Effects of humidity in the laboratory on thin-layer chromatography of insecticides

In this laboratory, thin-layer chromatography (TLC) is used for the separation and tentative identification of organo-chlorine insecticide residues in biological material. In the course of our work it became evident that R_F values were lower in winter than in summer, with the result that methoxychlor, dieldrin and heptachlor epoxide were poorly separated.

An investigation was initiated to determine the influence of humidity at ambient temperature on the activity of the adsorbent. Other workers[1,2] have studied the effects of humidity on R_F values; however, they preconditioned thin-layer plates for hours in constant humidity chambers. This communication shows the effect of humidity in the laboratory on the activity of the adsorbent when the plates are exposed for only a short time, in the normal course of spotting the sample.

Experimental

The standard technique of ascending TLC[3] was employed. Aluminum oxide G (Merck) was layered 250 μ thick on 20 × 20 cm glass plates, activated at 80° for 30 min and stored over anhydrous $CaSO_4$.

Before spotting, the humidity in the laboratory was equilibrated with a portable humidifier; for high humidities (60–90 %) a steam bath was also employed. The humidity was determined with a sling psychrometer before each run. The plates were spotted with 3 μg quantities (1 μg/μl) of each insecticide (Table I) separately

TABLE I

EFFECT OF RELATIVE HUMIDITY IN THE LABORATORY ON R_F VALUES

Aluminum oxide G plates exposed to the humidity for 10 min, temperature 24° ± 1°. The results are the mean of three determinations.

Compound	R_F × 100 *at relative humidity*					
	14 %	*22 %*	*42 %*	*60 %*	*70 %*	*90 %*
Aldrin	65	68	68	74	74	92
DDE	54	57	60	70	70	92
DDT	34	38	42	57	60	85
DDD	15	18	22	36	41	73
Heptachlor epoxide	8	11	14	25	30	60
Dieldrin	7	8	10	19	23	50
Methoxychlor	5	5	5	11	16	35

and as a composite. Each plate was exposed for exactly 10 min to the atmosphere in the laboratory, then placed in a lined tank and developed with Skellysolve B (mainly *n*-hexane) for a distance of 10 cm from origin line. Chromatoplates were sprayed with silver nitrate solution[4] and irradiated with two germicidal U.V. lights.

Results and discussion

The results in Table I show that the R_F values were affected by the humidity in the laboratory; the activity of the adsorbent decreased as humidity increased.

The effect of humidity was more pronounced for some compounds than it was for others. The data were subjected to an analysis of variance which confirmed these conclusions and indicated a coefficient of variability at 2.5 %.

Fig. 1 shows that methoxychlor, dieldrin, and heptachlor epoxide were poorly separated at 14 % relative humidity but were fully resolved at 60 % as shown in Fig. 2; however, DDE and aldrin were not clearly separated. When the humidity exceeded 70 % spots began to tail and became diffused.

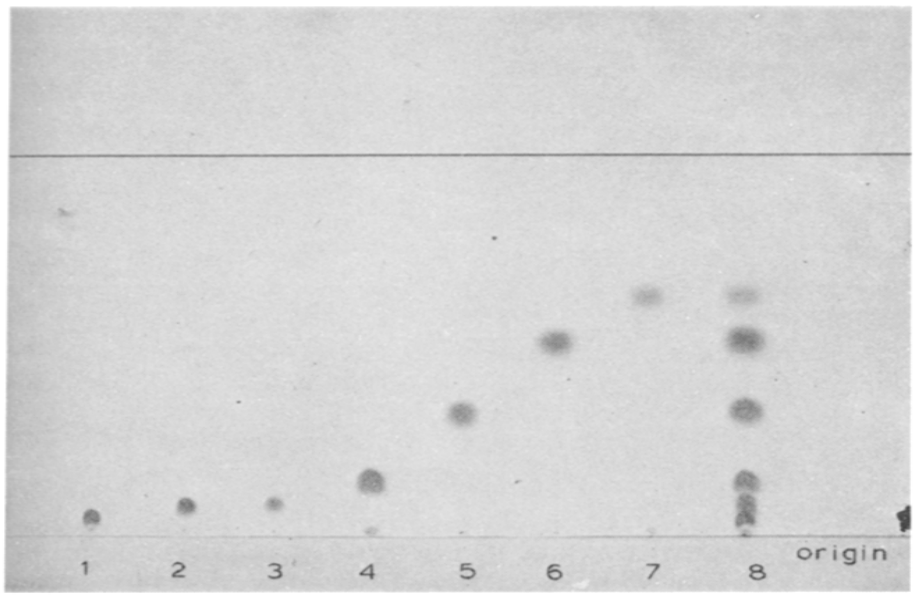

Fig. 1. Chromatogram of insecticides run at 14 % relative humidity. Plate: aluminum oxide G. Solvent: Skellysolve B. Temperature: 24°. Compounds: 1 = methoxychlor; 2 = dieldrin; 3 = heptachlor epoxide; 4 = DDD; 5 = DDT; 6 = DDE; 7 = aldrin; 8 = mixture of compounds.

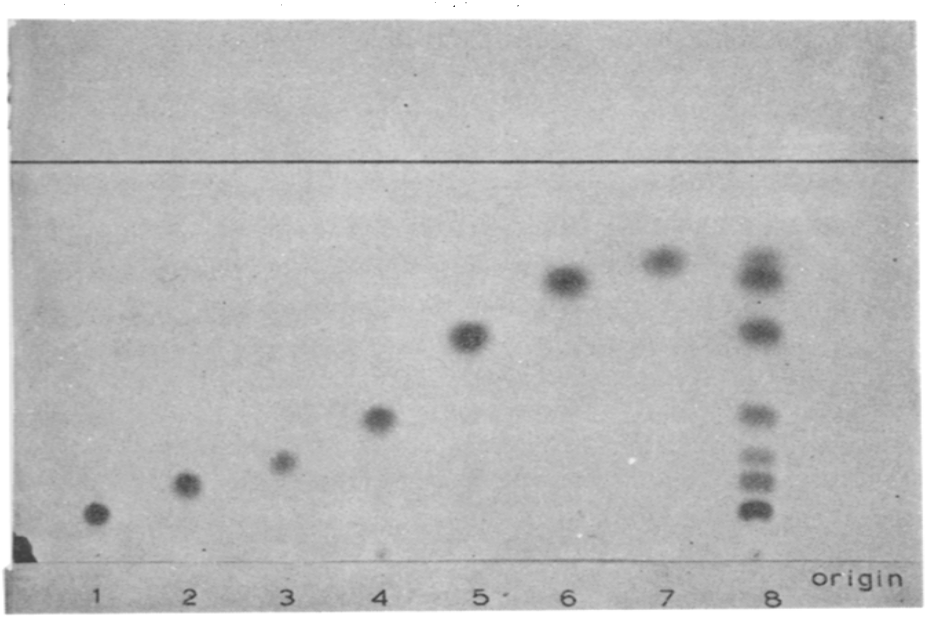

Fig. 2. Chromatogram of insecticides run at 60 % relative humidity. Conditions and code identification of compounds are as in Fig. 1.

These results show the importance of close control of laboratory humidity when using TLC for the separation of organo-chlorine insecticides.

United States Department of the Interior, Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Md. (U.S.A.) WILLIAM L. REICHEL

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Circular thin-layer chromatography of quinomycin antibiotics and a simple recording method of spots in the ultraviolet region

The circular technique in thin-layer chromatography (TLC) was established by STAHL[1] in 1958. However, it has not been applied extensively, presumably because it is more time-consuming and the advantages of a circular technique are rarely needed in TLC. In the course of structural studies on quinoxaline antibiotics[2], we found that a circular TLC was excellent for separating the minor components of this group of antibiotics. Furthermore, it was shown that the recording method for a visible chromatogram by a common letter copying machine[3] can be extended to record U.V.-absorbing zones.

Quinomycin antibiotics are closely related peptide lactones containing quinoxaline; the components A, D, B_0, B, E, and C differ in their N-methyl amino acid parts[2]. After many attempts to separate these components, TLC on aluminum oxide, with the lower layer of the solvent system: ethyl acetate–*sym.*-tetrachloroethane–water (3:1:3, v/v)[4], was found to be useful, but did not separate the whole mixture (Fig. 1). Since these antibiotics have a very limited solubility, it was difficult to find a better solvent system. Therefore, we tried the circular technique with this solvent system. A thin-layer plate (20 × 20 cm) with a hole (2 mm diameter) in the center was prepared. Samples were applied as narrow zones or small spots on a small circle around the hole. A cotton wick was attached to the hole, and the plate was placed on a petri dish containing the solvent, which had previously been allowed to equilibrate in an enclosed chamber. STAHL's process A[1] was, of course, available, but, in general, a better chromatogram was obtained by the above method. The developed zone could be detected by iodine vapour[5] or Dragendorff's reagent, but the most sensitive method was when a U.V. lamp (Mineralight, made by Ultra-Violet Prod. Inc.) was used to illuminate the aluminium oxide GF_{254} (Merck) layer. These antibiotics have U.V. absorption maxima