Dry Ice Homogenization Procedure for Fish Samples in Pesticide Residue Analysis

A technique for homogenization of fish and other biological samples with resilient tissues is described. The sample is frozen, sectioned into small pieces, and ground with dry ice in a blender. This method fragments the embrittled tissue into a fine homogenate without the aid of a desiccant.

Accurate sampling of large organisms for residue analysis is difficult to achieve since such samples are not homogenous and pesticides are not uniformly distributed throughout the tissues. A costly method of achieving accuracy is to extract all or a major portion of a sample. However, most investigators use homogenization of the tissues, assuming that a portion of the sample will be representative of the whole.

Many authors have reported homogenization techniques for biological samples. That of Davidow (1950), typical of the laborious procedures commonly used, required that the sample be ground with anhydrous sodium sulfate by use of a mortar and pestle. Kallman *et al.* (1962) reported the use of a blender to grind tissue with sodium sulfate. An interesting technique was reported by Parker *et al.* (1965) in which they used dry ice as a grinding aid. In this communication we describe a method, based on the use of dry ice, by which fish are pulverized into a finely powdered homogenate.

MATERIALS AND EQUIPMENT

Blender container and motor unit—A 1-l. Waring stainless-steel container was modified by enlarging the vent hole to $^1/_2$ in. and changing the pitch on the lower pair of blades from $+2°$ to $-15°$. These changes allowed the CO_2 gas to escape and assured efficient blending. The multispeed Waring base was rated at 120 v and 800 watts.

Miscellaneous equipment—Other supplies needed include: a heavy knife or meat cleaver; a 2-lb mallet; a hardwood cutting board; plastic bags; dry ice; styrofoam container; leather gloves; and a freezer.

METHOD

Whole fish are frozen to facilitate cutting and grinding, as well as to preserve the sample. Remove the sample from the freezer, weigh, and slice it into pieces using the knife and mallet. For optimum blending, the sliced sections should not exceed the following dimensions: $^1/_2 \times {}^1/_2 \times 1$ in., cross, sagittal, and longitudinal sections, respectively. Break the dry ice into $^1/_2$ in. chunks, place them into the blender, and pulverize. Enough dry ice should be used to equal or slightly exceed the weight of the tissue sample. Hold a cloth or large lab wipe over the vent hole in the blender top to prevent loss of material. (CAUTION: Hold the cloth or lab wipe in place with a leather glove to prevent frostbite.) Add the sample and begin blending at low speed. When the sample is blending smoothly, increase to maximum blending speed. Continue blending until the sample is reduced to a fine powder. The total time required will depend on the size of the sample, but will usually be from 3 to 5 min. Sample size with the 1-l. container can vary from 30 to 200 g. A 4-l. container may be used for samples up to 800 g.

Pour the pulverized mixture into a plastic bag, close the top of the bag with a rubber band, and store the comminuted sample in a freezer overnight. This allows complete sublimation of the dry ice, which takes approximately 7 hr. After overnight storage the sample is ready for extraction.

DISCUSSION

Complete sample homogenization is essential when accurately quantitating pesticides in biological samples, especially fish, where the fat is heterogeneously distributed (Love, 1962).

Homogenizing whole fish with a desiccant (considered standard procedure by many researchers) is a tedious task. Often, the sample requires several refreezings and regrindings before the resilient tissues, *i.e.*, skin, muscles, and cartilage, can be completely macerated. Calculations are complicated by having to contend with a desiccant factor and the chemical properties of the desiccant. The dry ice procedure eliminates the desiccant and keeps the frozen tissue brittle and vulnerable to fragmentation.

A large fish weighing approximately 300 g was homogenized and five 30-g subsamples were taken for pesticide analysis (DDE, DDD, and DDT). The analytical results obtained agreed with alternate grinding procedures (Fish-Pesticide Research Laboratory, 1969). Neither the weight of the fish nor the way the plastic bags are sealed apparently affects the dissipation of the dry ice.

Certain precautions should be exercised when employing the dry ice technique. Since breathing a 10% or higher con-

centration of CO_2 can cause death (Merck, 1960), the dry ice (or samples intermixed with dry ice) should not be stored in a poorly ventilated room like a walk-in freezer unless adequate precautions are taken. Gloves should be worn to avoid frostbite when handling the dry ice.

LITERATURE CITED

Davidow, B., *J. Ass. Offic. Agr. Chem.* **33,** 886 (1950).
Fish-Pesticide Research Laboratory, Columbia, Mo. 65201, unpublished data, 1969.
Kallman, B. J., Cope, O. B., Navarre, R. J., *Trans. Amer. Fish. Soc.* **91,** 14 (1962).
Love, R. M., "The Physiology of Fishes," pp. 406–407, Academic Press, Inc., New York, N. Y. (1962).
Merck Index, 7th Ed. p. 211, Merck and Co., Inc., Rahway, N. J. (1960).
Parker, K. D., Dewey, M. L., DeVries, D. M., Lau, S. C., *Toxicol. Appl. Pharmacol.* **7,** 719 (1965).

P. E. Benville, Jr.[1]
R. C. Tindle[2]

Bureau of Sport Fisheries and Wildlife
Fish-Pesticide Research Laboratory
Columbia, Mo. 65201

[1] Duty Station, Fish-Pesticide Research Laboratory, P.O. Box 98, Tiburon, Calif. 94920.
[2] To whom correspondence should be addressed.

Received for review June 15, 1970. Accepted July 13, 1970. The use of proprietary names in manuscript is for purposes of identification only and does not suggest endorsement of these products by the Bureau of Sport Fisheries and Wildlife, or the U. S. Department of the Interior.