DDD Poisoning in a Loon and the Identification of the Hydroxylated Form of DDD

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In December 1973, a common loon (Gavia immer) was found in a soybean field in Madison County, Mississippi. The bird was alive when found but was lying on its back and died shortly thereafter. Autopsy findings and results of chemical residue analysis are reported here.

METHODS

The autopsy was performed as described by MULHERN et al. (1970).

The carcass (after removal of skin, feet, wings, liver, and gastrointestinal tract) was ground and homogenized in a Hobart food cutter. A 10-g aliquot of the carcass and the entire brain were mixed separately with anhydrous sodium sulfate in a blender and extracted for 7 hours with hexane in a Soxhlet apparatus. An aliquot of the extract containing not more than 0.5 g of lipid was cleaned up on a Florisil column. Organochlorine pesticides and polychlorinated biphenyls (PCB's) were separated on a silicic acid column and analyzed by gas chromatography (GC) on a 4/6% SE-30/QF-1 column. The brain and carcass were analyzed for p,p'-DDE, p,p'-DDD, p,p'-DDT, dieldrin, heptachlor epoxide, mirex, oxychlordane, cis-chlordane and/or trans-nonachlor, cis-nonachlor, hexachlorobenzene and PCB. This analytical procedure has been described in detail by CROMARTIE et al. (In press). Identities of the pesticides in the carcass and brain were confirmed on an LKB 9000 gas chromatograph-mass spectrometer (GC-MS). Because GC-MS analysis indicated the presence of compounds other than those listed above, the silicic acid column eluate of the carcass extract was further studied by GC on (1) a 3% XE-60 column to separate o,p'-DDE and DDMU and (2) a 3% OV-275 column to separate o,p'-DDD and DDMS. To resolve DDMU from heptachlor epoxide, an aliquot of the silicic acid column eluate was separated into four zones by thin layer chromatography (TLC) and pesticides were eluted from each zone by hot benzene (MULHERN 1968).

RESULTS AND DISCUSSION

The loon was a subadult female weighing 4 lb with fairly well-developed pectoral muscles but without subcutaneous, abdominal, and coronary fat. No gunshot wounds or fractures were found. Gross lesions were absent, and no pathogenic bacteria were isolated from the liver or the heart.

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Tissue sections of the stomach, proventriculus, lung, liver, small intestine, thyroid, kidney, and pancreas were examined and found normal. Because the entire brain was saved for chemical analysis, brain sections were not examined. Small ring- and droplet-like inclusions were noted in the liver cells similar to those reported in birds experimentally poisoned by DDT or other agents (ORTEGA 1966).

Based on the autopsy findings and the high level of DDD (200 ppm) in the brain (Table 1), the cause of death can be presumed to be DDD poisoning. This conclusion is supported by the work of STICKEL et al. (1970) who found DDD residues of 65 ppm or higher in the brains of 95 percent of experimentally dosed cowbirds dying of DDD poisoning. It is noteworthy that the poisoning of this loon occurred 1.5 years after the moratorium on DDT and DDD usage.

Further study of the carcass extract after zonal separation by TLC revealed the presence of an unknown compound in the first zone with a GC retention time slightly longer than that of DDT, having four chlorine atoms ($C_{14}H_{10}Cl_4O$) and the following mass spectrum:

m/e 334 (4 Cl, 0.31%, M^+), m/e 251 (2 Cl, 91%, M-$CHCl_2$), m/e 235 (2 Cl, 11%, M-CH_3Cl_2), m/e 139 (1 Cl, 100%, M-$C_7H_6Cl_3$), m/e 111 (1 Cl, 34%, M-$C_8H_6Cl_3O$).

The o,p'-DDT metabolite [1,1-dichloro-2-(2-chloro-3-hydroxyphenyl)-2-(4-chlorophenyl) ethane] (FEIL et al. 1973) was ruled out as a possibility after comparison of its spectrum with that of the unknown. Moreover, the corresponding p,p'-DDT metabolite was also ruled out on the assumption that it would have a mass spectrum similar to that of the o,p'-form. Review of the published mass spectra of compounds structurally related to DDD (SPHON AND DAMICO 1970) showed a marked similarity between the mass spectrum of the unknown and that of Kelthane [1,1-bis-(p-chlorophenyl)-2,2,2-trichloroethanol]. The four-chlorine analogue of Kelthane [1,1-bis-(p-chlorophenyl)-2,2-dichloroethanol] or dichloro Kelthane (DCK) was synthesized by the following method:

> Dissolve 4 g chromium trioxide in 4 ml distilled water and dilute to 75 ml with glacial acetic acid. Add 0.5-0.6 g DDD and heat at 100-105°C for 2 hours. Transfer the solution to a 500-ml separatory funnel; add 200 ml distilled water and extract with 200 ml hexane or iso-octane. Discard the aqueous phase and filter through two layers of heavy (Whatman #3) filter paper. Concentrate the filtrate to 2-3 ml, mix with 2-3 g Celite 545, and allow remaining solvent to evaporate. Fill a 17- x 200-mm chromatographic column to a depth of 13-14 cm with 80-200 mesh alumina (Alcoa F-20) activated at 180°C for 2 hours. Add the Celite mixture to the top of the column and compact with a flattened glass rod. Elute the DDD, dichlorobenzophenone (DCBP), and other oxidation products except DCK from the column with 100 ml 5% ethyl acetate in iso-octane (V/V). Elute the DCK with 35 ml reagent-grade ethyl acetate and evaporate to dryness by

warming under a stream of air. To the solvent-free residue add 3 ml iso-octane; warm to dissolve, and then chill at -10°C to crystallize the DCK. Decant supernatant, and dry crystals. The melting point of DCK is 106°C. If necessary, recrystallize using 40% water in methanol.

Both the mass spectrum and retention time of DCK and the unknown were identical. Because of the high concentration of DDD in these tissues, this unknown should be classed as a hydroxylated form of DDD. To our knowledge, this is the first time this metabolite has been reported in an environmental sample.

TABLE 1

Pesticide residues in common loon tissue - ppm, wet weight

Compound	Carcass	Brain
p,p'-DDE	680	130
p,p'-DDD	1400	200
p,p'-DDT	10	2.1
Dieldrin	0.18	N.D.[1/]
p,p'-DDMU	67	N.A.[2/]
p,p'-DCBP	5.0	N.D.
p,p'-DDMS	34	N.A.
o,p'-DDD	33	N.A.
o,p'-DDE	55	N.A.
DCK	26	N.A.
Heptachlor epoxide	N.D.	N.D.
Mirex	N.D.	N.D.
Oxychlordane	N.D.	N.D.
cis-Chlordane[3/]	N.D.	N.D.
cis-nonachlor	N.D.	N.D.
Hexachlorobenzene	N.D.	N.D.
PCB	180	15

[1/] N.D. = none detected
[2/] N.A. = not analyzed
[3/] and/or trans-nonachlor

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