probably results in minimal degradation of the chemically stable organochlorines. While the levels of some contaminants may have decreased over time, those determined in this study should not have undergone dramatic degradation.

5.11 ASSESSMENT OF ANALYTICAL TECHNIQUES

As indicated in chapter 3.5, the owl carcasses were initially analyzed at the TSL. The analytical problems encountered resulted from the application of an inappropriate technique to a matrix which was very difficult to clean up. Lipid contents of some samples were high enough that application of the equivalent of 10 g of sample extract (up to 320 mg of lipid) would have overloaded a 10 g florisil clean up column. A substantial amount of lipid would have found its way into the final extract and caused considerable GC contamination. Even samples which did not apparently overload the florisil clean up column could have contaminated the GC, as was subsequently discovered during

re-analysis. The quantity of lipid (extracted from 5 g samples) which eluted with the relatively more polar insecticides (heptachlor epoxide, oxychlordane, and dieldrin) was sufficient to contaminate a megabore column equipped GC unless the sample was diluted considerably.

The experience at the TSL made it evident that a technique could not be applied without first evaluating its suitability to the sample to be analyzed. It became evident that quality assurance can be a problem when samples are submitted to a laboratory for analysis despite the declared adherence to an internal quality assurance program. Analysis of tissues for organochlorines requires that appropriate procedures be developed and followed consistently. It is difficult to be confident that samples are being analyzed properly unless a researcher exercises a certain amount of control over the analyses.

The TSL's analytical difficulties prompted the evaluation of a number of sample clean up methods by the author. Evaluation of available methods extended the duration of the study considerably. It was initially assumed that extracts could be cleaned up appropriately simply by ensuring that the adsobent column was not overloaded with lipid. Extracts with lipid contents calculated not to overload a clean up column were eluted through both florisil and alumina columns with a solvent system which removed the polar insecticides. Lipids eluted in both the PCB (fraction 1) and the polar

insecticide (fraction 2) fractions but only those in fraction 2 contaminated the GC. Use of 2 adsorbent columns with the first acting to remove the bulk of the lipid was also attempted.

Various combinations of alumina, florisil, reverse phase C-18, and silica gel columns were used but lipids with a polarity similar to that of the chlordanes and dieldrin co-eluted with these insecticides. A freeze-out technique reduced total sample lipids but did not remove the polar lipids of concern. Liquid-liquid partitioning (Tessari et al. 1980) was combined with florisil column clean up but did not remove more lipid than did a single adsorbent column and required considerably more time and solvent. Neither saponification or thin layer chromatography were capable of removing the coextractives.

The method used to analyze Great Horned Owls and their eggs for organochlorine contaminants was a variation of a standard method (Holden and Marsden 1969) adapted to deal with the lipid levels present in these tissues. The single alumina clean up column used in this study did not remove co-extractives from fraction 2 in a satisfactory manner. The second fraction was therefore diluted 8-9 times, depending on a sample's lipid content, to ensure that injections could be made without adversely affecting the GC. Reasonable detection limits were maintained by analyzing fraction 2 using the analytical system's maximum sensitivity.

It is evident that adsorbent and polarity based clean up techniques are not in and of themselves appropriate for removing the co-extractives present in these tissues. Gel permeation chromatography (Tessari et al. 1980) is the method of choice for removing the bulk of the lipid present in a sample. Its capacity for lipids extends the life of both the GC column and the detector, improves the quality of chromatograms and therefore the accuracy of quantification and improves detection limits since sample sizes can be increased if deemed necessary. Further clean up using columns packed with an adsorbent also allows the sample to be fractionated. Identification and quantification of compounds is simplified if PCBs are separated from most of the other organochlorines and if toxaphenes (if present) are separated from PCBs. Samples analyzed in this study were fractionated to separate PCBs and p,p'-DDE from the chlordanes and dieldrin. Further fractionation is desireable to separate co-eluting compounds such as heptachlor epoxide and oxychlordane.

If PCBs are expected to be present in a sample the gas chromatograph should be equipped with a high resolution capillary column. Megabore columns, such as that used in this study, are not capable of resolving the complex mixtures of organochlorines found in biological samples. Various PCBs and pesticides co-elute making proper identification and quantification of compounds present at

low levels difficult or impossible. HRGC is also necessary if quantification of total PCBs is to be improved by reference to individual congeners rather than a few select peaks. PCB quantification using peak 138, which is present in approximately equal amounts in Aroclors 1254 and 1260, can exaggerate PCB levels by up to 100% (R. Turle 1988 pers.commun.) Congener specific quantification is also necessary if an accurate assessment of PCB toxicity to an organism is to be made since toxicity of individual congeners varies considerably (Maack and Sonzogni 1988, Tanabe 1988, Safe et al. 1985). Identification of congeners is also neccesary to monitor the fate of PCB mixtures (Van der Oost et al. 1988). Coplanar PCBs are normally present in samples at very low levels but should be identified to improve PCB toxicity assessment.

The low levels of dieldrin and heptachlor epoxide/oxychlordane detected in samples analyzed during this study suggest that while levels of these compounds can be monitored as part of routine analysis for other organochlorine insecticides, it may be appropriate to de-emphasize these chemicals so that others may be focused on. This does not neccesarily apply to other bird species but could be justified in the case of non-migratory, terrestrial predators such as the Great Horned Owl. Some researchers (Kubiak et al. 1989, Tanabe 1988) indicate that coplanar and other MFO inducing PCBs can pose more of a long term toxic

threat to wildlife than do dioxins or furans. The MFO inducing PCBs are less toxic than the more toxic dioxins and furans but can be present in relatively high amounts and thereby pose more of a threat to certain organisms. Toxaphenes were identified in the 2 samples analyzed by high resolution chromatography but were present at levels well below those which affect Mallard embryo and duckling survival (Hoffman and Eastin 1982, Haseltine et al. 1980). This is expected since toxaphenes have less of a tendency to bioaccumulate in terrestrial ecosystems than in aquatic ecosystems (Haseltine et al. 1980). It may therefore be of interest to analyze aquatic birds for toxaphenes.

Duplicate muscle samples, and spiked muscle and egg samples were analyzed to ensure the internal consistency of extraction and clean up procedures. Recovery studies provide a general indication of the extent to which a particular compound is being recovered. While duplicate analysis provides some basis for monitoring analytical integrity it is expensive in terms of time and materials. Internal recovery standards (added to a sample before it is extracted) are a simple, inexpensive way of monitoring the sample losses which can occur during extraction and clean up. If internal standards are combined with high and low level recovery studies the number of duplicates needed to ensure internally consistent analysis can be reduced.

Chapter VI

SUMMARY AND CONCLUSIONS

A number of raptors and fish-eating birds have experienced population declines in North America. Some of these species have not yet recovered and might still be affected by persistent environmental contaminants. Great Horned Owls are relatively abundant in Manitoba. Road killed owls had been collected since 1982 and a good selection of carcasses was available for analysis. Addled eggs had been collected in Saskatchewan, and stored in the Canadian Wildlife Service's tissue bank in Ottawa, since 1967. Twelve of these were made available for analysis. As predators positioned at the top of their terrestrial food chain, these owls should be a good indicator of the extent to which their food chain is contaminated by PCBs, organochlorine insecticides, and mercury.

The analytical problems experienced during the course of this study were considerable. HRGC revealed a number of coextractives which could have interfered with the analysis carried out on a megabore column. They have particularly affected the reliability of low level results. The analytical pitfalls presented when low levels of environmental contaminants are sought make caution advisable when interpreting the results of this or any similar study.

Analysis for PCBs, p,p'-DDE, heptachlor epoxide/oxychlordane, dieldrin, and mercury (egg samples were not analyzed for mercury) indicated that Great Horned Owl contamination levels were generally low. Mean PCB, organochlorine insecticide, and mercury levels were similar to those found in European owls but were substantially lower than those present in owls and other birds collected in more contaminated parts of the United States. Mean and maximum muscle tissue levels of organochlorines and mercury were below concentrations which have affected other species. Future studies should be more representative of the whole province. Rather than expanding sample collection efforts by taking live birds from regions from which "biologically lost" material is unavailable, blood samples could be collected and analyzed if a species is amenable to live trapping.

Mean egg PCB, p,p'-DDE, heptachlor epoxide/oxychlordane, and dieldrin concentrations (in eggs collected during the 1980s) were below those at which biologically significant eggshell thinning occurs in sensitive species. One egg had p,p'-DDE concentrations which could have caused shell thinning though the significance of any thinning would be minor unless Great Horned Owls are very susceptible. Concentrations of contaminants in the post-DDT eggs were generally lower than those present in eggs of aquatic birds and usually similar to those in raptor and owl eggs

collected in Manitoba during the latter 1980s. Owls could have experienced significant shell thinning during the 1960s as 3 of 6 eggs contained levels which cause significant thinning in sensitive species.

Neither sex or maturity affected residue levels present in Great Horned Owls. PCB, p,p'-DDE, heptachlor epoxide/oxychlordane, and dieldrin levels present in owl eggs did not decrease between 1967/68 and the early to mid-1980s though all the organochlorines, save PCBs, showed a tendency to decrease. Larger sample sizes are needed to clarify trends. The residue levels present in the non-migratory Great Horned Owls suggest that their terrestrial food chain is generally subject to low levels of contamination by organochlorine insecticides, PCBs, and mercury.

Some coplanar PCBs are present in the owls analyzed though their potential toxic significance is unclear since the most toxic of these were not quantified. Future research might consider these toxic PCBs, dioxins, and dibenzo-furans as well as other organochlorines. This is necessary so that the extent of contamination of the terrestrial environment by these chemicals can be assessed. Toxaphene, which was present at low levels in these terrestrial predators preferentially accumulates in aquatic ecosystems. Fish-eating birds could be analyzed for toxaphenes to monitor the levels of this contaminant in

Manitoba's aquatic ecosystems. Some of the more acutely toxic organophosphate and carbamate insecticides might also be monitored though (depending on the compound of concern) such a monitoring program would require that samples be analyzed soon after collection. Blood samples may be the most appropriate substrate for analysis of these relatively unstable compounds in threatened species. Mercury appears to be present at background levels in Great Horned Owls. This indicates that levels of this metal have declined to background in the owl's terrestrial food chain.

It is evident that the appropriateness of analytical techniques should be considered before techniques are used for biologically dissimilar tissues. Standard methods must be assessed in light of the nature of the sample being analyzed. Failure to do so can be very costly both in terms of time, analytical equipment, and the reliability of results. Reliable information about contamination by toxic chemicals is necessary if appropriate management decisions regarding species which might be threatened by contaminants are to be made.

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Appendix A

Common Loon - *Gavia immer* (Brünnich)

Red-necked Grebe - *Podiceps grisegena* (Boddaert)

Fulmar - *Fulmarus glacialis* (Linnaeus)

Manx Shearwater - *Puffinus puffinus* (Brünnich)

Northern Gannnet - *Morus bassanus* (Linnaeus)

American White Pelican - *Pelecanus erythrorhyncus* Gmelin

Brown Pelican - *Pelecanus occidentalis* Linnaeus

Double-crested Cormorant - *Phalacrocorax auritus* (Lesson)

Snowy Egret - *Egretta thula* (Molina)

Black-Crowned Night-Heron - *Nycticorax nycticorax* (Linnaeus)

Cackling Goose - *Branta hutchinsii* (Ridgway)

Black Duck - *Anas rubripes* Brewster

Mallard - *Anas platyrhynchos* Linnaeus

Osprey - *Pandion haliaetus* (Linnaeus)

Black-eared Kite - *Milvus migrans* (Bonaparte)

Bald Eagle - *Haliaeetus leucocephalus* (Linnaeus)

White-tailed Sea Eagle - *Haliaeetus albicilla* (Linnaeus)

Cooper's Hawk - *Accipiter cooperii* (Bonaparte)

Swainson's Hawk - *Buteo swainsoni* Bonaparte

Red-tailed Hawk - *Buteo jamaicensis* (Gmelin)

Ferruginous Hawk - *Buteo regalis* (Gray)

Rough-legged Hawk - *Buteo lagopus* (Pontoppidan)

Golden Eagle - *Aquila chrysaetos* (Linnaeus)

American Kestrel - *Falco sparverius* Linnaeus

Merlin - Falco columbarius Linnaeus

Peregrine Falcon - Falco peregrinus Tunstall

Prairie Falcon - Falco mexicanus Schlegel

Ring-necked Pheasant - Phasianus colchicus Linnaeus

Chicken (domestic) - Gallus sp.

Bonaparte's Gull - Larus philadelphia (Ord)

Ring-billed Gull - Larus delawarensis Ord

Herring Gull - Larus argentatus Pontoppidan

Red-billed Gull - Larus novaehollandiae scopulinus (Ord)

Forster's Tern - Sterna forsteri Nuttall

Atlantic Puffin - Fratercula arctica (Linnaeus)

Ring Dove - Streptopelia risoria (Linnaeus)

Mourning Dove - Zenaida macroura (Linnaeus)

Barn Owl - Tyto alba (Scopoli)

Eastern Screech Owl - Otus asio (Linnaeus)

Eagle Owl - Bubo bubo (Linnaeus)

Burrowing Owl - Athene cunicularia (Molina)

Great Gray Owl - Strix nebulosa (Forster)

Tawny Owl - Strix aluco (Linnaeus)

Long-eared -Asio otus (Linnaeus)

Short-eared Owl - Asio flammeus (Pontoppidan)

Hawk Owl - Ninox strenua (Gould)

Robin - Erithacus rubecula (Linnaeus)