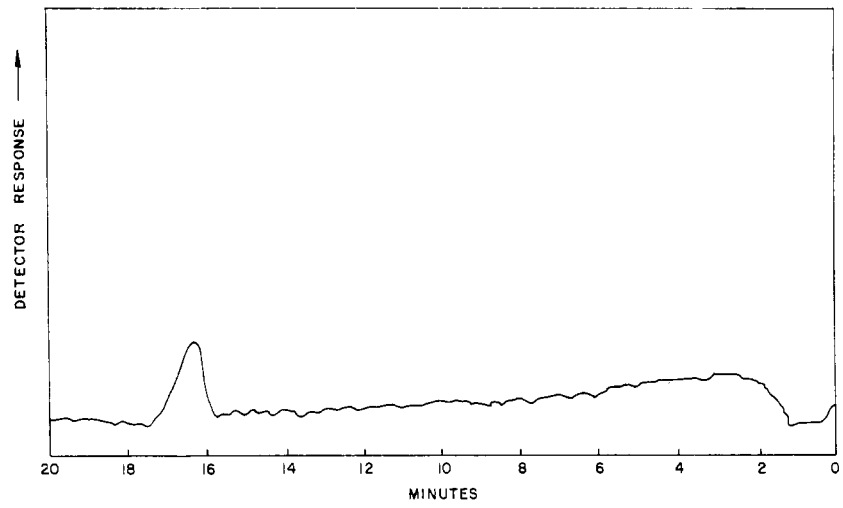

Figure 6. Chromatogram of chlorpromazine metabolites and decomposition products

and an oxidation oven, both feeding into the same titration cell. The cell may be of the Ag/Ag^+ type or the I_2/I^- type with gold-plated electrodes. Each oven contains two paired columns. One pair is packed with a nonpolar stationary liquid (silicone DC-200), and the second pair with a polar liquid (silicone QF—1+SE30). Thus it is possible to check retention times on both nonpolar and polar columns, and at the same time verify elemental composition by using the oxidation and reduction modes with either the silver or iodine cells. This combination affords excellent proof of identity in many cases.

The relative sensitivity of this method to various classes of pesticides when the sum of phosphorus, chlorine, and sulfur is measured is shown in Table III. Some of the organic phosphates such as ronnel and trithion yield responses equivalent to those obtained from chlorinated pesticides. Practical working sensitivity is of the order of 0.1 μg. for most compounds. With the Model C-200 microcoulometer it should be possible to obtain sensitivities substantially better than this. However, some modifications will probably have to be made for the measurement of PH_3 because of the changes in design of the titration cell, and the low solubility of this gas in the electrolyte.

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PHOSPHORESCENCE

Phosphorimetric Study of Some Common Pesticides

H. A. MOYE and J. D. WINEFORDNER
University of Florida, Gainesville, Fla.

The phosphorescence characteristics of 52 pesticides (including several known degradation products) are surveyed. Thirty-two of these phosphoresce sufficiently that excitation spectra, emission spectra, decay times, analytical curves, and limits of detection can be tabulated. The other 20 compounds did not give detectable phosphorescence excitation and emission spectra for 10^{-2} M ethanolic solutions.

As a preparation for work on crop residues of pesticides the application of phosphorimetry to the analysis of these residues was studied. Phosphorimetry has previously been used to determine the three major tobacco alkaloids (*6*), drugs in biological fluids (*5, 7, 8*), and air pollutants (*3*).

Experimental

Apparatus. All phosphorimetric measurements were taken with the Aminco Bowman spectrophotofluorometer (No. 4-8202) with the phosphoroscope attachment (No. C27-62140, American Instrument Co., Inc., Silver Spring, Md.). The mercury-xenon lamp (No. 416-993) was used for all quantitative measurements, while the xenon lamp (No. 416-992) was used to record all spectra. The quantitative measurements were made with the slit program: *A* 3 mm., *B* 4 mm., *C* 4 mm., *D* 3 mm., and *E* 3 mm., and the spectra with the slit program: *A* 3 mm., *B* 0.5 mm., *C* 0.5 mm., *D* 3 mm., and *E* 0.5 mm.

All spectra were recorded with a Moseley X-Y recorder (No. 135-A, F. L. Moseley Co., Pasadena, Calif.)

Reagents. All compounds were either analytical grade, obtained from major pesticide manufacturers, or technical grade, which had been redistilled or recrystallized until they appeared as one spot when chromatographed on a thin layer of silica gel. All compounds were stored at near 0° C. in a refrigerator before use.

Absolute ethanol (Union Carbide Corp.) purified as previously described

(7), was used as the phosphorimetric solvent. Stock ethanolic solutions of each compound were prepared. Solutions of lower concentrations were prepared by successive dilution. Ethanol was used as the solvent because it is inexpensive, it can be prepared in a highly pure state, it freezes to a clear, rigid glass at 77° K., and most pesticides are soluble in it.

Procedure. Prior to any series of measurements, the phosphorimeter was calibrated (8). Analytical curves or relative phosphorescence signal *vs.* compound concentration were obtained as previously described (5, 7). The phosphorescence intensity signal owing to the compound was obtained by subtracting the background owing to the phosphorescing impurities in the ethanol for each sample measured. From practical considerations, the lowest concentration that could be confidently measured was that which gave a reading of 1 scale division (1% of full scale) over that of the background. Because the ethanolic background was precise to ±0.5 scale division, this procedure was possible. The ethanolic background for any combination of excitation and emission wavelengths was about 10% or less of full-scale on the most sensitive setting of the photomultiplier microphotometer. Therefore the limit of detection was defined as that concentration which gave a reading of 1 scale division over the background on the most sensitive scale on which the background could be measured.

The lifetime, τ, was measured by shutting off the exciting radiation with a manual shutter and plotting the phosphorescence signal *vs.* time with the X-Y recorder. The response of the recorder prevented any measurements of τ's shorter than 0.2 second.

Results and Discussion

In Table I, the phosphorescence emission and excitation peaks (uncorrected for instrumental response), the phosphorescence lifetimes, the approximate ranges of concentration over which near-linear analytical curves result, and the limits of detection of 32 pesticides (and several metabolic products) are given. The low limits of detection and the extensive range of concentrations over which analytical curves can be used should be emphasized. Compounds which gave no detectable phosphorescence and so are not listed in Table I were: chlordan, endrin, heptachlor, lindane, methyl parathion, malathion, Thimet, Thiodan, Delnav, HE-OD, HHDN, aldrin, dieldrin, Telodrin, 3-amino-1,2,4-triazole, Phosphamidon, U. C. 21149 (4), dimethoate, dimethoate acid, and dimethoate oxygen analog.

Several of the compounds listed in Table I have great phosphorimetric sensitivities but considerably poorer sensitivities by other methods of analysis. In these cases, the application of phosphorimetry seems particularly ideal. For example, Co-Ral and *p*-nitrophenol had phosphorimetric detection limits less than 50 picograms per ml. Other methods of determining Co-Ral and *p*-nitrophenol are substantially less sensitive. The most sensitive method for Co-Ral has been gas chromatography using the electron capture detector—for example, Bonelli, Hartman, and Demick (2) have detected as little as 300 picograms of Co-Ral using the electron capture detector. Anderson, Adams, and MacDougall (1) were able to detect concentrations of Co-Ral in the microgram per milliliter range in animal tissues using fluorometry. Spectrophotometric methods are even less sensitive. Because of the great phos-

Table I. Phosphorescence Characteristics of 32 Pesticides and Related Compounds

Compound[a]	Excitation[b] Maximum, Mμ	Emission[b] Maximum, Mμ	Lifetime, Seconds	Limit of[c] Detection, Nanograms/ Ml.	Useful Range of Analytical[d] Curve, Mole/Liter
DDT (*p,p'*)	270	420	0.2	0.67	1.9×10^{-9}–7.4×10^{-4}
DDD (*p,p'*)	265	415	0.2	1.28	4.0×10^{-9}–1.3×10^{-3}
DDE (*p,p'*)	270, 245	425	0.2	0.25	1.0×10^{-9}–8.8×10^{-4}
Kelthane	285	515	0.2	0.56	1.5×10^{-9}–7.1×10^{-4}
Methoxychlor	275	380, 395, 360	0.7	0.45	1.3×10^{-9}–9.6×10^{-5}
Chlorobenzilate	275, 255	415, 425, 445 400, 480	0.2	0.98	3.0×10^{-9}–1.2×10^{-3}
Toxaphene	240, 280	390	1.9	18.6	4.5×10^{-8}–7.5×10^{-3}
Kepone	260	410	1.25	998.0	2.0×10^{-6}–9.2×10^{-3}
Sulfenone	275	390, 375	0.2	0.51	2.0×10^{-9}–9.1×10^{-5}
Tedion	295, 285	410	0.2	0.18	5.0×10^{-8}–6.3×10^{-5}
Orthotran	260	395, 375	<0.2	2.45	8.0×10^{-9}–7.5×10^{-4}
Parathion	360	515, 490	<0.2	8.2	3.0×10^{-8}–9.0×10^{-4}
Ronnel	300	475	<0.2	0.64	2.0×10^{-9}–1.0×10^{-3}
Co-Ral	335	510, 490	<0.2	0.036	1.0×10^{-10}–8.3×10^{-6}
Diazinon	275	395, 375	5.0	3.03	1.0×10^{-8}–1.1×10^{-3}
Guthion	325, 220, 315	420, 400	0.6	63.5	2.0×10^{-7}–7.5×10^{-3}
Trithion	305	430	<0.2	0.27	8.0×10^{-10}–8.5×10^{-5}
Aramite	285	400	3.3	0.34	1.0×10^{-9}–1.1×10^{-4}
Isolan	285	395	1.6	211.0	1.0×10^{-6}–1.3×10^{-2}
Sevin	300	510, 475 485, 550	2.0	4.0	2.0×10^{-8}–1.0×10^{-3}
Zectran	285	440	0.45	5.5	2.5×10^{-8}–7.6×10^{-4}
Bayer 44646	290, 260	460	0.60	11.6	6.0×10^{-8}–6.3×10^{-4}
Bayer 37344	275	435	<0.2	9.8	5.0×10^{-8}–7.4×10^{-3}
NIA 10242	285	400	1.6	0.66	3.0×10^{-9}–7.3×10^{-4}
U. C. 10854	270	385	2.9	1.9	1.0×10^{-8}–1.2×10^{-3}
Imidan	305	440, 420	0.75	0.60	2.0×10^{-9}–8.5×10^{-5}
2,4,5-Trichlorophenoxyacetic acid	300	480	<0.2	0.51	2.0×10^{-9}–9.5×10^{-4}
2,4-Dichlorophenoxyacetic acid	290	495	<0.2	4.4	2.0×10^{-8}–8.6×10^{-4}
p-Chlorophenol	290	505	<0.2	23.2	1.8×10^{-7}–1.1×10^{-2}
2,4,5-Trichlorophenol	305, 280	485	<0.2	3.0	1.5×10^{-8}–6.7×10^{-4}
p-Nitrophenol[e]	355	520, 495	<0.2	0.024	1.7×10^{-10}–5.0×10^{-4}
1-Naphthol	320	475, 495, 520	1.15	0.25	1.7×10^{-9}–8.4×10^{-4}

[a] Compounds listed in groups according to structure.
[b] Quantitative measurements made at first wavelength listed.
[c] Result of three separate measurements.
[d] Range of concentrations over which analytical curves are approximately linear.
[e] Measured in ethanol made basic with 2 drops of diethylamine per 10 ml. of ethanol.

phorimetric sensitivity of *p*-nitrophenol, it should be possible to determine low concentrations of parathion, which can be hydrolyzed to *p*-nitrophenol. Low concentrations of methyl parathion (methyl parathion showed no detectable phosphorescence) can also be determined by hydrolysis to *p*-nitrophenol. Because of the similarities in the structures and the great differences in the phosphorescence sensitivities of methyl parathion, parathion, and *p*-nitrophenol, several additional studies were carried out to prove that *p*-nitrophenol impurity was not responsible for the phosphorescence of parathion. An ether solution of the parathion sample used for these studies was shaken with aqueous base which would extract any *p*-nitrophenol. Then the aqueous base was acidified and re-extracted with ether. This solution was clear (ether solutions of *p*-nitrophenol are yellow) and was not phosphorescent, indicating no *p*-nitrophenol impurity in the parathion.

The phosphorimetric determination of the carbamates (Sevin, Zectran, Bayer 44646, Bayer 37344, NTA 10242, and U.C. 10854) and of the phosphate Imidan also appears promising. All of these, except possibly Imidan, should be relatively insensitive to the electron capture detector. At the present time, colorimetric methods are used for the determination of the carbamates. The colorimetric limits of detection are always in the microgram per milliliter range. Work in this laboratory has already been done on the determination of low concentrations of *p*-nitrophenol in urine. In addition, work is now being initiated on the phosphorimetric determination of carbamate residues on agricultural crops. The great sensitivities of many of the other pesticides and related compounds in Table I should result in many more agricultural applications. However, a poor cleanup of the sample and a poor separation of the pesticide from the sample can result in serious errors and much higher limits of detection than those given in Table I. The limits of detection in Table I are for the ultimate detection limits when the background is completely a result of the solvent.

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RESIDUE DETECTION

Tracer Study of Residues from 2-Chloro-6-(trichloromethyl)pyridine in Plants

CARL T. REDEMANN, ROBERT T. MARTIN, JANET D. WIEN, and JOYCE G. WIDOFSKY

Bioproducts Department, The Dow Chemical Co., Walnut Creek, Calif.

THE ANNOUNCEMENT of the N-Serve nitrogen-extending agent by The Dow Chemical Co. aroused interest in several problems related to 2-chloro-6-(trichloromethyl)pyridine, the active ingredient in this product. One of these topics was the detection and identification of any chemical residues which might occur in crop plants grown on soil subsequent to treatment with N-Serve. The work which resulted from this study is presented here.

Methods and Materials

2 - Chloro - 6 - (trichloromethyl)-C^{14}-pyridine. 2-Chloro-6-(trichloromethyl)-C^{14}-pyridine was prepared as described in a previous publication (*2*). The specific activity was 1 millicurie per millimole.

6-Chloropicolinic Acid. The reference sample of 6-chloropicolinic acid was prepared by the acid hydrolysis of 2-chloro-6-(trichloromethyl)pyridine, as described previously (*2*).

Treated Fertilizer. Ammonium phosphate fertilizer was treated by moistening with a 2% solution of 2-chloro-6-(trichloromethyl)-C^{14}-pyridine in ethyl ether, followed by air-drying at room temperature for 2 minutes prior to application to the soil.

Planting. Three-gallon metal cans, $9^1/_2$ inches in diameter, were filled to within 2 inches of the top with soil. Three-inch deep furrows were opened diametrically along the surface of the soil, and enough treated ammonium phosphate was added to provide 100 pounds per acre of nitrogen and 2 pounds per acre of 2-chloro-6-(trichloromethyl)-C^{14}-pyridine.

The furrows were immediately filled with soil, and the soil was seeded to a single crop. For tomatoes, transplants rather than seeds were planted in the cans of treated soil.

Subsequently, all crops were grown to maturity in the greenhouse under maintenance which avoided leaching of the soil.

Varieties. Golden cross Bantam corn was used. The carrots were Imperator. The lettuce was New York Special. The tomatoes were Pearson. The oats were unnamed.

Radioautographs. Radioautographs