Rapid Thin-layer Chromatographic Screening for Parathion in Canned Peaches Without Cleanup

by Daniel E. Ott, Fred E. Hearth, and Francis A. Gunther
Department of Entomology
University of California, Riverside, California

The extensive use of parathion as an insecticide on commercially grown peaches in California has created a need for a rapid screening method for the insecticide in the marketable crop. Using the method of KOVACS (1), with only slight modifications with respect to activation time of thin-layer chromatography (TLC) plates and the use of a simplified developing tank, concentrates of extractives of commercially canned peaches in methylene chloride stripping solution may be chromatographed and subjected to a series of chromogenic spray reactions to yield in about one hour qualitative and semi-quantitative information in the range 0.5 to 11 p.p.m. of residual parathion.

This procedure could serve as a cleanup step prior to final quantitative determinations by such analytical methods as oscillopolarography by the procedure of HEARTH et al. (2), automated analysis after the manner of OTT and GUNTHER (3), or gas chromatography according to the procedure of GIUFFRIDA and IVES (4).

Screening procedures for other pesticide residues in other substrates involving TLC without prior cleanup may be found in the reports by MORLEY and CHIBA (5,6) and OTT and GUNTHER (7).

A rapid automated screening procedure for technical grade

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parathion in canned peaches has been reported by OTT and GUNTHER (8).

Method

Reagents

(a) TLC adsorbent.--Aluminum Oxide G. Warner-Chilcott Laboratories (formerly Research Specialties Co.), Richmond, Calif.

(b) Immobile phase.--15% *N,N*-dimethylformamide in diethyl ether.

(c) Developing solvent.--Methylcyclohexane (practical grade). Eastman Kodak Co., Rochester, N. Y.

(d) Chromogenic reagents.--(i) Dye solution: dissolve 100 mg. of tetrabromophenolphthalein ethyl ester (Eastman Kodak Co.) in 50 ml. of redistilled acetone. (ii) Silver nitrate solution: dissolve 0.5 g. of silver nitrate in 25 ml. of distilled water, dilute to 100 ml. with acetone. (iii) Citric acid solution: dissolve 5 g. of citric acid in 50 ml. of distilled water, dilute to 100 ml. with acetone.

Procedure

Preparation of TLC plates.--Prepare TLC plates 0.5-mm. thick in the usual manner from a slurry of 30 g. of aluminum oxide G in 35 ml. of distilled water. Allow the plates to air dry approximately 15 minutes then activate at 110° C. for 30 minutes. Plates may be used immediately upon cooling or stored in a dry place for future use.

Sample preparation and TLC.--Concentrate 20 ml. of a methylene chloride stripping solution of finely ground drained canned peaches

(1 g./2 ml.) to approximately 1 ml. in a Kuderna-Danish evaporative concentrator (9) over a steam bath. Finally concentrate to a volume suitable for TLC application (100 µl. or less; equivalent to extractives from 10 g. of peaches) with a gentle jet of clean, dry air while warming the tube to about 40° C. in a water bath. Vertically score the TLC plate into channels for each spot to be developed and prepare a hole in the adsorbent at the origin of the spot to prevent "flaking" as discussed by CHIBA and MORLEY (6) and by HEARTH *et al*. (2). Horizontally score the plate 10 cm. above the origin. Spot the entire concentrate on the TLC plate with a 100-µl. syringe or disposable micropipette. [Application time for many samples is considerably shortened by the use of one or more multiple-spotting devices as described by OTT and GUNTHER (3).] Also, spot on the same plate an appropriate control, a fortified control, and a standard. Prepare a fortified control by first spotting the desired amount of parathion in *n*-hexane solution followed by overspotting of a control concentrate, or, more realistically, a parathion standard is added directly to a control stripping solution prior to concentration.

Expose the spotted and air dried plate 20 to 30 minutes to the vapors only from the immobile phase in a closed, filter paper-lined TLC development tank: support the plate out of direct contact with the solvent mixture or place it in the empty side of a divided-bottom tank. This procedure permits extremely uniform saturation of the adsorbent with the immobile phase and was shown to give superior results to either the dipping procedure outlined by KOVACS

(1) or an alternate method of spraying the prepared plate with the immobile solvent prior to immersion in methylcyclohexane. At the end of the vapor saturation period, carefully introduce the developing solvent to a depth of about 0.5 cm. into the empty side of the tank or place the plate in a separate filter paper-lined tank containing the developing solvent and develop to the previously scored 10-cm. line.

Spray the air dried plate uniformly with the dye solution; the entire plate turns bright blue. Follow by a spray of the silver nitrate solution, wait 2 minutes, and finally spray the plate with the citric acid solution. An immediate background color change to yellow is produced with the last spray, while the R_f area containing parathion becomes lavender. Extractives from 10 g. or less of control peaches per spot yield no detectable blue or lavender areas.

KOVACS (1) has reported that the spot color produced with this insecticide is vivid blue and fades irreversibly after 30 to 40 minutes. However, we have found that color may be restored with a fine spray of water as much as 24 hours later.

Results and Discussion

Rapid comparative identification of parathion in fortified canned peaches from a lower limit of 0.5 p.p.m. up to 11 p.p.m. is possible by this method, which gives no discernible chromogen-reactive background in unfortified controls. This reported upper limit is not an absolute maximum but rather the highest fortification examined.

Parathion standards exhibit R_f values in the range of 0.70 to

0.76 while spots from fortified controls range from R_f 0.65 to R_f 0.74. This slight lag caused by the presence of peach extractives requires the inclusion of appropriately placed fortified controls on the same plate with test samples.

The successful application of this method for the detection of parathion residues in peaches strongly suggests the use of the method for rapid screening for Diazinon, malathion, parathion, Systox (thiono), Trithion, and other sulfur-containing organophosphorus insecticide residues in other crop substrates. TLC identification of these insecticides in the presence of extractives from strawberries, apples, and certain vegetable crops has already been reported by KOVACS (1).

Summary

Rapid screening for parathion above 0.5 p.p.m. in canned peaches is possible by thin-layer chromatography including a selected colorimetric spray detection system which does not respond to the natural components in the substrate. Approximately one hour is required from spotting of plate to interpretation of results.

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