Micro Scale Alkali Treatment for Use in Pesticide Residue Confirmation and Sample Cleanup

by Susan J. V. Young and Jerry A. Burke
Division of Chemical Technology
Bureau of Foods
Food and Drug Administration
Washington, D. C. 20204

Procedures involving alkali treatment for dehydrochlorination of certain organochlorine pesticides and saponification of fats have long been employed in pesticide residue analysis. In 1942 Brand and Busse-Sunderman (1), and in 1946 Soloway et al. (2), studied rates of dehydrochlorination of DDT. Mills (3) used refluxing alcoholic KOH in the cleanup of fatty foods for paper chromatographic detection of alkali-stable organochlorine pesticides. Klein and Watts (4) used alcoholic NaOH to dehydrochlorinate o,p'- and p,p'-DDT, p,p'-TDE, and Perthane prior to gas chromatographic separation of the respective olefins. These investigators called attention to several earlier uses of alkali dehydrochlorination in pesticide residue chemistry. Recent literature contains numerous references to application of this treatment in pesticide residue analyses, including an adaptation for use in a pre-gas chromatographic (GLC) column (5).

In spite of the knowledge of this reaction we have observed that it is not fully and effectively utilized by residue laboratories for forming derivatives, gaining information for identity confirmation, or obtaining better cleanup of troublesome extracts. This is probably because the procedure has not been described in detail for simple micro scale application in multiresidue analysis.

The purpose of this work was (1) to arrive at optimum and convenient parameters of the alkali treatment in order to obtain rapid dehydrochlorination resulting in product solutions suitable for analysis by GLC; (2) to obtain complete yield and recovery of olefins from a number of bis(phenyl) chloroethane pesticides; (3) to determine the effect of the treatment on several important pesticide and industrial chemicals; (4) to describe in detail the procedure for routine application in the residue analytical laboratory.

METHOD

Reagents and Apparatus

(a) Potassium hydroxide - Anhydrous pellets.
(b) Ethanol - USP 95%.
(c) Hexane - Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories, Inc. 1953 S. Harvey St., Muskegon, Mich. 49442).

Bulletin of Environmental Contamination & Toxicology,
Vol. 7, No. 2/3, 1972 © Springer-Verlag New York Inc.

(d) *Micro condenser* - 19/22 §; K-569250 (Kontes Glass Company, Vineland, N. J. 08360).

(e) *Concentrator tube* - Mills type, 19/22 § with stopper, 10 ml graduated in 0.1 ml up to 1.0 ml; K-570050 (Kontes Glass Company).

(f) *Alkali dehydrochlorination reagent* - Dissolve 2 g KOH in 100 ml ethanol.

(g) *Ethanol - water, 1 + 1* - Combine equal parts by volume of distilled water and ethanol.

(h) *Gas chromatograph* - Equipped with electron capture detector and 6' x 4 mm id glass column containing either (*1*) 10% DC-200 or (*2*) 1:1 mixture of 15% QF-1 + 10% DC-200 on 80-100 mesh Chromosorb W(HP). *Operating conditions*: N_2 flow 120 ml/min; temperatures, column and detector 200°C, injector 225°C; concentric design electron capture detector operated at dc voltage to cause 1/2 full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} amp.

Procedure

Accurately pipet, into a 10 ml Mills tube, 2 ml of a petroleum ether solution of sample extract [6% or 15% Florisil eluate (6)] containing concentrations of pesticides suitable for subsequent GLC analysis. Add 1 ml of 2% ethanolic KOH and a few carborundum chips and fit the tube with a micro condenser. [Note: Avoid getting alkali on the ground glass joint· light greasing of joint with silicone lubricant may prevent sticking.] With a test tube clamp, hold the tube over an opening in the steam bath in such a manner that gentle boiling occurs. When the volume has been reduced to about 1 ml, insert the tube completely into the steam bath opening and heat vigorously for 15 minutes or until the volume reaches 0.2 ml. Remove tube from the steam. If a precipitate has formed, as is often the case with extracts containing fatty substances, add a few drops of 2% ethanolic KOH and warm gently in steam with swirling until the precipitate dissolves. After the solution has cooled slightly, add about 2 ml ethanol-H_2O (1 + 1). Allow solution to reach room temperature and pipet 1 or 2 ml hexane into tube. Stopper tube with ground glass, invert, shake vigorously for about 30 seconds, and allow solvent layer to separate sharply. With microliter syringe, carefully withdraw aliquot of upper layer for determination by GLC. [Note: Separation of phases should be sharp so that solution withdrawn for GLC analysis will be free from alkali.]

Discussion

Development of Method

Initial experimentation was performed to establish the reaction conditions which would give complete and rapid dehydrochlorination of *p*,*p*'-DDT, *o*,*p*'-DDT, *p*,*p*'-TDE, *o*,*p*'-TDE, methoxychlor, and Perthane and which would permit complete recovery of the respective olefins. Work was done to determine the effect on olefin formation of fatty substances not removed during sample cleanup and the capacity of the reaction to eliminate fatty substances.

Several considerations found necessary for practical and reliable use of the alkali treatment have been incorporated into the method and are briefly discussed. The steam bath was chosen as a source of heat because of its ready availability and convenience. The Mills reaction tube was fitted with a micro condenser to eliminate losses due to volatilization, which often occurred in the absence of the condenser. Both KOH and NaOH have been used to the satisfaction of previous investigators. The more frequent use of KOH by other workers and its higher solubility in ethanol made it the choice for this work. An alkali concentration of 2% has been widely used, and was found ideal for treatment of aliquots of cleaned-up sample extracts containing quantities of bis(phenyl) chloroethane pesticides ranging from a few nanograms to 100 μg. In order to provide sufficient reflux time and temperature it was necessary that the initial volume of ethanol be in excess of 0.5 ml. When smaller volumes were used, dehydrochlorination was usually incomplete. Emulsions often occurred during extraction of the olefin into hexane after saponification of fatty substances. The use of ethanol + water (1 + 1) instead of water as the diluent resulted in a sharp separation of hexane and aqueous layers. Recoveries of olefins were not adversely affected if the ethanol content was less than about 70%. Less than 30% ethanol did not adequately enhance separation of the two layers. The non-volatile fatty substance transferred to acetonitrile by partitioning a petroleum ether solution of butterfat with acetonitrile was used in tests to evaluate the effects of fatty substances on the reaction. Experiments in which varying volumes of 2% ethanolic KOH were used to saponify 350 mg portions of this butterfat showed that each 1 ml of 2% KOH would saponify about 50 mg of the fat. When the weight of fatty substances exceeded about 50 mg, complete dehydrochlorination of Perthane (40 μg) and methoxychlor (4.0 μg) was not obtained. However, p,p'-DDT (8.0 μg) was completely dehydrochlorinated in the presence of 100-120 mg of fat. Additional experimentation showed that complete dehydrochlorination of methoxychlor and Perthane did not occur until the fat was completely saponified; these were the bis(phenyl) chloroethanes most resistent to dehydrochlorination. Quantities of Perthane and p,p'-DDT up to 100 μg in the presence of not more than 50 mg butterfat were readily dehydrochlorinated with 1 ml of 2% KOH at steam bath temperature; dehydrochlorination of larger amounts of pesticide was not attempted. Hexane, because of its higher boiling point and greater ease of drawing into a microsyringe, was used instead of petroleum ether, to extract the olefin after reaction in order to avoid possible errors in quantitation.

Effect on Selected Chemicals

The dehydrohalogenation reaction, as described under "Method", was applied to p,p'-DDT (0.8 μg), o,p'-DDT (0.8 μg), p,p'-TDE (2.0 μg), o,p'-TDE (0.4 μg), methoxychlor (4.0 μg), and Perthane (40 μg). Quantities shown in parentheses were chosen for ease in GLC determination. The pesticides were treated individually in petroleum ether, in 6% ethyl ether/petroleum ether Florisil eluates (6) containing the equivalent of 6 g of kale, and in petroleum ether containing 30-60 mg of fatty substances extracted from butter by partitioning (6) between petroleum ether and acetonitrile. Gas chromatography with electron capture detection, operated as described under "Method", was used for all determinations.

Each pesticide was completely altered in each of the solution types, i.e., none of the parent compound remained after treatment as described under "Method". Percent recoveries of the respective olefins were calculated according to the following equation:

$$\frac{\text{wt. olefin compound determined by GLC}}{\text{wt. parent compound represented in aliquot to GLC}} \text{ X}$$

$$\frac{\text{wt. parent compound/mol. wt.}}{\text{wt. olefin compound/mol. wt.}} \text{ X } 100$$

Recoveries of olefins approximated 100% and ranged from 86% for *p,p'*-DDE and *o,p'*-TDE olefin in petroleum ether to 110% for *p,p'*-DDE in the extract from butter. Two olefin derivatives, the *cis* and *trans* isomers, are formed from *o,p'*-TDE (7). These have identical retention times on the two GLC columns used in this work and were quantitated as a single compound.

Several additional common organochlorine pesticides and polychlorinated biphenyls (PCB) were subjected to the described alkali treatment. All tests were made with petroleum ether solutions of the chemical under study. The quantity of each chemical was chosen for ease of determination by GLC and is given in parentheses.

Polychlorinated biphenyls ranging from 21 to 60% average chlorine content were stable to this treatment. Complete recoveries were obtained for the commercial PCB mixtures, Aroclors 1221 (16 μg), 1232 (16 μg), 1242 (16 μg), 1254 (8 μg), and 1260 (8 μg). Recoveries of unchanged aldrin (0.4 μg), dieldrin (0.4 μg), and endrin (0.4 μg) ranged from 70 to 90%. No alteration products were detected.

Heptachlor (0.4 μg) and heptachlor epoxide (0.4 μg) were markedly affected; recoveries of the original compound ranged from 30 to 50%. Minor GLC peaks were observed on the 10% DC-200 column at retention times relative to aldrin of 1.63 after treatment of heptachlor epoxide and 0.59 and 0.93 after treatment of heptachlor.

The alkali treatment completely eliminated lindane (0.2 μg) and the alpha (0.2 μg), beta (0.2 μg), and delta (0.2 μg) isomers of BHC. Following the reaction, only small early eluting gas chromatographic peaks, presumably from trichlorobenzenes, were observed.

About 40% of mirex (4.0 μg) remained unchanged after reaction; sometimes a minor GLC peak appeared at a retention time relative to aldrin of 1.83 on the 10% DC-200 column.

Endosulfan I and II, treated separately, were completely eliminated. Each isomer gave a single alteration product with retention time relative to aldrin of 1.82 on the 10% DC-200 column and 2.23 on the 1:1 10% DC-200/15% QF-1 column. The peak height of the alteration product was approximately one-tenth the peak height of the parent compound. A structure for this derivative has been proposed (8).

Endosulfan sulfate also was completely eliminated. Two alteration products were obtained with retention times relative to aldrin of 0.28 and 0.38 on the 10% DC-200 column.

Dicofol (1.0 μg) was completely eliminated but only 65% of the major alteration product, 4,4'-dichlorobenzophenone, was recovered. A minor peak was observed in the chromatogram at a retention time relative to aldrin of 1.71 on the 10% DC-200 column. The 4,4'-dichlorobenzophenone (2.0 μg) was not affected by treatment with alkali.

The products resulting from alkali treatment of toxaphene (10.0 μg) gave a multicomponent chromatogram but consisting of components with earlier retention times than toxaphene itself.

The electron capture GLC responses to sulfur (20 μg), frequently encountered in residue analysis at retention times relative to aldrin of 0.23, 0.55, and 1.13 on the 10% DC-200 column, were eliminated by the alkali treatment.

Application of Method

The most obvious use of the alkali treatment is to form the olefins of bis(phenyl) chloroethane pesticides for confirmation of residue identity. The complete yield and recovery of the olefin derivative makes possible quantitative confirmation of a residue of the parent pesticide. For example, a residue of *p,p*'-DDT can be quantitated before alkali treatment and verified by quantitation as *p,p*'-DDE after treatment.

In addition the GLC retention time region of the reacted compound can be examined for presence of peaks from unreacted and presumably interfering substances. We have found the alkali treatment especially useful in connection with the real or suspected presence of residues of PCB. In this case the characteristic olefin derivatives of *p,p*'-DDT, *o,p*'-DDT, and *p,p*'-TDE can be formed and the GLC retention time region underlying the parent compounds can be examined. The stability of PCB to alkali, with no change in the GLC pattern, is a characteristic which can be readily utilized in confirmation of the identity of this complex residue. The high recovery of unreacted dieldrin, endrin, and aldrin following alkali treatment likewise can be of value in the confirmation of identity of these pesticides.

TABLE 1

Compounds Treated with Alkali According to the Described Procedure

Compound	Approximate Amount Altered, %	Major Alteration Products Detectable by GLC	Approximate Amount of Parent Compound Changed to Alteration Product, %
p,p'-DDT	100	p,p'-DDE	100
o,p'-DDT	100	o,p'-DDE	100
p,p'-TDE	100	p,p'-TDE olefin	100
o,p'-TDE	100	cis o,p'-TDE olefin trans o,p'-TDE olefin	100
Perthane	100	Perthane olefin	100
methoxychlor	100	methoxychlor olefin	100
dicofol	100	4,4'-dichlorobenzophenone	65
4,4'-dichlorobenzophenone	0	----	---
lindane	100	presumably trichlorobenzenes	---
α,β,δ isomers of BHC	100	presumably trichlorobenzenes	---
endosulfan I	100	1.82 DC-200[a,b] 2.23 mixed[c]	---
endosulfan II	100	1.82 DC-200[a,b] 2.23 mixed[c]	---
endosulfan sulfate	100	0.28, 0.38 DC-200[a]	---
mirex	60	1.83 DC-200[a]	---
heptachlor	50-70	0.59, 0.93 DC-200[a]	---
heptachlor epoxide	50-70	1.63 DC-200[a]	---
aldrin	10-30	----	
dieldrin	10-30	----	
endrin	10-30	----	
toxaphene	apparently greatly altered	substances with earlier RRT_A than toxaphene	---

TABLE I (continued)

Compound	Approximate Amount Altered, %	Major Alteration Products Detectable by GLC	Approximate Amount of Parent Compound Changed to Alteration Product, %
polychlorinated biphenyls: Arochlors 1221, 1232, 1242, 1254, and 1260	0	----	---
sulfur: 0.23, 0.55, 1.13 DC-200[a]	100	----	---

---

[a] Retention time relative to aldrin on the 10% DC-200 column, described under "Reagents and Apparatus (h)".

[b] Structure elucidated by Greve and Wit (8).

[c] Retention time relative to aldrin on the 1:1 QF-1 + 10% DC-200 column, described under "Reagents and Apparatus (h)".

Cleaned-up extracts of some samples may contain non-pesticidal substances which give rise to electron capture response. Other extracts may contain fatty substances, not removed by the clean-up, which can prohibit application of some tests, e.g., thin layer chromatography. This is particularly true of the 15% ethyl ether/petroleum ether Florisil column eluate (6) for non-fatty samples such as carrots and fatty samples such as some fish. Electron capturing substances present in carrots must be eliminated before determination of dieldrin and/or endrin residues. Treatment with alkali serves well for this purpose. Extracts of fatty samples may require treatment to eliminate both electron capturing substances and non-volatile fatty substances. In many instances thin layer chromatography or microcoulometric GLC cannot be accomplished prior to alkali treatment. Electron capture responses to sulfur, often a source of annoyance to the residue chemist, are also eliminated by this treatment.

These common uses of the alkali treatment serve to illustrate its value to the residue chemist. The directions for laboratory application outlined here should enhance the use of this procedure of long standing.

References

1. BRAND, K. and BUSSE-SUNDERMAN, A., Berichte, 75 B 1819 (1942).
2. SOLOWAY, S. B., SCHECHTER, M. S., and JONES, H. A., Soap and Sanitary Chemicals, 1946 Blue Book, 18th Ed., 215 (1946).
3. MILLS, P. A., J. Ass. Offic. Anal. Chem. 42, 734-740 (1959).
4. KLEIN, A. K. and WATTS, J. O., J. Ass. Offic. Anal. Chem. 47, 311-316 (1964).
5. MILLER, G. A. and WELLS, C. E., J. Ass. Offic. Anal. Chem. 52, 548-553 (1969).
6. Pesticide Analytical Manual, Vol. I, Food and Drug Administration, Washington, D. C., 2nd Ed., 1968; Revised July 1969, July 1970, and April 1971.
7. KRAUSE, R. T., Private Communication, Food and Drug Administration, Washington, D. C., May 1970.
8. GREVE, P. A. and WIT, S. H., J. Agr. Food Chem. 19, 372-374 (1971).