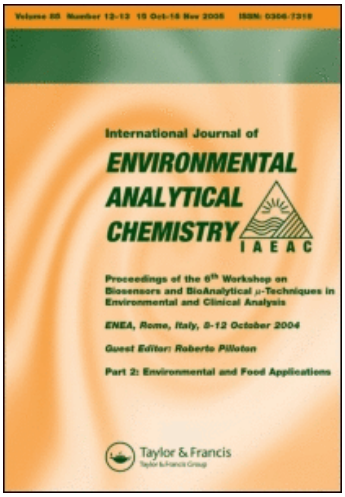


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

A New Method for the Detection of Organophosphorus Pesticides by *in situ* Fluorometry on Thin-Layer Chromatograms

G. L. Brun[a]; D. Surette[a]; V. Mallets[a]
[a] Department of Chemistry, Université de Moncton, New Brunswick, Canada

To cite this Article Brun, G. L. , Surette, D. and Mallets, V.(1973) 'A New Method for the Detection of Organophosphorus Pesticides by *in situ* Fluorometry on Thin-Layer Chromatograms', International Journal of Environmental Analytical Chemistry, 3: 1, 61 — 71
To link to this Article: DOI: 10.1080/03067317308071068
URL: http://dx.doi.org/10.1080/03067317308071068

Intern. J. Environ. Anal. Chem. 1973, Vol. 3, pp. 61–71

Printed in Great Britain

A New Method for the Detection of Organophosphorus Pesticides by *in situ* Fluorometry on Thin-Layer Chromatograms†

G. L. BRUN, D. SURETTE, and V. MALLET‡

Department of Chemistry, Université de Moncton, Moncton, New Brunswick, Canada

(*Received October 19, 1972*)

KEY WORDS: *In situ* fluorometry, pyrolysis, thin-layer chromatography, organophosphorus pesticides.

A new technique for rendering certain organophosphorus pesticides fluorescent has been developed which requires heating the chromatoplate at an optimum temperature for a definite period of time. Some of the pesticides that give positive results are: menazon, coumaphos, azinphosmethyl, and MARETIN. Menazon and coumaphos are of particular interest since both compounds are difficult to detect by gas chromatography. Fluorescence is produced from other organophosphorus pesticides by spraying the chromatogram with a strong base prior to heating.

The principal advantage of the technique is that the fluorescence is generated ***in situ*** without the use of a spray reagent and fluorescence quantitation should thus be greatly facilitated. The technique is simple, rapid, and affords excellent sensitivity. Visual detection limits ranging from 0.001 mcg to 0.1 mcg have been obtained.

† Presented at the Symposium on Recent Advances in the Analytical Chemistry of Pollutants, Halifax, N.S., August 23–25, 1972.

‡ To whom correspondence should be addressed.

INTRODUCTION

Gas chromatography continues to be of major importance in residue analysis, and high-speed liquid chromatography is rapidly becoming an established technique for pesticide analysis.[1] However, the need for a simple and inexpensive means of determining pesticides and other organic pollutants still exists. A combination of thin-layer chromatography and fluorescence spectroscopy appears very appropriate in this respect.

Ragab[2] was one of the first to apply fluorogenic spray reagents to the detection of organophosphorus compounds on silica-gel chromatograms, although the fluorescence was never measured. Similar fluorogenic reagents were later developed by Belliveau *et al.*[3] for a variety of sulfur-containing pesticides, and visual detection limits ranging from 0.3 to 0.1 mcg per spot were reported. The compound to be detected was treated with bromine vapours producing hydrobromic acid. The plate was then sprayed with a non-fluorescent chelate solution, and, as the chelating agent was displaced in acid media, fluorescence was produced.[4]

The technique was further improved by Frei and Mallet[5] who used salicyl-2-aldehyde-2-quinolylhydrazone (SAQH) as chelating agent and manganese as complexing metal. The method was then applied to the quantitative analysis of azinphosmethyl (Guthion) in natural water samples[5] and in blueberries.[6]

pH-sensitive fluorogenic spray reagents were introduced by Belliveau and Frei[4,7] in connection with sulphur-containing pesticides previously treated with bromine vapours. The fluorogenic spray reagent was dichlorodicyanobenzoquinone (DDQ) which produced blue fluorescent spots on a non-fluorescing silica-gel layer. 3-Hydroxyflavones, *viz.*, fisetin, robinetin and flavonol were also shown to be pH-sensitive.[8]

Flavones have also been used as fluorogenic spray reagents for organothiophosphorus insecticides spotted on cellulose layers.[9–11] Production of fluorescence in this case was attributed to an enhancement of the fluorescence of the flavone in the presence of the more-polar pesticide spot.

One of the most recent studies involving organothiophosphorus pesticides was carried out by Bidleman and Frei[12] who used a non-fluorescent palladium chelate as spray reagent. The reaction was based on the affinity of palladium ions for sulphur atoms, and upon spraying the chromatogram the fluorescent chelating agent was liberated. This particular technique was definitely an improvement over previous experiments, both in terms of sensitivity and selectivity.

In all of the above cases, however, the fluorescence was derived indirectly from the spray reagent. In the present work, a technique has been developed

whereby the fluorescence is derived directly from the pesticide to be determined. The only necessary criterion for the production of fluorescence is a certain degree of aromaticity within the compound under investigation.

EXPERIMENTAL

Chemicals and apparatus

Most of the pesticides investigated were analytical standards. Coumaphos and MARETIN, however, were of technical grade and were purified by multiple recrystallizations in ethanol. A list of the pesticides used in this study and the manufacturers, as given by Kenaga and Allison,[13] is provided in Table I.

All the solvents used were either spectranalysed or pesticide grade (Fisher Scientific Co., Montreal, Canada). Silica-gel H (Brinkman Instruments, Rexdale, Canada) was used as chromatographic adsorbent.

A VIS-UV Chromatogram Analyzer (Farrand Optical Co. Inc., New York, U.S.A.) with motorized monochromators was used to record the fluorescence spectra. The source was provided by a 150-W high-pressure xenon arc lamp. A # 7–54 filter (230–420 nm) was used in the exciter drawer with a # 3–73 filter (405–800 nm) in the analyzer leg. Attenuators of 0.625 in. in diameter were used with both filters. Entrance and exit slits measuring 10 nm were also employed. The photomultiplier detector consisted of a 1-P21 tube.

For visual observation of the fluorescence a longwave (366 nm), 230-W, mercury u.v. lamp (BLAK-RAY, B-100A) was employed.

General procedure

The adsorbent was prepared by shaking 30 g of Silica-gel H with 80 ml of distilled water in a 250-ml stoppered flask. The plates (20 × 20 cm) were then coated to a thickness of 250 microns with a Desaga TLC applicator and then left to dry at room temperature. The plates were not activated.

Standard pesticide solutions of 1000 ppm (w/v) were prepared in methylene chloride. A plate was then spotted with 2 mcg of each pesticide by means of 2-mcl spotting capillaries. The chromatograms were developed in a 5:1 (v/v) mixture of *n*-hexane and acetone following a procedure utilized by Askew *et al.*[14] After development the plates were dried with a cold air stream and then heated at different temperatures: 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250°C for periods of 5, 10, 20, and 30 min, respectively. After heating, the plates were cooled in air, and examined under u.v. light.

When the optimum temperature and time of heating the plate were known, the fluorescence spectra were recorded. The excitation spectra were recorded

TABLE I

Organophosphorus pesticides investigated

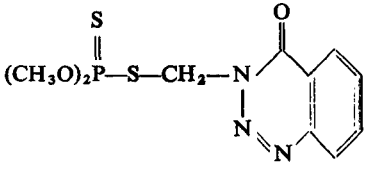
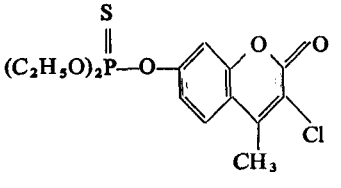
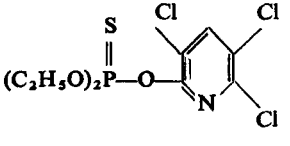
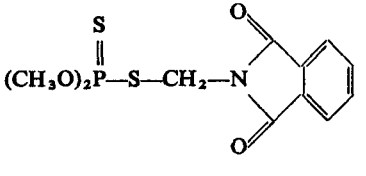
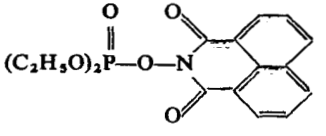
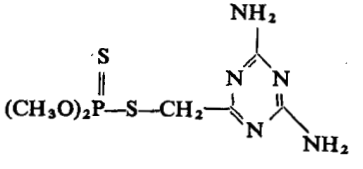
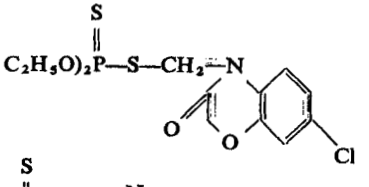
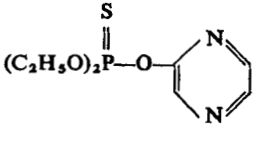
Pesticide and manufacturer	Chemical name	Structure
Azinphosmethyl (Chemagro Corp.)	O,O-dimethyl-S-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl) phosphorodithioate	
Coumaphos (Chemagro Corp.)	O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) O,O-diethyl phosphorothioate	
Dursban (Dow Company)	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	
Imidan (Stauffer)	O,O-dimethyl S-phthalimidomethyl phosphorodithioate	

TABLE I (continued)

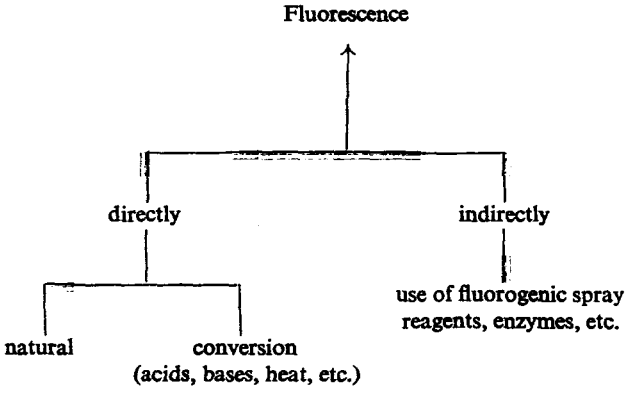
Pesticide and manufacturer	Chemical name	Structure
MARETIN (Chemagro Corp.)	N-hydroxynaphthalimide diethyl phosphate	
Menazon (Imperial)	S-[(4,6-diamino-s-triazin-2-yl) methyl] O,O-dimethyl phosphorodithioate	
Phosalone (Rhodia)	S-[(6-chloro-2-oxo-3-benzoxazolinyl) methyl] O,O-diethyl phosphorodithioate	
ZINOPHOS (American Cyan)	O,O-diethyl O-2-pyrazinyl phosphorothioate	

by placing the analyzer monochromator at a wavelength of 550 nm and scanning the excitation monochromator. The procedure was repeated after lowering the wavelength of the analyzer monochromator by 20 nm each time until a maximum excitation peak was obtained. The emission spectra were recorded by setting the excitation wavelength at 300 nm and scanning the analyzer monochromator, then increasing the excitation wavelength by 20 nm for each scan until a maximum emission peak was reached.

The visual detection limits were obtained under the optimum conditions for each fluorescing species. Dilution series of each pesticide were prepared in *n*-hexane from the original stock solutions and 1-mcl aliquots were spotted each time.

RESULTS AND DISCUSSION

Fluorescence of organic compounds can be obtained in two ways: directly and indirectly (Scheme 1).



SCHEME 1

In the indirect approach, the compound is rendered fluorescent by the use of fluorogenic spray reagents,[2–12] enzyme inhibition,[15] fluorescence labelling,[16] etc. In the direct approach, the material is either naturally fluorescent or it can be converted into species which fluoresce. The advantage is that the fluorescence is derived from the original compound. The number of organic pesticides which are naturally fluorescent is very restricted. Many compounds, however, do possess the necessary degree of aromaticity and may be converted to fluorescent species by a variety of methods.

In this study a number of 'aryl' (including some heterocyclic) derivatives of organophosphorus pesticides have been chosen (Table I). One example is

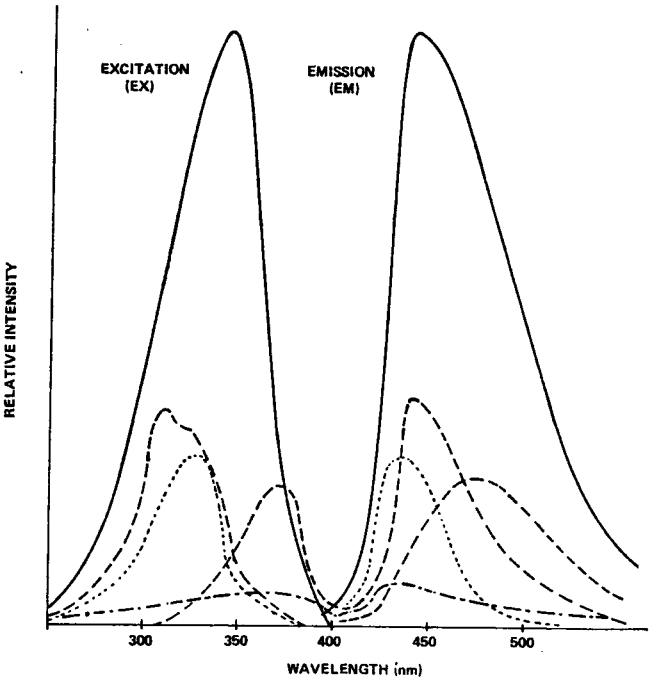


FIGURE 1 Fluorescence spectra of coumaphos: ———, heated at 200°C for 20 min; — — —, sprayed with NaOH and then heated at 200°C for 20 min; - - - - -, at room temperature; · — · —, background of the plate.

TABLE II

Fluorescence spectral data

Conditions	Wavelength (nm)			
	Coumaphos		MARETIN	
	EX	EM	EX	EM
a) At room temperature	325	434	364	482
b) Heated at 200°C for 20 min	344	440	352	435
c) Sprayed with NaOH and then heated at 200°C for 20 min	310 372	441 474		
d) Sprayed with KOH and then heated at 200°C for 20 min; the chromatoplate is covered with a glass plate.	——		368	508

coumaphos, the fluorescence spectra of which are illustrated in Figure 1 under various experimental conditions. The compound is almost non-fluorescent on silica-gel layers, but when heated at a high temperature for some time, a large increase in fluorescence intensity is observed. There is also a bathochromic shift in the wavelengths of maximum excitation and emission. When the chromatoplate is sprayed with 1.0N aqueous NaOH and heated at 200°C for 20 min, the fluorescence intensity is lowered and the spectra indicate two distinct absorption and emission peaks (Table II), probably resulting from a mixture of fluorescent species produced on heating.

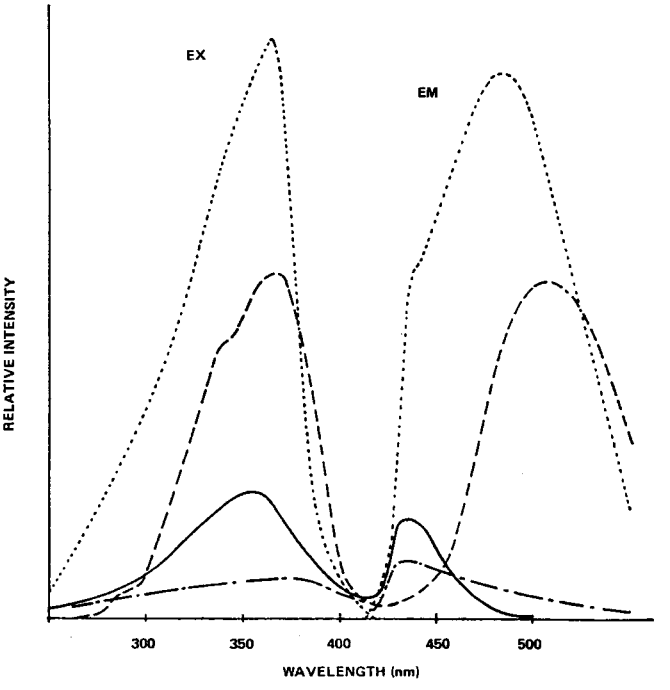


FIGURE 2 Fluorescence spectra of MARETIN.

MARETIN demonstrates just the very opposite of coumaphos (Figure 2). At room temperature the compound is very fluorescent, but upon heating (200°C for 20 min) the fluorescence intensity is quenched to yield a very intense red spot under u.v. light. When the chromatoplate is treated with 1.0N aqueous KOH and heated for 20 min at 200°C, the initial bluish green fluorescence changes to a yellowish green with a considerable shift in emission wavelength (*cf.* Table II). It is important to note, for the cases of both

coumaphos and MARETIN, the variety of excitation and emission wavelengths that can be measured under different conditions. This aspect adds a great deal of selectivity to the technique.

Azinphosmethyl is absolutely non-fluorescent on silica-gel layers but when heated at 200°C for 30 min fluorescence is produced. The emission spectrum consists of two peaks when the excitation wavelength is set at 400 nm (Figure 3). As the excitation wavelength is lowered the emission peak at longer wavelength disappears, at the expense of a stronger peak at 442 nm.

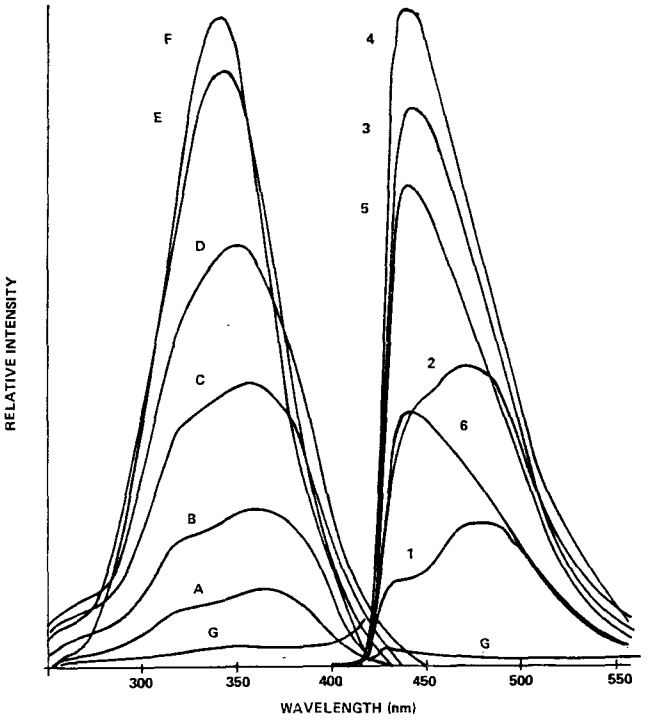


FIGURE 3 Fluorescence spectra of azinphosmethyl when heated at 200°C for 30 min.

Scan of excitation			Scan of emission		
		EM (nm)			EX (nm)
A	—	540	1	—	400
B	—	520	2	—	380
C	—	500	3	—	360
D	—	480	4	—	340
E	—	460	5	—	320
F	—	440	6	—	300

The background of the plate is negligible under these conditions. The reason for a doublet peak is probably the formation of a mixture of fluorescent species upon heating, as was the case with coumaphos.

A complete list of the pesticides showing positive results is given in Table III. With the exception of coumaphos and MARETIN which are both fluorescent (coumaphos only slightly) on silica-gel layers at room temperature, all the others have to be heated at specific temperatures for any fluorescence to show up. Interesting results were obtained with menazon, a few nanograms of which can be detected. This compound, however, is difficult to detect by gas chromatography, as also is coumaphos.[14]

TABLE III

List of pesticides giving positive results

Pesticide	Optimum conditions		Wavelength (nm) of maximum		Visual detection limit (mcg)
	Temperature (°C)	Time (min)	EX	EM	
Azinphosmethyl	200	30	342	442	0.3
Coumaphos	200	20	344	440	0.001
Dursban	225	30	358	458	0.06
Imidan	225	30	345	505	1.0
MARETIN	Room temperature		364	482	0.008
Menazon	225	30	370	475	0.009
Phosalone[a]	200	120	370	489	0.04
ZINOPHOS[b]	225	30	365	450	0.2

[a] After heating the chromatoplate, it is left exposed to the air for at least 48 hr.
[b] The chromatoplate is covered with a glass plate while heating.

The fluorescence of phosalone shows up only after a prolonged period of time following the preliminary heating period. In the case of ZINOPHOS, the chromatoplate had to be covered with a glass plate while heating. Fluorescence was also obtained for Dursban and Imidan. It is to be noted that for each pesticide listed in Table III, the wavelengths of excitation and emission are very selective under the conditions specified.

Other organophosphorus pesticides, *viz.*, M-1703, diazinon, fenthion, Folithion, Proban, Thiophos and Trithion, were investigated but the results were negative.

CONCLUSIONS

The technique just described provides a new approach to the analysis of pesticides and other organic pollutants. The fluorescence is produced without the use of fluorogenic spray reagents, hence background fluorescence is practically nonexistant. Another advantage stems from the fact that added selectivity is introduced from the different excitation and emission wavelengths obtained by varying the operating conditions. The technique becomes especially important with certain compounds such as menazon and coumaphos, which are difficult to detect by other means.[14]

A large number of pesticides other than organophosphorus derivatives are at present under investigation. Preliminary results indicate that many may be rendered fluorescent in this manner. The important characteristic again is that each fluorescing species produced possesses its own excitation and emission wavelengths. It is also intended to investigate the reaction mechanism wherever possible. The task is a very complex one, since often many species are produced upon pyrolysis.

Acknowledgements

This work was supported by grants from the National Research Council of Canada and the Canada Department of Agriculture.

References

1. J. J. Kirkland (Ed.), *Modern Practice of Liquid Chromatography* (Wiley Interscience, New York, 1971).
2. M. T. H. Ragab, *J. Ass. Offic. Anal. Chem.* **50,** 1088 (1967).
3. P. E. Belliveau, V. Mallet, and R. W. Frei, *J. Chromatogr.* **48,** 478 (1970).
4. P. E. Belliveau and R. W. Frei, *Chromatographia* **4,** 193 (1971).
5. R. W. Frei and V. Mallet, *Intern. J. Environ. Anal. Chem.* **1,** 99 (1971).
6. R. W. Frei, V. Mallet, and M. Thiébaud, *Intern. J. Environ. Anal. Chem.* **1,** 141 (1971).
7. R. W. Frei and P. E. Belliveau, *Chromatographia* **5,** 296 (1972).
8. R. W. Frei, V. Mallet, and C. Pothier, *J. Chromatogr.* **59,** 135 (1971).
9. V. Mallet and R. W. Frei, *J. Chromatogr.* **54,** 251 (1971).
10. V. Mallet and R. W. Frei, *J. Chromatogr.* **56,** 69 (1971).
11. V. Mallet and R. W. Frei, *J. Chromatogr.* **60,** 213 (1971).
12. T. F. Bidleman, B. Nowlan, and R. W. Frei, *Anal. Chim. Acta* **60,** 13 (1972).
13. E. E. Kenaga and W. E. Allison, *Bull. Ent. Soc. Amer.* **16,** 68 (1970).
14. J. Askew, J. H. Ruzicka, and B. B. Wheals, *Analyst* **94,** 275 (1969).
15. C. E. Mendoza, *Residue Rev.* **43,** 105 (1972).
16. J. F. Lawrence and R. W. Frei, *J. Chromatogr.* **66,** 93 (1972).