

## Organochlorine Insecticide Residues in Amphibians and Reptiles from Iowa and Lizards from the Southwestern United States<sup>1</sup>

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Ecosystems are generally contaminated with low levels of organochlorine insecticide residues. Numerous reports of residues in biota include relatively little data for amphibians and reptiles. To help fill this void, specimens of amphibians and reptiles were collected from 2 agricultural areas of Iowa, and lizards were collected from nonagricultural areas in Arizona, New Mexico, and Texas. Iowa specimens came from a region with past use of organochlorine insecticides, particularly DDT as a general purpose insecticide and aldrin and heptachlor for control of soil-insect pests.

CULLEY and APPELGATE (1967) estimated insecticide concentrations in 3 species of lizards (checkered whiptail, Cnemidophorus tessellatus; western whiptail, C. tigris; and little striped whiptail, C. inornatus) collected from agricultural and desert areas in the vicinity of Presidio, TX. Residue data, presented as a composite for all 3 species of lizards and as mean concentrations of p,p'-DDE (ppm) in 5 or 6 samples, were: 0.1 to 7 (tail muscle), 0.3 to 3.8 (brain tissue), 0.1 to 3.8 (liver), 5.4 to 46 (coelem fat bodies), and 0.1 to 7.7 (stomach contents). There seemed to be little difference in concentrations between samples from various sites in cotton fields or

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from the desert peripheral to cotton fields. Concentrations decreased up to 9 miles from cotton fields; thereafter they remained essentially static. Identical mean concentrations of p,p'-DDE (3.4 ppm) were found in muscle tissue of 4 gravid and 4 nongravid female lizards (species not given) collected in the same field; eggs from the gravid females contained 16 ppm p,p'-DDE.

MEEKS (1968) applied <sup>36</sup>Cl-DDT (0.2 lb/acre) to a marsh at the edge of Lake Erie and measured residues in plants and animals. Mean residues (ppm) in fat tissues of northern leopard frogs, Rana pipiens pipiens, were about 0.03 and in bullfrogs, Rana catesbeiana, about 0.4, 1 year after application. One common snapping turtle, Chelydra serpentina serpentina, caught 15 months after the DDT application had about 13 in the fat and lower residues in other tissues. High residues also were found in fat of 7 midland painted turtles, Chrysemys picta belli, and 2 Blanding's turtles, Emydoidea (=Emys) blandingii (average about 3.1 and 4.2, respectively). Fat always contained higher residues than any other tissues in northern water snakes, Natrix sipedon sipedon (e.g., about 2.2, 1 year after application). These residue values are converted to wet weight values from dry weight and conversion values given in the report.

KORSCHGEN (1970) reported organochlorine insecticide residues in 1 bull snake, Pituophis sayi, 2 eastern garter snakes, Thamnophis sirtalis, and 42 American toads, Bufo americanus americanus, collected in 1967 in 2 cornfields near Malta Bend (Saline Co), MO that had been treated with aldrin for 15 and 16 years of the past 17 years. Average residues (ppm) of DDT, DDE, and DDD (combined) and of dieldrin were approximately: 1 bull snake, 0.3 and 1.2; 2 garter snakes, 0.5 and 12.4; 2 adult toads, 0.1 and 1.4; and 40 young toads, 0.1 and 4.6, respectively, based on wet weights of the tissues analyzed.

FLEET et al. (1972) reported residues of DDT and dieldrin in snakes collected from aquatic-riparian habitats in 2 agroecosystems near College Station, TX. One sampling area on the floodplain of the Brazos River (Burleson Co) is used extensively in cotton production; the other sampling area on the floodplain of the Navasota River (Brazos Co) is partially wooded and used primarily for cattle grazing. Extensive use of insecticides had never occurred in the second area. Residue analyses were made on fat bodies from 33 specimens of 10 species. Residues (ppm) of p,p'-DDE and dieldrin were much higher in snakes collected in the Brazos area (36 to 1009 and 0.9 to 14, respectively) than in snakes collected in the Navasota area (0.2 to 15 and 0.1 to 0.3, respectively). Insecticide residues seemed to be highest in those species whose food is most closely associated with water. Precipitation runoff presumably carries residues into aquatic environments. Distribution of residues is also affected by magnification within food chains.

DIMOND et al. (1975) reported DDT residues (ppm) in frogs, toads, and snakes from Maine forests sprayed with DDT at 1 lb/acre. Frog species were mostly the green frog, Rana clamitans, plus a few specimens of the northern leopard frog; the pickerel frog, Rana palustris; and R. catesbeiana. Samples were analyzed as mixed-species pools. Total DDT residues decreased from 0.61 to 0.15, 1 and 10 years after a single application of DDT. Data for American toads were 2.39 to 0.33, 1 and 7 years after a single application of DDT and 1.69 to 0.06 for eastern garter snakes, 1 and 10 years after a single application of DDT. OWEN and WELLS (1976) found background residues of p,p'-DDE (ppm) in fat samples from red-eared (3 specimens), Chrysemys scripta, and midland painted (2 specimens) turtles of about 4.6 and 2.7, respectively. The turtles were collected in an area of northwest Tennessee (Reelfoot Lake) where DDT had been used for agricultural purposes.

FLEET and PLAPP (1978) reported an overall decline in DDT residues in fat samples from aquatic snakes from 1971 (FLEET et al. 1972) to 1974-75. The snakes were collected in a cotton agroecosystem near College Station (Burlison Co), TX. The collection area was adjacent to cropland, which, from the late 1940's through 1960's was treated repeatedly with DDT and other insecticides to control cotton insects. An increase in p,p'-DDE/p,p'-DDT ratios, an absence of p,p'-DDD, and essentially no dieldrin were reported for the 1974-75 samples compared with the 1971 samples.

## MATERIALS AND METHODS

### Specimen collection and preparation

Twenty-one American toads were collected in Ames (Story Co), IA on May 17, 1974. Seven northern leopard frogs; 18 northern water snakes; 1 red-sided garter snake, Thamnophis sirtalis parietalis; 1 smooth green snake, Opheodrys vernalis; 3 western plains garter snakes, Thamnophis radix haydeni; 1 common snapping turtle; and 1 midland painted turtle were collected near Tripoli (Bremer Co), IA in the floodplain of the Wapsipinicon River on May 3 and 8, and June 5, 1974. Both Iowa collection areas are agricultural with major emphasis on corn and soybean production. Specimens were brought to the laboratory, measured from snout to vent, and decapitated. A ventral incision was made, fat removed, and stored in cardboard (ice cream) cartons at -10°C. Toads and frogs contained very little fat; therefore, total viscera samples were taken for analysis. Eggs were removed from gravid females and stored. The means and ranges of wet sample weights are shown in Table 1. Individual analyses were made of all samples.

Thirty-five lizards were collected between May 24 and 26, 1974 at the following locations: (1) Chiricahua Mountains, Silver Point (Cochise Co), AZ, elevation 1,890 m, Sonoran

transition zone, essentially a rocky and loose sand habitat with scattered clumps of creosotebush, chino grama grass, and burro brush; 1 Chihuahua whiptail lizard, Cnemidophorus exanguis; 2 sagebrush lizards, Sceloporus graciosus; 2 side-blotched lizards, Uta stansburiana; 3 tree lizards, Urosaurus ornatus; 1 western whiptail lizard; and 12 Yarrow's scaly lizards, Sceloporus jarrovi; (2) Malpais lava flows 5 miles east of Grants (Valencia Co), NM, elevation 1,676 m, dominant vegetation was leather weed, mesquite, dog cactus, and seepwillows; 1 tree lizard; (3) Study Butte (Brewster Co), TX, elevation 1,341 m, loose sand washes with scattered creosotebush, mesquite, and ocotillo; 8 greater earless lizards, Holbrookia texana; 1 New Mexican whiptail lizard, Cnemidophorus neomexicanus; and 2 western whiptail lizards; and (4) Terlingua (Brewster Co), TX, elevation 1,646 m, in a rocky canyon; 2 fence lizards, Sceloporus undulatus. Lizards were collected by "noosing" and then stunning them with heavy rubber bands. The lizards were preserved on ice until they were frozen (-10°C) at Ames, IA.

The lizards were thawed before analysis. All viscera were removed, weighed, and transferred to a mortar for grinding with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The carcass was weighed and cut into small pieces before transferring to a mortar for grinding with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Individual analyses were made of all lizards. These specimens contained so little fat it was necessary to analyze total carcass and viscera samples.

#### Residue analyses

Sample extraction, liquid-liquid partitioning, and liquid chromatography procedures followed those described by THOMPSON (1977), Section 5,A,(1), for the analysis of human or animal adipose tissue. A sample was macerated with Na<sub>2</sub>SO<sub>4</sub> and the fat was isolated by repetitive extractions with petroleum ether. Organochlorine insecticide residues were extracted from the fat with acetonitrile and then partitioned back into petroleum ether by aqueous dilution of the acetonitrile extract. The petroleum ether extract was concentrated to 5 ml with a Kuderna-Danish (K-D) evaporator and transferred to an activated Florisil<sup>®</sup> column for successive elutions with 6% and 15% ethyl ether/petroleum ether. The respective eluates were both concentrated to suitable volumes with K-D evaporators and the final extracts examined by electron capture, gas-liquid chromatography.

A Packard, Model 7821, gas chromatograph equipped with a dual-column oven and electron capture detectors was used to analyze the 2 eluates from the Florisil column. Operating temperatures were: injector 230°C, column 190°C, detector 215°C, and outlet 190°C. Two different glass columns were used to verify the results. One column (4 mm i.d. x 122 cm) was packed with 4% SE30 and 6% OV210 on Chromosorb<sup>®</sup> WHP 80/100 mesh. A second column (4 mm i.d. x 183 cm) was packed with 5% OV210 on Chromosorb WHP 80/100 mesh. Data in Tables 1 and 2 are the average of residues detected with the 2 columns. The carrier gas was

TABLE 1

Mean sample weights and organochlorine insecticide residues in wildlife from Iowa, 1974. Dashes indicate samples were not available. Blanks indicate samples do not apply for that category. DDE = p,p'-DDE; HO = heptachlor epoxide. Numbers in parentheses show the range of values.

| Wildlife        | Sex              | No. of<br>speci-<br>mens | Weights<br>(g)       | Residues (ppb)      |                | No. of<br>samples | Weights<br>(g) | Eggs |                     |               |               |              |
|-----------------|------------------|--------------------------|----------------------|---------------------|----------------|-------------------|----------------|------|---------------------|---------------|---------------|--------------|
|                 |                  |                          |                      | DDE                 | Dieldrin       |                   |                | DDE  | Dieldrin            |               |               |              |
|                 |                  |                          |                      | HO                  |                |                   |                |      |                     |               |               |              |
| American toads  | F                | 4                        | 9.45<br>(9.10-10.27) | N <sup>a</sup>      | 8              | N                 | -              | -    | -                   |               |               |              |
|                 | M                | 17                       | 3.85<br>(2.02-6.23)  | 19<br>(N-176)       | 10<br>(N-33)   | N                 | -              | -    | -                   |               |               |              |
|                 | F                | 4                        | 1.50<br>(0.19-4.29)  | N                   | N              | N                 | -              | -    | -                   |               |               |              |
|                 | M                | 3                        | 0.25<br>(0.04-0.71)  | N                   | N              | N                 | -              | -    | -                   |               |               |              |
| Snakes          |                  |                          |                      | Fat                 |                |                   |                |      |                     |               |               |              |
|                 | Northern water   | F                        | 13                   | 2.68<br>(1.23-5.14) | 79<br>(N-300)  | 50<br>(N-161)     | 26<br>(N-101)  | 9    | 4.20<br>(2.86-8.62) | 60<br>(N-274) | 49<br>(N-150) | 15<br>(N-61) |
|                 |                  | M                        | 5                    | 2.84<br>(0.34-3.72) | 215<br>(N-625) | 132<br>(98-193)   | 25<br>(N-47)   |      |                     |               |               |              |
|                 | Red-sided garter | F                        | 1                    | 4.06                | 36             | 99                | 26             | 1    | 6.21                | 52            | 26            | 42           |
| Smooth green    | F                | 1                        | 0.55                 | N                   | 116            | 34                |                | -    | -                   | -             | -             |              |
| Western plains  | F                | 1                        | 1.11                 | 112                 | 118            | 51                |                | 1    | 6.49                | 31            | 41            | 13           |
| garter          | M                | 2                        | 1.95                 | 10                  | 26             | 6                 |                |      |                     |               |               |              |
| Turtles         |                  |                          |                      | (N-19)              | (N-52)         | (N-13)            |                |      |                     |               |               |              |
| Common snapping | F                | 1                        | 0.90                 | N                   | N              | N                 |                | 1    | 3.22                | N             | N             | N            |
| Midland painted | M                | 1                        | 2.32                 | 18                  | 74             | N                 |                |      |                     |               |               |              |

<sup>a</sup>None detected.

TABLE 2

Mean sample weights and organochlorine insecticide residues in lizards from the southwestern United States, 1974. DDE = p,p'-DDE. Numbers in parentheses show the range of values.

| Lizards              | No. of samples | Carcass             |                | Viscera                          |                |
|----------------------|----------------|---------------------|----------------|----------------------------------|----------------|
|                      |                | Weights (g)         | Residues (ppb) | Weights (g)                      | Residues (ppb) |
|                      |                |                     | DDE            | DDE                              | Dieldrin       |
| Arizona              |                |                     |                |                                  |                |
| Chihuahuah whiptail  | 1              | 3.94                | 34             | 1.35                             | 30             |
| Sagebrush            | 2              | 3.18<br>(2.71-3.65) | N              | 0.45<br>(0.38-0.51)              | N              |
| Side-blotched        | 2              | 1.01<br>(0.95-1.08) | N              | 0.30<br>(0.25-0.35)              | N              |
| Tree                 | 3              | 1.55<br>(1.29-1.82) | N              | 0.43<br>(0.27-0.69)              | N              |
| Western whiptail     | 1              | 6.75                | N              | 1.69                             | N              |
| Yarrow's scaly       | 12             | 4.95<br>(2.99-9.73) | N              | 1.43<br>(0.56-6.84) <sup>b</sup> | 2<br>(N-30)    |
| New Mexico           |                |                     |                |                                  |                |
| Tree                 | 1              | 2.12                | N              | 0.83                             | N              |
| Texas                |                |                     |                |                                  |                |
| Greater earless      | 8              | 4.08<br>(2.36-7.93) | 2<br>(N-15)    | 1.10<br>(0.56-2.52)              | 22<br>(N-148)  |
| New Mexican whiptail | 1              | 2.89                | 227            | 0.69                             | 246            |
| Western whiptail     | 2              | 3.90<br>(3.20-4.61) | N              | 0.75<br>(0.67-0.83)              | N              |
| Fence                | 2              | 1.61<br>(1.59-1.64) | N              | 0.38<br>(0.31-0.45)              | N              |

<sup>a</sup>None detected.

<sup>b</sup>Pregnant female.

prepurified nitrogen with flow rates of 112 and 60 ml/min for columns 1 and 2, respectively. Organochlorine insecticides (analytical standards) were used to confirm retention times with the two gas chromatography columns. Approximately 5 picograms/ $\mu$ l of DDE, dieldrin, or heptachlor epoxide could be detected accurately by these methods. The sensitivity level of detection was near 1 ppb for the 3 compounds listed in the results. Recoveries of from 85 to 95% of these compounds were attainable at this level of detection. There was no evidence of polychlorinated biphenyl or other electron-capturing compounds with these procedures and samples.

## RESULTS

Only residues of DDE and dieldrin in viscera of toads and of DDE, dieldrin, and heptachlor epoxide in fat and eggs from reptiles were found in specimens collected in Iowa (Table 1). These residues are similar to or less than residues found by other workers (MEEKS 1968, KORSCHGEN 1970, FLEET et al. 1972, DIMOND et al. 1975, FLEET and PLAPP 1978). The highest residues were found in snakes; residues in toads and frogs were absent or very low, probably owing to the analysis of viscera samples. Higher residues in snakes, including their eggs, than in toads and frogs seem to be consistent with the higher position of snakes in the food chain. There was no consistent trend in residues between sexes within species.

Only low or nondetectable residues of DDE and dieldrin were found in carcass and viscera samples from lizards collected in the southwestern United States (Table 2). One exception was a New Mexican whiptail lizard collected in Texas. Even though different species are involved, higher and more frequent residues of DDE and dieldrin were found in samples from Iowa where organochlorine insecticides had been used for 25 years than were found in samples collected in areas of the southwestern United States where these insecticides had been used infrequently, if at all.

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