Bull. Environ. Contam. Toxicol. (1995) 54:817–824

Environmental Contamination and Toxicology

Dicofol and DDT Residues in Lizard Carcasses and Bird Eggs from Texas, Florida, and California

D. R. Clark, Jr.,[1] E. L. Flickinger,[1,*] D. H. White,[2] R. L. Hothem,[3] A. A. Belisle[4]

[1]National Biological Service, Southern Science Center, Brazos Field Station, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843, USA
[2]National Biological Service, Southeastern Biological Science Center, Southeast Research Group, School of Forest Resources, University of Georgia, Athens, Georgia 30602, USA
[3]National Biological Service, California Pacific Science Center, Davis Field Station, c/o Department of Wildlife and Fish Conservation Biology, University of California, Davis, California 95616, USA
[4]National Biological Service, Patuxent Environmental Science Center, Laurel, Maryland 20708, USA

Received: 28 June 1994/Accepted: 31 October 1994

Dicofol is an organochlorine agricultural pesticide used to control mites. The principal commercial dicofol product is known as Kelthane™. More than 70% of dicofol product (about 3 million lb or 1.4 million kg) sold annually in the U.S. is applied in California, Arizona, Texas, and Florida. Florida citrus and California cotton receive more than half the total (Clark 1990). In laboratory studies, dicofol, which is structurally similar to DDT, had adverse impacts on reproduction in fish (deformed larvae, delayed hatching), birds (reduced eggshell thickness, reduced hatchability), and mammals (reduced insemination rate, reduced pregnancy rate, failure to produce young) (see Clark 1990 for review). Overall, the reproduction of birds seems less sensitive to dicofol than to DDE. However, in birds, dietary concentrations of dicofol between 1 and 10 μg/g (wet weight) fed to captive adult females caused such problems as eggshell thinning, reduced hatching success, or reduced fertility in American kestrels (*Falco sparverius*) (Fry et al. 1988, Clark et al. 1990) and eastern screech-owls (*Otus asio*) (Wiemeyer et al. 1989). In spite of these laboratory findings, there have been no intensive field investigations of possible reproductive effects of dicofol on wild birds. Such studies must wait until field residue data are sufficient to identify populations with high exposure. If dicofol accumulates in birds in the field and heavily exposed populations can be identified, then their reproduction can be studied. Residues have not been reported from reptiles. Analytical screening of wildlife tissue samples for organochlorine chemicals only rarely includes dicofol, and this may explain why the relative hazard of dicofol to wildlife populations is poorly known.

We selected bird eggs for sampling because other published studies have investigated the reproductive effects of dicofol on captive birds (Fry et al. 1988, Wiemeyer et al. 1989, Clark et al. 1990) and because other investigators previously have sampled bird eggs from wild populations (see Clark 1990 for summary). We also collected lizards for analysis because lizard movements are much more restricted than those of birds, thus potentially subjecting lizards to heavier and more continuous exposure.

Present address: 2701 Leary Lane, No. 37, Victoria, Texas 77901, USA

Correspondence to: D. R. Clark

Our objectives were (1) to learn more about the extent to which dicofol residues accumulate in lizard carcasses and bird eggs in geographical areas where use is high, and (2) to use published data from laboratory studies of birds to judge whether the residue concentrations we found may threaten reptile or bird reproduction in contaminated populations. We also evaluated other recovered residues in the DDT group regarding possible effects.

MATERIALS AND METHODS

Samples were collected in 1988 from areas of high dicofol use. Samples analyzed included lizard carcasses from Texas, Florida, and California and bird eggs from Texas and California. Lizards were collected by hand or shot with dust shot from 0.22 caliber cartridges. Single eggs were collected from individual nests.

Texas lizards were 10 spotted whiptails (*Cnemidophorus gularis*) collected from citrus orchards in Cameron and Hidalgo Counties in June 1988. Six whiptails were collected June 10 in the middle of orchard no. 7; the other four were collected June 29-30 on the edges of orchards no. 2, 3, or 7. Dicofol was applied in late April (orchards no. 2 and 3) or early May and on June 1, 1988 (orchard no. 7), at 1.7-2.0 kg of active ingredient/ha (= 1.5-1.8 lb/A). Florida lizards were eight six-lined racerunners (*C. sexlineatus*) and two green anoles (*Anolis carolinensis*) collected from a citrus orchard at Haines City, Polk County, on May 24, 1988. Dicofol was last applied June 20, 1987, at 2.8 kg of active ingredient/ha (= 2.5 lb/A). California lizards were six side-blotched lizards (*Uta stansburiana*) and four western fence lizards (*Sceloporus occidentalis*) collected from cotton growing areas in Kings County June 17-20, 1988. All California lizards were collected near (within 0.4 km) cotton; dicofol was last applied June 23, 1987, at 1.7 kg of active ingredient/ha (= 1.5 lb/A) adjacent to one of four sites where lizards were collected, but spray dates and amounts near the other sites are unknown.

Texas bird eggs were five great-tailed grackles (*Quiscalus mexicanus*), four white-winged doves (*Zenaida asiatica*), and two white-tipped doves (*Leptotila verreauxi*) collected from citrus orchards (orchards no. 4 and 6) in Cameron and Hidalgo Counties on June 8-10, 1988. Dicofol was last applied in April 1988 at 2.0 kg of active ingredient/ha (= 1.8 lb/A). California bird eggs were seven black-necked stilts (*Himantopus mexicanus*), three American avocets (*Recurvirostra americana*), two northern harriers (*Circus cyaneus*), two American bitterns (*Botaurus lentiginosus*), two killdeers (*Charadrius vociferus*), two cinnamon teal (*Anas cyanoptera*), and one mallard (*A. platyrhynchos*) collected from cotton growing areas in Kings, Fresno, and Kern Counties between May 20 and June 21, 1988, except for one killdeer egg collected April 15, 1988. All California eggs were collected near dicofol-treated fields (all were within 8 km, 9 of 19 were within 1.5 km). In 15 cases, the nearest field had been sprayed with dicofol in June-July of 1987, in three cases it was sprayed in May-June of 1988, and in one case the spray date was

unknown. Spray application rate was 1.1 kg of active ingredient/ha (= 1.0 lb/A).

Egg contents were removed from shells, placed in chemically cleaned glass jars, frozen, and shipped to the Patuxent Wildlife Research Center where they were kept frozen until chemical analysis. Lizards were collected and frozen prior to shipment to Patuxent where they were thawed, measured, sex determined, examined for reproductive condition, and prepared as carcasses by removal of skin, head, feet, tail, and gastrointestinal tract.

Analytical procedures were modified from Krynitsky et al. (1988). All samples were analyzed for *o,p'*-dicofol, *p,p'*-dicofol, *o,p'*-dichlorobenzophenone (DCBP), *p,p'*-DCBP, *o,p'*-monodechlorinated dicofol (DCD), *p,p'*-DCD, *o,p'*-chloro-DDT (cl-DDT), *p,p'*-cl-DDT, *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT. The limit of detection was 0.1 µg/g. All residue concentrations are reported on a µg/g (parts per million) wet weight basis.

Both *o,p'*- and *p,p'*- isomers of DCBP, DCD, and DDE may also be decomposition products formed during the injection of *o,p'*- and *p,p'*-dicofol and cl-DDT. The gas chromatograph (Hewlett Packard 5890 series II) was calibrated by injecting *o,p'*- and *p,p'*-dicofol at several levels and measuring the amounts of corresponding DCBP and DCD isomers formed. Residue amounts of DCBP and DCD were corrected for any amounts formed from dicofol during injection; values for *p,p'*-DDE were reported without correction because no *p,p'*-cl-DDT was detected. A procedural blank was carried through with every 10 samples to correct for background contamination. Duplicate samples and spiked controls were also included to monitor analytical precision and accuracy. Average recoveries were 97% for *o,p'*-dicofol, 97% for *p,p'*-dicofol, 92% for *o,p'*-DCBP, 93% for *p,p'*-DCBP, 90% for *o,p'*-DCD, 85% for *p,p'*-DCD, 107% for *o,p'*-cl-DDT, 101% for *p,p'*-cl-DDT, 100% for *p,p'*-DDE, 100% for *p,p'*-DDD, and 99% for *p,p'*-DDT. Mean residue concentrations are geometric because of skewness in the residue data. Residues that were not detected (nd) entered the calculations as one-half the detection limit.

RESULTS AND DISCUSSION

Altogether six compounds were found in the samples: *p,p'*-dicofol, *p,p'*-DCBP, *p,p'*-DCD, *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT. Within each lizard species, means for snout-vent length (SVL) and residues did not differ significantly ($p > 0.05$) between the sexes and so the data were combined (Table 1). Dicofol residues were highest in spotted whiptail lizards from the Lower Rio Grande Valley of Texas with one carcass containing 12 µg/g dicofol, 15 µg/g DCD, and 0.79 µg/g DCBP (Table 1). All whiptails from each of two Texas citrus orchards (no. 2 and 7) contained dicofol residues; a single lizard from a third orchard (no. 3) contained only DDE. Dicofol also occurred in a six-lined racerunner lizard from a Florida citrus orchard (Table 1), but the amount was small

Table 1. Residues of dicofol and DDT in lizard carcasses. Means for snout-vent length (SVL) are common averages; means for residues are geometric. All lizards were reproductively active adults except 1 male (SVL 54 mm) and 1 female (SVL 66 mm) racerunner.

		Residue (µg/g wet weight)		
	SVL (mm)	*p,p'*-DDE	*p,p'*-DCD	*p,p'*-dicofol
Whiptail lizard carcasses, Texas, n = 10[a]				
Mean	85.5	0.443	0.868	0.712
95% C.I.	78.8-92.2	0.284-0.692	0.250-3.01	0.219-2.31
Range	72-97	0.14-0.96	nd[b]-15	nd-12
Racerunner lizard carcasses, Florida, n = 8[c]				
Mean	66.2	1.00		
95% C.I.	61.3-71.2	0.220-4.56		
Range	54-75	nd-9.6		nd-0.20
Anole lizard carcasses, Florida, n = 2[d]				
Data	55, 55	nd, 0.14		

[a] SVL was not available for two lizards because shooting separated vertebrae. *p,p'*-DDE present in all 10, *p,p*-DCD and *p,p*-dicofol in 9 of 10 lizards. Sample contained 4 males and 6 females. Lizard with maximum concentrations of *p,p'*-DCD and *p,p'*-dicofol also contained 0.79 ppm of *p,p'*-DCBP and was the only lizard containing this residue. [b] "Not detected" = below detection limit (0.1 µg/g wet weight). [c] Sample contained 4 males and 4 females. *p,p'*-DDE present in 7 of 8 lizards, only 1 contained *p,p'*-dicofol. [d] Sample contained 2 males.

(0.20 µg/g) and seven other racerunners and two green anoles from the same orchard contained no dicofol residues; eight of 10 lizards contained DDE. Lizards from sites near California cotton fields contained no residues of either dicofol or DDT.

The only bird egg containing dicofol residues was from a black-necked stilt nest adjacent to a California cotton field. This egg (Table 2) contained a trace of DCBP (0.026 µg/g) and appeared also to contain a trace of dicofol (0.12 µg/g), although the dicofol could not be confirmed. Single eggs from six other stilt nests as well as from 11 nests of six other species--northern harrier, American bittern, American avocet, killdeer, cinnamon teal, mallard--contained only DDE, except that one harrier also contained DDD, and DDT (Table 2). One cinnamon teal egg lacked any detectable residues.

Table 2. Residues of dicofol and DDT in bird eggs. Means are geometric. All eggs contained *p,p'*-DDE except one teal.

			p,p'-DDE (µg/g wet weight)		
Species	State	n	Mean	95% C.I.	Range-Data
Grackle	Texas	5	2.20	0.408-11.9	0.37-15
Stilt[a]	California	7	2.79	1.13-6.92	0.53-9.6
Avocet	California	3	0.478	0.0148-15.5	0.13-2.1
Harrier[b]	California	2	4.07	- -	2.4, 6.9
Bittern	California	2	0.222	- -	0.17, 0.29
Killdeer	California	2	1.71	- -	1.4, 2.1
Teal	California	2	- -	- -	nd[c], 0.24
Mallard	California	1	- -	- -	0.51

[a] One egg also contained 0.12 ppm *p,p'*-dicofol that could not be confirmed by GC/MS but 0.026 µg/g of *p,p'*-DCBP was confirmed present. [b] One egg also contained 0.77 µg/g of *p,p'*-DDD and 0.30 µg/g of *p,p'*-DDT. [c] "Not detected" = below detection limit (0.1 µg/g wet weight).

Single eggs from five Texas nests of great-tailed grackles all contained only DDE (Table 2), while six eggs of two species of doves (white-winged and white-tipped) lacked any detectable residues.

The highest concentration of DDE was 15 µg/g in a Texas grackle egg (Table 2). Concentrations of 9.6 µg/g were found in a California stilt egg (Table 2) and in a Florida racerunner carcass (Table 1). The highest DDE concentration for a raptor was 6.9 µg/g in a California harrier egg, which also contained 0.77 µg/g DDD and 0.30 µg/g DDT (Table 2). DDE was found in all but one of 19 California bird eggs, but it was not present in any of 10 lizard carcasses from the same area. DDE was present in all 10 Texas lizard carcasses and in eight of 10 Florida lizard carcasses (Table 1).

The highest concentrations of dicofol residues previously reported in free-living wildlife were 1.8 µg/g dicofol, 1.2 µg/g DCBP, and 1.5 µg/g DCD in an eastern screech owl egg from central Florida (Clark 1990). Residues in the carcass of a whiptail (Table 1), however, exceeded the screech owl dicofol value by 6.6 times and the DCD value by 10 times; amounts of DCBP were similar. Whether these maximum amounts of dicofol (12 µg/g) and DCD (15 µg/g) in the whiptail lizard are enough individually or together to affect reproduction is not known. Note that while Wiemeyer et al. (1989) found no overall effect on reproductive success, carcasses of screech owls that produced eggs with thinned shells contained 5.4-7.8 µg/g dicofol and 3.4-6.6 µg/g DCD. Because these amounts are less than those found in the most contaminated lizard carcass, effects on a lizard population might be worthy of investigation if such concentrations are common and persistent among many

individuals. That egg-laying reptiles may be vulnerable to eggshell-thinning compounds was indicated when populations of oviparous, but not viviparous, snakes were apparently extirpated by the related chemical DDE (Fleet et al. 1972, Fleet and Plapp 1978); however, there are no direct experimental data concerning such chemical effects on reptile reproduction.

Factors that may have affected dicofol residue concentrations in Texas lizards (Table 1) are confounded but include orchard, location within orchard, and time between spray and collection. However, the three lizards containing the highest dicofol residues were collected only 9 d after spraying. Bird eggs collected from Texas orchards about 6 wk post-spray contained no dicofol residues. Among California bird eggs, only a single egg collected on the day of spraying and adjacent to the sprayed field contained dicofol, and only a trace amount. This egg may have been contaminated by pesticide drift. Other eggs with nearby sprays that occurred about 1 mon before collection lacked dicofol residues. The trace amount of dicofol in a lizard from a Florida orchard (Table 1) suggests persistence of almost a year post-spray. In general, it appears that relatively high dicofol residues may accumulate, at least in lizards, but dissipate rapidly. Rapid dissipation would reduce the likelihood of chronic effects. However, in the case of the sampled population of Texas whiptail lizards, the high dicofol residue levels in June occurred during the reproductive season as evidenced by three females with enlarged ovarian follicles or oviductal eggs. In Texas the reproductive season of this species extends from late April to late July (Ballinger and Schrank 1972).

Maximum DDE concentrations in some species of bird eggs--6.9 µg/g in a harrier egg and 9.6 µg/g and 7.7 µg/g each in stilt eggs from California, and 15 µg/g in a grackle egg from Texas (Table 2)--are at least as high as mean concentrations that caused impaired reproduction in such species as the bald eagle, *Haliaeetus leucocephalus* (Wiemeyer et al. 1984), black-crowned night-heron, *Nycticorax nycticorax* (Henny et al. 1984), and white-faced ibis, *Plegadis chihi* (Henny and Herron 1989). There are no data for lizards with which to compare the extreme value of 9.6 µg/g found in a Florida racerunner (Table 1). Possible effects of DDE on populations of these bird and lizard species are unknown, but effects would depend on both mean concentrations of DDE and species sensitivity.

Lack of both DDE and dicofol residues in California lizards may result only because they were collected from soil where neither DDT nor dicofol had ever been applied, whereas Florida and Texas lizards were collected in or on the edge of orchards and probably had lived their entire lives on soil formerly treated with DDT and more recently with dicofol. It is worth investigating whether lizards present under these conditions are genetically resistant to the effects of DDE or dicofol or both. Resistance to DDT residues has long been known in populations of fish (Vinson et al. 1963) and frogs (Boyd et al. 1963) and to endrin--

another organochlorine--in mice (Webb et al. 1973). Resistance in groups with a cleidoic egg, such as oviparous lizards, oviparous snakes, turtles, crocodilians, or birds, however, has not been reported.

In summary, relatively high dicofol residues occurred in some lizards in Texas citrus orchards in early summer when female lizards were producing eggs. Concentrations may have peaked within days of dicofol application and dissipated within a few weeks. Whether reproduction might be affected at the population level depends on the reproductive sensitivity of lizards to such dicofol concentrations. Resistance of reptiles to DDE and dicofol might reasonably be investigated in the lizard population we sampled. The data from bird eggs were too few for conclusions, but to detect peak residue loads, future dicofol studies should concentrate on predaceous bird species likely to feed within the treated agricultural habitats, and collecting of eggs for analysis should begin within days after spray application.

Acknowledgments. We thank G. H. Heinz, P. F. P. Henry, W. A. Link, and J. W. Spann for critical reviews of the manuscript.

REFERENCES

Ballinger RE, Schrank GD (1972) Reproductive potential of female whiptail lizards, *Cnemidophorus gularis gularis*. Herpetologica 28:217-222

Boyd CE, Vinson SB, Ferguson DE (1963) Possible DDT resistance in two species of frogs. Copeia 1963:426-429

Clark DR Jr (1990) Dicofol (Kelthane) as an environmental contaminant: A review. US Fish Wildl Serv, Fish Wildl Tech Rep 29, 37 pp

Clark DR Jr, Spann JW, Bunck CM (1990) Dicofol (Kelthane)-induced eggshell thinning in captive American kestrels. Environ Toxicol Chem 9:1063-1069

Fleet RR, Clark DR Jr, Plapp FW Jr (1972) Residues of DDT and dieldrin in snakes from two Texas agro-ecosystems. BioScience 22:664-665

Fleet RR, Plapp FW Jr (1978) DDT residues in snakes decline since DDT ban. Bull Environ Contam Toxicol 19:383-388

Fry DM, Bird DM, Shutt L, Ditto M, Rosson B (1988) Eggshell thinning and embryo toxicity of dicofol miticide to American kestrels. P 56 in Ninth annual meeting (abstracts), Society of Environmental Toxicology and Chemistry, November 13-17, 1988, Arlington, VA

Henny CJ, Herron GB (1989) DDE, selenium, mercury and white-faced ibis reproduction at Carson Lake, Nevada. J Wildl Manage 53:1032-1045

Henny CJ, Blus LJ, Krynitsky AJ, Bunck CM (1984) Current impact of DDE on black-crowned night-herons in the Intermountain West. J Wildl Manage 48:1-13

Krynitsky AJ, Stafford CJ, Wiemeyer SN (1988) Combined extraction-cleanup column chromatographic procedure for the determination of dicofol in avian eggs. J Assoc Off Anal Chem 71:539-542

Vinson SB, Boyd CE, Ferguson DE (1963) Resistance to DDT in the

mosquito fish, *Gambusia affinis*. Science 139(3551):217-218
Webb RE, Hartgrove RW, Randolph WC, Petrella VJ, Horsfall F Jr (1973) Toxicity studies in endrin-susceptible and resistant strains of pine mice. Toxicol Appl Pharmacol 25:42-47
Wiemeyer SN, Spann JW, Bunck CM, Krynitsky AJ (1989) Effects of Kelthane on reproduction of captive eastern screech-owls. Environ Toxicol Chem 8:903-913
Wiemeyer SN, Lamont TG, Bunck CM, Sindelar CR, Gramlich FJ, Fraser JD, Byrd MA (1984) Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs--1969-79--and their relationships to shell thinning and reproduction. Arch Environ Contam Toxicol 13:529-549