1998

Patterns of Organochlorine Pesticide Contamination in Neotropical Migrant Passerines in Relation to Wintering Range and Wintering Habitat

Jeffrey A. Klemens '98
Illinois Wesleyan University

Patterns of Organochlorine Pesticide Contamination in Neotropical Migrant Passerines in Relation to Wintering Range and Wintering Habitat.

Jeffrey A. Klemens[1], R. Given Harper[1], Jeffrey A. Frick[2], Angelo P. Capparella[3], Heidi B. Richardson[1]

Department of [1]Biology and [2]Chemistry, Illinois Wesleyan University, Bloomington, IL 61702-2900

[3]Department of Biological Sciences, Illinois State University, Normal, IL 61790-4120

Abstract

Seventy-two Neotropical migrant passerines were analyzed for the presence of 17 organochlorine pesticide residues (aldrin; alpha-BHC; beta-BHC; gamma-BHC; delta-BHC; p,p'-DDD; p,p'-DDE; p,p'-DDT; dieldrin; endosulfan I; endosulfan II; endosulfan sulfate; endrin; endrin aldehyde; heptachlor; heptachlor epoxide; methoxychlor) using standard methods of gas chromatography applied to bird tissues. Individuals were collected along the Mississippi River during May 1996 and represent 11 species (Catharus ustulatus; Geothlypis trichas; Protonotaria citrea; Mniotilta varia; Contopus virens; Dendroica petechia; Empidonax minimus; Passerina cyanea; Myiarchus crinitus; Vireo gilvus). The contaminants most frequently detected were p,p'-DDE, dieldrin, and heptachlor epoxide. No significant difference was found between male and female birds, or between birds that wintered in one of two broad habitat types. Levels of all three pesticides, however, were significantly higher for those birds that predominantly winter in Northern South America than for those birds that predominantly winter in Central America and Mexico.

Introduction

Organochlorine compounds (OCs) may impair reproduction and cause developmental defects in vertebrates of many taxa (see review by Colburn et al. 1993). Industrialized and developing nations alike have released many of these compounds into the environment in great quantities over the last several decades, and, to some unknown degree, their use continues in developing nations (Mora and Anderson 1991). These compounds are highly persistent and mobile in the atmosphere; therefore , they can be incorporated into biomass far from their point of application (Rapaport 1985). Simonich and Hites (1995) recently demonstrated that organochlorine contaminants, including several pesticides, are ubiquitous in tree bark samples collected from around the world, including remote locations in South America. Standley and Sweeney (1995) documented OC contamination in tree bark and mayfly larvae from the Parque Nationale de Guanacaste in Costa Rica.

Estimating the degree to which OCs have entered the environment is extremely difficult because few data exist on their release. Wildlife assays may prove to be particularly useful in determining the extent of environmental OC contamination. In some cases, such assays can show definitively that OC contamination is occurring in an area. Also, quantifying contaminant levels in wildlife can provide information on how and to what extent a contaminant is being incorporated into the biomass of an ecosystem. This type of information is important in determining the threat the contaminant poses to organisms at all trophic levels.

The first step in designing wildlife assays is the selection of an appropriate group of study organisms. We believe that the passerine songbirds are a suitable taxon for wildlife assays for several reasons. They are middle trophic level organisms, they are widely distributed globally, and OC contamination of less than 10 ppb can be detected in individuals. Passerines from around the world are often collected for use as museum study specimens (Remsen 1995), and the carcasses can be made available for pesticide analysis.

Neotropical migrants (passerines that winter in the Caribbean, Mexico and Central and South America but breed in the United States and Canada) are of particular interest as study organisms. First, we believe that OC contamination is widespread in Neotropical migrants. In a previous study we detected contamination in 19 of 21 individuals representing nine species (Harper et al. 1996). Due to this high occurrence of contamination and the diversity of wintering habitats and localities represented by Neotropical migrants, they can provide a great deal of information about patterns of OC contamination throughout the Americas. In addition, the use of Neotropical migrants also provides researchers with the opportunity to study a two-continent system without traveling extensively. Furthermore, some Neotropical migrant species are believed to be in decline (see review by Robinson 1997), and organochlorine pesticide contamination has been suggested as a possible cause for these declines (Gard et al. 1993, Block et al. 1995, Gard et al. 1995).

The purpose of this study was to search for patterns of OC contamination in eleven species of Neotropical migrants which were selected to represent a diversity of wintering habitats and wintering ranges. Wintering range is important to determine possible sources

of OC contamination. Comparison of wintering habitat is important because different habitats may contain varying levels of contaminant, even within a geographical region. It is also important to compare males and females within a given species, because some species exhibit sex-specific habitat selection on their wintering grounds (Lopez Ornat and Greenberg 1990).

Methods

Collection and Preparation of Birds

Seventy-two Neotropical migrants were either salvaged as television tower kills or collected in mist nets. Tower kills were collected on 16 May 1996 in Livingston Co., IL. Mist netting was conducted from 14 May 1996 to 22 May 1996 at the Keithsburg Division of the Mark Twain National Wildlife Refuge in Mercer County, IL, USA. The birds were put on ice or dry ice at the time of collection and were later transferred to a -80° C freezer for storage until they were thawed and prepared as museum skin specimens. The skinning procedure results in the skin, feathers, distal limbs, bill, partial braincase and stomach being excluded from analysis. All other parts of the carcass were included in the analysis, although the contents of the digestive tract were rinsed out in order to prevent contamination by recently ingested materials, which may have been contaminated with OC pesticides. All skins were deposited in the Illinois State University Department of Biological Sciences bird collection. Sex was determined by direct examination of gonads All birds used in this study were classed as "After Hatch Year", which was based upon the

US Fish and Wildlife Service Bird Banding Laboratory method of assigning individuals collected after 1 January as AHY. After preparing the study skin, the carcass, including all retrievable subcutaneous fat, was refrozen until chemical analyses could be performed.

Residue analysis

At the time of analysis the carcass was thawed and the digestive tract, except the stomach, was rinsed with deionized water. Sodium sulfate (not exceeding 50% of the weight of the carcass) was added to the carcass which was then ground into a paste with a tissue homogenizer. The mixture was then transferred to a soxhlet thimble and extracted with hexane (approximately 150 ml, pesticide-grade, Fisher) for 15-24 h. The extract was concentrated to under 5 ml and then transferred to a chromatography column containing Florisil® (20g, 60-100 mesh, activated at 130°C for 16 h) and sodium sulfate (1-2 cm). The column had been washed with hexane (~40 ml). The column was eluted with 200 ml portions of 6% diethyl ether in hexane (fraction 1), 15% diethyl ether in hexane (fraction 2), and 50% diethyl ether in hexane (fraction 3). These elutions were collected, concentrated to about 5 ml using a rotary evaporator and rediluted to 10.0 ml in a volumetric flask.

Each fraction was analyzed by gas chromatography with a Hewlett Packard (HP) 6890 series gas chromatograph equipped with two Ni^{63} electron capture detectors operated at 300°C. One microliter injections were made with an autosampler (HP 18596-C) into a split/splitless injector operated at 230°C. The analyte was separated on two different fused silica capillary gas chromatograph columns using helium as the carrier gas.

A 30-m DB-35 (0.32-mm inside diameter) served as the primary column for pesticide quantification, while a 30-m DB-1701 (0.32-mm inside diameter) was used for pesticide confirmation. The oven temperature was ramped from an initial temperature of 150°C to 200°C at a rate of 8°C/min. The temperature was ramped to 290°C for 7 min. Data were collected and analyzed with HP environmental analysis software. Peak areas from eight calibration standards were used to calculate response factors. Curve fit was performed by linear regression, and linearity of each calibration curve was verified by determining the coefficient of determination of the line formed by the eight response factors for each pesticide. The coefficient of determination was always greater than 95%. The average response factor from the calibration curve was used to quantify detections and standards. In all cases the retention times of the peaks closely matched the standards. Most of the chemicals selected for analysis have been analyzed in studies of other organisms (DeWeese et al. 1986; Fyfe et al. 1990; Elliott and Martin 1994). The chemicals assayed for were alpha-BHC, gamma-BHC, beta-BHC, heptachlor, delta-BHC, aldrin, heptachlor epoxide, endosulfan I, p,p'-DDE, dieldrin, endrin, p,p'-DDD, endosulfan II, p,p'-DDT, endrin aldehyde, endosulfan sulfate and methoxychlor. Positive identification of pesticides was made when sample retention times were within ± 0.05 min of the average retention time of the calibration standards on both columns.

Birds examined

The eleven species examined were Catharus ustulatus, Swainson's thrush (n=9); Contopus virens, eastern wood pewee (n= 2); Dendroica petechia, yellow warbler (n=6);

Empidonax minimus, least flycatcher (n= 5); Geothlypis trichas, common yellowthroat (n= 10); Mniotilta varia, black and white warbler (n=10); Myiarchus crinitus, great-crested flycatcher (n=5); Pheucticus ludovicianus, rose-breasted grosbeak (n=5); Passerina cyanea, indigo bunting (n=10); Protonotaria citrea, prothonotary warbler (n=5); and Vireo gilvus, warbling vireo (n=5).

These species were assigned to habitat and range groups based on information pooled from a number of sources (Briskie 1994; Curson et al. 1994; DeGraaf and Rappole 1995; Kricher 1995; Lanyon 1997; McCarty 1996; Payne 1992; Rappole et al. 1993; Ridgely and Tudor 1989, 1994; Stotz et al. 1996). These groupings are illustrated in Table 1.

Statistical analysis

All concentrations that fell below our detection limits (about 0.01 ng/g for a larger bird, about 0.1 ng/g for a smaller bird) were treated as zeros for the purpose of statistical analysis. Statistical analyses were performed only for levels of p,p'-DDE, dieldrin, and heptachlor epoxide, as no other pesticides occurred in enough birds to make meaningful comparisons. Because the data were not normally distributed, they were transformed before analysis: p,p'-DDE was transformed to natural log ([DDE]+1); dieldrin and heptachlor epoxide were square root transformed {([dieldrin]+0.5) ([heptachlor epoxide]+0.5)}. Statistical analyses were conducted using a three-way ANOVA that compared mean OC concentration in birds of different sexes and with different wintering habitats and wintering ranges (Sokal and Rohlf 1995). Analyses were made using SPSS

software (SPSS Inc. 1993). Tests were run independently for each of the three compounds examined.

Results

Sixty-six of the 72 birds, representing all species examined, contained at least one of six organochlorine pesticide residues. These six compounds were the only ones detected (Table 2). Six individuals of 4 species were not contaminated with detectable OC levels.

There were no significant differences between male and female birds in levels of p,p'-DDE, dieldrin, or heptachlor epoxide (Table 3). Likewise, there were no significant differences between birds from scrub and forest habitats in levels of p,p'-DDE, dieldrin or heptachlor epoxide (Table 3). Non-South American wintering birds, however, had significantly higher levels of p,p'-DDE, dieldrin and heptachlor epoxide than South American wintering birds (Table 3, Figure 1). There were no significant interactions among any of the dependent variables.

Discussion

Our findings demonstrate that organochlorine pesticide contamination is ubiquitous in the Neotropical migrant passerines examined in our study. These results were consistent with those of Harper et al. (1996) both in terms of occurrence and level of contamination. Levels of contamination ranged across six orders of magnitude, while

average levels were lower than those reported in other studies (DeWeese et al. 1986, Baril et al. 1990, Fyfe et al 1990, Mora and Anderson 1991). These studies, however, examined areas where pesticide use was known to occur.

We did not see a difference between pesticide levels in male and female birds. Although sex-specific habitat selection is known to occur in Neotropical migrants, it has only been documented for a few species. Comparing levels in males and females of all species was necessary to maintain reasonable sample size, but probably obscured any pattern that may exist. Although we detected no differences in pesticide contamination between birds that winter in different habitats, our habitat categories were very broad, and probably did not reflect the actual habitat differences experienced by different migrant species. Stotz (1996), for example, categorizes Neotropical habitat types into 3 major habitats and 42 sub-habitats.

Although those birds that winter predominantly in Central America exhibited significantly higher levels of pesticide contamination than those that winter predominantly in South America, it is still unclear where Neotropical migrants are acquiring their pesticide load. There are three possible sources of pesticide contamination for Neotropical migrant passerines: breeding grounds, wintering grounds, and the migration route. Our findings do not allow us to draw definitive conclusions about the source of organochlorine pesticide contamination in Neotropical migrant passerines. However, we can rule out the breeding grounds as the sole source of contamination by the following argument. Our working hypothesis has been that two species with comparable feeding ecologies and body sizes that live in the same region for a given period of time will acquire similar levels of

contamination from that region. If breeding grounds were the sole source of contamination, we would therefore expect to see no difference in pesticide levels in South American wintering migrants and non-South American wintering migrants. Since we do see significant differences, these migrants must be picking up differential levels of pesticide contaminants on their respective wintering grounds and/or migratory routes.

Analysis of the potential differences in contamination within wintering grounds and along migratory routes is complicated by the fact that the migratory routes of South American wintering migrants, for the most part, include the migratory routes and wintering grounds of the non-South American wintering migrants (DeGraaf and Rappole 1995, Rappole et al. 1993). Another potential complication is that migrating birds often stop to rebuild fat reserves needed for migration. These feeding stops can result in increases in body mass of up to 10% in a single day (see review by Moore 1992). If migrating birds are exposed to pesticides along the migratory route, they may acquire these chemicals at a higher rate than wintering birds due to the rapid accumulation of biomass.

Future work should focus on examining the resident birds of North America, South America, Central America and Mexico. By comparing levels in migrants to levels in year-round residents, perhaps we can gain a clearer understanding of the sources of OC contamination in Neotropical migrants.

Acknowledgments

Heartfelt thanks to Anne Bartuszevige, Virginia Flanagin, Neva Laurie and Mark Wieland for many hours of work in the lab and to Dr. Sheryl Soukup for reviewing manuscripts and attending presentations.

Literature cited

Baril A, Elliott JE, Somers JD, Erickson G (1990) Residue levels of environmental contaminants in prey species of the peregrine falcon, *Falco peregrinus*, in Canada. Canadian Field-Naturalist 104:273-284

Block WM, Finch DM, Brennan LA (1995) Single-species versus multiple-species approaches for management, pp. 461-476 *In:* Martin TE and Finch DM (eds) Ecology and management of Neotropical migratory birds: a synthesis and review of critical issues. Oxford Univ. Press

Briskie JV (1994) Least Flycather (Empidonax minimus), *In:* Poole A and Gill F (eds) The Birds of North America, No. 99. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Colburn T, Vom Saal FS, Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspectives 101:378-384

Curson J, Quinn D, Beadle D (1994) Warblers of the Americas. Houghton Mifflin Co.

DeGraaf RM, Rappole JH (1995) Neotropical migratory birds: natural history, distribution, and population change. Comstock Publishing Assosciates

DeWeese LR, McEwen LC, Hensler GL, Petersen BE (1986) Organochlorine contaminants in passeriformes and other avian prey of the peregrine falcon in the western United States. Environ Toxicol Chem 5:675-693

Elliott JE, Martin PA (1994) Chlorinated hydrocarbons and shell thinning in eggs of (*Accipter*) hawks in Ontario, 1986-1989. Environ Pollut 86:189-200.

Fyfe RW, Banasch U, Benavides V, De Benavides NH, Luscombe A, Sanchez J (1990) Organochlorine residues in potential prey of peregrine falcons, *Falco peregrinus*, in Latin America.. Canadian Field-Naturalist 104:285-292

Gard NW, Hooper MJ, Bennett RS (1993) Effects of pesticides and contaminants on Neotropical migrants, pp 310-314 *In:* Finch DM and Stangel PW (eds) Status and management of Neotropical migratory birds. US Fish Wildl Serv Gen Tech Report RM-229

Gard NW, Hooper MJ, Bennett RS (1995) An assessment of potential hazards of pesticides and environmental contaminants, pp 294-310 *In:* Martin TE and Finch DM (eds) Ecology and management of Neotropical migratory birds: a synthesis and review of critical issues. Oxford Univ. Press

Harper RG, Frick JA, Capparella AP, Borup B, Nowak M, Biesinger D, Thompson CF (1996) Organochlorine Pesticide Contamination in Neotropical Migrant Passerines. Arch Environ Contam Toxicol 31:386-390

Kricher JC (1995) Black-and-white warbler (*Mniotilta varia*) *In:* Poole A and Gill F (eds) The Birds of North America, No. 158. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Lanyon WE (1997) Great crested flycatcher (*Myiarchus crinitus*) *In:* Poole A and Gill F (eds) The Birds of North America, No. 300. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Lopez Ornat A, Greenberg R (1990) Sexual segregation by habitat in migratory warblers in Quintana Roo, Mexico. Auk 107:539-543

McCarty JP (1996) Eastern wood-pewee (*Contopus virens*) *In:* Poole A and Gill F (eds) The Birds of North America, No. 245. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Moore FR, Kerlinger P (1990) Stopover and fat deposition by North American wood-warblers (Parulinae) following spring migration over the Gulf of Mexico. Oecologia 74: 47-54

Moore FR, Simons TR (1992) Habitat suitability and stopover ecology of Neotropical migrant landbirds pp 345-355 *In:* Hagan JM III, Johnston DW(eds) Ecology and conservation of Neotropical migrant landbirds. Smithsonian Institution Press, Washington, DC

Mora MA, Anderson DW (1991) Seasonal and geographical variation of organochlorine residues in birds from northwest Mexico. Arch Environ Contam Toxicol 21:541-548

Payne RB (1992) Indigo bunting (*Passerina cyanea*) *In:* Poole A and Gill F (eds) The Birds of North America, No. 4. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Rapaport RA. Urban NR, Capel PD, Baker JE, Looney BB, Eisenreich SG, Gorham F (1985) New DDT inputs to North America: atmospheric deposition. Chemosphere 14:1167-1173

Rappole JH, Morton ES, Lovejoy TE III, Ruos JL (1993) Aves migratorias en los Neotropicos. PR Donnelly & Sons Co.

Remsen JV Jr. (1995) The importance of continued collecting of bird specimens to ornithology and bird conservation. Bird Conservation International 5:145-180

Ridgely RS, Tudor G (1989) The birds of South America Vol. 1 the oscine passerines. University of Texas Press

Ridgely RS, Tudor G (1989) The birds of South America Vol. 2 the sub-oscine passerines. University of Texas Press

Robinson SK (1997) The case of the missing songbirds. Consequences 3(1):3-15

Standley LJ, Sweeney BW (1995) Organochlorine pesticides in stream mayflies and terrestrial vegetation of undisturbed tropical catchments exposed to long-range atmospheric transport. J N Am Benthol Soc 14(1):38-49

Simonich SL, Hites RA (1995) Global distribution of persistent organochlorine compounds. Science 269:1851-1854

Sokal RR, Rohlf FJ (1995) Biometry, third edition. W.H. Freeman and Company

SPSS Inc., Narusis MJ (1993) SPSS for windows base systems user's guide release 6.0. SPSS Inc.

Stotz DF, Fitzpatrick JW, Parker TA, Moskovits DK (1996) Neotropical birds: ecology and conservation. University of Chicago Press

Table 1. Species groupings by wintering range

	Wintering Range	
	Central America	South America
Wintering Habitat Forest	*Mniotilta varia* *Vireo gilvus* *Myiarchus crinitus* *Protonotaria citrea*	*Catharus ustulatus*
Scrub	*Passerina cyanea* *Geothlypis trichas* *Dendroica petechia* *Empidonax minimus*	*Contopus virens* *Pheucticus ludovicianus*

Table 2. Occurrence of organochlorine contamination

Compound	Number of contaminated individuals	Number of contaminated species	Low (ng/g)	High (ng/g)
DDE	65	11	0.02	3540
Dieldrin	54	11	0.2	170
Heptachlor epoxide	44	11	0.22	73
DDT	6	5	0.0052	98
DDD	4	4	3.3	52
Endosulfan I	1	1	1.1	1.1

Number of contaminated individuals is the number of individuals that contain the compound. *Number of contaminated species* is the number of species where at least one individual of the species contains that pesticide. *Low* and *High* are the lowest and highest levels of the compound detected across all species.

Table 3. Mean levels of organochlorine contamination by sex, wintering habitat, and wintering range.

	OC level	n	F	p
Sex				
DDE			0.130	0.720
Male	120 ± 150	48		
Female	46 ± 18	24		
Dieldrin			0.643	0.426
Male	18 ± 8	48		
Female	15 ± 7.5	24		
Heptachlor epoxide			0.150	0.700
Male	11 ± 4.3	48		
Female	11 ± 5.3	24		
Habitat				
DDE			0.491	0.486
Forest	37 ± 15	34		
Scrub	150 ± 190	38		
Dieldrin			0.023	0.880
Forest	13 ± 4.3	34		
Scrub	21 ± 10	38		
Heptachlor epoxide			0.245	0.623
Forest	9.0 ± 3.0	34		
Scrub	12 ± 5.5	38		
Range				
DDE			24.3	0.000
SA*	5.6 ± 4.3	16		
CA**	120 ± 131	56		
Dieldrin			4.56	0.037
SA	8.1 ± 6.7	16		
CA	20 ± 7.2	56		
Heptachlor epoxide			4.13	0.046
SA	4.5 ± 3.8	16		
CA	13 ± 3.8	56		

OC level is the non-transformed mean ± 95% confidence interval. Means and 95% confidence intervals are for non-transformed data; F and p values reported are for transformed data.

*SA denotes those birds that primarily winter in South America

**CA denotes those birds that primarily winter in Central America and Mexico

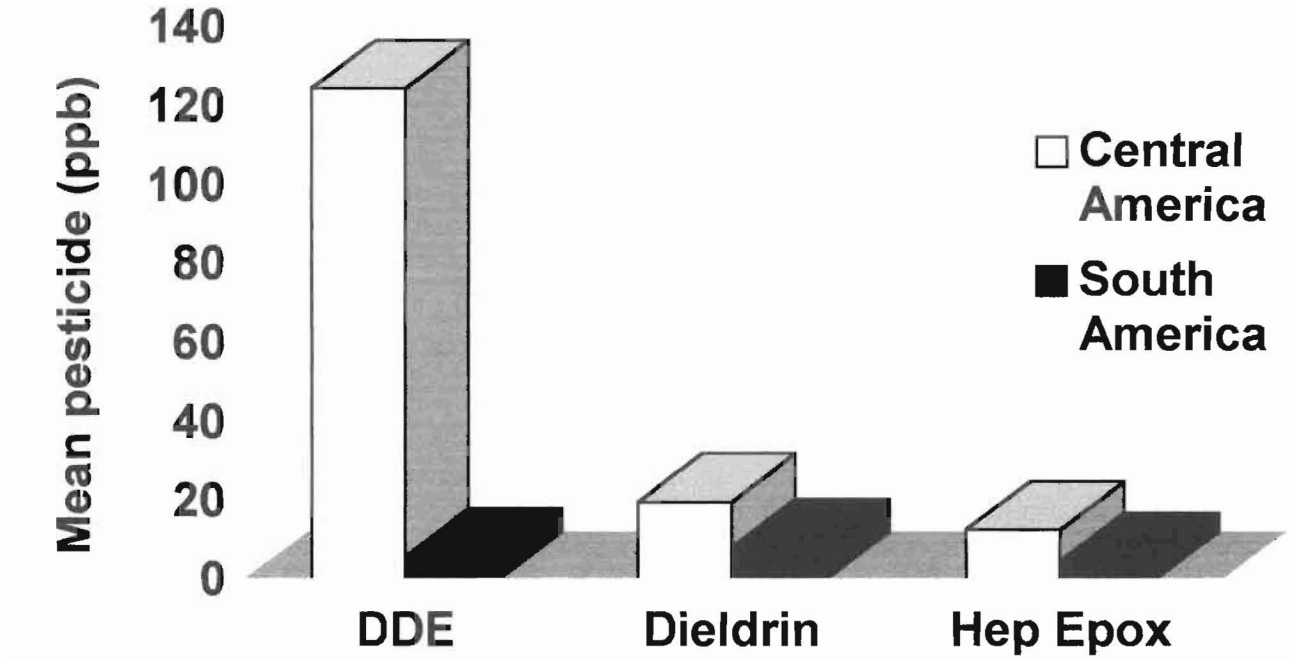

Figure 1. Mean levels of organochlorine contamination by wintering range. DDE, Dieldrin, and Hep Epox represent the untransformed mean levels of p,p'-DDE, dieldrin, and heptachlor epoxide, respectively.