Improved gas chromatographic systems for determining organochlorine pesticide residues in wildlife

The use of gas–liquid chromatography to determine and identify the organochlorine pesticide residues in avian tissue and eggs is well established and documented. Evidence obtained from the use of a single gas chromatographic column is insufficient in many cases and it is recommended[1] that samples should be examined on at least two different stationary phases. Those commonly used are silicone oils or elastomers such as E301 or SE52, and Apiezon L grease, the differing polarities of these substances conferring variations in their separatory characteristics. The performances of both these types of column can be improved by use of silanized Chromosorb G[2] as the support in place of Celite 545; this results in far less tailing of peaks and reduces decomposition of pesticides on the columns. The higher bulk density of Chromosorb G necessitates some reduction in the loading of the stationary phase in order that the pesticides of greater retention volume are still eluted within a reasonable time. It is still desirable to include a little Epikote in the stationary phase to facilitate separation of the BHC isomers and further to prevent decomposition of some compounds. The performance of Apiezon L columns can also be improved by using glass columns and applying the stationary phase to the support under conditions in which contact with air is limited, as described later. Apiezon L columns made in this way, using benzene as solvent, give negligible decomposition of *p,p'*-DDT, good separation of *p,p'*-TDE from *p,p'*-DDT and of *o,p'*-DDT from *p,p'*-DDE, and in many ways such columns have similar separation characteristics to the Oronite Polybutene 128 column reported by RICHARDSON[3].

These two types of column, in conjunction, can thus resolve a number of difficulties, *e.g.* dieldrin coincides with p,p'-DDE on silicone columns and with *p,p'*-DME (1,1-di-4-chlorophenyl-2-chloroethylene) on the Apiezon column, but the true dieldrin figure can be calculated using data obtained from both columns. However, certain problems associated with the gas chromatographic separation of organochlorine pesticide residues still remain. For some wildlife samples, particularly avian tissue and eggs, the peaks at the retention times of *p,p'*-DDT and *p,p'*-TDE are misleadingly high due to co-extracted interfering compounds which pass through most normal clean-up processes. Thin-layer chromatography can be used[4] to resolve this problem since these interfering compounds, subsequently referred to as "avian compounds", show appreciably greater R_F values on silica gel chromatoplates than *p,p'*-DDT and *p,p'*-TDE. Subsequent elution of the appropriate thin-layer area followed by re-examination by GLC has often shown these pesticides to be present in much smaller amounts than indicated initially.

This procedure is, however, time-consuming and in order to retain the relative speed of using GLC only, a different stationary phase was sought on which the retention times of *p,p'*-DDT and *p,p'*-TDE would be radically different from those of the avian compounds. The behaviour of these compounds on silica gel layers indicated that they were less polar in nature than the pesticides, and that therefore a polar stationary phase might be applicable. As such a column would need to be in continuous service in this Laboratory, a low bleed-off rate was another required characteristic.

The cyanosilicone GE-XE60 used by GOULDEN *et al.*[5] in their multicolumn

TABLE I

RELATIVE RETENTION TIMES (DIELDRIN = 100):

Column (1): Silicone Gum GE-SE 52 1.3%, Epikote Resin 1001 0.15% on Chromosorb G (DMCS treated, acid washed, 70 to 80 or 80 to 100 mesh). 2 ft. U-tube column in copper ($^1/_4$ in. O.D.) or glass ($^1/_8$ in. I.D.). Working temperature 160°.
Column (2): Apiezon L grease 1.3%, Epikote Resin 1001 0.2% on Chromosorb G (DMCS treated, acid washed, 80 to 100 mesh). 2 ft. U-tube in glass ($^1/_8$ in. I.D.). Working temperature 190°.
Column (3): Silicone GE-XE 60 1.3%, Epikote Resin 1001 0.13% on Chromosorb G (DMCS treated, acid washed, 70 to 80 mesh). 6 ft. U-tube in glass ($^1/_8$ in. I.D.). Working temperature 200°.

Stationary phase (1) *Silicone Gum GE-SE 52*		(2) *Apiezon L*		(3) *Cyanosilicone GE-XE 60*	
Hexachlorobenzene	15	Diazinon	14	Hexachlorobenzene	12
α-BHC	16	α-BHC	18	Diazinon	18
Diazinon	22	Hexachlorobenzene	25	α-BHC	26
γ-BHC	22	γ-BHC	25	Heptachlor	26
β-BHC	28	Malathion	27	Aldrin	28
Heptachlor	30	Heptachlor	34	γ-BHC	39
Aldrin	37	β-BHC	34	Heptachlor epoxide	65
Malathion	51	Parathion	41	*p,p'*-DME	68
Parathion	59	Aldrin	44	Malathion	76
Heptachlor epoxide	59	Heptachlor epoxide	59	Endosulfan A	78
Endosulfan A	79	Endosulfan A	90	*p,p'*-DDE	81
p,p'-DME	79	*p,p'*-DME	94	Parathion	85
Dieldrin	100	Dieldrin	100	Dieldrin	100
p,p'-DDE	103	*p,p'*-DDE	120	β-BHC	100
Endrin	120	Endrin	120	Avian compound	107
o,p'-DDT	145	*o,p'*-DDT	155	*o,p'*-DDT	115
Endosulfan B	157	Endosulfan B	157	Endrin	117
Avian compound	164	*p,p'*-TDE	188	Avian compound	143
p,p'-TDE	175	Avian compound	195	*p,p'*-DDT	194
Avian compound	209	Avian compound	234	*p,p'*-TDE	206
p,p'-DDT	216	*p,p'*-DDT	244	Endosulfan B	218
Avian compound	234	Avian compound	266	Avian compound	230
Avian compound	390	Avian compound	294		
		Avian compound	520		

spectrochromatograph had been found promising for organophosphorus compounds[6] and was thought likely to be suitable for this purpose. A column packing was prepared by dissolving the cyanosilicone, together with a little Epikote, in chloroform and applying this solution to the Chromosorb G under conditions in which contact with air was limited. This was achieved by warming the mixture in a 250 ml round-bottomed flask over a gentle source of heat while rotating the flask rapidly and irregularly enough to ensure thorough mixing; a mechanical rotary evaporator gave unsatisfactory results. When all the solvent appeared to have gone, the flask was heated in an oven at 100° for half an hour. Some of the solution of the stationary phase was held back and used to rinse the clean glass tubing which was then allowed to dry out before being packed. This method of preparing the column tubing has been found to help minimize tailing of chromatogram peaks generally. Retention times for a column prepared in this way are given in Table I and, for comparison, retention times on a silicone gum GE-SE52 column and an Apiezon L column are also given.

On the cyanosilicone GE-XE60 column, the relative retention times of the earliest avian compounds are 107 and 143 (dieldrin = 100) whilst the peak corresponding to the next commonly occurring avian compound has a relative retention

time of 230. Thus with relative retention times of 194 and 206 respectively, *p,p'*-DDT and *p,p'*-TDE appear on an otherwise long flat part of the chromatogram between these earliest and later avian compound peaks. Although not completely separated one from the other, they can be easily detected and their levels estimated in extracts which contain avian compounds.

Coupled with its reversal of the usual dieldrin and *p,p'*-DDE retention times, this column is a very useful addition to the usual silicone and Apiezon columns already in operation. It has been used for the detection and estimation of small amounts of endrin in a series of samples containing much larger amounts of *p,p'*-DDE, normally impossible on existing systems without resort to the thin-layer method referred to above.

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Gas chromatography of 3,4-methylenedioxyphenyl carbamates

The gas chromatographic behavior of simple and N-substituted carbamates[1–3] and of 3,4-methylenedioxyphenyl derivatives[4] has been previously described. Several groups of workers have investigated the efficacy of uniting the herbicidal activity of carbamates with the synergistic action observed in the presence of 3,4-methylenedioxyphenyl (MDO-phenyl) derivatives via the preparation of 3,4-MDO-phenyl carbamates[5–10]. Analysis of such compounds utilizing thin-layer chromatography has been reported earlier[11]. The documentation of analysis of 3,4-MDO-phenyl derivatives via various colorimetric means and by infrared spectrophotometry, and of 3,4-MDO-phenyl synergists by paper and thin-layer chromatographic techniques are given in this latter reference.

The present study describes the gas chromatographic behavior of a selection of 3,4-MDO-phenyl carbamates and the relation of elution data to structural features of the aryl carbamate substituents.