

Effects of Fixation on the Extraction of Dieldrin and p,p'-DDT from Muscle Tissue

by KARL H. DEUBERT and JAYNE S. TIMMERMAN

*Laboratory of Experimental Biology
University of Massachusetts
Cranberry Station
East Wareham, Mass. 02538*
and

LAWRENCE R. McCLOSKEY*

*Department of Biology
Walla Walla College
College Place, Wash. 99324*

INTRODUCTION

The ubiquitous nature of chlorinated hydrocarbon insecticides and related compounds makes the analysis of environmental samples from remote areas especially interesting. In few cases, however, is immediate processing of those samples possible. Freezing is widely accepted as a means of preservation. But if freezers or dry ice are not available, chemical fixatives must be used as preservatives.

Fixatives are intended to stabilize proteins to maintain, as much as possible, the general form of a tissue. Their effects, if any, on the extractability of chlorinated hydrocarbon insecticides, are poorly known. In the literature only two relevant comments were found (COX, 1970; FRENCH and JEFFERIES, 1971).

In our laboratory a number of samples of fixed tissues were analyzed for dieldrin and p,p'-DDT without knowledge of possible effects of the fixatives on the accuracy of the procedure. The samples, originally taken for gonad index studies, were unique (McCLOSKEY and DEUBERT, in press), and the alternative to analysis with possibly "built-in" error would have been loss of information. To test the reliability of the extraction procedure, we studied the effects of the fixatives on the extraction of dieldrin and p,p'-DDT.

METHODS and MATERIALS

Samples of muscle tissue were taken from codfish, smelt and brook trout and fixed in formalin (approximately 4 percent) fixative and Bouin's fixative (50 ml formalin, 150 ml saturated aqueous solution of picric acid, 10 ml glacial acetic acid). Frozen subsamples were used as references.

Extraction of pesticide residues was carried out as previously described (McCLOSKEY and DEUBERT, in press).

Since the samples were not homogenized prior to fixation, non-uniform distribution of insecticide residues in the tissues could have affected the residue amounts in the subsamples. To compensate

* Person to whom reprint requests should be directed.

TABLE 1

Insecticide residues in muscle tissues after fixation.
Quantities in ppm relative to wet weight of fixed tissue.

Time after Fixation	Smelt			Cod			Trout	
	Fixative	Dieldrin	p,p'-DDT	Dieldrin	p,p'-DDT	Dieldrin	p,p'-DDT	p,p'-DDT
Prior to Fixation		0.014	0.054	0.005	0.026	0.017	0.030	
1 Day	Formalin Bouin	0.022 0.019	0.051 0.039	0.006 0.012	0.011 0.032	0.019 0.022	0.042 0.032	
	Mean Frozen	0.021 0.027	0.045 0.056	0.009 0.008	0.022 0.020	0.021 0.026	0.037 0.041	
1 Week	Formalin Bouin	0.028 0.031	0.036 0.044	0.009 0.017	0.020 0.024	0.032 0.018	0.019 0.034	
	Mean Frozen	0.030 0.020	0.040 0.052	0.013 0.009	0.022 0.027	0.025 0.021	0.027 0.036	
3 Weeks	Formalin Bouin	0.012 0.020	0.072 0.014	0.009 0.010	0.026 0.026	0.018 0.020	0.043 0.034	
	Mean Frozen	0.016 0.018	0.043 0.052	0.010 0.007	0.026 0.026	0.016 0.013	0.034 0.042	

for this possible variable, eight individual smelt and trout were cut into pieces of about five grams each. Five of these were selected at random and used as one subsample. In testing codfish tissue, we used but one piece. For analysis the fixed subsamples were blotted with filter paper previously extracted with n-hexane, and homogenized in a Waring blender. Aliquots containing one to two grams fat were used for extraction.

Extraction and analysis were done in duplicate.

RESULTS and DISCUSSION

The results are presented in Table 1. Apparently, fixatives used in these experiments and under the specified conditions had little or no effect on the amounts of dieldrin and p,p'-DDT residues extracted from the fixed tissues. This is in accordance with previously obtained results (COX, 1970; FRENCH and JEFFERIES, 1971). FRENCH and JEFFERIES worked with tissue containing larger quantities of pesticides. Our results indicate that the use of formalin or Bouin's mixture does not affect the extractability of p,p'-DDT and dieldrin at the low levels occurring in environmental samples. As well, our results indicate that tissue samples fixed in Bouin's solution for histological studies can still be used for dieldrin and DDT analysis.

Variations in our figures are probably due to the small quantities of residues involved.

Changes in weight during fixation were not determined.

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Smithsonian Institution issued to Dr. L. R. McCloskey, and by Hatch Project 293.

REFERENCES

- COX, J. L.: Science 170, 71 (1970).
- FRENCH, M. C., and D. J. JEFFERIES: Bull. Envir. Contam. & Tox. 6, 460 (1971).
- MCCLOSKEY, L. R., and K. H. DEUBERT: Bull. Envir. Contam. & Tox. 8, in press.