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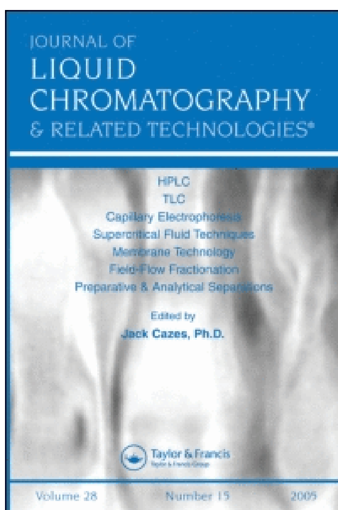

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Measurement Of Ethiofos (Wr 2721) In Plasma: Preliminary Pharmaco-Kinetics in the Beagle

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MEASUREMENT OF ETHIOFOS (WR 2721) IN PLASMA: PRELIMINARY PHARMACOKINETICS IN THE BEAGLE

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ABSTRACT

A specific, sensitive high performance liquid chromatographic method for ethiofos [S-2-(3-aminopropylamino)ethyl phosphorothioate, WR 2721, I] in plasma is described. The detection limit is 0.05 µg/mL (0.23 µM). Application of the method to the development of pharmacokinetic parameters following IV administration of the drug to beagle dogs is demonstrated. Presented are pharmacokinetics of unchanged drug in plasma on 10-min constant-rate infusion of 150 mg/kg to two dogs, two studies in each dog. Following the cessation of drug infusion the plasma concentration versus time profile was best described by a two-compartment pharmacokinetic model. Mean pharmacokinetic parameters were: terminal elimination half-life = 16.0 min, volume of central compartment = 120 mL/kg and clearance = 11.0 mL/min/kg.

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INTRODUCTION

Ethiofos [S-2-(3-aminopropylamino)ethyl phosphorothioate, WR 2721, I)] is a radioprotective drug being investigated by the U.S. Army, the National Cancer Institute and others. As part of a program with the U.S. Army Medical Research and Development Command, we have been determining plasma concentrations of this compound following IV administrations to beagle dogs.

We previously reported a sensitive, precise analytical method for determination of the drug in plasma (1,2). The method, using high performance liquid chromatography (HPLC) with fluorescence detection, was used in preliminary intravenous dosing studies of the drug in which a rapid rate of disappearance over a thousand-fold concentration range (>1000 to <1 μg/mL) was observed. However, an apparent terminal phase with a long half-life was noted in these studies with very low drug levels being measured for extended periods (up to 6 days) (2). Because the possibility of low-level interferences generated by the drug dosing itself is always present in such experiments, a separation more selective for unchanged material was sought.

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Investigations of the HPLC separation of I and its homolog, S-3-(4-aminobutylamino)propyl phosphorothioate (WR 80855, II), from endogenous plasma components have shown that dramatic changes in selectivity may be induced by changing the polar modifier used in the mobile phase. This observation was exploited in the development of a more selective HPLC system and its application is reported herein.

$NH_2CH_2CH_2CH_2NHCH_2CH_2SPO(OH)_2$

I

$NH_2CH_2CH_2CH_2CH_2NHCH_2CH_2CH_2SPO(OH)_2$

II

MATERIALS AND METHODS

Apparatus

A Waters Associates Model 244 Liquid Chromatograph and a Laboratory Data Control (LDC) modular HPLC system were used. Each was fitted with a Rheodyne Model 7125 injector (50 μL loop), an LDC Fluoromonitor III fluorescence detector and a Waters Associates RCM-100 Radial Compression Module with a 5-μm μBondapak C-18 cartridge. The detector excitation source was a 340-380-nm low-pressure mercury and phosphor conversion lamp. A 370-nm bandpass filter and a 418-700-nm cutoff filter controlled excitation and emission wavelengths. The analytical column was protected by a Whatman, Inc. guard column dry-packed with Whatman, Inc. Pellicular ODS C-18.

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Reagents

USP absolute ethanol was purchased from U.S. Industrial Chemicals Company and HPLC-grade acetonitrile from J. T. Baker Chemical Company. The alkyltriethylammoniumphosphates, pentyl-, hexyl-, heptyl-, octyl- and dodecyltriethylammonium phosphate, were purchased from Regis Chemical Company as 0.5M solutions in water. Fluorescamine was purchased from Aldrich Chemical Company and fresh solutions (5 mg/mL) were prepared weekly from reagent-grade acetone which had been stored over 4A molecular sieves. The Walter Reed Army Institute of Research furnished I as the trihydrate (Lot AX, Bottle BK02762) in >99.0% purity as well as the internal standard II as the monohydrate (Bottle AG49364).

Sample Preparation

Plasma obtained by centrifugation of whole blood samples was chilled in polyethylene vials, the internal standard was added (in 0.05M sodium borate-potassium chloride pH 10 buffer solution) and the mixture was quick-frozen in a Dry Ice/isopropyl

alcohol bath. Typical sample volumes were 150 µL of plasma and 150 µL of internal standard. The time from sample drawing to freezing was standardized and explicit. Samples were stored at -75°C until time of analysis. Immediately after thawing at room temperature the samples were treated with 200 µL of a 0.05M sodium borate-potassium chloride buffer (pH 7.60) and while the mixture was being agitated with a vortex mixer, 250 µL of the fluorescamine reagent was added. After mixing for about 60 seconds, an additional 250 µL portion of reagent was added and agitation was continued 20-30 seconds. The resulting mixture was pressure-filtered through a 0.45-µm filter and 50 µL of the clear filtrate was injected onto the HPLC column.

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Pharmacokinetic Studies in Beagles

The laboratory animal facilities and animal care program of Southwest Research Institute have been reviewed and accredited by the American Association for Accreditation of Laboratory Animal Care according to standards set forth in ''Guide for the Care and Use of Laboratory Animals,'' DHEW Publication NIH 78-23.

Healthy, 1-year-old, male beagle dogs weighing about 15 kg were purchased from Laboratory Research Enterprises, Inc., Kalamazoo, Michigan. The animals were dosed intravenously via the cephalic vein with a 0.9% saline solution containing I (100 mg/mL) over a 10-minute period. The dose was exactly 150 mg I (anhydrous basis) per kg body weight. Blood samples (3 mL) were withdrawn into EDTA Vacutainers™ from a cannula placed in the jugular vein. Each sample was immediately chilled in an ice/water bath and centrifuged. The plasma samples were treated as described above.

Data Analysis

The data from each experiment were fitted to one-, two- and three-compartment open pharmacokinetic models with zero-order input of drug into the central compartment at a rate of 15 mg/kg/min for 10 minutes and first-order elimination from the central com-

partment. Only data through 120 minutes were included because the concentration at later times were below the limit for accurate quantitation (0.1 μg/L). The data were weighted by the reciprocal of the observed concentration. Kinetic parameters relatable to physiologic phenomena, i.e., volumes and clearances, were obtained iteratively using the digital computer program NONLIN (3). An F-test (4) was used to assess statistical differences among the models.

RESULTS AND DISCUSSION

Analytical Method

We previously reported that preliminary dosing studies indicated a lengthy terminal phase for the drug following administration to beagle dogs. Subsequent dosings using a modified analytical procedure did not show the presence of drug past 2 to 4 hours at a detection limit of 0.05 μg/mL. Although analysis of plasma samples taken prior to dosing did not indicate an interference, it is conceivable that an interference could have developed post-administration, possibly due to the dosing itself. It was shown that neither the thiol metabolite of the drug nor its symmetrical disulfide interfered. Investigation of a homologous series of alkyltriethylammonium phosphates (alkyl = pentyl, hexyl, heptyl, octyl and dodecyl) produced separations of the drug-plasma system with widely varying selectivities, but only one of the series gave a mobile phase system which was highly selective for both unchanged drug and the homolog used as internal standard. A mobile phase of dodecyltriethylammonium phosphate at a concentration of 0.01M in a mixture of acetonitrile:ethanol:water (20:8:72) at pH 2.8 and a flow of 2.0 mL/min allowed I and II to be separated from each other and from endogenous materials in beagle plasma in <30 minutes (Figure 1). Absence of interference was demonstrated by analysis of a plasma blank (Figure 2). With this system the lower limit of detectability was 0.05 μg I/mL plasma with acceptable precision (CV <10%) obtainable down to about 0.1 μg/mL.

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FIGURE 1. Chromatographic trace of beagle plasma spiked with 1.0 µg/mL of both ethiofos and WR 80855. Attenuation = 5X

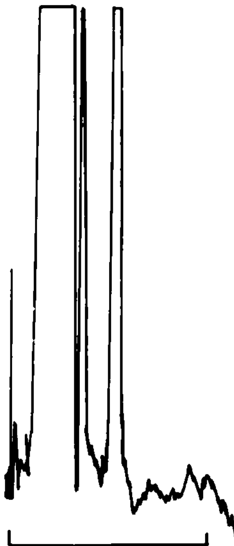

FIGURE 2. Chromatographic trace of beagle plasma blank. Attenuation = 5X

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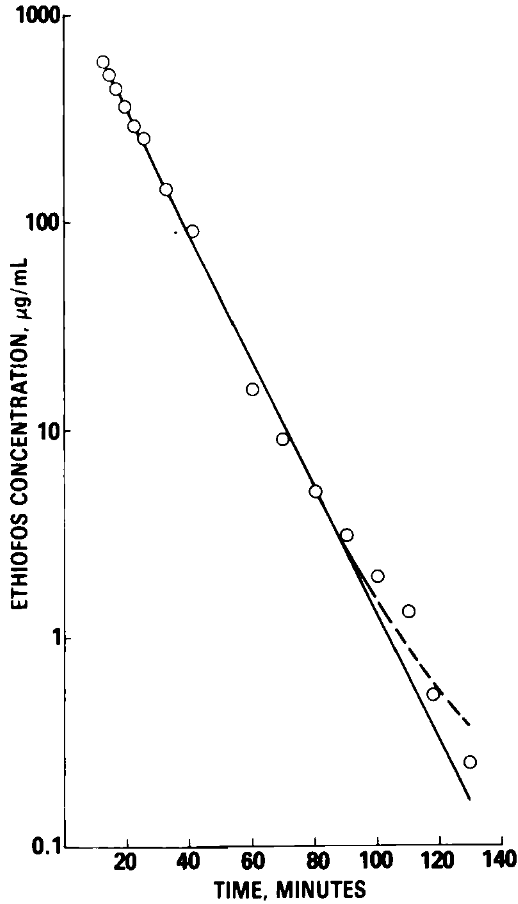

FIGURE 3. Ethiofos Pharmacokinetics--Experiment 1
The solid line is the concentration-time profile predicted by the one-compartment model. The dashed line is the portion of the concentration-time profile predicted by the two-compartment model that differs from that predicted by the one-compartment model

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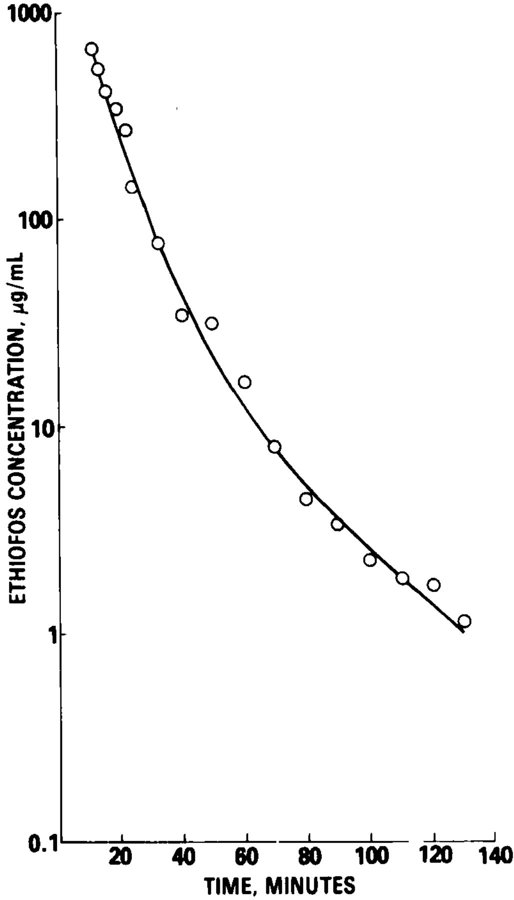

FIGURE 4. Ethiofos Pharmacokinetics--Experiment 2
The solid line is the concentration-time profile predicted by the two-compartment model

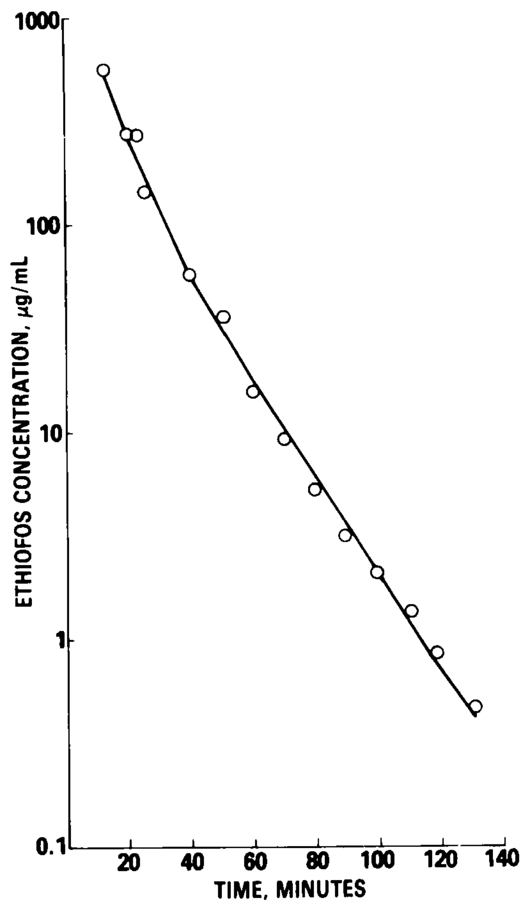

FIGURE 5. Ethiofos Pharmacokinetics--Experiment 3
The solid line is the concentration-time profile predicted by the two-compartment model

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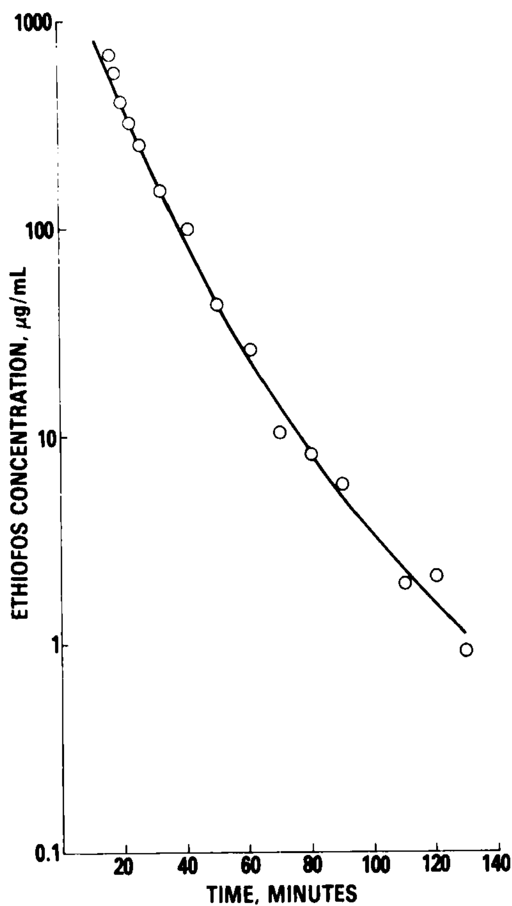

FIGURE 6. Ethiofos Pharmacokinetics--Experiment 4
The solid line is the concentration-time profile predicted by the two-compartment model

Pharmacokinetics

In contrast to earlier results (2) which indicated a lengthy terminal phase, plasma levels dropped below the limit of reliable quantitation in less than 2 hours following three of the dosings and in less than 4 hours following the fourth. These observations suggest that previous results were biased by the presence of low-level interferences. Concentration-time profiles for the dosing experiments are presented in Figures 3-6.

Data sets for Experiments 2, 3 and 4 were adequately described (F-test) by the two-compartment model (Table 1).
However, the two-compartment fit to the data from Experiment 1 did not result in a statistically better description of the concentration profile. In spite of this the two-compartment model provided a better visual description of the data (Figure 6). The parameters for the models of best fit for all four experiments are summarized in Table 2. Because of the ambiguity in models for Experiment 1, parameters for both the one- and two-compartment models for this data set are given in Table 3.

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TABLE 1
Weighted Sum of Squared Deviations for Fitted Data

Experiment Number	Number of Compartments: 1	2	3
1	10.51[a]	9.84	9.79
2	259.74	105.91[b]	105.91
3	99.83	47.85[c]	47.88
4	172.96	103.17[d]	103.18

[a] $F_{2,13} = 0.40$, $p>0.05$. Two-compartment model gave slightly better visual fit.
[b] $F_{2,13} = 9.44$, $p<0.0005$
[c] $F_{2,11} = 5.97$, $p<0.025$
[d] $F_{2,13} = 4.40$, $p<0.05$

TABLE 2
Ethiofos Pharmacokinetic Parameters

Parameter	Dog CSX-1 Experiment 1	Dog CSX-1 Experiment 4	Dog BD-13 Experiment 2	Dog BD-13 Experiment 3
V_c, mL/kg	158	119	106	133
V_p, mL/kg	-	12.6	24.4	28.6
Cl_D, mL/min/kg	-	0.480	0.905	2.10
Cl_E, mL/min/kg	11.1	9.28	11.3	12.4
α, min^{-1}	-	0.0853	0.119	0.130
β, min^{-1}	0.0698	0.0348	0.0332	0.0528
$T_{1/2\alpha}$, min	-	8.12	5.82	5.33
$T_{1/2\beta}$, min	9.93	19.9	20.9	13.1

V_c - volume of central compartment
V_p - volume of peripheral compartment
Cl_D - distributional clearance
Cl_E - elimination clearance
α - distribution phase macro rate constant
β - elimination phase macro rate constant
$T_{1/2}$ - half-life

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TABLE 3
Comparison of Parameters for Experiment 1
for the One- and Two-Compartment Models

Parameter	Number of Compartments 1	Number of Compartments 2
V_c, mL/kg	159	158
V_p, mL/kg	-	24.4
Cl_D, mL/min/kg	-	0.0770
Cl_E, mL/min/kg	11.1	11.1
α, min^{-1}	-	0.0701
β, min^{-1}	0.0698	0.00313

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