# Organochlorine Pesticide Residues Associated with Mortality: Additivity of Chlordane and Endrin

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Mortality may be correlated with residue levels of organochlorine pesticides in the brains of exposed animals. Experimental studies have compared brain residues of animals that died with those of survivors of identical or similar exposure. Based on the results of such studies, lethal residue levels that may be used for diagnostic purposes have been established for some compounds.

DALE et al. (1962) correlated the concentration of DDT with signs of poisoning in rats that were fed 200 parts per million (ppm) in the diet for 90 d and then partially starved for 10 d. DALE et al. (1963) further correlated the severity of signs of poisoning with brain residues of DDT resulting from a single oral dose (150 mg/kg) in rats.

The validity of using brain residues of DDT and DDD for diagnosing cause-of-death in birds was demonstrated by STICKEL et al. (1966). Brain residues of DDT and DDD from dead or dying birds exposed to DDT were similar and were higher than brain residues of birds surviving exposure (STICKEL et al. 1966; STICKEL and STICKEL 1969). Rarely did cowbirds (Molothrus ater) fed 500 ppm DDT in the diet die with less than 30 ppm DDT + DDD in the brain. Though the relative contribution of DDT and DDD toward lethality was not determined, the investigators attributed mortality to the presence of residues of both of these chemicals in the brain. STICKEL et al. (1966) also presented supporting evidence from other reports which included residue levels of DDT and DDD in brains of birds collected from die-offs in the field. HILL et al. (1971) determined lethal ranges of DDT + DDD residues in brains of experimentally-treated songbirds. Brains of house sparrows (Passer domesticus) contained 18.0-37.9 ppm; bobwhite quail (Colinus virginianus), 16.5-37.9 ppm; cardinals (Cardinalis cardinalis), 16.6-24.0 ppm; and blue jays (Cyanocitta cristata), 12.0-19.6 ppm.

STICKEL et al. (1969) concluded from results of experimental studies with Japanese quail (Coturnix coturnix) and from reports of others that brain residues of dieldrin exceeding 4-5 ppm were indicative of dieldrin-caused mortality in birds and mammals. Based on such experimental studies and residues of dieldrin detected in birds apparently dying from exposure in the environment, dieldrin has been implicated as the causative factor in some



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bald eagle (Haliaeetus leucocephalus) mortality in the United States (REICHEL et al. 1969, COON et al. 1970, MULHERN et al. 1970, BELISLE et al. 1972, CROMARTIE et al. in press).

ROBINSON (1969) reviewed the effects of organochlorine insecticides on bird populations in Britain. He concluded from laboratory and field residues in tissues of poisoned birds that dieldrin (HEOD) and heptachlor epoxide were probably additive in their lethal effects. The residues of dieldrin in tissues (livers and brains) of poisoned birds were lower when heptachlor epoxide was also present.

Tissues of birds suspected of dying from poisoning rarely contain only one pesticide residue. In order to interpret the relative lethal effects of organochlorine insecticides in the environment, we must understand the quantitative aspects of additivity. This study was designed to provide information on possible additive lethal effects of chlordane and endrin.

## MATERIALS AND METHODS

Male and female bobwhite quail (14-wk-old) were randomly divided into 4 groups. All groups were fed turkey maintenance mash with 1% (w/w) propylene glycol mixed into the diet. Pesticides were dissolved in the propylene glycol and then mixed into the diet. Eight birds received 10 ppm chlordane (technical grade) in the diet for 10 wk. Twenty quail were treated with 10 ppm chlordane for 10 wk followed immediately by 10 ppm endrin (98% pure) in the diet. A fourth group (N=20) received 10 ppm endrin in the diet. After 9 or 10 d on clean food, survivors which appeared healthy were sacrificed by decapitation. The first 2 birds to die in any treatment were arbitrarily eliminated from the experiment to reduce possible variability resulting from mortality not pesticidal.

Birds were skinned, and beaks, feet, wings, and intestinal tracts were removed. Brains were dissected and frozen prior to residue analysis of chlordane components and endrin. WARF Institute extracted and analyzed samples for pesticide residues. Brains were ground with about 20 g sodium sulfate, air-dried, and extracted in a pre-rinsed 33 x 94 mm Whatman Extraction Thimble on a soxhlet extractor for 8 h with 70 ml ethyl ether + 170 ml petroleum ether. An aliquot of the extract was cleaned up on a florisil column using a 3% ethyl ether in petroleum ether elution (135 ml total volume). The sample was then concentrated to an appropriate volume and 10µl or less injected into a Model 5360 Barber Colman Pesticide Analyzer for identification and quantitation.

Samples were analyzed by electron capture detection for endrin, oxychlordane, heptachlor, heptachlor epoxide, nonachlor, compound "C" (monochlorinated derivative of a pentachlorcyclopentadiene), compound "E" (α and β chlordane; 2:1 ratio), alpha

and gamma chlordane. Instrument conditions for analysis of residues were as follows:

1. Endrin: column, 190°C; injector, 225°C; detector, 240°C; 4 ft x 4 mm glass column packed with 5% DC-200 on 80/100 mesh Gas Chrom Q; carrier gas, nitrogen.

2. Oxychlordane and heptachlor epoxide: column, 195°C; injector, 225°C; detector, 245°C; 4 ft x 4 mm glass column packed with 11% OV-17 + QF1 on Gas Chrom Q, 80/100 mesh; carrier gas, nitrogen.

3. Other chlordane compounds: column 165°C; injector 220°C; detector, 220°C; 6 ft x 2 mm glass column packed with 3% OV-1 on 80/100 mesh Gas Chrom Q; carrier gas, nitrogen.

Brains (N=2) were spiked with endrin and chlordane and the efficiency of recovery determined. Endrin recoveries were 64.0 and 76.0%. Recoveries for technical chlordane in spiked brains were 81.8 and 92.5%. Analysis of endrin- and chlordane-treated food samples yielded 6.06 ppm and 9.4 ppm, respectively. The limit of detection based on a 1.0 g sample was 0.05 ppm.

Brains and carcasses were analyzed for lipid and moisture content. Moisture content was determined by weight loss of ground tissue after oven-drying (40°C) for 10-12 d. An aliquot of the sample extract was dried by evaporation of the solvent using a warm water bath and was then placed in an oven for 24 h at 40°C. The container was then removed, desiccated, weighed and percent lipid calculated.

Statistical comparisons between group means were made using the F-test and a probability of $p \leq 0.05$ was considered significant.

## RESULTS AND DISCUSSION

No mortality occurred among birds fed the control diet or those fed 10 ppm chlordane alone. Mortality among birds treated with 10 ppm endrin occurred on days 1 (N=1), 6 (N=7), 9 (N=5), and 10 (N=2). Mortality among birds of the chlordane-endrin treatment occurred on days 3 and 6-10 (N=14) of endrin exposure. Survivors were sacrificed on days 9 and 10 of endrin exposure.

All birds treated with endrin alone or with chlordane followed by endrin lost weight (Table 1). Controls and chlordane-treated birds did not lose weight, and lipid content of the carcasses was high. Chlordane-treated birds had lower carcass lipid content than did controls, but carcass mean weights were similar (controls=160.8 g; chlordane-treated=161.3 g). Moribund individuals had lost considerable body weight and contained much less body fat than did individuals that were not exhibiting signs of intoxication when sacrificed. Birds that died from

intoxication averaged weight losses of 32.2% (endrin-treated) and 31.4% (chlordane + endrin-treated) when compared with the control group (Table 1).

TABLE 1

Weight loss and lipid content (Mean % ± SE) of quail carcasses

| Treatment | | | | | |
|---|---|---|---|---|---|
| Chlordane | Endrin | Condition[a] | N | % Lipid | % Weight Loss |
| None | None | S | 4 | 4.69 ± 0.77 | -- |
| 10 ppm; 10 wk | None | S | 4 | 3.45 ± 0.56 | -- |
| None | 10 ppm; 6-10d | D | 13 | 0.43 ± 0.14 | 32.2 ± 2.4 |
| | | M | 1 | 0.73 | 68.6 |
| | | S | 3 | 2.73 ± 0.97 | 14.7 ± 1.1 |
| 10 ppm; 10 wk | 10 ppm; 6-10d | D | 12 | 0.39 ± 0.06 | 31.4 ± 3.0 |
| | | M | 3 | 0.27 ± 0.04 | 37.2 ± 11.7 |
| | | S | 3 | 2.78 ± 1.29 | 19.1 ± 3.5 |

[a]Condition: S=sacrificed, exhibiting no apparent signs of toxicity; D=dead; M=moribund or sick as evidenced by reduced activity and appetite.

The low lipid content of carcasses of moribund birds indicates that they were experiencing advanced symptoms of intoxication. As occurred with individuals that died of poisoning, the body lipids were depleted. Concurrent with fat mobilization brain residues of endrin were elevated (Table 2). STICKEL et al. (1973) discussed the relationships of body weight and lipid content as associated with brain residues of mirex in blackbirds. They demonstrated a direct relationship between percent of total (whole-body) lipids and percent of total µg mirex as represented in the brain. Brain lipids did not differ between dead blackbirds and survivors. My findings substantiate such a relationship, i.e., as body weight decreased and fat was mobilized, brain residues increased (Tables 1 and 2).

Brains of birds from the control treatment contained neither endrin nor chlordane components. Birds treated with chlordane alone revealed no endrin residues; those treated with endrin

alone contained no chlordane residues. Sublethal exposure to chlordane (10 ppm) resulted in low brain residues of heptachlor epoxide, nonachlor, oxychlordane, and compounds "C" and "E" (Table 2). Heptachlor epoxide, nonachlor, and oxychlordane are each toxic components or metabolites of technical grade chlordane.

Individuals that survived exposure to chlordane + endrin or endrin alone had lower brain residues of endrin than did birds that died (Table 2). Moribund individuals did not differ from the dead of the same treatment. Birds that died from endrin alone had brain residues ranging from 0.34-1.84 ppm; survivors ranged from 0.28-0.62 ppm. Some overlapping of brain residue ranges between dead and survivors is expected since there is a range of residues at death that reflects individual variability with regard to tolerance and extent of exposure. Evidence of weight loss (Table 1) among sacrificed birds indicates that some may have been approaching dangerous residue burdens in the nervous system, even though they exhibited no apparent signs of intoxication. In birds treated with chlordane followed by endrin the brain residues of endrin ranged 0.14-0.56 ppm among survivors and 0.17-1.25 ppm among dead. Brain residues of survivors were lower than those of dead birds and approached significance ($0.05<p<0.10$).

Birds treated with chlordane followed by endrin had considerably more chlordane residues in their brains than did birds treated with chlordane alone (Table 2). This suggests an uptake of chlordane components in brains of birds experiencing toxic effects from endrin exposure. The higher brain residues of chlordane compounds in dead and moribund birds indicates that as lipid mobilization occurred, increased residues (including endrin) accumulated in brain tissues.

Dead and dying quail treated with endrin + chlordane had significantly lower ($p<0.025$) brain residues than dead and dying of the endrin treatment. This difference can undoubtedly be attributed to the presence and accumulative toxic action of one or more of the chlordane components in the nervous system. The presence of toxic chlordane compounds effectively reduced by about 50% the minimum and by 38% the average endrin residues in brains of dead birds.

An accumulation of a single compound or its effects at a sensitive target site may be associated with death. This relationship has been demonstrated in birds (STICKEL et al. 1966, STICKEL et al. 1969) and mammals (DALE et al. 1962 and 1963) with organochlorines. It is logical to assume that different but closely related chemicals possessing the same mode of action would be accumulative in their toxic effect relative to their respective degrees of toxicity. Sublethal exposure of animals to cholinesterase (ChE) inhibitors increases the likelihood of toxic effects resulting from subsequent exposure to anticholinesterase agents. BENKE and MURPHY (1974) mentioned the importance of this phenomenon with regard to repeated human

TABLE 2

Residues (ppm ± SE[a]) recovered from brains of quail fed chlordane, endrin, chlordane followed by endrin, or a control diet.

| Treatment | | | | Brain residue | | | | |
|---|---|---|---|---|---|---|---|---|
| Chlordane | Endrin | Condition | N | Endrin | Heptachlor epoxide | Nonachlor | Oxychlordane | Others[b] |
| None | None | S | 4 | ND[c] | ND | ND | ND | ND |
| 10 ppm; 10 wk | None | S | 4 | ND | 0.05 ± 0.03 | 0.07 ± 0.01 | ND | 0.07 ± 0.01 |
| None | 10 ppm; 6-10d | D | 13 | 1.08 ± 0.12 | ND | ND | ND | ND |
| | | M | 1 | 1.02 | ND | ND | ND | ND |
| | | S | 3 | 0.43[d] ± 0.10 | ND | ND | ND | ND |
| 10 ppm; 10 wk | 10 ppm; 6-10d | D | 12 | 0.67[e] ± 0.09 | 1.03 ± 0.13 | 0.89 ± 0.13 | 0.40 ± 0.07 | 0.60 ± 0.05 |
| | | M | 3 | 0.69 ± 0.37 | 0.50 ± 0.27 | 0.32 ± 0.14 | 0.31 ± 0.14 | 0.21 ± 0.12 |
| | | S | 3 | 0.31[f] ± 0.13 | ND | 0.21 ± 0.06 | 0.16 ± 0.07 | 0.25 ± 0.03 |

[a] SE=standard error of the mean.
[b] Others=compound "C" and compound "E" (see text, Materials and Methods).
[c] ND=none detected; lower limit of sensitivity = 0.05 ppm based on a 1 g sample.
[d] Mean endrin residues in brains of sacrificed survivors are significantly less ($p<0.05$) than those of birds killed by endrin.
[e] Mean endrin residues in brains of birds killed by chlordane + endrin are significantly less ($p<0.025$) than those of birds killed by endrin alone.
[f] Mean endrin residues in brains of sacrificed survivors are less ($p<0.10$) than those of birds killed by chlordane + endrin.

occupational exposure and in fish species that exhibit a prolonged recovery period following ChE inhibition. The vulnerability of an animal to a toxic pollutant may thus be increased if the individual already carries a body burden of one or more closely related chemicals.

Tissues of most wild animals contain a variety of chemical residues which may include organochlorine or organophosphate pesticides, industrial pollutants such as PCB's, heavy metals, and others. When attempting to diagnose cause-of-death we must take into consideration the additive toxicity of closely related compounds. For instance, individuals with appreciable but sublethal residues of chlordane, dieldrin, endrin, and possibly other chemical pollutants may die after additional exposure to any other of these compounds. The question arises as to which chemical or chemicals may have caused mortality. The answer is not necessarily the chemical with the greatest residue, nor even one chemical alone; each may contribute relative to its toxicity. In this study mortality from endrin alone was associated with as little as 0.34 ppm in the brain; whereas in other studies mortality was caused by dieldrin when brain residues were about 10-fold greater (4.0-5.0 ppm). Until we can reliably measure the toxic effect of organochlorines at the target site (i.e., the biochemical lesion), we must be content with measuring and correlating the relative contribution of the residues in the target organ or tissues.

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