AN EVALUATION OF CONTAMINANT EXPOSURE OF THREE ENDANGERED BAT SPECIES IN VIRGINIA

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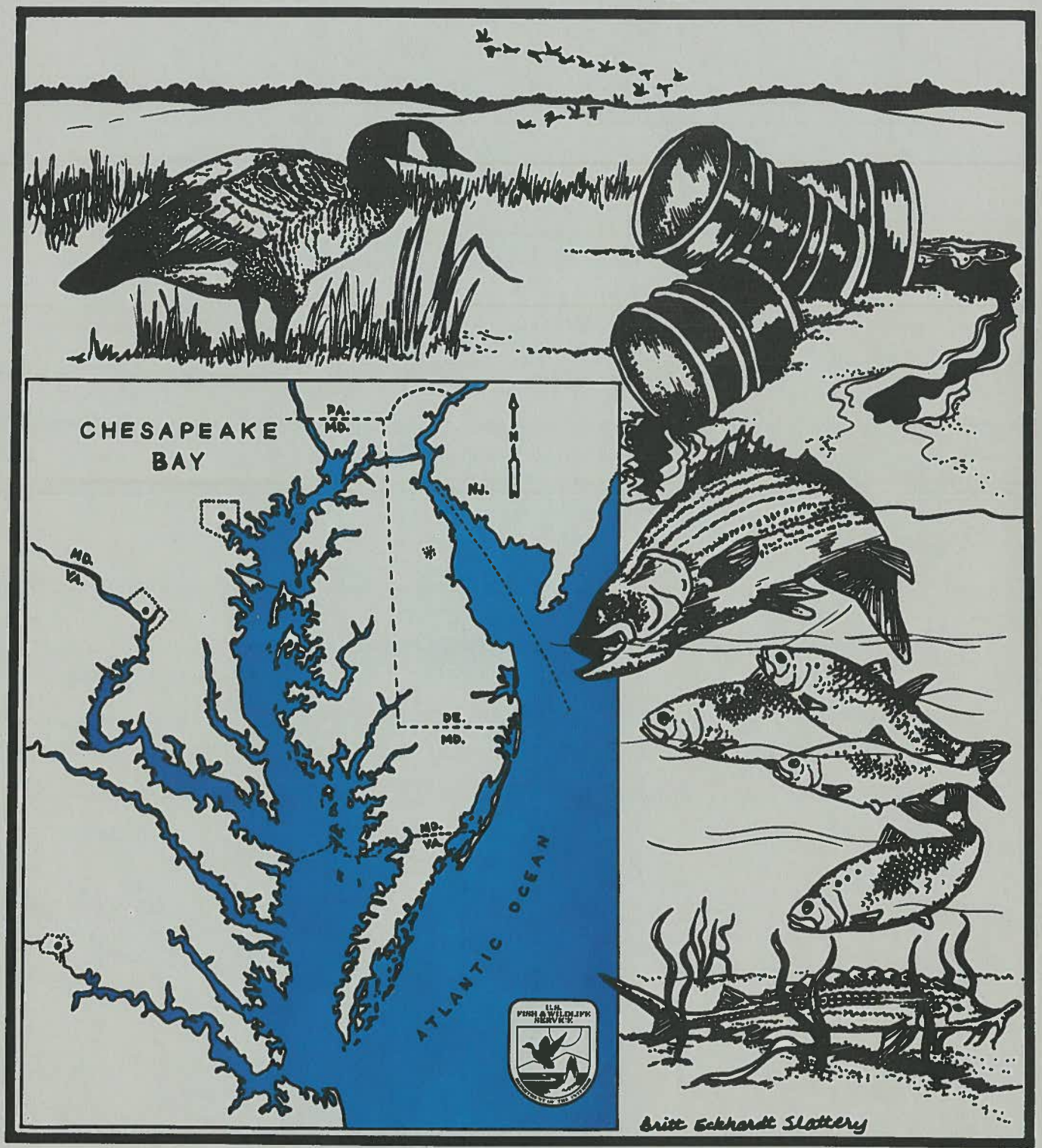

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An Evaluation of Contaminant Exposure of Three Endangered Bat Species in Virginia

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Title: **An Evaluation of Contaminant Exposure of Three Endangered Bat Species in Virginia.**

Abstract: A background contaminant study was conducted on bat guano and insects in Virginia caves to determine the possibility of exposure of endangered species of bats in Virginia to organochlorine pesticides, organophosphate pesticides, and metals. The three endangered species of bats in Virginia are the gray bat (Myotis grisescens), the Virginia big-eared bat (Plecotus townsendii) and the Indiana bat (Myotis sodalis).

Historic guano samples from a cave in Scott County, Virginia (Clinchport quadrangle, cave SP) tend to be higher in organochlorine compounds than recent samples from that cave, possibly indicating a source of exposure of these compounds to the bats has been eliminated. The study also provides data which can be used for future comparison in contaminant assessments in Virginia bats.

Key words: Environmental Contamination, Organochlorine Pesticides, Organophosphate Pesticides, Metals, Gray Bat (Myotis grisescens), Virginia Big-eared Bat (Plecotus townsendii), Indiana Bat (Myotis sodalis)

EXECUTIVE SUMMARY

There are three endangered species of bats in western Virginia, the gray bat (Myotis grisescens), the Virginia big-eared bat (Plecotus townsendii) and the Indiana bat (Myotis sodalis). Organochlorine pesticides and metals can bioaccumulate in bats, and are suspected to cause adverse effects during chronic exposure. Only one study on contaminants in bats in Virginia has been conducted to date, and no data has been available on contaminants in the three endangered bat species. The purpose of this study was to evaluate whether these endangered bat species have been or are being exposed to pesticides or metals, and to provide current baseline data from which future assessments can be made as to the relevance of contaminants and the health of the species.

Bat guano samples were collected from three caves in western Virginia and analyzed for metals and organochlorine compounds. Metals were found to be high in relation to uncontaminated samples from another study. Organochlorine compounds were below detection limits except for a historic sample in a Scott County cave (Clinchport quadrangle, cave SP). This may indicate that the bats in this cave had been exposed to organochlorine compounds, but that this exposure has decreased, possibly due to decreased use of these pesticides in the surrounding area.

Insect samples were collected near the caves and analyzed for organophosphate and carbamate compounds. Small sample sizes resulted in high detection limits; therefore it cannot be determined from this study whether these bats are being exposed to organophosphate or carbamate compounds from their food source.

This study provides data which can be used for future comparisons of contaminant levels in Virginia's bats. Further studies are needed to determine if these values are representative of bat colonies in general in Virginia, to pinpoint sources of contamination which are causing exposure to the endangered species of bats, and to determine if these levels of contamination adversely affects bats.

INTRODUCTION

Organochlorines and metals are known to bioaccumulate in bats (Clark et al. 1986, Clark et al. 1988). Elevated levels of dichlorodiphenyltrichloroethane (DDT), dieldrin and polychlorinated biphenyls (PCBs) have been detected in at least 25 species of bats from five countries (Clark 1981), and direct mortality from dieldrin in colonies of gray bats (Myotis grisescens) has been documented (Clark et al. 1978, Clark et al. 1983). Metals such as cadmium, mercury and lead have also been detected in elevated levels in many species of bats. High mercury levels were found in eastern pipistrelles (Pipistrellus sublflavus) collected along the North Fork Holston River in Virginia, the source apparently being wastewater from a chloralkali factory (Powell 1983). Although direct mortality in bats from organochlorines or metals has rarely been demonstrated, chronic affects on reproduction, acoustic behavior, and metabolism are suspected (Clark 1981).

There are three species of endangered bats in Virginia, the gray bat (Myotis grisescens), the Virginia big-eared bat (Plecotus townsendii) and the Indiana bat (Myotis sodalis). To date, other than the samples taken of the eastern pipistrelle (Pipistrellus subflavus), by Powell (1983) no contaminant data is available for bats in Virginia. In an effort to evaluate contaminant exposure to bats in Virginia without taking any endangered bats, guano samples were used to evaluate contaminant burdens. Clark et al. 1982 measured organochlorine pesticides in both bat carcasses and guano and found relationships

between contamination levels in the two media for dieldrin, heptachlor epoxide and dichlorodiphenyldichloroethylene (DDE). Although these relationships are variable and can not be used to predict contaminant levels in carcasses from those found in guano, they can be a warning signal for potential threats to bat populations. It is assumed that high levels of other pesticides and metals will similarly be related between levels found in guano to carcass levels and, therefore, can be used to determine if populations are being exposed to high levels of contamination.

The purpose of this study was to evaluate whether the three endangered species of bats have been or are being exposed to metals and/or organophosphate or organochlorine pesticides. The study will also provide data for comparisons with future studies as to the relevance of contaminants and the health and recovery of the species.

METHODS

Bat guano samples were collected from three caves in western Virginia, in Scott (Clinchport quadrangle, reference code cave SP, and Hilton quadrangle, reference code cave GB) and Tazewell (Burkes Garden quadrangle, reference code cave BE) Counties. Gray bats roost in these summer caves, along with other bat species. To prevent the disclosure of cave locations and human disturbance of the bat populations, caves will be discussed using reference codes only. Two samples of historic and recent guano were taken from cave SP, two recent guano samples were

taken from cave GB and one recent sample was taken from cave BE, as availability of guano allowed. Through coordination with the Virginia Department of Game and Inland Fisheries, researchers were identified with credentials and permits to enter caves and collect guano samples. The guano samples are composite samples from guano piles within the caves. Each composite sample was split into two separate chemically cleaned jars, one sample for metals analysis, and the other for organochlorine analysis. Recent samples were collected by scraping the top layer from the guano piles with a chemically cleaned teflon coated spatula and placing it in chemically cleaned glass jars. Historic samples were collected by inserting chemically cleaned glass tubes into the piles to obtain a core and then removing the guano that was between five and six inches deep in the piles and placing it in chemically cleaned glass jars. Compounds that were analyzed are listed in Appendix A.

Insects were chosen to screen for organophosphate exposure to bats because these compounds dissipate rapidly in guano, and therefore would not have been reflected in guano samples. Both insect samples were collected near cave BE using light traps. The jars in which the insects were trapped were chemically cleaned prior to their attachment to the light trap. The total contents in each collection jar was analyzed as one sample. The insect tissue analyses were performed at the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility, Laurel, Maryland. Samples were analyzed for the organophosphate and carbamate compounds listed in Appendix B.

Both guano and insect samples were frozen after collection. Percent moisture was analyzed on all samples and the metals were analyzed and reported on a dry weight basis. The organochlorine samples were analyzed and reported on a wet weight basis. The organochlorine analyses of bat guano were performed at Mississippi State Chemical Laboratory, Mississippi State University, and metal analyses of bat guano were performed at Hazleton Laboratories America, Inc., Madison, Wisconsin.

RESULTS AND DISCUSSION

Results of the organophosphate scans in the insect samples were all below the detection limits of 1.3 parts per million (ppm) (wet weight) in sample 1 (collected from cave BE, August 1, 1989) and 4.0 ppm (wet weight) in sample 2 (collected from cave BE, August 10, 1989). Results of the carbamate analysis in the insect samples were all below the detection limits of 2.5 ppm (wet weight) in sample 1 and 8.0 ppm (wet weight) in sample 2. These detection limits were higher than normal (0.5 ppm (wet weight) for organophosphates, and 1.0 ppm (wet weight) for carbamates), due to limited sample weight. Due to these analytical constraints, it cannot be determined whether the insect food base was contaminated with either organophosphates or carbamates.

Results of analyses for metals and organochlorines in bat guano are given in Tables 1 and 2 respectively. The data gives general

information on possible contaminant exposure to bats in Virginia, not specifically the three endangered species since other bat species are known to roost in these caves.

There is limited data from other studies to compare to these results. A guano sample from big brown bats (Eptesicus fuscus) in a cave near the Patuxent Wildlife Research Center, Laurel, Maryland contained 7.1 ppm lead, 0.54 ppm chromium, 340 ppm zinc, and 0.3 ppm cadmium (dry weight). These values are assumed to be relatively uncontaminated (Clark et al. 1986), and are all below the levels found in this study. Clark et al.'s 1986 study also analyzed guano from Florida caves where gray bats (Myotis grisescens) and southeastern bats (Myotis austroriparius) roost. These caves were near a source of metal contamination, and the guano levels are assumed to be elevated due to this exposure. The levels ranged from 3.4 to 6.1 ppm lead, 0.8 to 5.0 ppm chromium, 390 to 640 ppm zinc and 1.9 to 2.3 ppm cadmium (dry weight). These levels are generally in the range of or below levels in the current study.

From comparisons with the 1986 study (Table 3), it appears that metal levels in bat guano in these Virginia caves may currently be elevated compared to levels considered to be uncontaminated. There may be species differences in metal levels in guano, such as those evaluated in Clark et al.'s study versus this study, but these are the best data currently available for comparison. Only three locations were sampled on one occasion in this study so it is not known if these metals levels are representative of bats elsewhere in the state. It is also not known

if the levels of metals found in this study would adversely affect bats.

This study cannot determine the source of the elevated metal concentrations relative to the other study, but some possible sources include uptake of metals from contaminated drinking water or uptake through the food chain. Uptake through the food chain may begin with metals being deposited through atmospheric deposition onto plants which take up the metals and are eaten by insects, which are in turn eaten by the bats (Petit and Altenbach 1973). Another possibility is that the bats pick up metals from a distant source because bats migrate between summer caves and winter hibernacula, using different caves throughout the year.

Organochlorines are generally below detection limits in all samples, except historical samples from cave SP. This may indicate a past exposure of the bats roosting in cave SP to organochlorine pesticides which has now been reduced. This may reflect a reduction in use of these pesticides in the nearby areas. A guano sample from Cave Springs Cave, Morgan County, Alabama, in which organochlorines may have contributed to bat mortality, contained 3.4 ppm DDE, 1.1 ppm 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), 0.14 ppm dieldrin, 0.41 ppm heptachlor epoxide, 0.06 ppm oxychlordane, 0.17 ppm trans-nonachlor, 0.05 ppm cis-nonachlor, and 2.0 ppm PCBs all in dry weight (Clark, et al. 1988). These levels are all above the detected levels in the Virginia caves studied, except dieldrin, trans-nonachlor and total PCBs in the historic samples from cave SP.

CONCLUSIONS AND RECOMMENDATIONS

This data could be used for future comparisons to assess contamination problems or trends in guano samples. These current metal levels do seem to be elevated relative to uncontaminated samples from Maryland, possibly indicating current exposure of the bats to metals. It is not known if these levels adversely affect bats.

The high values of organochlorine compounds in historic samples from cave SP may indicate some source of organochlorine exposure to the bats in the past which has now been eliminated. This may be due to decreased use of these pesticides, some of which are no longer on the market.

Due to limited funding, small sample sizes were used and few sample sites were analyzed. In order to better evaluate possible contamination concerns for the endangered bats in Virginia, a more comprehensive study should be undertaken which includes many caves across the state, sufficient insect collections at each cave, and replication of samples at each site for statistical purposes. Because this study showed possible metal exposure to the bats through relatively high levels in the guano samples, future studies should focus on metals analysis and possible exposure pathways of metals to the bats. If problem areas are found with high metal concentrations in guano, follow-up studies can then be undertaken which would analyze metals in carcasses and give more specific information as to the threat to the bats caused by the metal exposure.

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Table 1. Metals detected in gray and big-eared bat guano samples in Virginia, August, 1989. Values represent concentrations in parts per million (ppm dry-weight).

FWS SITE #	GB1	GB2	SP1	SP2	SP1	SP2	BE
SAMPLE TYPE	R	R	R	R	H	H	R
% moist	83.70	87.60	71.60	53.60	76.20	70.70	71.80
COMPOUND							
As	3.31	3.79	5.99	4.53	0.97	1.60	4.61
Hg	0.791	1.282	0.567	NA	2.420	0.891	NA
Se	2.82	2.58	1.90	1.53	5.88	5.80	<0.35
Al	1306.75	2870.97	4260.56	6810.34	470.59	6348.12	2836.88
Sb	<6.13	<8.06	<3.52	<2.16	<4.20	<3.41	<3.55
Ba	20.55	34.11	52.82	69.18	52.94	139.59	50.35
Be	<0.31	<0.40	0.25	0.45	<0.21	0.34	<0.18
B	5.64	14.03	6.06	3.45	9.58	10.72	<1.77
Cd	2.21	1.45	2.68	1.16	5.29	2.42	2.13
Cr	2.02	5.08	8.77	4.53	3.28	9.15	3.69
Cu	126.38	151.61	113.38	68.97	290.34	177.13	109.22
Fe	1883.44	4556.45	7746.48	9073.28	2319.33	10068.26	5567.38
Pb	8.59	14.52	11.97	20.91	15.55	7.85	12.06
Mg	1190.18	927.42	25669.02	4547.41	5210.08	1750.85	8368.80
Mn	342.94	395.16	795.77	840.52	157.56	303.75	450.35
Mo	<3.07	<4.03	3.84	4.16	7.82	6.55	<1.77
Ni	<2.45	5.24	6.09	9.01	2.35	6.62	3.26
Ag	<3.07	<4.03	<1.76	<1.08	<2.10	<1.71	<1.77
Sr	90.80	21.61	44.37	24.35	29.41	45.05	9.26
Tl	<12.27	<16.13	<7.04	<4.31	<8.40	<6.83	<7.09
Sn	<3.07	<4.03	5.88	3.90	<2.10	17.88	7.77
V	3.50	5.48	10.28	10.43	3.19	10.07	3.83
Zn	619.63	830.65	570.42	308.19	1079.83	849.83	83.33

NA = insufficient sample weight for analysis.

< = below detection limit indicated.

FWS SITE# = U. S. Fish and Wildlife Service site number.
BE = Tazewell County cave (Burkes Garden quadrangle)
GB = Scott County cave (Hilton quadrangle)
SP = Scott County cave (Clinchport quadrangle)

SAMPLE TYPE: R = RECENT, H = HISTORIC

Table 2. Organochlorine compounds detected in gray and big-eared bat guano samples in Virginia, August 1989. Values represent compound concentrations (ppm) wet weight.

FWS SITE #	GB1	GB2	SP1	SP2	SP1	SP2	BE
SAMPLE TYPE	R	R	R	R	H	H	R
% MOIST	85.00	90.00	77.00	62.40	76.00	63.00	73.40
COMPOUND							
Oxychlordane	ND	ND	ND	ND	0.17	ND	ND
Hept. Epox.	ND	ND	ND	ND	0.13	ND	ND
t-Nonachlor	ND	ND	ND	ND	0.25	ND	ND
PCB's total	ND	ND	ND	ND	2.96	0.76	ND
p, p'-DDE	ND	ND	ND	0.03	0.63	0.08	0.04
Dieldrin	ND	ND	ND	ND	0.46	0.08	ND

* For a complete listing of scanned compounds refer to Appendix A.

ND = below detection limits.

Hept. Epox. = heptachlor epoxide.

FWS SITE# = Fish and Wildlife Service site number.
BE = Tazewell County cave (Burkes Garden quadrangle)
GB = Scott County cave (Hilton quadrangle)
SP = Scott County cave (Clinchport quadrangle)

SAMPLE TYPE: R = RECENT, H = HISTORIC

Table 3. Metals detected in bat guano samples from Virginia, Maryland, and Florida. Values represent concentrations in parts per million (ppm, dry weight).

FWS SITE #	MD	FLJ	FLG	GB1	GB1	GB2	SPR1	SPR2	SPH1	SPH2	BE
SPECIES	BB	G	G,SE	G,SE	G	G	G	G	G	G	BE
COMPOUND											
Cd	0.3	2.2	1.9	2.3	2.21	1.45	2.68	1.16	5.29	2.42	2.13
Cr	0.54	2.7	0.83	5.0	2.02	5.08	8.77	4.53	3.28	9.15	3.69
Pb	7.1	3.4	6.1	3.9	8.59	14.52	11.97	20.91	15.55	7.85	12.06
Zn	340	640	390	530	620	831	570	308	1080	850	83

SITES

MD = "Control" Cave in Maryland[1]
FL = Caves in Florida[1]
GB, SPR, SPH, BE = Caves in Virginia[2]

SPECIES

BB = Big Brown Bat (Eptesicus fuscus)
G = Gray Bat (Myotis grisescens)
SE = Southeastern Bat (Myotis austroriparius)
BE = Big-earred Bat (Plecotus townsendii)

[1] Clark, D.R., Jr., Wenner, A.S., and Moore, J.F. 1986. Metal residues in bat colonies, Jackson County, Florida, 1981-1983. Florida Field Naturalist 14:38-45.

[2] This study

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APPENDIX A - COMPOUNDS ANALYZED IN BAT GUANO SAMPLES

METALS

Arsenic (As)

Mercury (Hg)

Selenium (Se)

Aluminum (Al)

Antimony (Sb)

Barium (Ba)

Beryllium (Be)

Boron (B)

Cadmium (Cd)

Chromium (Cr)

Copper (Cu)

Iron (Fe)

Lead (Pb)

Magnesium (Mg)

Manganese (Mn)

Molybdenum (Mo)

Nickel (Ni)

Silver (Ag)

Strontium (Sr)

Thallium (Tl)

Tin (Sn)

Vanadium (V)

Zinc (Zn)

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ORGANOCHLORINE COMPOUNDS

HCB (Hexachlorobenzene)

α-BHC (benzene hexachloride)

Γ-BHC (benzene hexachloride)

β-BHC (benzene hexachloride)

δ-BHC (benzene hexachloride)

Oxychlordane

Heptachlor Epoxide

Γ-Chlordane

t-Nonachlor

Toxaphene

PCB's (Polychlorinated biphenyls(total))

o, p'-DDE (dichlorodiphenyldichloroethylene)

α-Chlordane

p, p'-DDE (dichlorodiphenyldichloroethylene)

Dieldrin

o, p'-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl) ethane)

Endrin

cis-nonachlor

o, p'-DDT (dichlorodiphenyltrichloroethane)

p, p'-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl) ethane)

p, p'-DDT (dichlorodiphenyltrichloroethane)

Mirex

APPENDIX B - ORGANIC COMPOUNDS ANALYZED IN INSECT SAMPLES

ORGANOPHOSPHATE COMPOUNDS

Acephate
Azinphos-methyl
Chlorpyrifos-dursban^{R}
Coumaphos
Demeton
Diazinon
Dichlorvos
Dicrotophos
Dimethoate
Disulfoton
Dursban^{R}
EPN (O-Ethyl O(4-nitrophenyl) phenylphosphonothioate)
Ethoprop
Famphur
Fensulfothion
Fenthion
Malathion
Methamidophos
Methyl Parathion
Mevinphos
Monocrotophos
Parathion
Phorate
Terbufos
Trichlorfon

CARBAMATE COMPOUNDS

Aldicarb
Carbaryl
Carbofuran
Methiocarb
Methomyl
Oxamyl

^{R} = Trade Name

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APPENDIX C

Laboratory Methods for

Organochlorine Analysis

Method 1. Analysis For Organochlorine Pesticides and PCBs In Animal and Plant Tissue.

Ten gram tissue samples are thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted with hexane for seven hours. The extract is concentrated by rotary evaporation; transferred to a tared test tube, and further concentrated to dryness for lipid determination. The weighed lipid sample is dissolved in petroleum ether and extracted four times with acetonitrile saturated with petroleum ether. Residues are partitioned into petroleum ether which is washed, concentrated, and transferred to a glass chromatographic column containing 20 grams of Florisil. The column is eluted with 200 ml 6% diethyl ether/94% petroleum ether (Fraction I) followed by 200 ml 15% diethyl ether/85% petroleum ether (Fraction II). Fraction II is concentrated to appropriate volume for quantification of residues by packed or capillary column electron capture gas chromatography. Fraction I is concentrated and transferred to a Silicic acid chromatographic column for additional cleanup required for separation of PCBs from other organochlorines. Three fractions are eluted from the silicic acid column. Each is concentrated to appropriate volume for quantification of residues by packed or megabore column, electron capture gas chromatography. PCBs are found in Fraction II.

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Method 4. Analysis For Aliphatic and Aromatic Hydrocarbons In Soil and Sediment.

Twenty gram soil or sediment samples are extracted with acetone, followed by petroleum ether, by allowing to soak one hour in each with intermittent shaking. A final acetone/petroleum ether extraction is done, and the extracts are combined, centrifuged, and transferred to a separatory funnel containing sufficient water to facilitate partitioning of residues into petroleum ether portion. The petroleum ether is washed twice with water and concentrated by Kuderna-Danish to appropriate volume for transfer to a 20 gram 1% deactivated silica gel column, topped with five grams neutral alumina. Aliphatic and polynuclear aromatic hydrocarbon residues are fractionated by eluting aliphatics from the column with 100 ml petroleum ether (Fraction I) followed by elution of aromatics using first, 100 ml 40% methylene chloride/60%petroleum ether, then 50 ml methylene chloride (Combined eluates, Fraction II). If needed, Fraction I containing aliphatics is subjected to additional cleanup by concentration and transfer to a deactivated (2% water) Florisil column. Aliphatic residues are eluted from the Florisil column using 200 ml 6% diethyl ether/94% petroleum ether. The eluate is concentrated to appropriate volume for quantification by capillary column, flame ionization gas chromatography. The silica gel Fraction II containing aromatic hydrocarbons is concentrated, reconstituted in methylene chloride, and subjected to gel permeation chromatographic (GPC) cleanup prior to quantification by capillary, flame ionization gas chromatography and fluorescence HPLC.

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Elution Profiles for Florisil, Silica Gel and Silicic Acid Column Separations

A. Florisil Column:

1. Fraction I (6% ethyl ether containing 2% ethanol, 94% petroleum ether)

   HCB, alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-nonachlor, toxaphene, PCB's, o,p'-DDE, alpha-Chlordane, p,p'-DDE, p,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, mirex, dicofol, endosulfan I (Split with FII).

2. Fraction II (15% ethyl ether containing 2% ethanol, 85% petroleum ether)

   dieldrin, endrin, dacthal, endosulfan I (split with FI), endosulfan II (split with FIII), endosulfan sulfate (split with FIII).

3. Fraction III (50% ethyl ether containing 2% ethanol, 50% petroleum ether)

   endosulfan II (split with FII), endosulfan sulfate (split with FII), malathion.

B. Florisil Mini-Column:

1. Fraction I (12 ml hexane followed by 12 ml 1% methanol in hexane)

HCB, gamma-BHC (25%), alpha-BHC (splits with FII), trans-nonachlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD (splits with FII), o,p'-DDT, p,p'-DDT, mirex, cis-nonachlor, cis-chlordane, trans-chlordane, PCB's, Photomirex and derivatives.

2. Fraction II (24 ml 1% methanol in hexane)

gamma BHC (75%), beta-BHC, alpha-BHC (splits with FI), delta-BHC, oxychlordane, heptachlor epoxide, toxaphene, dicofol, dacthal, endosulfan I, endosulfan II, endosulfan sulfate, octachlorostyrene, Kepone (with additional 12mls 1% methanol in hexane).

C. Silica Gel:

1. SG Fraction I (100 ml petroleum ether)

n-dodecane, n-tridecane, n-tetradecane, ocylcyclohexane, n-pentadecane, nonycyclohexane, n-hexadecane, n-heptadecane, pristane, n-octadecane, phytane, n-nonadecane, n-eicosane.

2. SG Fraction II (100 ml 40% methylene chloride in petroleum ether followed by 50 ml methylene chloride)

napthalene, fluorene, phenanthrene, anthracene, fluoranthrene, pyrene, 1,2-benzanthracene, chrysene, benzo [b] fluoranthrene, benzo [k] fluoranthrene, benzo [e] pyrene, benzo [a] pyrene, 1,2:5,6-dibenzanthracene, benzo

[g,h,i] perylene.

D. Silicic Acid:

1. SA Fraction I (20 ml petroleum ether)

   HCB, mirex

2. SA Fraction II (100ml petroleum ether)

   PCB's, p,p'-DDE (splits with SA III)

3. SA Fraction III (20 ml mixed solvent: 1% acetonitrile, 80% methylene chloride, 19% hexane)

   alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-chlordane, toxaphene, o,p'-DDE, alpha-chlordane, p,p'-DDE (splits with SAII), o,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, dicofol.