Radio-TLC was used to measure the chemical and biological release of ^{14}C-bound residues from soil treated with [^{14}C]*p,p'*-DDT (203) and microsomal oxidation of the herbicides EPTC and acetochlor and of the safener MG-191 in maize (204). Detection by radioscanning was done in the investigation of the degradation of [^{14}C]tebupirimphos under anaerobic aquatic conditions (205) and for TLC separation of soil-bound residues of cyprodinil (206).

C. Biological Methods of Detection

The use of biological methods in TLC is justified because they are highly specific and detection limits are lower than with other methods.

Enzyme inhibition methods include fluorescent detection, because cholinesterase, which is used as a substrate after hydrolysis, gives a fluorescent background. Such identification was investigated for about 150 pesticides (181). In the quantitative determination of OP insecticides the esterase was obtained from *Bacillus subtilis* cultures. The plate was sprayed with 1-thionaphthyl acetate and with a solution of 2,2'-azo(1-naphthol-8-chloro-3,6-disulfonic acid)–4,4'-diphenyl disulfide. The limit of detection was 0.1–5.0 ng (207). A variety of methods for the separation, activation, and quantification of OP insecticides using TLC with enzyme inhibition were examined, with special attention to activation with bromine, hypochloric acid, and *m*-chloroperbenzoic acid (208). Coupling chemical and physical methods with enzymatic inhibition tests allows for the detection of toxicologically active substances in situ (209). 4-Methylumbelliferone esters were used for low-level detection of OP pesticides and warfare agents. The limit of detection for OP compounds was 0.01–1.00 ng (210).

Herbicides having an inhibitory effect on photosynthesis can be detected by inhibition of the Hill reaction, which is highly selective and sensitive (1). The sensitivity of thiazafluoron determination in water by inhibition of the Hill photosynthesis reaction was 20 times greater than that of standard TLC methods. Chloroplast homogenate preparation and the chromatographic procedure were described (211). *Colletotrichum fragariae* was used as the indicator species for direct bioautographic assay of some natural fungicides (212). A spore suspension of *Botrytis cincera* in 1% potato dextrose agar was used for inhibition bioassay of antifungal activity (213).

D. Detection by Liquid Crystals

The liquid crystal method, which involves mapping the chromatogram by transferring organic substances from a TLC plate to a liquid crystal layer, was applied to warfare agents and pesticides separated on carbon layers. Liquid crystalline structure was disturbed by the pesticides incorporated, and light transmittance changed the pesticide spots, which enabled quantitative determination (214).

V. QUANTITATIVE DETERMINATION AND VALIDATION OF METHOD

Although thin-layer chromatography can still not compete with GC and HPLC in the quantitative determination of pesticides, more recent technologies such as video densitometry offer certain improvements in comparison with traditional slit-scanning densitometry. Validation was carried out on the video densitometric and slit-scanning determination of propham, chlorpropham, atrazine, diflubenzuron, tetramethrin, and α-cypermethrin for linearity, precision, and detection limit. A comparison of results showed that slit scanning is more sensitive and precise than video densitometry, but the RSD of 3.5–5.3% for the charge-coupled device (CCD) camera was acceptable. The main advantages of video technology were speed, excellent archiving capability, and the fact that data can be stored together, edited, and used for many tasks (215). A mixture of 10 pesticides was separated by two-dimensional (2-D) chromatography on cyano HPTLC plates with a polar mobile phase in the first dimension and a nonpolar mobile phase in the second dimension. Chromatograms were recorded with a color CCD camera and evaluated with Camag VideoScan software. In video densitometry, the parameters that should be adjusted to achieve maximum quantitative precision include camera settings and track settings. Two quantification modes were compared: scanning of the whole plate and scanning of manually defined bent tracks. Evaluation