1995

Organochlorine Pesticide Residues in the Neotropical Migratory Passerine Birds

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Approval Page
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by Birthe Borup

A PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR CHEMISTRY 499 AND HONORS IN CHEMISTRY

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1994

Appendix I

Sample ID #	Further ID	Weight of Bird	Weight of Sodium Sulfate	% of Sample Transferred to Soxhlet	Place of Death
1	B-294 TFS-13	19.317g	4.482g	91.365%	Site 1
2	APC-4236	5.780g	6.11g	93.35%	Normal, IL
3	B-312 APC-4232	17.881g	14.560g	95.330%	Site 1
4	B-303 TFS-21	6.398g	5.466g	93.695%	Site 1
5	TFS-10 B-291	18.212g	16.439g	91.937%	Site 2
6	APC-4235	18.870g	7.44g	96.35%	Normal, IL
7	B-290 TFS-10	16.950g	17.12g	86.73%	Site 2
8	B-321 JAD-8	11.945g	13.602g	94.289%	Site 4
9	B-301 TFS-19	13.81g	18.264g	95.81%	Site 3
10	B-323 JAD-10	11.148g	13.143g	94.969%	Normal, IL
11	TFS-23 B-305	11.378g	9.381g	94.47%	Site 2

Site 1: ca 5 miles WNW of Lexington, IL (McLean Co.)
Site 2: Vermilion Co.; ca 4 mi. SW of Fithian, IL, WICD-TV tower
Site 3: Piat Co.; ca 5 1/2 mi. W of Monticello, IL, WILL-TV tower
Site 4: 1/2 mile S of Bloomington, IL

Appendix II

Sample ID#	Common Name	Age	Pesticides Detected (ppb)
1	Gray catbird	adult	Heptachlor epoxide*: 5.7 Dieldrin: 12 4,4'-DDE: 4
2	American redstart	juvenile	Dieldrin: 4 4,4'-DDE: 9
3	Swainson's thrush	adult	4,4'-DDE: 1
4	Indigo bunting	adult	Heptachlor epoxide*: 2 Dieldrin: 2 4,4'-DDE: 3
5	Swainson's thrush	juvenile	no pesticides detected
6	Swainson's thrush	adult	Dieldrin: 0.4** 4,4'-DDE: 3
7	Swainson's thrush	juvenile	4,4'-DDE: 1
8	Ovenbird	juvenile	Heptachlor epoxide*: 8 Dieldrin: 11 4,4'-DDE: 5
9	Ovenbird	adult	Dieldrin: 4 4,4'-DDE: 16
10	Ovenbird	juvenile	Heptachlor epoxide*: 71 Dieldrin: 14 4,4'-DDE: 42 4,4'-DDT: 5
11	Ovenbird	adult	Heptachlor epoxide*: 3 Dieldrin: 4 4,4'-DDE: 25

* detected below detection limit

** traces of Heptachlor epoxide (20 ng/ml) are in pesticide grade hexane

Introduction

Historical Background

In 1874 Othmar Ziedler, a German chemist, was working on the synthesis and characterization of substituted aromatic hydrocarbons. In the process of his work he synthesized dichloro-diphenyl-trichloro-ethane, commonly known as DDT. It was not until 1930 that its use as an insecticide was detected by Paul Müller who found that it was effective against potato beetles (*Leptinotarsal declineata*) and clothes moths, (*Tineola besselliella*) (Dunlap 1981). Pure DDT is a white crystalline solid with a melting point of 109°C, and a vapor pressure of 0.025mPa at 20°C. Technical grade DDT is a white amorphous powder, consisting of a mixture of active 4,4'-DDT (65-80%), inactive 2,4'-DDT (14-21%), up to 4% DDD (1,1'-(2,2-dichloroethylidene) bis (4-chlorobenzene)), and traces of 2,2'-DDT. This mixture readily dissolves in xylene and tetraline (600 g/L), moderately dissolves in mineral oil and kerosene (50-80g/L), and is practically insoluble in water (1.2µg/ml) (Elvers et al. 1989).

The head of the Division of Entomology of the United States Department of Agriculture (USDA) in 1897 was alarmed by the transportation of insects into the USA. Foreign insects such as the gypsy moth (*Lymantria dispar*) from Europe, and the bollweevil (*Helicoverpa zea*) from Central America, which did not have natural predators in the US, caused severe problems as they multiplied and thrived undisturbed in their new home. Of the 72 most destructive insects in the US at the time, 36 were known to be imported and 6 were suspected to be foreign (non-endemic). At the time there was no effective way to fight the insect plague, and the increase in monocultures (i.e. the cultivation of a single plant species on an

extended plot of land), made the crops even more susceptible to insects (Dunlap 1981).

American farmers who tried to rid their fields of the growing insect problem experimented with many available options such as the "bug-killing machines", or days of fasting and prayer like the one declared by the governor of Missouri during the grasshopper epidemic in 1870 (Dunlap 1981). The first chemical insecticide used on a large scale was Paris Green, a copper acetoarsenite, which was used against the potato beetle in the 1870's (Dunlap 1981). Towards the end of the 1880s a newly established agricultural experiment station in the US was giving professional advice to worried farmers, and by the turn of the century a small, but growing, insecticide industry had established itself. By 1910 chemical insecticides were mostly used when combating insects on farms (Dunlap 1981).

During World War II, DDT became better known, especially through its first big field use - the Naples, Italy typhus epidemic of 1943-1944 (Dunlap 1981). The disease was spread by lice which DDT killed easily. By the time the epidemic was over, health officials had dusted over three million people with DDT, and it became standard US army issue in 1944 (Dunlap 1981). This new insecticide was light weight, inexpensive, very toxic to a broad range of insect species both by contact and by digestion, and apparently harmless to mammals. It was one of the most persistent poisons in the environment. A wall that was sprayed with DDT remained deadly to any insects that might happen to land on it up to six months after the first spraying (Marco et al. 1987). DDT was inexpensive because only a small amount was needed when spraying. It killed almost all of the mosquitos in an acre that was sprayed with only 1/4th of a pound of DDT dissolved in fuel oil (Marco et al. 1987).

DDT was introduced into the US in 1942, and was described as a "gift from heaven" and "a miracle poison" (Dunlap 1981). But the euphoria did not last long when bird watchers noticed a decline in the population of birds high in the food chain, and traced the cause back to DDT. In 1962 Rachel Carson wrote Silent Spring, which made the public aware of possible negative side effects of DDT on the ecosystems surrounding treated areas. This was the year of DDT's peak production (about $85*10^6$kg produced in the US alone).

In 1947 Congress passed the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). An amendment of this act was passed in 1972 which would later lead to severe restriction of the use of DDT and some other chlorinated pesticides in the US (Marco et al. 1987). The Environmental Protection Agency (EPA) withdrew its registration of DDT as of January, 1974 (Elvers et al. 1989). Canada, Japan, and most European countries have also restricted DDT to essential public health usage (Kirk-Othmer 1981). It is still used in many developing countries to control the malaria-carrying *Anophilus* mosquito and in delousing programs on humans to control typhus (Kirk-Othmer 1981).

DDT has saved and is still saving millions of lives, but its effectiveness is declining due to insects becoming resistant to the pesticide. Resistance to DDT in insects has been associated with increased activity of the detoxifying enzymes within the insect (Walker and Jefferies 1977).

Mode of Action

The peripheral sensory organs of insects are affected by DDT and its analogs (e.g. methoxychlor) (Kirk-Othmer 1981). DDT is absorbed into the lipoprotein matrix of the chitinous insect cuticle, which is aided by the high

fat solubility of the insecticide. As the DDT molecule enters a critical interface of the lipoprotein, such as the sodium gate, it creates a biochemical lesion in the nerve cell possibly by inhibiting Ca^{2+} ATPase. This prolongs the negative after-potential, creating multiple, repetitive firings of the nerve cells, which causes hyperactivity and convulsions in the affected organism. The result is death due to paralysis, metabolic exhaustion, or the production of an endogenous neurotoxin (Elvers et al. 1989).

DDT is a very resistant pesticide in the environment because of two factors. It neither photo-oxidizes easily (Elvers et al. 1989), nor does it biodegrade easily. DDT has spread throughout the whole world due to its high volatility, and it evaporates quickly from sprayed orchards and fields (Lloyd-Jones 1970). Within 16-20 weeks, half of the DDT sprayed on a field disappeared (Wheatley 1965), while during the same time-frame over 60% of the DDT sprayed on an apple orchard could not be accounted for (Stringer and Pickard 1968). Atmospheric dust contained an average of 41ppb chlorinated hydrocarbons in 1968 (Risebrough et al. 1968), which might explain the occurrence of DDT in the wildlife of isolated regions, such as the Antarctic (Lloyd-Jones 1970). Any DDT that is washed into marine or fresh water will also volatilize easily (Strachan et al. 1982). It did not biodegrade in fresh or marine water over a twelve week period, as demonstrated by the fact that it accumulated in sediments that were added to the water after this twelve week period (Strachan et al. 1982).

Residues of this pesticide have been found in virtually all recent samples of biological origin (Fabian et al. 1971). The metabolism of DDT is very complex (Figure1) (Chau and Lee 1987). In birds DDT dechlorinates slowly to form DDD (Strachan et al. 1982), which is further dechlorinated to

DDA (4,4'-dichlorodiphenylacetic acid), a predominant excretory metabolite (Elvers et al. 1989). *In vitro* studies suggest that porphyrins such as cytochrome P-450 (Walker 1969) and hemoglobin (Ecobichon and Saschenbrecker 1967) may be responsible for the dechlorination reactions of DDT to DDD. Because this process is inhibited by O_2 and CO, anaerobic conditions must first be established before reductive dechlorination can occur (Walker and Jefferies 1977). In muscles such conditions occur during prolonged exercise, but most of the time DDT dehydrochlorinates to form DDE (Strachan et al. 1982), which is even more toxic than DDT (Raloff 1994).

Once DDT has entered an organism it is difficult for that organism to get rid of it or its metabolites because they are fat soluble. Therefore the pesticide accumulates over the years of exposure. In Lake Michigan it was accumulated up to $3*10^6$ fold in lake trout (*Salvelinus namaycush*) (from 6ng/L of lake water to >20mg/kg of fish) (Elvers et al. 1989). By 1969 US citizens had an average of 2.3-4.0mg/kg of DDT stored in their body, and 4.3-8.0mg/kg of DDE (Elvers et al. 1989). Animals that are higher up in the trophic level are more affected than those that are herbivorous, because the food eaten by carnivores will contain, on average, more DDT in the fat depositions than plants. Organisms higher up in the trophic level live longer than those on the bottom, and therefore have a longer life span in which to accumulate the pesticides in their tissues. Thus it does not come as a surprise that the average amount of DDE found in piscivorous (fish eating) and insectivorous/ carnivorous birds of northwest Mexico was higher than that in granivorous species, since granivorous birds are lower in the trophic level than piscivorous and insectivorous/carnivorous birds (Mora and Anderson 1991).

The depletion of DDT in animal tissues after exposure was studied using young domestic chicks (*Gallus gallus*) (Donaldson et al. 1967). The highest concentration of DDT in the blood of the animals was observed right after the last contaminated food sample was given. For those chicks being fed continually, the blood DDT level fell slowly from that point as DDT was deposited in the fat tissue. For chicks not being fed after receiving their last contaminated feed, the amount of DDT in the blood doubled to twice the amount during the next 48 hours and then increased by 30% over the next 96 hours. This increase in DDT was due to the metabolism of adipose fat (a large DDT storage area) by the contaminated chicks. After this first starvation period, the concentration of DDT within the fat of both fed and starved chicks was analyzed. The higher concentration of DDT in the fat of the starved chicks points to the theory that a large amount of the mobilized DDT is redistributed within the body, rather than being excreted. The total DDT body burden did decrease for the starved chicks, but only after regaining their normal body weight did the DDT concentration also decrease more compared to that of the fed chicks. The dilution of DDT in the increasing amount of body fat is very important when considering pesticide body burden.

When fat tissue is mobilized during food deprivation, migration, or other forms of stress, DDT is released into the bloodstream, and it is then relocated into other parts of the body (Ecobichon and Saschenbrecker 1968 in Toxi. Appl. Pharm.). However, this redistribution within the body is not uniform. During stress, homing pigeons mobilized their adipocyte lipids, and thus released DDT into their bloodstream. They redistributed most of it into intracellular lipid droplets in muscle tissue and not into blood, brain, liver or heart (Findlay and Defreitas 1970). This suggests that the body

protects DDT-sensitive organs such as the central nervous system. Yet excess DDT in muscle cells can cause them to degenerate and can affect the peripheral regions of the central nervous system attached to the muscle tissue (Findlay and Defreitas 1970). Thus birds with more fat reserves are better able to store DDT in their body than underweight birds (Ecobichon and Saschenbrecker 1968 in Can. J. Phys. Pharm.).

DDT affects birds in many, often interdependent ways. Some of the documented effects are a decline in egg production, aberrant incubation behavior, delayed ovulation, mortality of breeding adults, embryotoxicity irrespective of eggshell deficiencies, mortality or aberrant behavior of recently hatched offspring (Blus 1982), endocrine system disruption (Colborn et al. 1993, McLachlan 1993), and eggshell deficiencies (Blus 1982). A 10-15% decrease in eggshell thickness has been observed in many avian species (Elvers et al. 1989), such as mallards (*Anas Platyrhynchos*) (Heath et al. 1969), and American kestrels (*Falco sparverius*) (Wiemeyer et al. 1970) which often leads to cracking of the eggs and thus death of the offspring as the mother tries to incubate the clutch. It was found that DDE concentrations of 10-40ppm in the feed of female mallards reduced the hatchability of incubated eggs by up to 75%. Most deaths occured during the final (fourth) week of incubation. Of the chicks that survived, none were crippled. DDT and DDD affected the mallards less severely (Heath et al. 1969). Risebrough *et al* suggest that the thinning of eggshells is due to inhibitory action of the pesticide on shell glands. DDT did not affect most other physiological processes in brown pelicans (*Pelecanus occidentralis*) whose eggshells decreased in thickness up to 95% (Risebrough et al. 1970). It has been found that DDE affects the activity of two shell gland enzymes, calcium-dependent adenosine triphosphate (Ca-ATPase) and carbonic

anhydrase (CA). Ca-ATPase transports calcium and carbonate from the blood to the calcifying egg, while CA is a mediator in the transport of carbonate and dicarbonate within the tissue (Miller et al. 1976).

The behavior of animals fed DDT and/ or DDE is also affected. Bobwhite quail (*Colinus virginianus*) fed grain containing 20ppm DDT made more errors in discrimination testing and in an operant conditioning chamber than did control animals (James and Davis 1965). Bengalese finches (*Lanchura striata*) exhibited abnormal aggressive behavior towards mates and offspring when fed grain containing 32-38ppm DDT. It was also noted in this study that the finches showed a delay in ovulation (Jefferies 1971). Delayed egg laying was also observed in ring doves (*Streptopelia risoria*) that were fed diets containing 10ppm DDT (Peakall 1970). The behavior of young mallard chicks whose parents were fed a diet containing 3ppm DDE, which was passed on to the offspring through the egg, also showed signs of aberrant behavior. The young ducklings were hyper-responsive to maternal calls, and were less responsive to frightening stimuli which tested their avoidance behavior (Heinz 1976).

Recently it has been discovered that many pesticides released into the environment have endocrine and reproductive system disrupting properties (e.g. chlordane, dieldrin, DDE (Colborn et al. 1993), DDT, PCB's (Colborn et al. 1993, McLachlan 1993)). DDT, DDE, kepone, heptachlor, dieldrin, mirex, toxophene, and some PCB's mimic the effects of naturally produced hormones like estrogen (Raloff 1994). Organisms are especially vulnerable before and right after birth, during which time these chemicals can exert irreversible damage (Colborn et al. 1993). The male offspring of women who took diethylstilbestrol (a synthetic estrogen) to prevent miscarriages, had a higher rate of cryptorchidism (undescended testes)

than normal (McLachlan and Newbold 1975). Of female mice treated with diethylstilbestrol during pregnancy, 60% of the male offspring were sterile, and 15 of the 24 male mice had testicular changes (McLachlan and Newbold 1975). The organs most affected by endocrine disrupting chemicals contain receptors for gonadal hormones (Colborn et al. 1993), and include mammary glands, fallopian tubes, uterus, cervix, and vagina for females; prostrate, seminal vesicles, epididymides, and testes for males; and external genitalia, brain, skeleton, thyroid, liver, kidney, and immune system of both sexes (Colborn et al. 1993). In general, these chemicals feminize males, over-feminize females (in some cases producing females with extra reproductive organs), and chronic exposure can lead to cancer in both sexes (Raloff 1994).

The children of women who consumed 2-3 fish from Lake Michigan for at least six years prior to giving birth in the past decade, had reduced birth-weight, smaller head circumference, shorter gestation, poorer autonomic and reflex functioning (Jacobsen et al. 1990), neonatal behavioral anomalies, poorer recognition memory, poorer short term memory, and poorer verbal and quantitative skills (Jacobson et al. 1985), compared to children whose mothers did not eat fish. It is believed that these problems are linked to high contaminations of pesticides in the Great Lakes. In 1994 PCB concentrations in eggs of bald eagles (*Haliaeetus leucocephalus*) around the great Lakes were greater than 120ppm (Raloff 1994).

PCB contamination in the Wadden Sea by the Netherlands has been linked to decreased populations of the common seal (*Phoca vitulina*), and the American mink (*Mustela vison*) (Reijnders 1986). Reproduction is interrupted after ovulation, and completely inhibited in the mink at

concentrations above 25μg per day (Reijnders 1986). Reproduction in bald eagles (*Haliaeetus leucocephalus*) declines if pesticide residues within their body is above 4-6ppm for PCBs or above 1ppm for DDE (Raloff 1994).

The amount of DDT residue found in the brain is most indicative of long term exposure (Lincer et al. 1970) because its accumulation in this sensitive organ is slow. It also indicates more clearly how much the birds are effected by the toxic pesticide in their body (Lincer et al. 1970). Hens that had been fed 250μg of DDT per day for 15 days showed no signs of toxicity, even though DDT levels in their liver was 126ppm, and that of their brain was 10ppm (Ecobichon and Saschenbrecker 1968 in Toxi. Appl. Pharm.). On the other hand, during a study on cockerels, those animals dying of DDT poisoning had a much lower amount of DDT in their body than the hens but the level of DDT in their brains was much higher. In some cases the stress associated with handling and weighing the birds was enough to initiate tremors and death (Ecobichon and Saschenbrecker 1968 in Toxi. Appl. Pharm.). It was found that a concentration of residues of both DDT and DDE greater than 35ppm was deadly (Ecobichon and Saschenbrecker 1968 in Toxi. Appl. Pharm., Stickel et al. 1966)

A study done on peregrine falcons (*Falco peregrinus*) in 1976-80 (Henny et al. 1982) indicated that most of their pesticide burden (primarily DDE) came from exposure in Latin America during their migration where DDT is still used to control malaria. The amount of DDE that first year spring migrants brought back from Latin America in 1979 compared to 1980 declined significantly (Henny et al. 1982). The use of organochlorine pesticides in Mexico declined to 20% during the 1980s after the usage of DDT was restricted by the government in 1978 (Mora and Anderson 1991). Yet a study done in 1991 on double-crested cormorants (*Phalacrocorax auritus*),

olivaceous cormorants (*Phalacrocorax olivaceus*), cattle egrets (*Bubulcus ibis*), great-tailed grackles (*Quiscalus mexicanus*), red-winged blackbirds (*Agelaius phoeniceus*), mourning doves (*Zenaida macroura*), and white-winged doves (*Zenaida asiatica*) in northwest Mexico found that the amount of DDE remained high (Mora and Anderson 1991). In 1991 Mexican free-tailed bats (*Tadarida brasiliensis*) seemed to pick up organochlorine pesticides (including p,p'-DDE) at Vickery Cave, Oklahoma (Thies et al. 1994), and red winged blackbirds, *Agelaius phoeniceus,* and tree swallows (*Tachycineta bicolor*) picked up organochlorine pesticides (including PCBs, mirex, p,p'-DDE, heptachlor epoxide, and dieldrin) in the Great Lakes and St. Lawrence River basin (Bishop et al. 1994). This is probably due to residues left over from intensive pesticide usage in these areas in the past (Thies et al. 1994, Bishop et al. 1994).

Extraction Procedure

A common procedure cited in the literature for extracting and analyzing the amount of DDT/DDE in biosamples was done by Cromartie et al. (Cromartie et al. 1975). The sample is collected, placed in a chemically cleaned glass container, and frozen for storage (Blus and Stafford 1980). Extended periods of times when the sample is kept at room temperature can lead to postmortem dechlorination of DDT by bacteria (Walker and Jefferies 1977). After 8 hours of storage at 20°C, 90% of the original DDT present was converted to DDD. In contrast, only 35% of the DDT was converted to DDD in 4 weeks when the sample was kept frozen at -20°C (Walker and Jefferies 1977). To prepare for analysis the sample is homogenized with anhydrous sodium sulfate in a blender to eliminate the water from the sample (Henny et al. 1989). Afterwards it is extracted with

hexane in a Soxhlet for seven hours (Henny et al. 1982). The hexane is heated to boiling, and the vapor condenses on the sides of the Soxhlet and runs down through the sample. The hot hexane extracts the fat and fat-soluble lipids since it is non-polar. Henny et al. (1982) suggests igniting the sodium sulfate for three hours at 675°C to minimize background interference. To further minimize interference a procedural background with sodium sulfate (i.e. a blank run) is suggested between every 12 samples (Henny et al. 1982). The lipids are removed from the pesticides by running the extract through a column containing partially deactivated Florisil (Blus et al. 1989), or they can be removed with Acetonitrile partitioning (Blus et al. 1974).

The sample is then concentrated to approximately 10ml. The pesticides and PCBs are seperated into four fractions using either silicAR (Henny et al. 1982) or Silica Gel (100-200 mesh, grade 923) (Henny et al. 1984) column chromatography. In either case the column can be topped with anhydrous sodium sulfate to extract any residual water. A commonly used solvent system suggested by Kaiser et al. (1980) is:

* fraction 1: 80ml of petroleum ether contains HCB and mirex
* fraction 2: 320ml of petroleum ether contains PCBs, PBBs, and mirex
* fraction 3: 275ml of 15% methylene chloride in hexane contains the remaining organochlorine compounds except endrin and dieldrin
* fraction 4: 200ml mixture of 1% acetonitrile, 19% hexane, and 80% methylene chloride contains endrin and dieldrin. Afterwards each fraction is concentrated to 10ml.

Gas chromatography is the best way to achieve good separation of the pesticides. The detector of choice is an electron capture detector because of its high sensitivity towards chlorinated pesticides. The radioactive

material used within the detector is Ni^{63} (Cromartie et al. 1975), which is a β emitter that ionizes the carrier gas. As long as no organic material comes off of the column, a constant current between the electrodes in the detector results. Once organic molecules that capture electrons (such as chlorinated compounds) pass through, the current is decreased (Skoog and Leary 1992). The column, usually a 1.5% OV-17/1.95% QF1 packed column (Henny et al. 1984), is kept at 190°C while the injection port is kept at 225°C and the detector is at 210°C (Reinert 1970). The gas flow rate of 5% methane in argon is set to 60ml/min through the column, but a capillary column can also be used. For confirmation of the identity of the substances coming off of the column, 10% of the samples are run on a GC - mass spectrometer (Blus and Stafford 1980). In a mass spectrometer the sample is gasified and ionized. The fragments of the compound are then separated on the basis of their mass-to-charge ratios, and the compound can thus be positively identified (Skoog and Leary 1992).

Current Study

Populations of neotropical migratory songbirds that nest in North America have been decreasing since the late 1970's (Sauer and Droege 1992). Robbins *et al.* (1989) conducted a study on 62 species of neotropical migrants in the United States east of the Mississippi and corresponding parts of Canada, by identifying and counting the number of birds seen along roads. Between 1966-1978, 24.2% of the species studied had a decrease in population size (9.7% were significant, $P<0.05$). This number increased to an alarming 71.0% (32.3% significant, $P<0.5$) from 1978-1987. Birds overwintering mostly in forests were more affected by these declines than those overwintering in shrubs (Robbins et al. 1989).

Most of the migratory birds breeding in eastern North America either fly across the Gulf of Mexico, along the Texas coast, or the Florida peninsula. Radar stations located along these coasts are able to pick up birds in migration, although they do not indicate the number of birds or the species of birds migrating. A study done on radar data taken in Lake Charles, Louisiana from 1965 to 1967 and from 1987 to 1989, found that the percentage of days in the spring (April 8 - May 15) in which birds took advantage of favorable weather decreased by almost 50% between the two time frames, indicating either a change in migratory behavior such as increased flock size, or different migration paths (Gauthreaux 1992), or that the net number of birds in migration may be decreasing (Rappole and McDonald 1994).

Two major hypotheses have been suggested to explain the decrease in population size of neotropical migrants. The first suggests that, because of increased brood parasitism, predation, and habitat fragmentation on the breeding grounds of these birds, reproductive success has declined (Terborgh 1989). Cowbirds (*Molothrus ater*) parasitize other bird species by laying their eggs in their nests (Terborgh 1989). The population of cowbirds has increased markedly since 1900. Because these birds find their food on open fields, they have had an increase in food supply as forests gave way to agriculture in the past 100 years. As large tracts of forests are fragmented, their edges are increased, exposing more nests to potential parasitism by cowbirds, which are not found deep in the forest. Neotropical migrants are especially affected because they are too small to fight off cowbirds, and they often do not recognize the threat because they were historically not parasitized (Terborgh 1989).

Opportunistic omnivorous predators (e.g. raccoons, *Procyon lotor*) thrive close to settlements where food supply is ample (e.g. in garbage cans). These predators will also feed on eggs and nestlings of birds, and Neotropical migrants are especially at risk because they are too small to fight off the predators, and many of them also breed on the ground (Terborgh 1989).

Habitat fragmentation increases both predation and parasitism (Litwin and Smith 1992), but this alone cannot completely explain the decline in population size of neotropical migrants. A study done at Sapsucker Woods, Ithaca, New York, from 1949 to 1980 showed that while populations of neotropical migrants declined (some even going extinct in the plot of land under study), resident birds increased in species richness and abundance (Litwin and Smith 1992).

If suitable breeding grounds are the limiting resource for neotropical migrants, then no suboptimum winter habitats should be occupied, because enough optimum habitats would be available (Rappole and McDonald 1994). Yet migrants have been found to use many different types of habitats in their wintering grounds, ranging from intact forests over secondary growth to open fields (Lynch 1992). Greenberg *et al.* however (Greenberg 1992), found that as long as a few patches of trees were available, forest migrants could overwinter in secondary growth habitats without a decline in body condition (as measured by total amount of body fat). According to this study, secondary growth habitat cannot be considered suboptimum, but the study does not account for possible increases in predation.

If the breeding grounds are the limiting resource for these birds, then optimum and sub-optimum habitats should be occupied, and floaters (i.e. animals that are physiologically capable of breeding, but do not because

they have no territory) should exist. Yet suboptimum plots (< 10 hectare) were not occupied by area-sensitive (i.e. forest interior) neotropical migrants (Freemark and Collins 1992), and the number of floaters seems to be decreasing (Rappole and McDonald 1994). On the other hand, in winter habitats the existence of birds without a territory has been documented through radio tracking of Wood thrushes (*Hylocichla mustelina*) which are territorial during winter (Rappole et al. 1992). The existence of floaters in breeding habitats would suggest, that optimum breeding habitats would be occupied every year as floaters obtain territories, but this has not been documented. Instead, a correlation has been found between droughts occurring during a breeding season (i.e. less reproductive success) and declines in the number of returning migrants the following year (Litwin and Smith 1992).

The second hypothesis suggested to explain the decrease in population size of neotropical migrants is a decrease in their wintering habitat (Rappole and McDonald 1994). Deforestation in Central and South America, where neotropical birds overwinter, has decreased suitable habitat needed by these birds. Apart from a few remaining stands, virtually all of the dry forest in the Pacific lowlands is gone (Hartshorn 1989). Of the ten tropical countries undergoing most massive deforestation between 1981 and 1985 (as measured through hectares lost per year), half are a part of Latin America; Brazil (1,480,000 hectares per year), Colombia (820,000 hectares per year), Mexico (595,000 hectares per year), Ecuador (340,000 hectares per year), and Peru (270,000 hectares per year) (World Resource Institute 1992). Over half of the Neotropical migrants overwinter in Mexico, Bahamas, Cuba, and Hispaniola, an area which is 1/5 to 1/10 the size of their breeding habitat. Thus, deforestation in these areas may have

a greater impact on population sizes than deforestation in North America (Terborgh 1989).

The steady decline of four Neotropical migrants in a tropical deciduous forest in southwestern Puerto Rico (the Guánica Forest) does not uphold the hypothesis that deforestation in the tropics is the cause for the decline in populations of neotropical songbirds. From 1986-1990 the populations of the northern parula (*Parula americana*), the prairie warbler (*Dendroica dominica*), the ovenbird (*Seiurus aurocapillus*), and the American redstart *Setophaga ruticilla*), declined steadily in the Guánica Forest, which has not been affected by deforestation. But if the decline of these four neotropical migrants was due to deforestation, then the population density within this untouched forest would have either increased as displaced birds from deforested areas attempted to overwinter in the Guánica Forest, or the density should have remained stable if territorial behavior occured (Faaborg and Arendt 1992). A similar observation was made in Rock Creek Park, in the District of Columbia, where the population of neotropical migrants decreased drastically, even though the size and constitution of the forest remained intact during the time of study (Terborgh 1989)

The effect of pesticides on the populations of neotropical migrants has received little study. Though DDT has been banned in the US since the 1970's (Elvers et al. 1989), residues of the pesticide are still in the environment. Many of the chlorinated pesticides (e.g. DDT, Hexachlorobenzene, and Aldrin) are still being used in Central and South America to control insect populations (FAO 1989). A large number of neotropical migrants feed on insects while overwintering in Latin America (Ehrlich et al. 1988), and can thus accumulate these pesticides in their

body. Birds feeding at lower trophic levels (e.g. fruit, grain) accumulate them to a lesser degree, because the concentration in their food will not be as high since bioaccumulation has occured to a lesser degree. During migration these birds mobilize their fat reserves as a source of energy. As mentioned earlier, any pesticides stored within these fat reserves will be transferred to the blood, where they can accumulate in the brain and result in the organism's death. During northward migration these birds are at risk of pesticide poisoning as they exert a lot of energy to fly along the Appalachian states, taking up to ten weeks for the trip (Terborgh 1989). But it is on their way south in the fall that they are especially at risk, because most fly over the Atlantic Ocean and use up most of their fat reserves (Terborgh 1989). The blackpoll warblers (*Dendroica striata*) for example, fly from Maine to Venezuela in 80-100 hours nonstop, without food, water, and rest. When they arrive in Venezuela they have used up all their fat reserves (Terborgh 1989).

In this study migratory neotropical passerines were analyzed for organochlorine pesticide levels. Correlations between age of birds, geographical nesting range, geographical wintering range, and pesticide levels within the birds could indicate possible sources of contamination.

Procedure / Methods

Extraction

Eleven migratory passerines were collected in central Illinois, USA, from 15 September 1993 to 11 September 1994. Species collected included one gray catbird (*Dumetella carolinensis*), one American redstart (*Setophaga ruticilla*), four Swainsons thrushes (*Catharus ustulatus*), one indigo bunting (*Passerina cyanea*), and four ovenbirds (*Seiurus aurocapillus*). Skin, feathers, and bones of the lower leg were removed, and the remains of each bird was frozen at -20 ^{o}C until ready for analysis. At time of analysis (September - November 1994) the digestive tract was removed and the entire carcass was homogenized with anhydrous sodium sulfate in a grinder. The dry mixture was then transferred to an extraction thimble and placed into a Soxhlet apparatus. Lipids and pesticides were extracted with approximately 100ml of pesticide-grade hexane for 15-24 hours. The extracts were stored in the refrigerator until cleanup.

Cleanup

Samples were concentrated to 10ml with a rotary evaporator and transferred to a chromatography column containing 20g of Florisil (60/100 Mesh activated at 130 ^{o}C for 16 hours) and topped with 1-2cm anhydrous sodium sulfate. Fractions 1, 2, and 3 were eluted with 6% ethyl ether: hexane (v,v), 15% ethyl ether: hexane (v,v), and 50% ethyl ether: hexane (v,v) respectively. Each fraction was concentrated to below 10ml with a rotary evaporator and then diluted to volume in a 10ml volumetric flask. Samples were transferred to amber containers to inhibit photolysis of

pesticides, and stored in the refrigerator until gas chromatography analysis was performed.

Gas chromatography analysis and verification

Illinois Wesleyan University did not have a gas chromatograph with an electron capture detector, and therefore the facilities at Illinois State University were used. Most literature specified the flow rate used, but the flow detector was disabled, after repeated trials I finally decided on a head pressure of 15 psi because it gave the most consistent results. Initially a temperature gradient was used. This gradient started at 150 ^{o}C and rose 10 degrees/ minute until it reached 230 ^{o}C, then the oven temperature stayed at 250 ^{o}C for 20 minutes. But the detector used was so sensitive to any temperature change, that the peak went off scale and did not come down until half an hour later. After repeated trials at various temperatures I found that 220 ^{o}C gave the best separation with very little peak spread.

Approximately two to three µl of each fraction was injected into the gas chromatograph at Illinois State University (HP 5890 A) fitted with a capillary column (HP-5, crosslinked 5% Phe Me Silicone, 25m*0.32*1.05µm film thickness) and equipped with an electron capture detector (HP G1223A Nickel63). The conditions for this gas chromatograph were as follows:

carrier gas: Helium
make up gas: Nitrogen
head pressure: 15 psi
isotherm with oven temperature: 220 ^{o}C
injector and detector: 250 ^{o}C
no purge
injection of 2-3µl
run time: 35 minutes

The settings on the integrator (an HP3396A) were:

zero:0
attenuation: 0
chart speed: 1
area reject: 10000
threshhold: 1
peak width: 0.04

However when the first sample was to be injected, a leak occured in the system close to the injection port and the instrument was not repaired by the time this study was conducted. Therefore all samples were sent to Daily Analytical Laboratories in Peoria, IL, not for verification of data as originally planned, but to obtain data in the first place. Daily Analytical Laboratories tested for pesticides listed in Table I. Detection limits are given in ng of pesticide per ml of hexane.

Table I

Pesticide	detection limit	Pesticide	detection limit
aldrin	3 ng/ml	Endosulfan I	3 ng/ml
alpha-BHC*	1 ng/ml	Endosulfan II	1 ng/ml
beta-BHC	1 ng/ml	Endosulfan sulfate	10 ng/ml
delta-BHC	1 ng/ml	Endrin	1 ng/ml
gamma-BHC**	1 ng/ml	Endrin aldehyde	10 ng/ml
4,4'-DDD	1 ng/ml	Endrin ketone	1 ng/ml
4,4'-DDE	1 ng/ml	Heptachlor	2 ng/ml
4,4'-DDT	1 ng/ml	Heptachlor epoxide	1 ng/ml
Dieldrin	1 ng/ml	Methoxychlor	1 ng/ml

* Benzenehexachloride
** Lindane

Results

All birds except a juvenile Swainson's thrush contained pesticide residues. The rest of the birds had 4,4'-DDE residues ranging from 1 to 9 ppb. Dieldrin was found in four out of six birds and ranged from 0.4 to 12 ppb. Pesticide-grade hexane used in this analysis contained trace amounts of heptachlor epoxide, but it was only detected in two out of six birds in concentrations ranging from 2 to 5.7 ppb. Extracts analysis for five birds are not available at this time, because due to instrumentation problems all samples had to be sent to Daily Analytical Lab. Results are summarized in Table II. No statistical analysis could be conducted due to the low sample size.

Table II

Species	Common Name	Age	Date of Collection	Sample ID #	Sex	Pesticides Detected (ppb
Catharus ustulatus	Swainson's thrush	juvenile	15 Sept. 1993	5	m	no pesticides detected
Catharus ustulatus	Swainson's thrush	juvenile	15 Sept. 1993	7	f	Results not available
Catharus ustulatus	Swainson's thrush	adult	11 May 1994	3	f	4,4'-DDE: 1
Catharus ustulatus	Swainson's thrush	adult	3 Sept. 1994	6	m	Dieldrin: 0.4** 4,4'-DDE: 3
Dumetella carolinensis	Gray catbird	adult	11 May 1994	1	f	Heptachlor epoxide*: 5. Dieldrin: 12 4.4'-DDE: 4
Passerina cyanea	Indigo bunting	adult	25 May 1994	4	f	Heptachlor epoxide*: 2 Dieldrin: 2 4,4'-DDE: 3
Seiurus aurocapillus	Ovenbird	juvenile	8 Sept. 1994	8	m	Results not available
Seiurus aurocapillus	Ovenbird	juvenile	11 Sept. 1994	10	f	Results not available
Seiurus aurocapillus	Ovenbird	adult	15 May 1994	9	m	Results not available
Seiurus aurocapillus	Ovenbird	adult	15 Sept.1994	11	m	Results not available
Setophaga ruticilla	American redstart	juvenile	2 Sept. 1994	2	m	Dieldrin: 4 4.4'-DDE: 9

** traces of Heptachlor epoxide (20 ng/L) are in pesticide grade hexane
* detected below detection limit

Discussion

For conservation purposes it is important to determine sources of pesticide contamination, and which birds are more susceptible to accumulating them, possibly through differing diets, different habitat types and different habitat ranges both in the breeding and wintering areas.

Diet

Birds that feed higher in the food chain are more susceptible to bioaccumulation of pesticides than if they fed lower in the food chain, because their food will be more contaminated, and they live longer thus having time frame in which to accumulate the pesticides (Mora and Anderson 1991). Except for the indigo bunting, no information was available on the winter diets of the birds used in this study. During the breeding season, the American redstarts diet consists mainly of insects, and rarely seeds and berries (Ehrlich et al. 1988). Both the Swainson's thrush and the gray catbird feed on insects, fruit, and spiders (Ehrlich et al. 1988). I assume they tend to feed at a lower trophic level and, therefore, should have lower pesticide concentrations in their body. Results of this study for DDE concentrations agree with this prediction, but the concentration of Dieldrin was much higher (12ppb) in the gray catbird, than in the American redstart (9ppb).

The indigo bunting feeds on insects (including caterpillars, grasshoppers, and beetles), fruit (e.g. berries), spiders, and seeds of grasses and herbs. During the winter its diet mainly consists of seeds of grasses, buds, and some insects (Payne 1992). According to its diet, the indigo bunting should have the lowest concentration of pesticides compared to the

other bird species. However, according to the data the Swainson's thrush had the lowest pesticide level, while the indigo bunting had the second lowest.

Habitat Type

The habitat type in the breeding and wintering range is also an important aspect to consider when assessing a birds possible susceptibility to pesticide accumulation. Birds living closer to agricultural areas (e.g. birds favoring edges of forests) will likely be more susceptible than those living farther away from contaminated areas (e.g. birds living deep within the forests).

During the breeding season, all four species used in this study could possibly breed near contaminated areas. The American redstart, the gray catbird, and the Swainson's thrush breed by forest edges (Ehrlich et al. 1988), whereas the indigo bunting breeds in brushy and weedy habitat next to openings such as cultivated land (Payne 1992). Furthermore, the breeding habitat of the gray catbird includes wooded suburbs, and the Swainson's thrush prefers orchards (Ehrlich et al. 1988). On the other hand, all four bird species also breed in possibly less contaminated areas. The American redstart is also found in deciduous and deciduous/coniferous woodland, and in second growth forest, while the gray catbird breeds in dense brush that often borders shrublands, swamps, and streams (Ehrlich et al. 1988) (though the latter two could be contaminated by runoff from agricultural land). The Swainson's thrush can be found in woodland and riparian thickets (Ehrlich et al. 1988), while the indigo bunting prefers deciduous forest where fallen trees have created a clearing (Payne 1992).

Information on winter habitat type preferences was only available for the indigo bunting, which winters in weedy fields and citrus orchards, savannas, weedy croplands (e.g. beanfields and ricefields), and low second growth forests (Payne 1992). Thus it is unclear if any difference in pesticide concentrations between these species may be due to different preferences in habitat type, especially because no information was available on habitat type during the wintering season for three of the four species.

Habitat Range

Pesticide levels in the environment also differ between geographical regions, as pesticide usage is more extensive in some countries than in others. Until 1981 DDT, hexachlorobenzene, and other chloro hydrocarbons were still extensively used in Latin American countries (e.g. El Salvador, Mexico, Argentina, and Suriname) (FAO 1989). In the US DDT was widely used until the late 1960's, and residues of it and other chlorinated pesticides were still being accumulated in North America by organisms as recently as 1991 (Thies et al. 1994, Bishop et al. 1994).

The Swainson's thrushes breeding range includes most of Canada and Alaska, and its wintering habitat range includes most of Central and South America (Figure 2). The American redstart breeds in the southern part of Canada and in most of central and eastern US (Figure 6). Both the gray catbird's (Figure 3) and the indigo bunting's (Figure 4) breeding range also includes most of the central and eastern US. The gray catbird's wintering range is the smallest of the four species, including Florida, parts of Mexico, and Nicaragua to Panama. The indigo bunting has a larger wintering range and is found from Mexico south to Colombia and

Venezuela, while the American redstart is found even farther south to northern Peru and northwest Brazil.

Heptachlor epoxide was only detected in the gray catbird and the indigo bunting. Their wintering habitat overlaps with the wintering habitat of the birds in which no heptachlor epoxide was found, and their breeding habitat also overlaps with that of the American redstart. Both species have a large wintering and breeding range. It is possible that the Swainson's thrushes and the American redstart lived in a different area than the birds containing heptachlor epoxide during the summer, and also that the Swainson's thrush wintered in a different area. Because the American redstart is a juvenile it did not migrate to its winter habitat. Therefore it is not known where (wintering and/or breeding habitat range) the indigo bunting and the gray catbird most likely accumulated the pesticide, because both their entire habitat range overlaps that of non-contaminated birds.

Heptachlor epoxide had been added by the manufacturer, in trace amounts (20ng/ml), to the hexane used for extraction. However, the concentration of heptachlor epoxide due to these trace amounts did not exceed 0.6 ng/ml in any of the analyzed fractions, and therefore was below the detection limit of 1 ng/ml. Furthermore, no heptachlor epoxide was detected in four bird samples. The heptachlor epoxide detected in the other two birds is, therefore, likely due to residues contained in the birds themselves, although the concentration level might be affected by the trace amounts.

The American redstart was the most contaminated bird, and because it was a juvenile, all of its pesticides must have come from its mother through the egg, or from contaminated food found in the breeding range.

This suggests that a large part of the pesticide contamination came from North America. In 1991 Mexican free-tailed bats in Oklahoma (Thies et al. 1994) and red winged blackbirds in the Great Lakes and St. Lawrence River basin (Bishop et al. 1994) also picked up organochlorine pesticide residues from these areas. In addition, the wintering range of the Swainson's thrush includes most of the overwintering ranges of the other birds, but it breeds farther north. Because it was the least contaminated of the analyzed birds, this suggests that most of the pesticide contamination stem from the US.

Age Class

The longer an organism lives, the more time it will have to accumulate pesticides. Therefore, older birds from the same species should be more contaminated than younger ones. In the Swainson's thrushes the older birds were contaminated, while in the younger bird no pesticide residues were detected. The juvenile American redstart had the highest DDE concentration (9ppb), and the second highest Dieldrin concentration (4ppb). It was also the youngest bird in which pesticides were detected (no pesticides were detected in the other analyzed juvenile), and thus had a short lifespan in which to accumulate the residues. The American redstart must have lived in a very contaminated area. Further research will show if this juvenile was an exception, or if the American redstart is highly vulnerable to pesticide accumulation.

Differences in Sex

Females are able to reduce some of their pesticide levels by passing them on to their offspring through the eggs (Bishop et al. 1994), and

therefore should have smaller pesticide concentrations compared to males of the same species. The adult female Swainson's thrush was less contaminated than the analyzed adult male of the same species. Yet it is unknown if the adult female ever bred, since it was captured during spring migration.

Possible Effects of Pesticide Concentrations

Dieldrin and DDE are known endocrine disrupters that mimic estrogen (Colborn et al. 1993). Although the concentrations at which these chemicals adversely affect neotropical migratory songbirds is unknown, the detected levels are cause for concern. The species with greatest decline are also the species in which the highest pesticide concentrations were found. According to breeding bird surveys from 1978 to 1988 the population of American redstarts in North America has decreased by 1.54%, while the population of the Swainson's thrush only declined by 0.05% (Sauer and Droege 1992). The population of the indigo bunting decreased by 0.7% in the years 1978 to 1987, while that of the gray catbird declined by 1.4% (Robbins et al. 1989). In this study the gray catbird was also much more contaminated by pesticides than the indigo bunting.

Because of the small sample size statistical analysis could not be performed, and it is unknown and unlikely that this data is representative of the entire population of each bird species. Differences in pesticide levels could be due to differences in age, and thus differences in time to accumulate the pesticides. In addition, the exact location within the breeding and wintering habitat range will have a significant impact on the concentrations of the pesticides within the birds.

In conclusion, pesticide levels of neotropical migratory songbirds are a cause for concern because they could be part of the reason for the observed decline in their populations. More birds must be analyzed to document possible sources of contamination. Further research should also be initiated to study what pesticide concentrations in the body of neotropical migratory songbirds disrupts their endocrine system, and thus if the observed pesticide levels is having an impact on the birds reproductive success.

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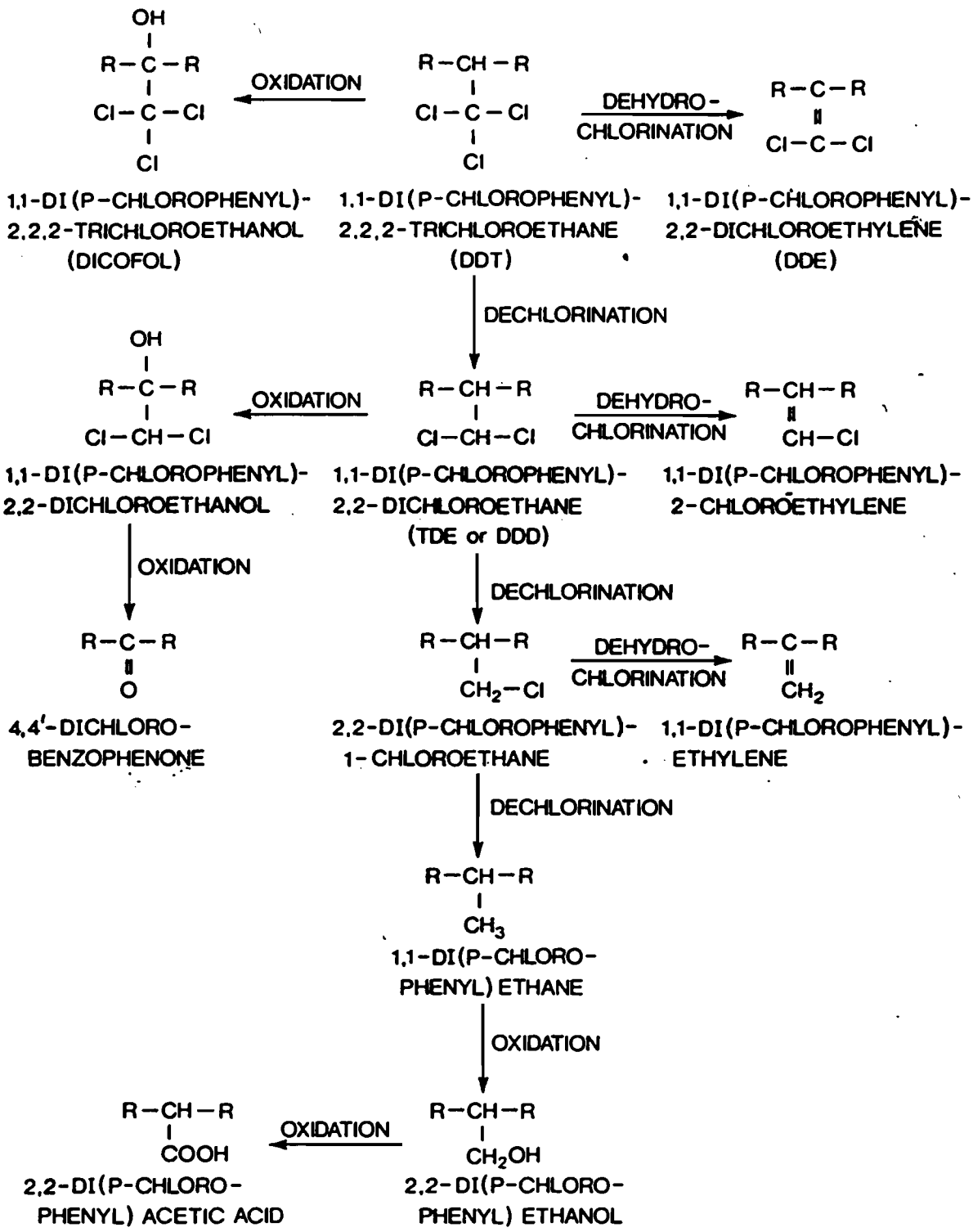
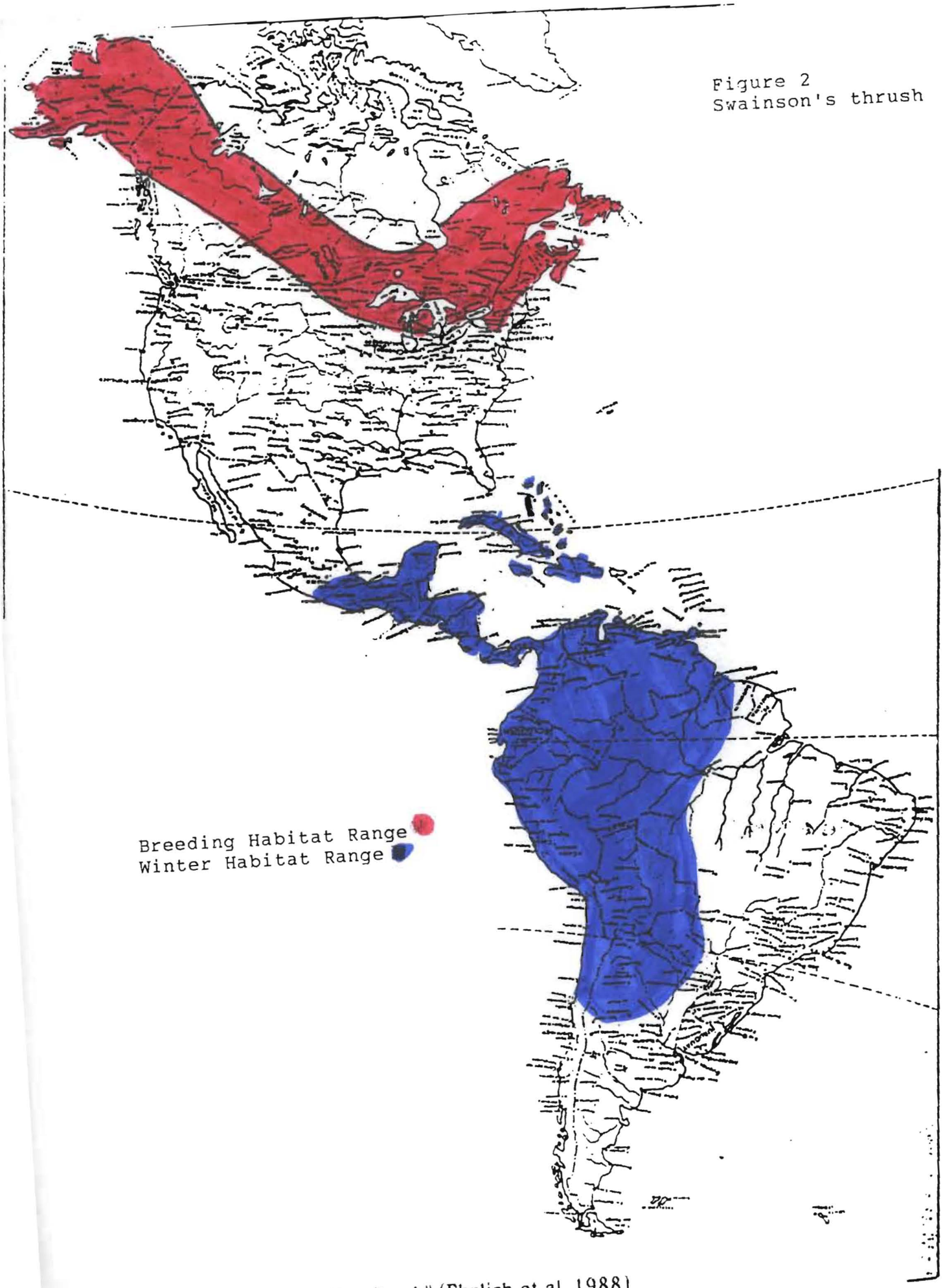


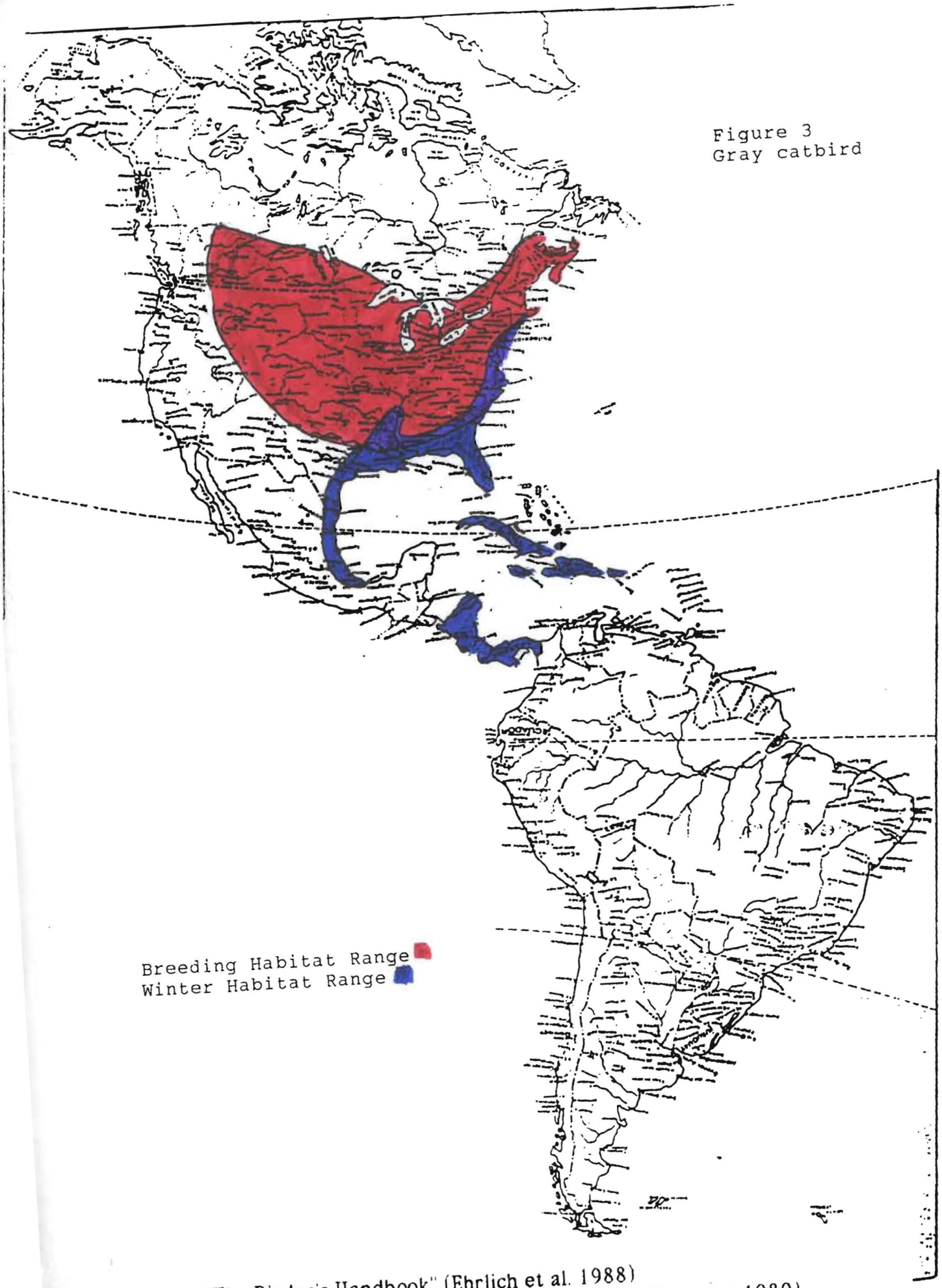
Figure 1: Major microbial degradation pathways of DDT (R-4-chlorophenyl) (Robbins et al. 1989).

Figure 2
Swainson's thrush



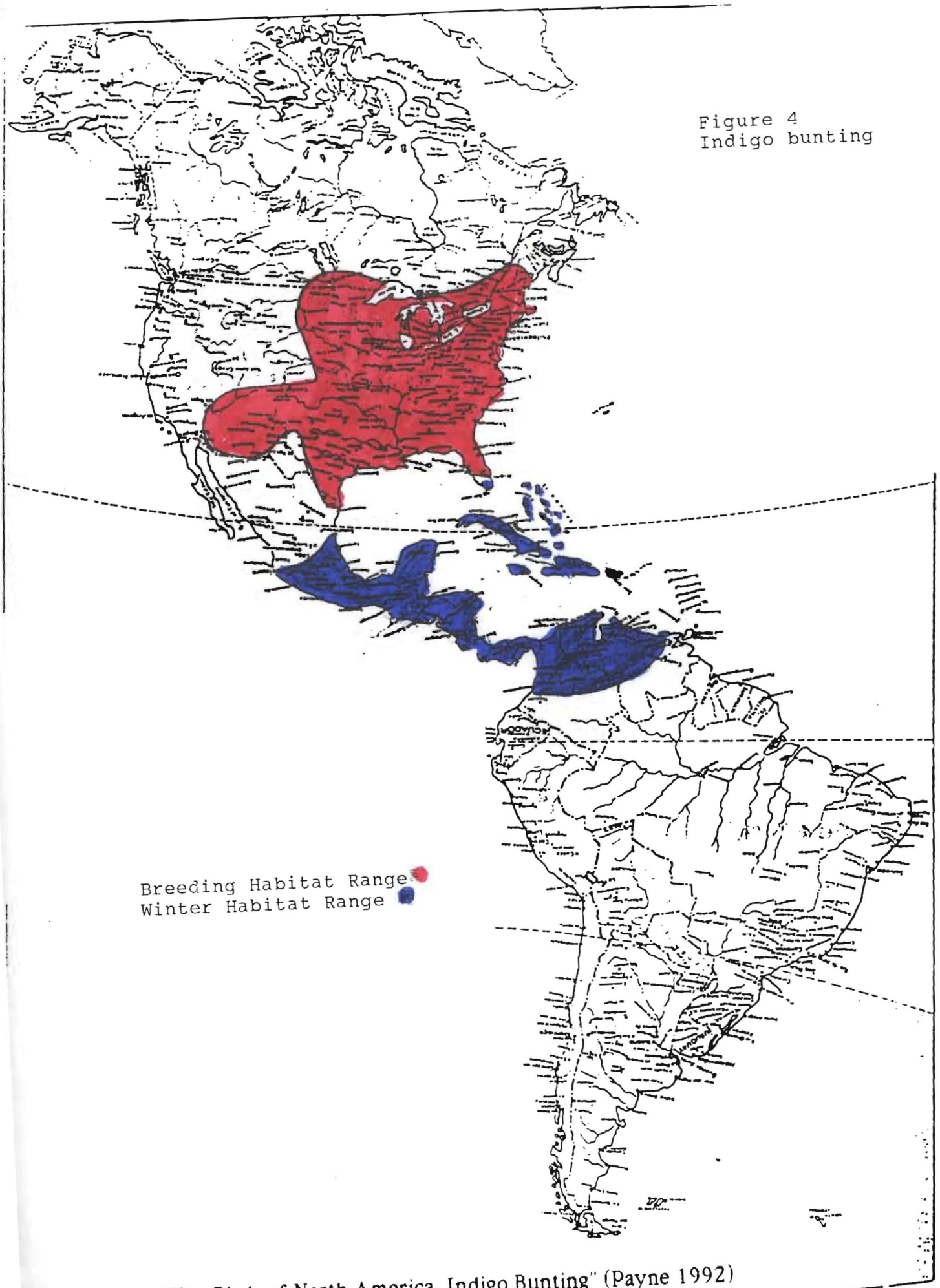
Adapted from "The Birder's Handbook" (Ehrlich et al. 1988)
and "A Field Guide to the Birds East of the Rockies" (Peterson 1980)

Figure 3
Gray catbird



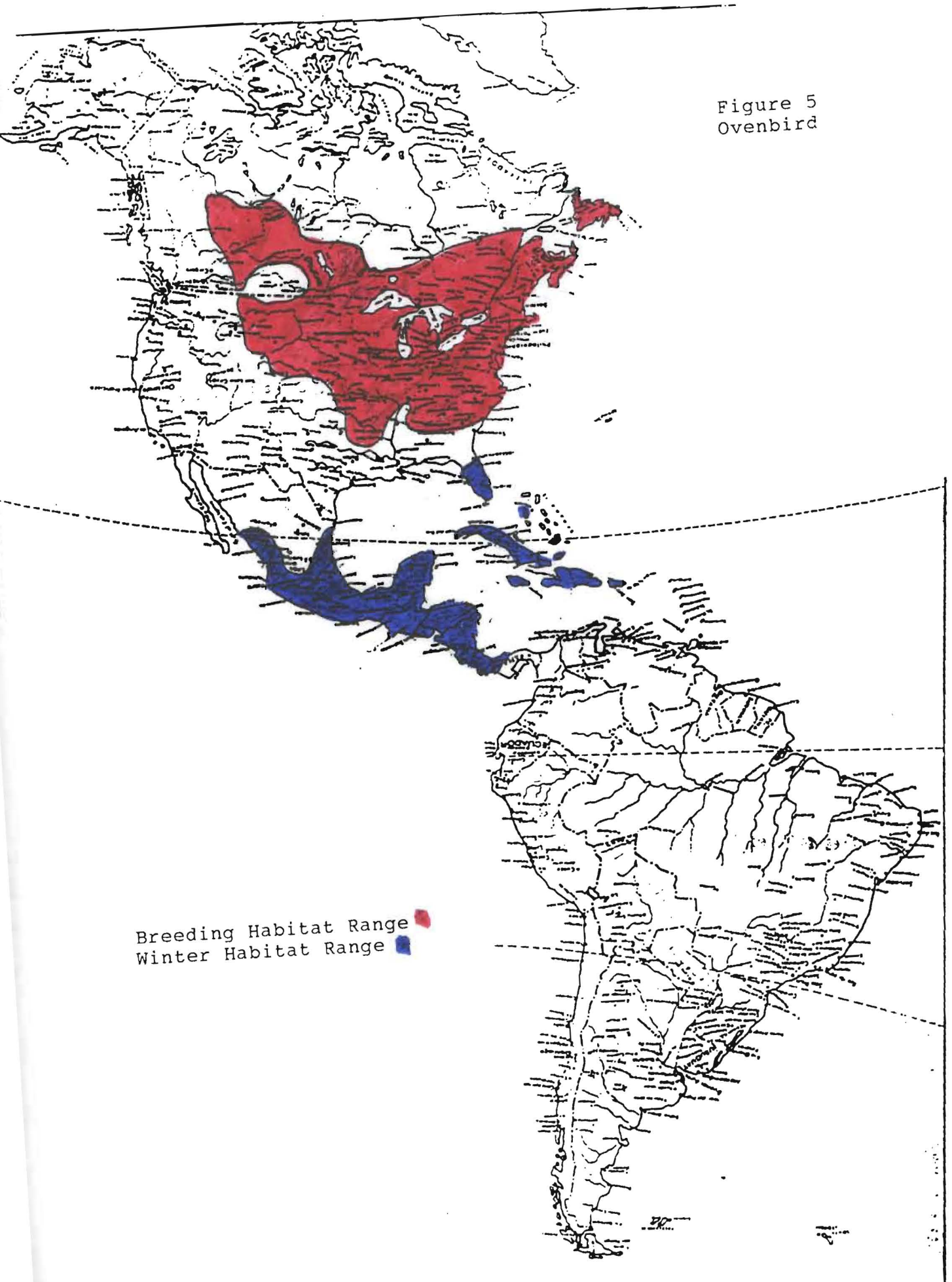
Adapted from "The Birder's Handbook" (Ehrlich et al. 1988)
and "A Field Guide to the Birds East of the Rockies" (Peterson 1980)

Figure 4
Indigo bunting



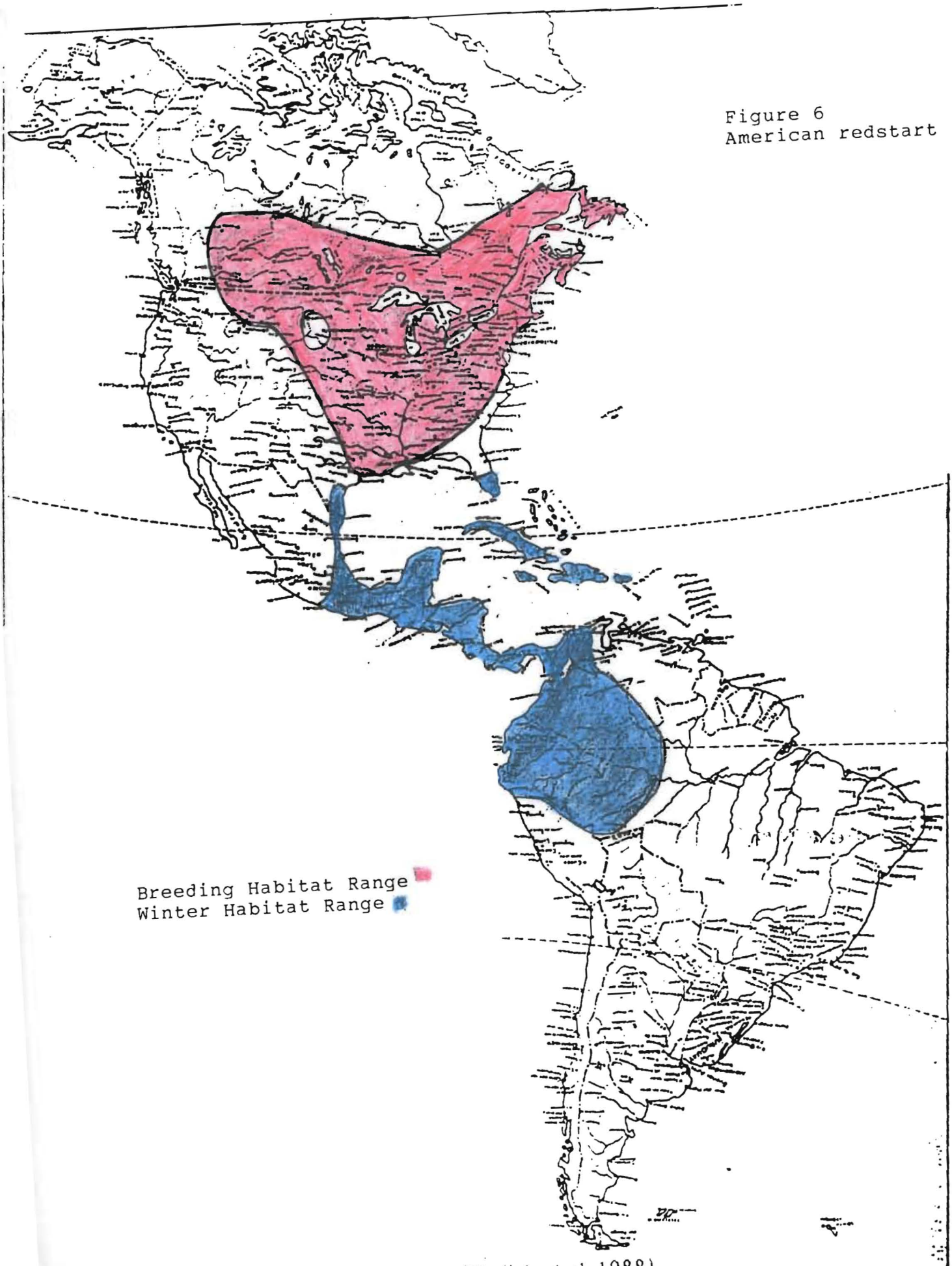
Adapted from "The Birds of North America, Indigo Bunting" (Payne 1992)

Figure 5
Ovenbird



Adapted from
"The Birds of North America, Ovenbird" (Vanhorn and Donovan 1994)

Figure 6
American redstart



Adapted from "The Birder's Handbook" (Ehrlich et al. 1988)
and "A Field Guide to the Birds East of the Rockies" (Peterson 1980)

Extract #1 Fraction #1

Appendix III: Sample #1 Chromatograph & Confirmation

Internal Standard Report

Data File Name : D:\HPCHEM\1\DATA\OCT20\030F0401.D
Operator : MMO
Instrument : ECD #2
Sample Name : 307-01 FRAC 1
Run Time Bar Code:
Acquired on : 21 Oct 94 09:27 AM
Report Created on: 26 Oct 94 10:10 AM
Last Recalib on : 07 JUN 94 11:36 AM
Multiplier : 10

Page Number : 1
Vial Number : 30
Injection Number : 1
Sequence Line : 4
Instrument Method: DUALPEST.MTH
Analysis Method : DUALPEST.MTH
Sample Amount : 0
ISTD Amount : 1

Sig. 1 in D:\HPCHEM\1\DATA\OCT20\030F0401.D

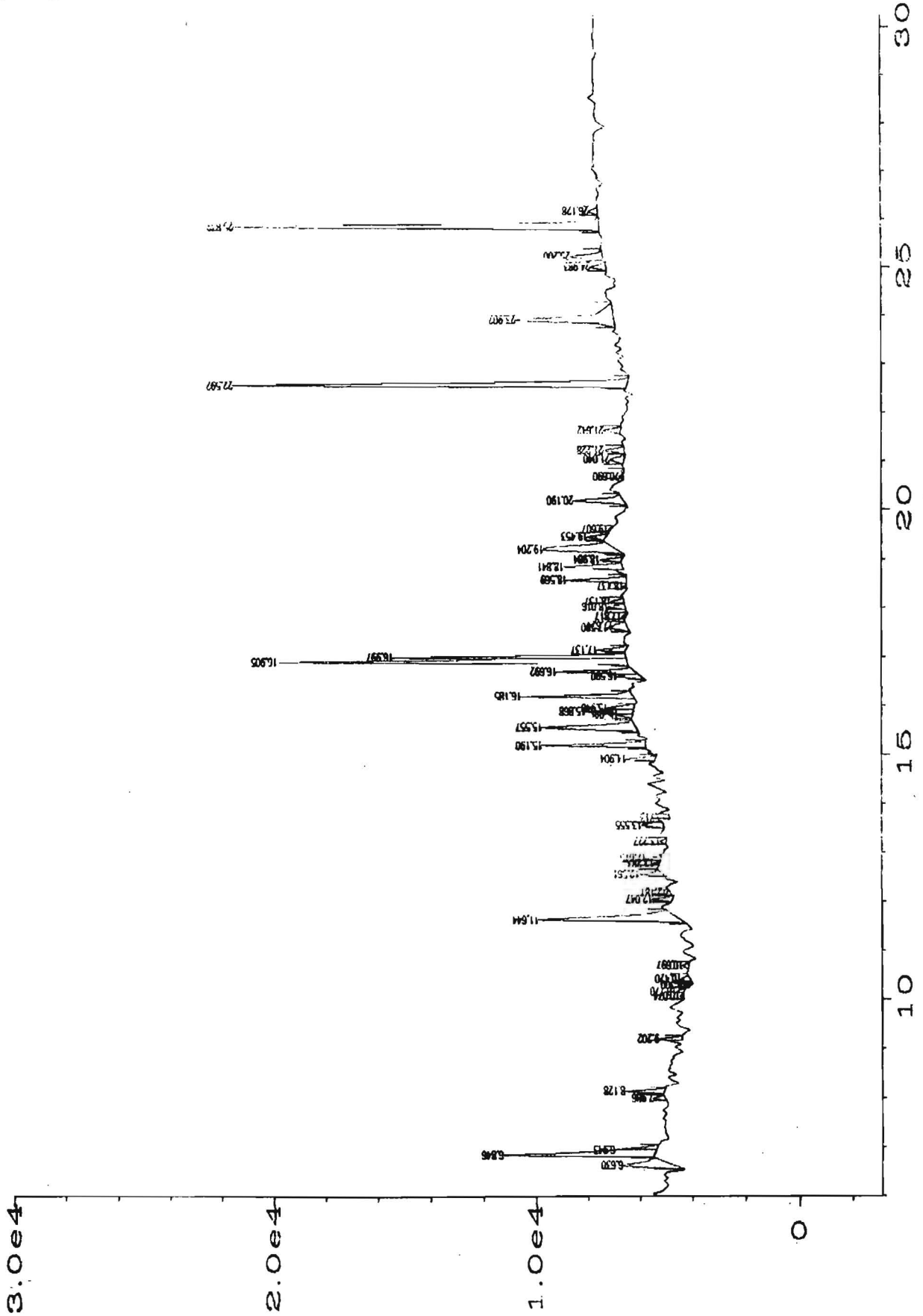
Ret Time	Area	Type	Width	Ref#	ug	Name
8.263	* not found *			1		Trifluralin
8.617	* not found *			1-R		TCMX (Surr)
9.202	2849	MM	0.053	1	0.256	Propachlor NC = Not Confirmed
10.170	1202	MM	0.061	1	0.00555	Hexachlorobenzene
10.559	* not found *			1		alpha-BHC
11.644	33013	MM	0.102	1	0.260	Gamma-BHC NC
11.884	* not found *			1		Beta-BHC
12.783	560	MM	0.046	1	0.00379	Heptachlor NC
12.875	1856	MM	0.058	1	0.0171	delta-BHC NC
13.713	276	MM	0.037	1	0.00167	Aldrin NC
15.190	14293	MM	0.060	1	0.102	Heptachlor Epoxide
16.187	* not found *			1		Endosulfan I
16.692	11486	VM	0.064	1	0.0731	p,p'-DDE
16.997	30535	VF	0.058	1	0.212	Dieldrin
17.983	* not found *			1		Endrin
18.254	* not found *			1		p,p'-DDD
18.507	* not found *			1		Endosulfan II
19.199	* not found *			1		p,p'-DDT & 0.173 ng
19.406	* not found *			1		Endrin Aldehyde
19.916	* not found *			1		Endosulfan Sulfate
21.040	3249	MM	0.100	1-R	0.0317	DBC (Surr)
21.727	* not found *			1		Methoxychlor
22.263	* not found *			1		Endrin Ketone
22.592	67936	BB	0.070	1-IR	10.000	Mirex
27.393	* not found *			1-R		DCB (Surr)

Time Reference Peak	Expected RT	Actual RT	Difference
2	8.617	* not found *	
21	21.079	21.040	-0.039
24	22.588	22.592	0.004
25	27.393	* not found *	

Could not find time reference peak:
No peak of Number 2's description at 8.617 + 0.041 - 0.041 min.
Could not find time reference peak:
No peak of Number 25's description at 27.393 + 0.041 - 0.041 min.
Not all time reference peaks were found
Not all calibrated peaks were found

Ext. #1 Fraction #1

Sig. 1 in D:\HPCHEM\1\DATA\OCT20\030F0401.D



Extract #1 Fraction #1 Confirmation

External Standard Report

Data File Name : D:\HPCHEM\1\DATA\OCT20\030R0401.D
Operator : MMO
Instrument : ECD #2
Sample Name : 307-01 FRAC 1
Run Time Bar Code:
Acquired on : 21 Oct 94 09:27 AM
Report Created on: 26 Oct 94 02:07 PM
Last Recalib on : 21 OCT 92 10:40 AM
Multiplier : 10

Page Number : 1
Vial Number : 30
Injection Number : 1
Sequence Line : 4
Instrument Method: DUALPEST.MTH
Analysis Method : PESTCON.MTH
Sample Amount : 0
ISTD Amount :

Sig. 2 in D:\HPCHEM\1\DATA\OCT20\030R0401.D

Ret Time	Area	Type	Width	Ref#	ug	Name
7.990	* not found *			1-R		TCMX(Surr.)
9.354	4727	MV	0.080	1	0.0153	HCB
9.469	7030	VM	0.083	1	0.346	Propachlor NC
11.533	* not found *			1		Gamma-BHC
12.187	2511	MM	0.059	1	0.00958	Heptachlor NC
12.869	* not found *			1		Aldrin
13.386	* not found *			1		Beta-BHC
14.027	* not found *			1		Delta-BHC
14.628	35346	BB	0.062	1	0.143	Heptachlor Epoxide
15.754	17419	VM	0.058	1	0.0834	p,p'-DDE
16.258	51411	BB	0.056	1	0.234	Dieldrin
16.832	* not found *			1		Endrin
20.356	* not found *			1-R		DBC(Surr)
24.508	* not found *			1-R		DCB(Surr)

NC / compounds outside RT window

Time Reference Peak	Expected RT	Actual RT	Difference
1	7.990	* not found *	
13	20.356	* not found *	
14	24.508	* not found *	

Could not find time reference peak:
No peak of Number 1's description at 7.990 + 0.050 - 0.050 min.
Could not find time reference peak:
No peak of Number 13's description at 20.356 + 0.050 - 0.050 min.
Could not find time reference peak:
No peak of Number 14's description at 24.508 + 0.050 - 0.050 min.
Not all time reference peaks were found
Not all calibrated peaks were found

Area Percent Report

Data File Name : D:\HPCHEM\1\DATA\OCT20\030R0401.D
Operator : MMO
Instrument : ECD #2
Sample Name : 307-01 FRAC 1
Run Time Bar Code:
Acquired on : 21 Oct 94 09:27 AM
Report Created on: 26 Oct 94 02:07 PM
Last Recalib on : 21 OCT 92 10:40 AM

Page Number : 1
Vial Number : 30
Injection Number : 1
Sequence Line : 4
Instrument Method: DUALPEST.MTH
Analysis Method : PESTCON.MTH
Sample Amount : 0

Extract #1 Frac. #1

Sig. 2 in D:\HPCHEM\1\DATA\OCT20\030R0401.D