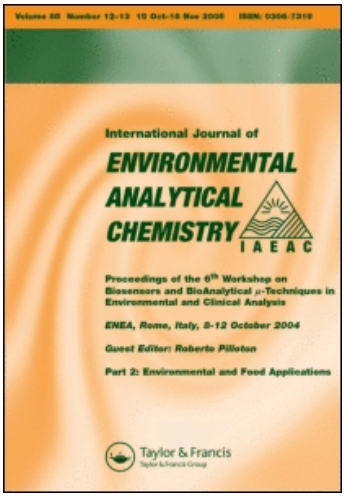

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GC-ECD Determination of Chlorpyrifos, its Oxon, and 3,5,6-Trichloro-2-Pyridinol in Soil, ELM Bark, and Litter Following Application for Control of the ELM Bark Beetle

Heng Jin[a]; G. R. B. Webster[ab]
[a] Department of Soil Science, The University of Manitoba, Winnipeg, MB, Canada [b] ORECL Research & Environmental Consulting Laboratories, Anne, MB, Canada

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GC-ECD DETERMINATION OF CHLORPYRIFOS, ITS OXON, AND 3,5,6-TRICHLORO-2-PYRIDINOL IN SOIL, ELM BARK, AND LITTER FOLLOWING APPLICATION FOR CONTROL OF THE ELM BARK BEETLE

HENG JIN and G. R. B. WEBSTER*

Department of Soil Science, The University of Manitoba, Winnipeg, MB, Canada R3T 2N2

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An analytical method has been developed for the simultaneous determination of residues of chlorpyrifos, and its main metabolites, the oxon and 3,5,6-trichloro-2-pyridinol in elm bark, litter, and soil following use in the control of the elm bark beetle, the principle vector of Dutch elm disease in the Canadian prairie provinces. The residues of chlorpyrifos, oxon, and pyridinol were extracted with methanol under acidic conditions from elm bark, litter, and soil, cleaned up by liquid-liquid partitioning, and simultaneously chromatographed by GC-ECD after the pyridinol had been derivatized with diazomethane. The average recoveries of chlorpyrifos, oxon, and pyridinol in all tested matrices in three replicates ranged from 90 to 102% with standard deviations of 0.4 to 8.2%.

Keywords: Analytical method; chlorpyrifos; oxon; pyridinol; elm bark; litter; soil; gas chromatography

INTRODUCTION

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) (Figure 1A), an organophosphate insecticide, is a broad-spectrum insecticide showing insecticidal activity against many insect and arthropod pests. It has successfully been utilized to combat such pests in the protection of human and animal health

*Corresponding author. Fax No.: +1-204-4225879, E-mail: webster@orecl.com
Current address: ORECL Research & Environmental Consulting Laboratories, P.O. Box 1101, 30 Dawson Road, Unit B, Ste. Anne, MB, Canada R5H 1C1

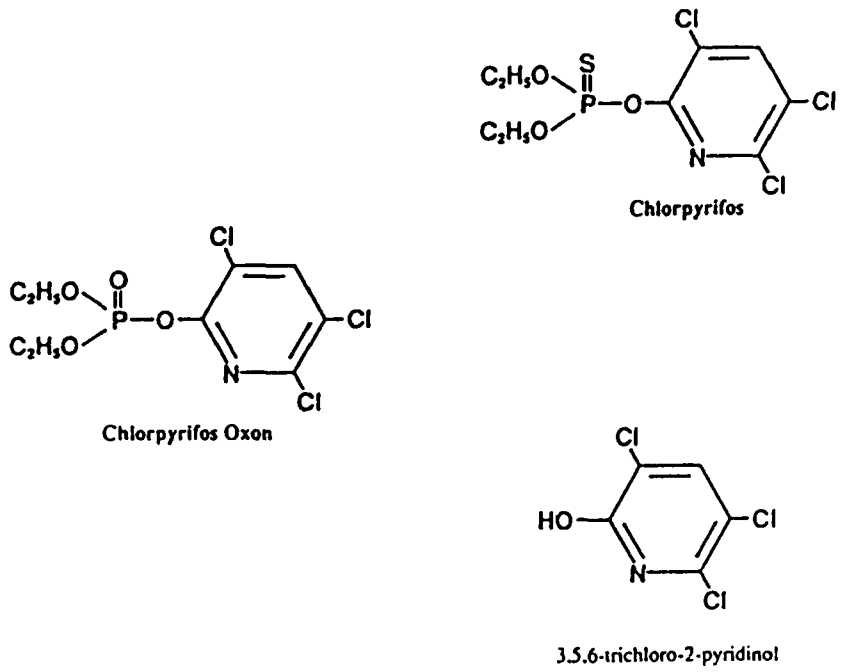

FIGURE 1 Chemical structures of chlorpyrifos, oxon, and pyridinol

and the production of food and fibre.[1,2] At present, chlorpyrifos is the registered insecticide for the control the elm bark beetle population in the integrated Dutch elm disease management program in urban areas and native elm stands in Canada.

A number of analytical methods for the determination of residues of chlorpyrifos alone, or in combination with its major metabolites, the oxon (Figure 1B) and/or the pyridinol (Figure 1C), in various environmental matrices had been developed previously.

Bowman and Beroza[3] reported a method to determine the residues of chlorpyrifos and its oxon in corn silage and grass. The method involved the extraction of the residues with benzene, cleanup of the extracts on a silica gel column, and determination of the residues by gas chromatography with flame photometric detection (GC-FPD). Struble and McDonald[4] developed methods to analyze chlorpyrifos and oxon in wheat plants and kernels using benzene extraction, silica gel column chromatography cleanup, and determination by GC-FPD. Mourer *et al.*[5] described a method for the determination of chlorpyrifos and pyridinol in dates. After extraction of residues with acetone, chlorpyrifos was cleaned up using Florisil and analyzed by gas chromatography with nitrogen-

phosphorus detection (GC-NPD). Pyridinol was derivatized with *bis*-(trimethylsilyl)-acetamide to form the pyridinol derivative and determined with a GC-Hall electrolytic conductivity detector (GC-HECD). Inman *et al.*[6] reported a method to analyze the residues of chlorpyrifos and pyridinol in peppermint hay and peppermint oil. The residues of chlorpyrifos and pyridinol were extracted with a mixture of hexane and 2-propanol. The residues of chlorpyrifos were cleaned up on a silica gel column and quantified by GC-FPD. The pyridinol was separated from the extraction solvent, cleaned up on acid alumina, derivatized with *N,O-bis*-(trimethylsilyl)-acetamide and analyzed by gas chromatography with electron capture detection (GC-ECD).

Braun[7] developed a method for determination of chlorpyrifos, oxon, and pyridinol in vegetable tissues. Samples were extracted with acetonitrile and the extracts were partitioned between benzene and aqueous sodium carbonate to separate the chlorpyrifos and oxon from pyridinol. Each fraction was individually cleaned up on silica gel which also served to fractionate chlorpyrifos from the oxon through hydrolysis of the oxon into the pyridinol. Chlorpyrifos was analyzed by GC-FPD. Residual pyridinol and pyridinol resulting from the hydrolysis of oxon in the silica gel column were derivatized with *N,O-bis*-(trimethylsilyl) acetamide and determined by GC-ECD.

Our present paper describes a simple analytical method for simultaneous determination of chlorpyrifos, oxon, and pyridinol residues in elm bark, litter, and soil following the use of chlorpyrifos in the control of the elm bark beetle, the principal vector of Dutch elm disease in the Canadian prairie provinces. Our new method has adapted previously reported GC-ECD methods and uses quantitative extraction under acidic conditions and simultaneous determination of the three analytes in the same chromatographic run.

EXPERIMENTAL

Chemicals

Methanol, ethyl ether, water, toluene, dichloromethane, and hydrochloric acid (pesticide residue analysis grade) were purchased from Baxter (Burdick & Jackson, Muskegon, MI, U.S.A.). Sodium sulfate (anhydrous) and sodium chloride (AR grade) were obtained from Mallinckrodt (Pointe-Claire, QC, Canada). Potassium hydroxide (ACS), and 1-methyl-3-nitro-1-nitrosoguanidine (97% purity) were from Aldrich (Milwaukee, WI, U.S.A.). Sodium carbonate (ACS) was from Fisher Scientific (Nepean, ON, Canada).

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) (99.5% purity), its oxon (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridylphosphate) (99% purity), and 3,5,6-trichloro-2-pyridinol (99.5% purity) were obtained from DowElanco (Midland, MI, U.S.A.).

Preparation of Diazomethane

Potassium hydroxide (2.3 g) was dissolved in 2.3 mL of water in a 125-mL conical flask, ethyl ether (25 mL) was added, and the flask was allowed to stand in an ice bath for 15 min. Diazomethane precursor (1-methyl-3-nitro-1-nitrosoguanidine) (1.5 g) was added gradually to the flask and slowly shaken until the reaction was complete. After the reaction had stopped, the ethyl ether layer containing the diazomethane was decanted into a container for storage. The diazomethane prepared in this way could be used for up to one week if stored in a freezer.

*(***Caution**: *Diazomethane and its precursor are potent mutagens and/or carcinogens. In addition, improperly handled diazomethane can pose an explosion hazard. Preparation of diazomethane must therefore be carried out behind a safety shield in a fume hood and the use of ground glass joints should be avoided. Diazomethane solution may be stored for up to a week in a closed container in a freezer.)*

Apparatus

The apparatus used included a rotary blender (Dynamics Corporation of America, New Hartford, CT, U.S.A.), extraction apparatus consisting of 50-mL round bottomed stainless steel centrifuge tubes (International Equipment Co., No. 613) which had been threaded and closed with stainless steel caps fitted with Teflon "O" ring gaskets, and stainless steel balls of approximately 1.75 cm diameter;[8] a wrist action shaker (model 75, Burrell Corporation, Pittsburgh, PA, U.S.A.); a centrifuge (International Centrifuge, model CS, International Equipment Co., Needham Heights, MA, U.S.A.); a rotary evaporator (Büchi, Flawil, Switzerland); and a gas chromatograph (Hewlett Packard 5890 - Series II) equipped with a auto sampler (Hewlett Packard Model 7673), a DB-5 capillary column (30 m × 0.32 mm), an electron capture detector, and a data processor [HP3365 II Chemstation (DOS Series)]. The GC parameters were as follows (all temperatures in °C): splitless injector, 200°; oven, 100° (0.5 min), 30°/min to 180° (10 min); detector, 350°; carrier gas, He 0.6 mL/min; precolumn pressure, 110 kPa; make-up gas, 5.12% argon in methane 60 mL/min; purge gas, He 1.5 mL/min.

Extraction

Soil, bark, and litter samples were homogenized in the rotary blender. The homogenized air-dried soil (15 g), ground elm bark (2.5 g), or ground air-dried litter (2.5 g) was weighed into the stainless steel centrifuge tubes containing two stainless steel balls.[8] Methanol (25 mL) and 0.6 N HCl (5 mL) were added. The tightly sealed capped tubes were mounted horizontally on the wrist-action shaker and shaken for 30 min. After shaking, the tubes were centrifuged for 20 min at 2900 rpm.

Liquid-Liquid Partition Clean-up

A 5 mL methanol extraction aliquot (equivalent to 2.5 g soil or 0.417 g litter or bark) was pipetted into a separatory funnel, combined with 50 mL 2% sodium chloride solution, 20 mL dichloromethane and 1 mL of 2.8 *N* HCl, and vigorously shaken for about 2 min. The dichloromethane phase was separated from the aqueous phase and drained through an anhydrous sodium sulphate layer into a 250-mL round bottomed flask. The remaining aqueous phase was extracted with 20 mL dichloromethane. The two dichloromethane extracts were combined and concentrated to approximately 0.5 mL on a rotary evaporator at 40°C and dry nitrogen was used to remove the dichloromethane completely and prepare the extract for the derivatization step.

Derivatization of Pyridinol

The pyridinol, which had been extracted with the chlorpyrifos and the oxon, required derivatization for GC analysis. The extract was quantitatively transferred from the round bottomed flask into a 15-mL graduated centrifuge tube using methanol, and concentrated to dryness under dry nitrogen. Four drops of methanol and 1 mL diazomethane in ethyl ether were added, mixed fully, and allowed to stand for 30 min at room temperature, and the solvent removed to dryness. Toluene (1 mL) was added and the mixture was diluted to an appropriate volume to facilitate analysis by gas chromatography.

Gas Chromatographic Analysis

The analytes were determined by GC-ECD; injection volume: 1.0 μL To determine the metabolites that required derivatization prior to GC analysis, standard solutions of the target analytes were passed through the same derivatization process as real samples and standard curves were made for each batch of samples to be analysed. The samples were analyzed as soon as they had been prepared.

TABLE I Recoveries of chlorpyrifos, oxon, and pyridinol in elm bark, litter, and soil

Analyte	*Matrix*	*Fortified Concentration (μg/g)*	*Recovery (%)*	*RSD (%)*
Chlorpyrifos	Elm bark	5	97	5.0
		50	94	4.2
		500	97	3.8
	Litter	1	97	1.9
		50	97	1.2
		1000	98	1.5
	Soil	1	102	2.1
		10	94	8.2
		30	97	1.5
Oxon	Elm bark	0.5	92	10.0
		1	92	5.3
		5	91	4.9
	Litter	0.5	96	2.8
		1	90	5.2
		5	98	1.6
	Soil	0.1	99	2.6
		0.5	95	5.7
		1	98	3.8
Pyridinol	Elm bark	1	97	1.5
		15	98	0.6
		30	91	0.6
	Litter	1	96	2.7
		10	96	2.7
		100	99	0.4
	Soil	1	97	3.4
		8	93	4.2
		15	96	2.7

Fortification

Recoveries of chlorpyrifos, oxon, and pyridinol were determined in triplicate at appropriate levels in elm bark, litter, and soil. The fortifications were made by adding appropriate amounts of chlorpyrifos and oxon in acetone, and pyridinol in methanol into the homogenized, weighed ground elm bark, litter, or soil samples to give designated fortification levels. The fortified samples were allowed to stand 1 h to let the solvent evaporate and then subjected to the procedures outlined above. The fortification levels of individual analytes in different matrices depended on the actual residue levels of the analytes in the samples with the range of fortification concentrations covering that of the residue concentration found in the actual samples.

RESULTS AND DISCUSSION

Table I presents the ball-mill extraction recoveries of chlorpyrifos, oxon, and pyridinol fortified in elm bark, litter, and soil at various levels. When elm bark

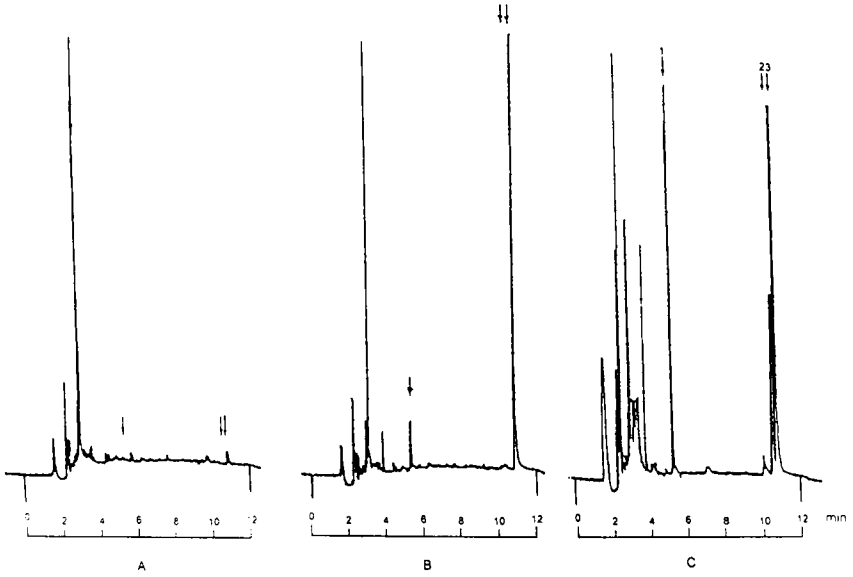

FIGURE 2 Typical GC-ECD chromatograms of untreated bark control (A), treated bark (B), and standards (C) of pyridinol, oxon, and chlorpyrifos

was fortified with chlorpyrifos at levels of 5, 50, and 500 μg/g, the average recoveries were 94 to 97% with the relative standard deviations (RSDs) of 3.8 to 5%. Approximately 97% recovery (RSDs 1.2 to 1.9%) was achieved from litter fortified with chlorpyrifos at 1.0, 50, and 1000 μg/g. The fortification recoveries of chlorpyrifos in soil were 94 to 104% at the levels of 1.0, 10, and 30 μg/g (RSDs 1.5 to 8.2%).

The average recoveries of oxon were 91 to 92% (RSDs 4.9 to 10%) in elm bark and 90 to 98% (RSDs 1.6 to 5.2%) in litter when these two matrices were fortified at 0.5, 1.0, and 5.0 μg/g, and 95 to 99% (RSDs 2.6 to 5.7%) in soil when fortified at 0.1, 0.5, and 1.0 μg/g.

The average recoveries of pyridinol were 91 to 98% (RSDs 0.6 to 1.5%) in elm bark at fortification levels of 1.0, 15, and 30 μg/g; 96 to 99% (RSDs 0.4 to 2.7%) in litter at 1.0, 10, and 100 μg/g; and 93 to 97% (RSDs 2.7 to 4.2%) in soil at 1.0, 8.0, and 15 μg/g.

Typical gas chromatograms for the analytical standards of methylated pyridinol, oxon and chlorpyrifos, control elm bark, and samples of elm bark treated with chlorpyrifos are shown in Figure 2. Typical gas chromatograms for the control litter, and litter sample taken near the treated elm trees are displayed in

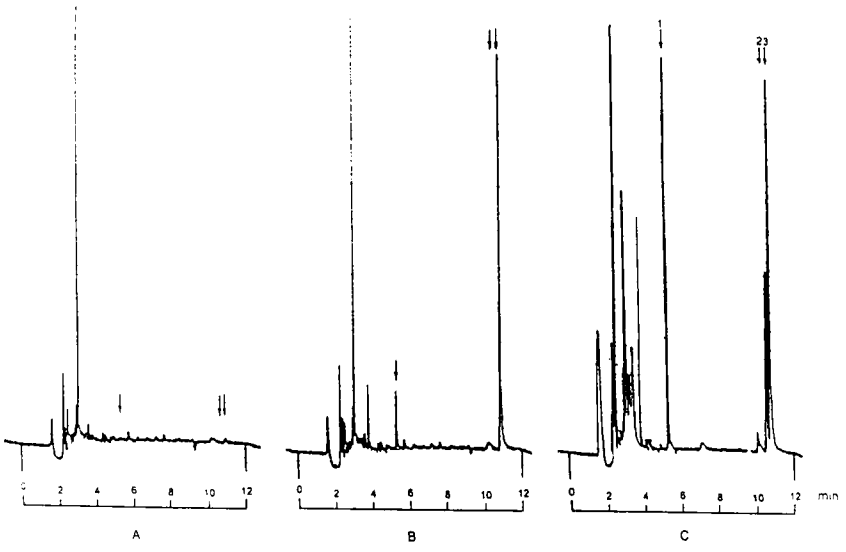

FIGURE 3 Typical GC-ECD chromatograms of untreated litter control (A), treated **litter** (B), and standards (C) of pyridinol, oxon, and chlorpyrifos

Figure 3. Typical gas chromatograms of control soil, and the soil samples taken near the treated elm trees are shown in Figure 4. The retention times, the minimum detectable amounts on the gas chromatograph, and the detection limits of methylated pyridinol, oxon, and chlorpyrifos in the elm bark, litter, and soil are listed in Table II. No significant interfering peaks existed at the retention times of the target analytes.

The attainment of good resolution between chlorpyrifos and oxon and high reproducibility and sensitivity of the GC method to the oxon make separation of chlorpyrifos and oxon prior to analysis unnecessary. In earlier analytical methods, determination of residues of chlorpyrifos, oxon, and pyridinol normally required that the pyridinol first be separated from the mixture of chlorpyrifos and pyridinol through liquid-liquid partition by adjusting the solution pH.[5,7] The oxygen analogue was separated from the parent chlorpyrifos by passing the extract through a silica gel column which decomposed the oxon into pyridinol.[7] The pyridinol was then derivatized prior to gas chromatographic analysis. Thus, the chlorpyrifos, oxon, and pyridinol in one sample had to be analyzed by GC separately. The simultaneous analysis of chlorpyrifos, oxon, and pyridinol is much simpler and more productive.

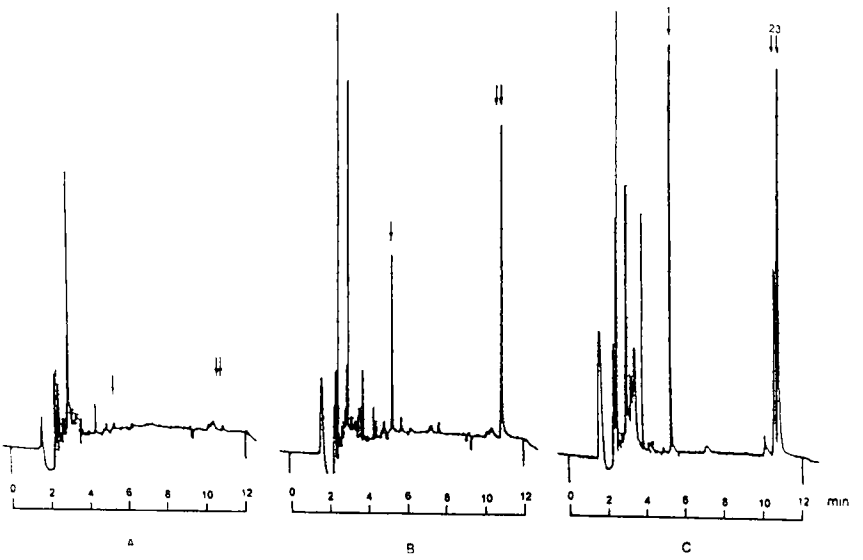

FIGURE 4 Typical GC-ECD chromatograms of untreated soil control (A), treated soil (B), and standards (C) of pyridinol, oxon, and chlorpyrifos

CONCLUSIONS

The new analytical procedure described is quantitative and reproducible and has been shown to be suitable for the analysis of residues of chlorpyrifos in environmental samples related to the control of the elm bark beetle, the principal vector of Dutch elm disease.

Acknowledgements

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TABLE II The retention times, minimum detectable amount, and detection limits of the target analytes in different matrices

Analyte	*Retention time (minutes)*	*Minimum detectable amount (ng)*	*Detection limit (μg/g)*		
			Elm bark	*Litter*	*Soil*
Chlorpyrifos	11.6	0.002	0.024	0.024	0.004
Oxon	10.8	0.004	0.048	0.048	0.008
Pyridinol	5.4	0.002	0.024	0.024	0.004

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