A Rapid and Reliable *in situ* Spectrophotofluorometric Method for the Analysis of Co-Ral and Bayrusil in Lake and Sewage Water

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Co-Ral, also known as coumaphos, has proven to be very effective for the control of parasites attacking and infesting domestic animals. Residue analysis has been accomplished by several methods, namely: photofluorometry in solution (ANDERSON et al. 1956); colorimetry (CALBORN and MANN 1960); and by gas chromatography (MARTIN 1971). Bayrusil was introduced in 1969 as an insecticide for the control of biting and sucking insects and a GLC method is available for residue analysis (MARTIN 1971). However, none of these pesticides has been analysed by fluorescence measurements directly on thin-layer chromatograms.

In an earlier publication (Brun and MALLET 1973), a method was described by which Co-Ral could be made to fluoresce on silica-gel layers simply by heating the chromatogram at a specific temperature for a definite period of time. Fluorescence spectral data were given and measurement of the fluorescence was carried out directly from the thin-layer chromatogram. In a subsequent study (BRUN and MALLET 1973), similar results were obtained with Bayrusil.

In this study, it was intended to apply some of the results obtained earlier to the actual analysis of practical samples. For this purpose, lake and sewage water was collected and spiked with known amounts of the pesticides for further analysis.

EXPERIMENTAL

Chemicals and Apparatus. Analytical grade Co-Ral was obtained from Chemagro Corporation and relatively pure Bayrusil was furnished by Bayer. Silica-gel H was used as thin layer adsorbent. A VIS-UV Chromatogram Analyser (Farrand Optical Co. Inc.) with motorized monochromators was used to measure the fluorescence spectra. The instrument was equipped with a 1-P28 photomultiplier detector tube along with interference filters #7-54 (230-420 nm) and # 3-75 (405-800 nm) in the excitation and analyser legs, respectively. Reducing apertures of 0.005 in. were

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used with both filters. A Turner Fluorometer Model 111 (G.K. Turner Associates) with TLC attachment was used for quantitative measurements. Filters #7-60 (300-400 nm) and 2A (> 415 nm) were placed in the entrance and exit slits, respectively.

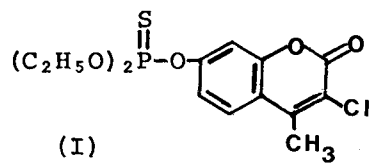
General Procedure: Extraction. The pesticide is extracted from 1000 ml of water by shaking vigourously with three successive portions of 50 ml of n-hexane in a 2000 ml separatory funnel. The combined organic phases are dried over 20 g of anhydrous sodium sulfate. The solvent is evaporated with a flash evaporator to a volume of approximately 10 ml at a temperature of 25-35°C. The remaining solvent is transfered to a Concentratube (Lab Research Co.) with two portions (5 ml) of n-hexane. The solvent is then further evaporated to a volume of around 10 µl.

Chromatography. The concentrate is spotted 2 cm from the bottom of a thin-layer chromatogram(250 µ) with a 10 µl microsyringe. The chromatogram is also spotted with the appropriate standards. The plate is then eluted at a distance of 10 cm using a 7:2 (v:v) solution n-hexane: acetone.

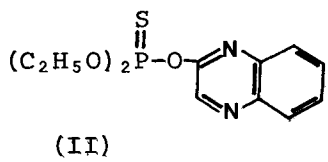
Detection and Fluorescence Measurements. For Co-Ral the fluorescence is produced by heating the chromatogram at a temperature of 200°C for 20 minutes. In the case of Bayrusil, the plate is first sprayed with a solution of aqueous KOH (1.0 N) and then heated at 100°C for 30 minutes. The chromatograms are left to cool for at least 15 minutes before measurement. The fluorescent spots are usually scanned in a direction perpendicular to the development, depending on the position of the interfering co-extractives. However, at very low concentrations or when too many interfering substances are present, the plate may be scanned in the direction of the development.

RESULTS AND DISCUSSION

The fluorescence spectral data for Co-Ral (I) and Bayrusil (II) were described in a previous paper (BRUN and MALLET 1973).



(I)



(II)

Excitation:	344 nm	356 nm
Emission:	440 nm	440 nm

It was found that under the experimental conditions, instrumental limits of detection were 0.001 µg for Co-Ral and 0.008 µg for Bayrusil. Reproducibility at these concentrations, however, is found to be poor and for practical purposes it is better to work with concentrations approximately 10 times higher with relative standard deviations around 5% for Co-Ral and 2% for Bayrusil.

The better reproducibility obtained with Bayrusil is attributed to its greater stability under UV light as shown in Fig. 1. While performing visual observations with Co-Ral, precautions should be taken not to expose the chromatogram to UV light for too long. The fluorescence is not affected by visible light in both cases.

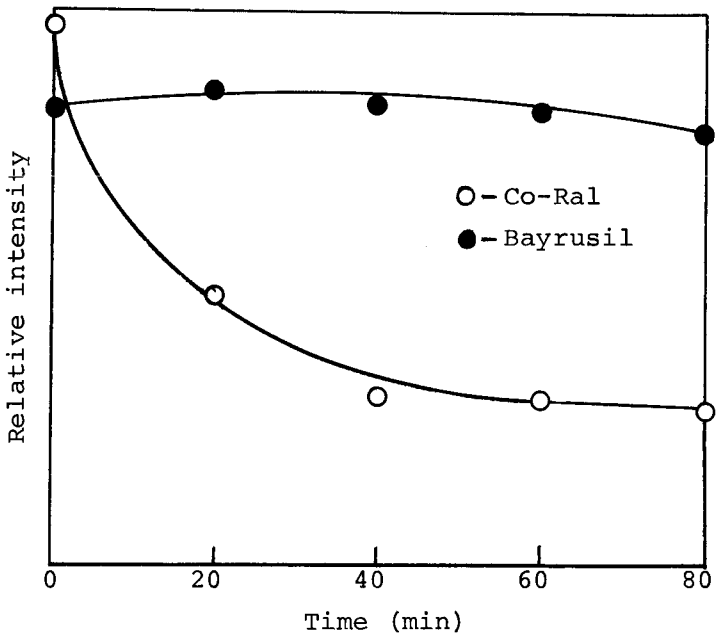


Fig. 1 Fluorescence stability of Co-Ral (0.1 µg) and Bayrusil (1.0 µg) under UV light.

Fig. 2 illustrates a linear relationship between concentration and relative intensity with Co-Ral. Sensitivity is not very good in the region between 0.002 and 0.01 µg but as was mentioned earlier, reproducibility is not suitable at that concentration range. A similar behavior is observed with Bayrusil.

Recovery data for Co-Ral and Bayrusil in water samples are given in Table 1. Co-Ral can be detected quite readily at the 0.1 ppb level in ordinary lake water. In sewage water, problems are encountered at concentrations lower than 0.1 ppb. The problem is even worse with Bayrusil in sewage water and it is thought that the loss is due in part to the formation

TABLE 1

Recovery of Co-Ral and Bayrusil from lake and sewage water

Concentration (ppb)	% Recovery[A]			
	Co-Ral		Bayrusil	
	L.W.	S.W.	L.W.	S.W.
10	86	80	94	55
1.0	100	90	86	54
0.5	104	92	D	D
0.1	108	72	D	
0.01	86	D		

A - Average of three extractions
D - Dectable visually at that concentration
L.W. - Lake water; S.W. - Sewage water

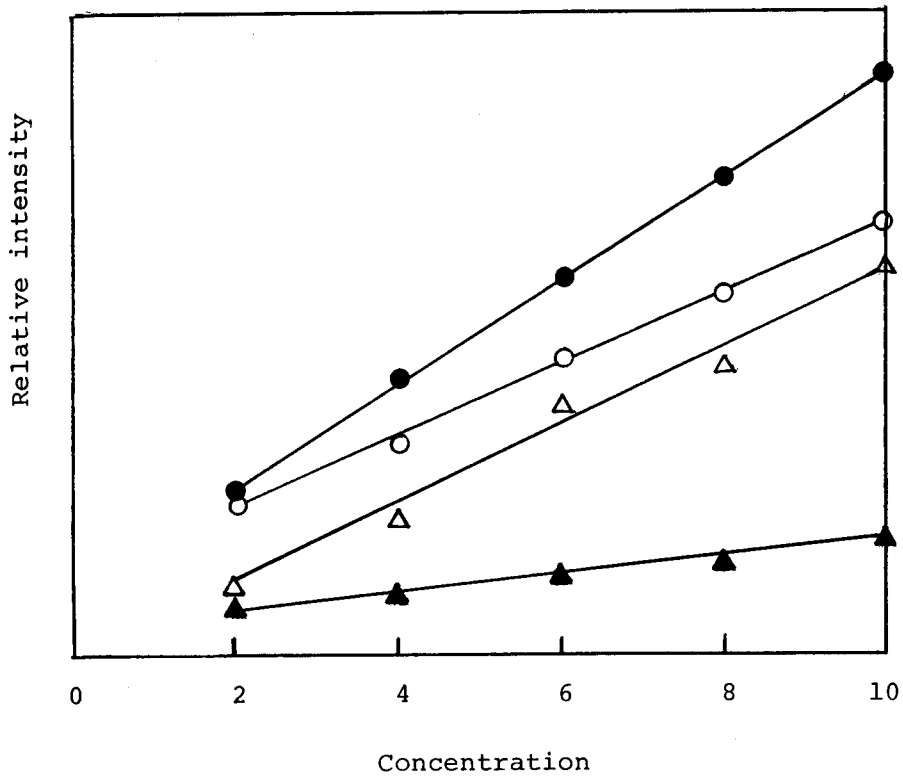


Fig. 2. Calibration curves with Co-Ral
(○): 2-10 µg; (●): 0.2-1 µg;
(△): 0.02-0.1 µg; (▲): 0.002-0.01 µg

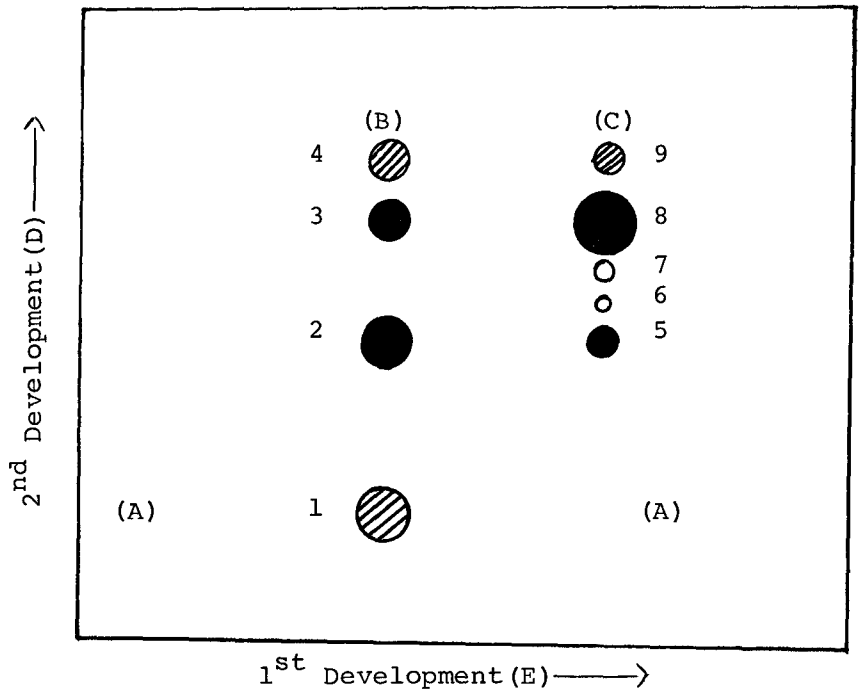


Fig. 3 Separation of the fluorescent species obtained from Co-Ral after heat treatment.

A - Original spot (10 µg); B - Separation of Co-Ral after heat treatment; C - Separation of technical Co-Ral (10 µg); D - Elution in hexane-acetone 3:1 (v/v); E - Elution in hexane-acetone 9:1 (v/v).

TABLE II

Spectral data of fluorescent species of Co-Ral after heat treatment.

Spot	Wavelenght of maximum (nm)	
	EX	EM
1	335	420
2 and 5	340	433
3 and 8	342	424
4 and 9	340	405

of emulsions during the solvent extraction. Other extraction procedures are presently being investigated to improve recoveries. In the case of Co-Ral, recoveries are less at 10 ppb than they are at 1.0 ppb because of solvent saturation at the greater concentration.

The mechanism of production of fluorescence has not been investigated fully but it is thought that fluorescence results from the degradation of the pesticide into one or more fluorescent species upon heat treatment. For instance, when two-dimensional chromatography is carried out with a sample of Co-Ral after heat treatment, at least four distinct fluorescent species can be detected under UV light (Fig. 3).

Some of the fluorescent species can be partly identified by comparison to those resulting from the separation of a spot of technical Co-Ral. Spots 2, 3 and 4 have similar R_f values and fluorescence spectral data (Table II) than spots 5, 8 and 9 respectively. Spots 2 and 5 are believed to be Potasan, which according to WASLESKI (1966), is a common by-product in the synthesis of Co-Ral. Potasan is different from Co-Ral, in that the 3-chloro substituent is absent. Spot 8 corresponds to pure Co-Ral. Therefore, spot 3 is part of the original sample of Co-Ral which did not degrade after heat treatment. No attempts were made to identify the other spots.

The present data indicate the feasibility of using in situ fluorometric techniques for the analysis of certain organophosphorous pesticides in water. The procedure does not necessitate preliminary clean-up and interfering co-extractives which fluoresce only in solution are also avoided. An advantage over other in situ fluorometric techniques is that fluorogenic spray reagents are not used and the fluorescence produced is very selective. Problems encountered with Bayrusil in sewage water have to do with the extraction procedure and not the method of detection. The problem is expected to be solved very soon.

ACKNOWLEDGEMENTS

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REFERENCES

ANDERSON, C.A., J.M. Adams and D. MacDougall, J. Agr. Food Chem., 7, 256 (1956).

CALBORN, M.C. and H.D. Mann, J. Econ. Ent., 53, 263 (1960).

MARTIN, H., "Pesticide Manual", British Crop Protection Council, Worcester, England (1971).

BRUN, G.L. and V. Mallet, Intern. J. Environ. Anal. Chem., 3 (1), 61 (1973).

BRUN, G.L. and V. Mallet, J. Chromatogr., 80, 117 (1973).

WASLESKI, D.M., J. Agr. Food Chem., 14, 156 (1966).