COMPARISON OF METHODS OF PRESERVING TISSUES FOR PESTICIDE ANALYSIS

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(Received May 27, 1983)

Abstract. Formalin preservation, freezing, spoiling followed by freezing, and phenoxyethanol were compared in terms of concentrations of DDT, DDD, DDE, endrin, and heptachlor epoxide measured in brain, liver and carcass of birds fed dietary dosages of pesticides and in spiked egg homogenate. Phenoxyethanol proved to be an unsatisfactory preservative; the amount of "extractable lipid" was excessive, and measurements of concentrations in replicates were erratic. Concentrations of residues in formalin-preserved and frozen samples did not differ significantly in any tissue. Percentage lipid in brains and eggs, however, were significantly lower in formalin-preserved samples. Samples of muscle and liver that had been spoiled before freezing yielded less DDD, and muscle samples yielded more DDT than formalin-preserved samples. We conclude that formalin preservation is a satisfactory method for preservation of field samples and that the warming and spoiling of samples that may occur unavoidably in the field will not result in misleading analytical results.

1. Introduction

Freezing remains the customary way of storing samples for residue analysis, but it often is impossible for collectors in the field. Even in the laboratory, freezing creates serious problems of freezer space and presents constant worries about freezer failure and thawing of samples in shipment. Also, it is known that some microbial action continues in frozen samples, and this might cause a gradual degradation of residues. For these reasons, samples from several of our studies were preserved in a 4 percent formaldehyde solution. Many workers have used this technique in the field and several favorable laboratory tests have been reported (Cox, 1970; French et al., 1971; Deubert et al., 1973).

The percentage of lipid extracted from brains, however, proved to be consistently lower after formalin preservation than after freezing. The possibilities of 2-phenoxyethanol as described by Nakanishi et al. (1969) also seemed promising. A test therefore was planned to compare four methods of preserving and storing tissues: freezing, formalin, phenoxyethanol, and spoilage followed by freezing (to learn what happens when tissues remain unfrozen for considerable periods).

The chemicals selected were: p,p'-DDT, which metabolizes into DDE and DDD; endrin, which is said to degrade rapidly in living animals; and heptachlor, which changes rapidly to heptachlor epoxide in animal tissues. Dosage levels were selected with the goal
of having realistic but readily measurable residues of each chemical. Although these chemicals are no longer used extensively in the United States, they are used in other countries, including wintering areas of birds that breed in the United States. Organochlorine residues continue to occur in environmental samples and the need for monitoring continues (Fleming et al., 1983).

2. Experimental Methods

Dietary dosages of heptachlor, dieldrin, and endrin were fed to adult male grackles (Quiscalus quiscula), resulting in natural incorporation of chemicals into the tissues and producing samples comparable to those from the field. Birds were fed heptachlor and p,p'-DDT at 5 ppm (dry weight) in the diet for 9 days, June 15–24, 1971. For the last 3 days of this period, endrin was added to the diet at 4.36 ppm. Chemicals were dissolved in Wesson oil and blended into diet of turkey starter crumbles. Birds were sacrificed with chloroform vapor on June 24 and dissected at once. They appeared healthy and were in good flesh and fat.

Upon dissection, each brain was bisected longitudinally and each half was randomly assigned to a different preservation method, with the restriction that the two halves be assigned to the same two methods only twice. The same random assignment procedure and restrictions were applied to liver and breast muscle samples, except that these tissues were cut into strips, then into chunks and alternate chunks were assigned to two preservatives. Breast muscle also was trimmed of all visible fat before subdivision. All samples were weighed to 0.1 mg before preservation. Residue readings were based on these weights. Brain samples weighed 1.0 to 1.5 g, liver samples 1.0 to 1.9 g, and muscle samples 3.6 to 6.3 g. The jars used had a capacity of 34 ml and were nearly filled when fluid was used.

A 4% formaldehyde solution was made by diluting chemically pure formalin with distilled water. One part of 2-phenoxyethanol was used with 50 parts of distilled water. The freezer operated at −17 to −14 °C. The samples to be spoiled were left open in the laboratory for about 2 hr and were then kept loosely capped in a refrigerator at 7 °C for 21 days, when mold appeared on tissues; jars then were sealed and frozen.

Egg samples, in contrast to tissue samples, were spiked directly, providing a comparison of preservation methods, but very likely resulting in a higher percentage recovery than would occur with naturally incorporated chemicals. Contents of commercial hens’ eggs were stirred and put through a 3-mm-mesh sieve to remove the thick parts, chiefly chalazae. Of the fluid egg, 250 g was weighed into a 1000 ml beaker. One ml of hexane containing DDT, endrin, and heptachlor was added to provide spiking at the rate of 4 ppm (wet weight) of each chemical. The mixture was stirred mechanically to assure distribution of chemical. Approximately 11 g of the mixture was poured into each of 12 small, tared jars and weighed to 0.1 mg. Three of the jars were then filled with formalin and three with phenoxyethanol. Three were promptly frozen. Three that were designated for spoiling were left open in the laboratory for about 2 hr and were then placed in a refrigerator at 7 °C, on August 4, 1971. The bottles were kept loosely capped.