The Preservation of Biological Tissue for Organochlorine Insecticide Analysis

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Introduction

The collection and transport of biological material from field experiments and contamination surveys of distant areas for subsequent organochlorine analysis has proved a difficulty for many workers. For example, George and Frear (1), studying organochlorine contamination in the Antarctic, had to carry insulated containers with continually replaced dry ice and place their samples in deep-freeze whenever this was possible on their journey. Because of the need to examine further the organochlorine contamination in areas (including tropical areas) distant from deep-freezes and dry ice, the possible use of preservatives was studied at Monks Wood. The two preservatives used were aqueous solutions of formaldehyde and pehnol. Both are readily available and may be transported in concentrated form and neither has the disadvantages of alcohol (see Discussion). Changes in sample weight and pesticide residue composition in avian liver tissue preserved in both materials have been studied over a period of one year.

Materials and Methods

Reagents: Phenol and formaldehyde were both reagent grade chemicals. Hexane, acetone, anhydrous sodium sulphate, sand and aluminium oxide were all purified in this laboratory and tested by Gas-Liquid chromatography (G.L.C.) before use.

Instruments: The gas chromatographs used were the Perkin Elmer Model 452 fitted with an electron-capture detector system and an all glass injection system and column.

Sample preparation: Two pigeons were force fed with gelatin capsules containing a mixture of 5 mg Dieldrin (HEOD) and 10 mg pp'-DDT (both in crystalline form) at the rate of one a day for five days. Following the final dose the birds were killed by cervical dislocation and the livers removed immediately. These were then finely sliced, mixed together and the total mass divided into ten approximately equal portions which were weighed (approximately 2 g each). The liver specimens were placed in 2 oz (57 g) glass jars and two were analysed immediately to provide zero hour information. A volume of 25 ml of a 4% solution of formaldehyde (10 ml of 40% formalin + 90 ml glass distilled water) was added

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to four samples and a similar amount of 10% Phenol (10 g phenol crystals (hydroxybenzene - C_6H_5OH) dissolved and made up to 100 ml with distilled water) was added to the remaining four. The eight samples were then allowed to stand at room temperature (Min: 10°C; Max: 27°C) for the period of the experiment.

Sample analysis: Samples from each treatment were analysed at 5 days, 10 days, 4 months and one year after addition of the preservative. In both treatments tissue was removed from the preservative and lightly blotted with filter paper before weighing and residue analysis. The remaining preservative was also analysed for pesticide content.

Extraction and Clean Up Procedure: Tissue samples were first ground with sand and anhydrous sodium sulphate and then extracted with a hot mixture of redistilled hexane and acetone to a final volume of 250 ml. The final extract was reduced to 2 ml on a hot water bath (80°C) and this volume added to a dry column of 5 g 5% deactivated aluminium oxide covered with 5 g of anhydrous sodium sulphate. The pesticides were eluted with 25 ml of hexane which was used for G.L.C. analysis. The remaining preservatives were shaken with 2 x 10 ml hexane which was collected, reduced to 2 ml and treated as above.

Gas-Liquid Chromatography Conditions: Two G.L.Cs were used. One was fitted with a 3 ft (90 cm) column packed with 3% Apiezon L + 0.3% Epikote on Diatomite CQ 100-120 mesh and the other with a 3 ft column packed with Silicone + Epikote on Diatomite CQ 100-120 mesh. Both ovens were operated at 188°C and a nitrogen carrier flow of 100 ml per minute was used. Quantification was by measurement of peak height compared to that of standards.

Results

As expected, the weight of the tissue in both treatments changed over the one year period of storage (Table 1). The formaldehyde preserved group showed a slow decrease in weight with a final loss of 10% in a year. The changes in the phenol preserved group, on the other hand, were more erratic changing from a loss to a final gain.

TABLE 1

Effect of Preservative on Tissue Weight

Sampling Date	4% Formaldehyde	10% Phenol
Zero hour	-	-
5 days	- 1%	- 8%
10 days	- 1%	- 12%
4 months	- 6%	- 4%
1 year	- 10%	+ 20%

Residue determination (Table 2) on both groups of tissue revealed that the pesticides and metabolites are both stable and extractable when compared with the zero hour sample, particularly in the phenol. Unlike phenol, the formaldehyde preservation does not completely inhibit the post mortem convertion of pp'-DDT to pp'-TDE. Only 10.5% of the original pp'-DDT was present after one year's storage in this preservative. However, the total DDT material present was not very different. If the pp'-TDE present is multiplied by 1.11 as suggested by Jefferies and Walker (2), to convert it back to the original pp'-DDT the residues of pp'-DDT in the two zero hour samples are 31.7 and 30.3 ppm and that in the one year formaldehyde sample is 28.9 ppm.

TABLE 2

Residue levels in tissue after preservation (expressed in parts per million wet weight) (N/D none detected - below 0.01 ppm)

Sampling Date	4% Formaldehyde				10% Phenol			
	HEOD	ppDDE	ppTDE	ppDDT	HEOD	ppDDE	ppTDE	ppDDT
Zero Hour	4.90	8.69	12.40	17.98	4.21	8.00	11.00	18.21
5 days	4.85	9.40	11.10	18.84	4.40	8.15	10.99	19.50
10 days	4.81	8.24	10.10	18.10	4.73	8.90	13.10	17.00
4 months	4.44	8.10	20.40	6.30	4.31	8.37	9.89	18.30
1 year	4.63	8.24	24.30	1.89	4.64	8.80	10.40	17.80
	(Preservative N/D)				(Preservative N/D)			

The final hexane extracts from the phenol group contained small crystals of phenol which did not interfere with the residue determination but continual analysis of this type of specimen may shorten the working life of the column or detector.

There were no detectable levels of organochlorines in the residual preservative in either case.

Discussion and Conclusion

The continued breakdown of pp'-DDT to pp'-TDE in the livers stored in formaldehyde is of interest. This was found to occur in Bengalese finch (Lonchura striata) livers stored under deep freeze conditions of - 14°C (2). The present experiment supports the finding that this breakdown continues after death and that most of the TDE found in wildlife specimens is of post-mortem origin. This breakdown is probably due to bacterial action as it is unlikely that this would be completly inhibited by the concentration of formaldehyde used. It has been demonstrated that the bacteria Proteus vulgaris (3), Serratia marcescens and Escherichia coli (4) can all bring about the reductive dechlorination of DDT to TDE. Phenol, although antiseptic, also does not inhibit all bacterial

action. The present lack of breakdown may be due to a higher concentration of preservative or an inhibition of the bacteria responsible for the action.

The two preservatives used in the present test are both suitable for use in the field. Both can be transported in concentrated form and diluted in the field. Although weighing of the specimens at the time of collection is obviously an advantage, in extremely difficult conditions a portable balance could be dispensed with in the case of formaldehyde as the weight change is small and predictable. The post-mortem breakdown of the pp'-DDT to pp'-TDE in the formaldehyde is unfortunate but can be corrected. Such a breakdown also occurs rapidly in deep-freeze conditions (2), as does weight change through 'freeze-drying'. Thus this is not an extra disadvantage not normally faced in organochlorine analysis. The use of a more concentrated formaldehyde solution may reduce this condition. Both materials have many advantages over alcohol, which has to be used in more concentrated form thus increasing the original bulk carried, and is subject to a high evaporation rate in the tropics and causes customs difficulties. Its most troublesome feature is that it is an organochlorine extractant and would entail analysis of the alcohol as well as the specimen. This is not necessary with either phenol or formaldehyde.

On balance, this laboratory has decided to use a 4% solution of formaldehyde for the preservation of tissue from field experiments or for the transport of specimens by air or sea freight when the time delay precludes the use of dry ice. Although almost as good, phenol was rejected on account of possible column difficulties and weight changes.

References

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