In our experience, any compound, including proteins, containing an aromatic ring system may be readily detected in trace amounts by these procedures. The great advantage of this procedure is that the compounds are not destroyed or altered in any way. The fluorescence and phosphorescence are easily visible with the naked eye.

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Methyl yellow as a spray reagent in the paper chromatography of chlorinated hydrocarbon pesticides

N,N-Dimethyl-*p*-phenylazoaniline (methyl yellow) has been reported to undergo a color change when a chloroform solution of the dye is irradiated with X-rays[1]. This reaction suggested a possible application in the detection of chlorinated hydrocarbon pesticides on paper chromatograms. When the chromatograms were sprayed with a solution of methyl yellow and exposed to ultra-violet radiation, the pesticides appeared as red spots against a yellow background. Fourteen pesticides containing chlorine were tested and all were easily detected by the reagent.

The spray reagent was prepared by dissolving 100 mg of methyl yellow in 60 ml of ethanol in a 100 ml volumetric flask. Twenty-five ml deionized water was added and the solution brought to volume with ethanol. Paper chromatograms were prepared by spotting known amounts of the pesticide solution (1 μg/μl) on Whatman No. 1 chromatographic paper (8 × 8 in.). The papers were then impregnated with a 5 % solution of cottonseed oil in ethyl ether[2], and developed with pyridine–water (6:4, v/v).

Upon removal from the chromatographic tank, the chromatograms were dried in air and sprayed with the methyl yellow solution until the paper appeared uniformly wet. After being again dried in air, the sheets were finally exposed for five minutes to 30 W of ultra-violet radiation. The ultra-violet source, consisting of two 15 W 20-in. germicidal lamps in a reflector housing, was placed 2 to 4 in. above the paper chromatograms.

Minimum amounts of chlorinated pesticides which could be detected ranged from 2 to 8 μg per spot (Table I). All pesticides tested could be detected above a level of 8 μg in the chromatographic system described, the spots ranging from 5 to 10 mm in diameter. The red color formed by the methyl yellow and pesticide was quite

intense at first but gradually faded over a period of one hour. Additional exposure to ultra-violet radiation did not restore the color. However, the sprayed chromatograms could be held as long as two days before being exposed to ultraviolet radiation with no loss of the original intensity of the spots.

TABLE I

SENSITIVITY OF SPRAY REAGENT

*Pesticide**	*Minimum amount detectable μg***
Toxaphene	4
DDT	2
Aldrin	4
Chlordane	4
Endrin	4
Heptachlor	4
Methoxychlor	4
Perthane	4
Rothane	4
2,4-D	8
Dieldrin	4
Chlorobenzilate	8
Kelthane	2
Lindane	4

* Standard solutions of pure chlorinated hydrocarbon pesticides.
** Spots ranged 5–10 mm in diameter.

Although hexane, alcohol and acetone were good solvents for methyl yellow, aqueous ethanol (25 % water) was the most suitable and gave the most sensitive spray reagent. Addition of 2-phenoxyethanol, which was reported to increase the sensitivity of the $AgNO_3$ spray reagent[3], gave no improvement in either sensitivity or the time required for the appearance of the spots. The methyl yellow spray reagent, although not as sensitive as the $AgNO_3$ reagent, has the advantage that it is not affected by impurities in the paper as is the $AgNO_3$ reagent. Furthermore, the "curtain" effect which appears with the $AgNO_3$ reagent and which masks spots found near the solvent front, is eliminated so that the spots having high R_F values are clearly visible.

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