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Note

Sequential thin-layer chromatography of leptophos and related compounds

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Leptophos is a new organophosphorus insecticide that causes delayed neurotoxicity in hens and farm animals, similar to that reported for tri-*o*-cresyl phosphate (TOCP)[1,2]. This compound has been implicated also in the poisoning and paralysis of some workers in the Texas factory where it was manufactured[3]. Unlike most of the other organophosphorus compounds, leptophos is relatively persistent in the environment[4].

Gas–liquid chromatographic (GLC) analysis, using 5% OV-17 on 80–100 mesh Gas-Chrom Q, was utilized to determine leptophos and its oxygen analog (leptophos oxon), with a flame-photometric detector[5]. However, the GLC sensitivity to the oxygen analog is invariably lower than that to its parent compound. Because of their instability, however, other possible degradation products of leptophos were, in general, not successfully recovered. The compound 4-bromo-2,5-dichlorophenol was converted into a trimethylsilyl ether derivative and determined with ^{63}Ni electron capture detector[6].

This paper reports a rapid thin-layer chromatographic (TLC) method which offers fast and convenient analyses of leptophos and its possible degradation products.

EXPERIMENTAL

Chemicals

Leptophos [O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate], its related compounds and the radioactive materials used in this experiment were provided by Velsical (Chicago, Ill., U.S.A.). The following analytical grade compounds were investigated: leptophos, desbromoleptophos [O-(2,5-dichlorophenyl) O-methyl phenylphosphonothioate], 4-bromo-2,5-dichlorophenol, leptophos oxon [O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonate], O-methyl phenylphosphonothioic acid (MPPTA), O-methyl phenylphosphonic acid (MPPA) and phenylphosphonic acid (PPA). Two radioactive chemicals were used: [^{14}C]phenylleptophos [O-(4-bromo-2,5-dichlorophenyl) O-methyl [^{14}C]phenylphosphonothioate] (specific activity 6.23 mCi/mmole) and [^{14}C]phenoxyleptophos] [O-(4-bromo-2,5-dichloro[^{14}C]phenyl) O-methyl phenylphosphonothioate (specific activity 7.34 mCi/mmole) (New England Nuclear, Boston, Mass., U.S.A.).

Thin-layer plates

Gelman type SA, ITLC, silicic acid-impregnated glass fiber sheets (Gelman, Ann Arbor, Mich., U.S.A.) were used.

The solvents used were: a, acetonitrile–water–ammonia (40:9:1) and b, *n*-hexane–diethyl ether (9:1).

Procedure

Aliquots (10 μl) of acetone solutions (1 μg/μl) of leptophos and related compound were spotted on the glass-fiber sheet. Chromatograms were developed in a lined, pre-equilibrated tank; up to 6 cm with solvent a, then to 16 cm with solvent b. The respective regions were identified by visualization in an iodine chamber.

The radiochemical purities of [^{14}C]phenyl- and [^{14}C]phenoxyleptophos were evaluated using the same sequential ITLC system. For the characterization of impurities, a mixture of leptophos and structurally related compounds was added to each of the [^{14}C]leptophos solutions and the chromatography of each solution was subsequently determined. The standards were detected by their color in iodine vapor. The sheets were cut into 5-mm strips, placed in scintillation vials, and vigorously mixed with a scintillation medium. The scintillation solvent was composed of toluene–ethylene glycol monomethyl ether (2:1, v/v) containing 5 g of 2,5-diphenyloxazole (PPO) and 200 mg of 1,4-bis[2(5-phenyloxazolyl)benzene] (POPOP) per l. Radioactivity was determined using a Beckman Model LS-100 liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Leptophos causes delayed neurotoxicity in hens when administered orally[1,2] or dermally[7], similar to the neurotoxic effect of TOCP[8]. Whereas ingested leptophos is rapidly metabolized and excreted in the mouse (a species non-susceptible to delayed neutoxicity of organophosphorus compounds)[9] it is slowly metabolized and excreted in the hen (a susceptible species)[10]. Species selectivity has been attributed to differences in pharmacokinetics and metabolism of leptophos. It is therefore important to develop a simple method, with good accuracy, suitable for the identification of leptophos degradation products in biological and nonbiological systems.

The R_F values (average of three developments) of leptophos and related compounds in two single solvents and two-sequential solvent systems are listed in Table I. These compounds could be classified into two groups according to their separation of ITLC: non-polar compounds —leptophos, desbromoleptophos and leptophos oxon; and polar compounds— MPPTA, MPPA and PPA. 4-Bromo-2,5-dichlorophenol showed a behavior intermediate between non-polar and polar. Generally, these two classes can be resolved from each other; however, the individual compounds within each group may not be readily separated.

The best solvent system for the separation of polar compounds from each other as well as from non-polar compounds was solvent a. In this system all non-polar compounds moved together and too fast with the solvent front ($R_F = 0.98$) to interfere with the polar compounds. The polar substances moved in good separable distances from each other with the following R_F values: MPPTA 0.74, MPPA 0.14, and PPA

TABLE I

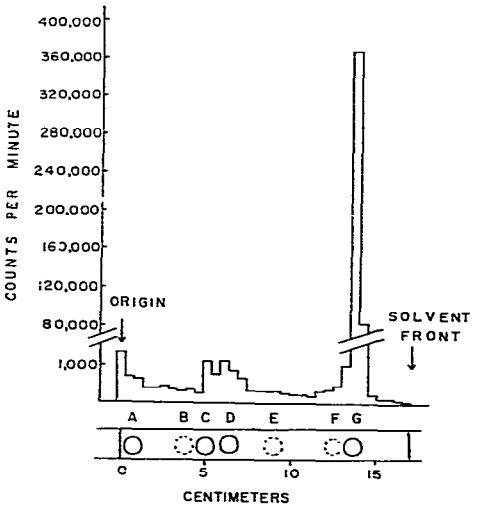
R_F VALUES FOR LEPTOPHOS AND RELATED COMPOUNDS ON ITLC SHEETS USING SINGLE AND SEQUENTIAL SOLVENT SYSTEMS

Solvents: a, acetonitrile–water–ammonia (40:9:1); b, *n*-hexane–diethyl ether (90:10). Sequential solvent system: solvent a for 6 cm, and solvent b for 16 cm.

Compound	*Solvent a*	*Solvent b*	*Solvent a followed by solvent b*
Leptophos	0.98	0.79	0.86
Desbromoleptophos	0.98	0.77	0.77
Leptophos oxon	0.98	0.10	0.45
4-Bromo-2,5-dichlorophenol	0.80	0.36	0.54
MPPTA	0.74	0.00	0.30
MPPA	0.14	0.00	0.26
PPA	0.01	0.00	0.00

0.01. The intermediate 4-bromo-2,5-dichlorophenol had an R_F value of 0.80. On the other hand, when solvent b was used, the non-polar compounds moved and separated from the stationary polar compounds. The R_F values for these non-polar substances were as follows: leptophos 0.86, desbromoleptophos 0.77 and leptophos oxon 0.45. The R_F value for 4-bromo-2,5-dichlorophenol was 0.54.

By employing a two-solvent sequential TLC system, which developed the ITLC sheets first with solvent a for 6 cm followed by solvent b for 16 cm, a good resolution of all compounds tested was obtained (Table I). It is of interest to report



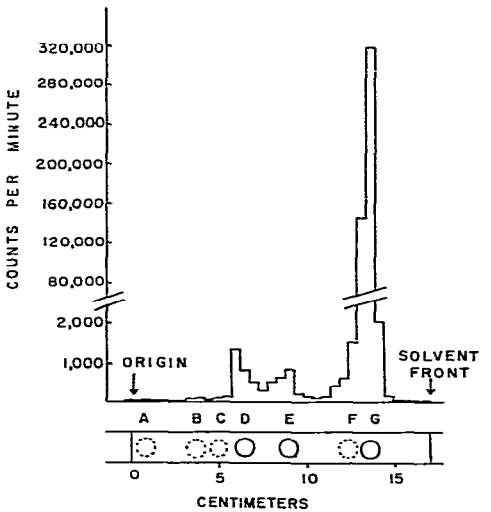


Fig. 1. Chromatogram and scan for [^{14}C]phenyl leptophos, using Gelman Type SA, ITLC silicic acid-impregnated glass fiber sheets, following sequential elution with solvent a for 6 cm and solvent b for 16 cm. A = PPA; B = MPPA; C = MPPTA; D = leptophos oxon; E = 4-bromo-2,5-dichlorophenol; F = desbromoleptophos and G = leptophos.

Fig. 2. Chromatogram and scan for [^{14}C]phenoxyleptophos using Gelman Type SA, ITLC silicic acid-impregnated glass fiber sheets, as described in legend of Fig. 1.

that a fresh solution of leptophos oxon in acetone always gave one spot, while old acetone solutions gave 2 or 3 spots of which one corresponded to leptophos oxon. These results indicate the instability of leptophos oxon and the necessity for the use of mild conditions for the extraction, clean-up and analysis of leptophos breakdown products in different systems.

The radiochemical purity of two ^{14}C-labeled leptophos compounds was evaluated using the 2-solvent sequential TLC system. [^{14}C]Phenylleptophos was found to be 98.24% pure (Fig. 1). The impurities were identified by SLTC to be PPA (0.94%), MPPA (0.25%) and leptophos oxon (0.57%). The purity of [^{14}C]phenoxyleptophos was determined to be 98.85%, with 0.51% leptophos oxon and 0.64% 4-bromo-2,5-dichlorophenol as impurities (Fig. 2).

The radioactive purity of [^{14}C]phenyl- and [^{14}C]phenoxyleptophos has been determined using this system which utilized Gelman Type SA, ITLC silicic acid-impregnated glass fiber sheets.

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