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Degradation of [^{14}C]DDT on Silica Gel G chromatograms under laboratory conditions

In metabolic studies with small amounts of pesticides in marine animals¹⁻³ we used [^{14}C]DDT, which was purified by thin-layer chromatography (TLC) on silica gel before use. We observed the formation of polar degradation products from [^{14}C]DDT on silica gel chromatograms under laboratory conditions. Degradation of DDT has been reported to occur on silica gel by the action of sunlight in the presence of photosensitizing agents⁴ and on aluminium oxide layers using UV light⁵. The findings on this subject reported in this paper may be of importance in eliminating a possible source of error in the interpretation of degradation pathways of DDT.

Experimental

[^{14}C]DDT, 19.1 mCi/mmole, was obtained from the Radiochemical Centre, Amersham, Great Britain. Unlabelled DDT, DDD, DDE and DDA were kindly supplied by Fa. Geigy, Basel, Switzerland. Solvents, Silica Gel G and Silica Gel GF₂₅₄ were of analytical grade from Merck, Darmstadt, G.F.R. The coating materials were applied to 20 × 20 cm glass plates by the usual method to give a thickness of 250 μm . Two solvent systems were used: (A) *n*-heptane with 1% of acetone⁶; (B) benzene-methanol-acetic acid (80:14:7). The solvents were allowed to move 10 cm from the starting point. [^{14}C]DDT was dissolved in ethanol to give a final concentration of 1 μg per 5 μl . Unlabelled DDT, DDE and DDD were used as reference substances in a mixture containing 30 mg of each in 10 ml of *n*-hexane; 5- μl aliquots were used as standards. DDA was used in the same concentration but in acetone solution. Examination and irradiation with UV light at 254 nm and 366 nm, respectively, were carried out in the Fluotest apparatus (Quarzlampengesellschaft Hanau, G.F.R.). Radioactive compounds on the chromatograms were scanned with a Desaga scanner. Spotting of the substances with a Hamilton syringe and development of the chromatoplates were carried out under the following conditions: (1) in the dark with only a minimum amount of light; (2) under laboratory daylight; and (3) under a fluorescent lamp. In the case of UV irradiation, the spotted chromatoplates were treated before development and in this way the [^{14}C]DDT was exposed to UV light at the point of application. The chromatoplates were first developed in solvent A and scanned. After a subsequent run of the same chromatogram in solvent B, it was scanned again. Between the first scan and the development in solvent B the chromatograms were stored in the dark for various periods of time.

Results and discussion

TLC of [^{14}C]DDT on Silica Gel G and Silica Gel GF₂₅₄ under the above conditions gave rise to the formation of polar substances. The most effective was shortwave UV light, even after 15-min periods, as used in the detection of separated compounds. Under these conditions four components were probably formed (Table I and Fig. 2). Component IV remained at the starting point in solvent A and seemed

TABLE I

CHROMATOGRAPHY OF [^{14}C]DDT AND UNLABELLED DDA, DDD, DDT AND DDE

Compound	R_F values	
	In solvent A	In solvent B
DDA	0.0	0.7
DDD	0.25	0.9
DDT	0.36	0.9
DDE	0.55	0.9
Component I	0.0	0.0
Component II	0.0	0.7
Component III	0.0	0.8
Component IV (?)	0.0	0.9

to combine with DDT in solvent B. The other conditions yielded in general one spot, which remained at the starting point in solvent A, and which probably consisted of components I and IV (Fig. 1). When chromatograms developed in solvent A were stored in the dark for up to four weeks and subsequently devel-

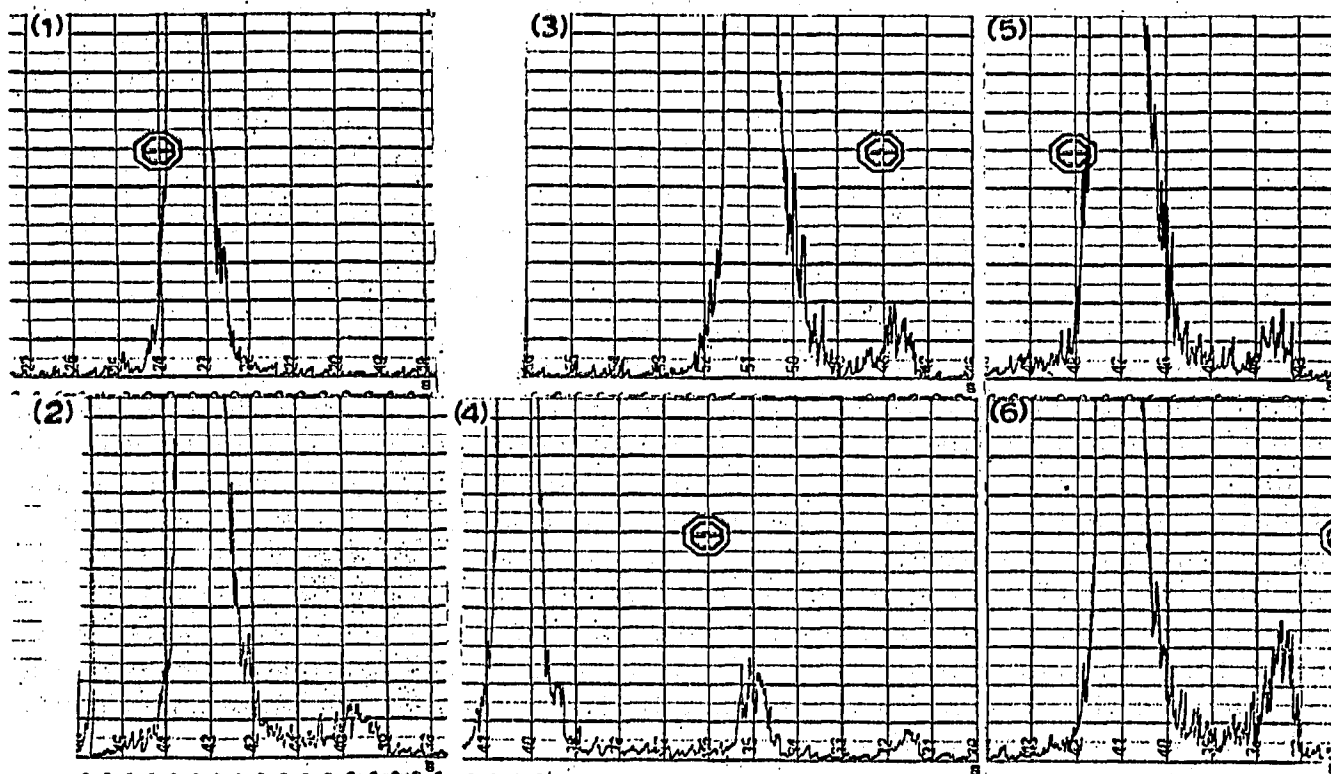


Fig. 1. Radioactive scans of chromatograms from $1 \mu\text{g}$ of [^{14}C]DDT spotted on the indicated layers and exposed to various conditions before development. S = Starting point. 1 = Silica Gel GF₂₅₄ in the dark, solvent A, no storage. 2 = Silica Gel G, 2 h in daylight, solvent A. A fluorescent lamp produced the same effect. 3 = Silica Gel GF₂₅₄, 2 h under fluorescent lamp, solvent A. 4 = Chromatogram from 3 after storage for four weeks in the dark and subsequent development in solvent B. Formation of component I at the position of DDT in solvent A. 5 = Silica Gel G, 2 h under UV light of wavelength 366 nm, solvent A. 6 = Same procedure as in 5 but on Silica Gel GF₂₅₄.

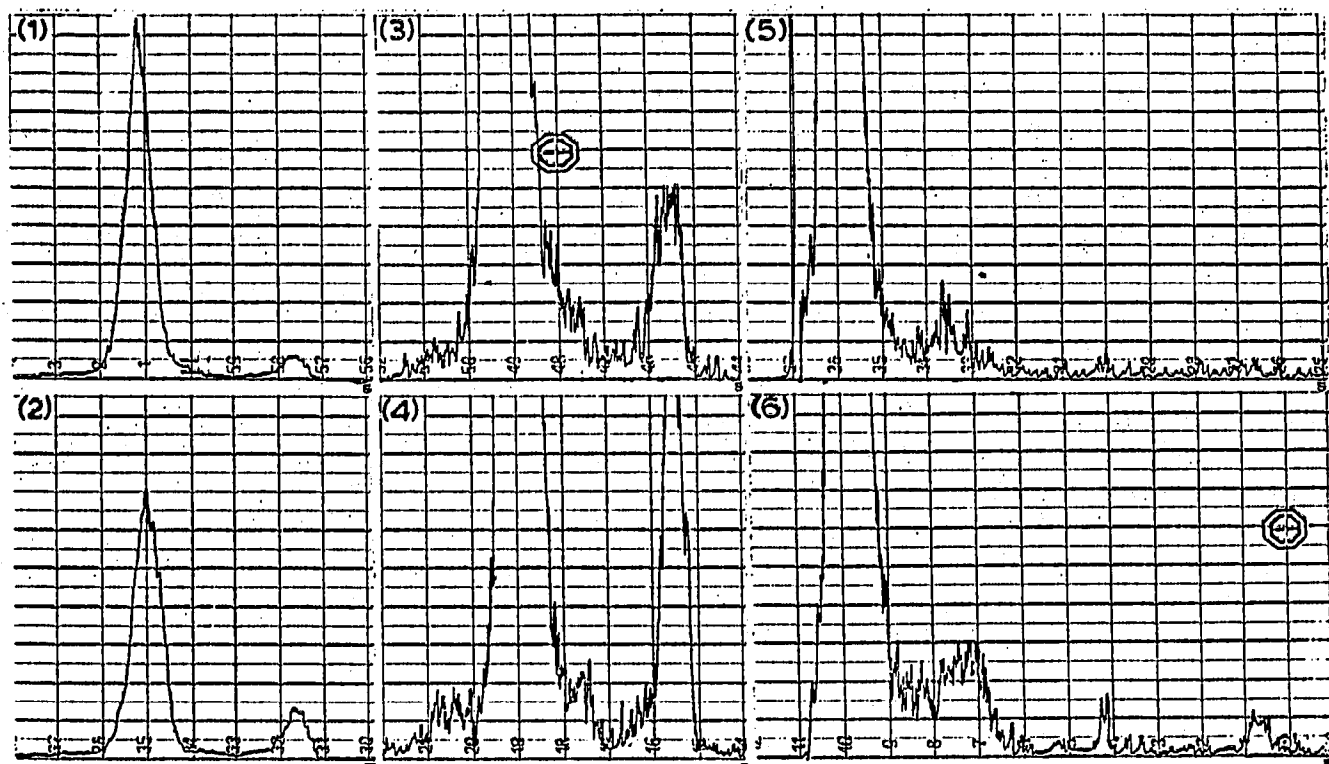


Fig. 2. Radioactive scans of chromatograms from $1 \mu\text{g}$ of $[^{14}\text{C}]\text{DDT}$ spotted on the indicated layers and exposed to UV light of wavelength 254 nm. S = Starting point. 1 = Silica Gel GF₂₅₄, 15 min under UV light, solvent A. 2 = Silica Gel GF₂₅₄, 60 min under UV light, solvent A. 3 = Chromatogram from 1 but with 10-fold resolution during scanning. 4 = The same as in 3 but with chromatogram from 2. 5 and 6 = Chromatograms from 1 and 2, with subsequent development in solvent B without prior storage.

oped in solvent B, the results indicated the probable formation of component I during storage. Furthermore, very small amounts of radioactive by-products appeared at the positions of DDE and DDD, but a more extensive identification has not been performed. The nature of the photodegradation products is not yet known.

Studies by a more sensitive method such as radioscanning led to the conclusion that in the investigation of metabolic or other degradation pathways with $[^{14}\text{C}]\text{DDT}$ the evaluation of silica gel chromatograms will not be conclusive if appropriate precautions are not followed, such as working in the dark and repeated purification of the $[^{14}\text{C}]\text{DDT}$ batches to remove interfering material.

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