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Evaluation Of Microdosing Strategies For Studies In Preclinical Drug Development: Demonstration Of Linear Pharmacokinetics In Dogs Of A Nucleoside Analogue Over A 50-Fold Dose Range

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EVALUATION OF MICRODOSING STRATEGIES FOR STUDIES IN PRECLINICAL DRUG DEVELOPMENT: DEMONSTRATION OF LINEAR PHARMACOKINETICS IN DOGS OF A NUCLEOSIDE ANALOGUE OVER A 50-FOLD DOSE RANGE

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[1]Abbreviations: AMS, accelerator mass spectrometry; AUC, area under the plasma concentrations versus time curve; CLp, plasma clearance; C_{max}, maximum concentration in plasma; HPLC, high performance liquid chromatography; I.V., intravenous; LC-MS/MS, liquid chromatography tandem mass spectrometry; LSC, liquid scintillation counting; PK, pharmacokinetics; P.O., oral; T_{max}, time of occurrence of maximum concentration in plasma; $T_{1/2\_,}$ alpha half life; $T_{1/2\_,}$ beta half life; $T_{1/2\_,}$ gamma half life; Vd_{ss}, steady-state volume of distribution.

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ABSTRACT

The technique of accelerator mass spectrometry (AMS) was validated successfully and utilized to study the pharmacokinetics and disposition in dogs of a preclinical drug candidate (Compound A), after oral and intravenous administration. The primary objective of this study was to examine whether Compound A displayed linear kinetics across sub-pharmacological (microdose) and pharmacological dose ranges in an animal model, prior to initiation of a human microdose study. The AMS-derived disposition properties of Compound A were comparable to data obtained via conventional techniques such as LC-MS/MS and liquid scintillation counting analyses. Thus, Compound A displayed multiphasic kinetics and possessed low plasma clearance (4.4 mL/min/kg), a long terminal elimination half-life (19.4 hr) and high oral bioavailability (82%). Currently there are no published comparisons of the kinetics of a pharmaceutical compound at pharmacological versus sub-pharmacological doses employing microdosing strategies. The present study thus provides the first description of the pharmacokinetics of a drug candidate assessed under these two dosing regimens. The data demonstrated that the pharmacokinetic properties of Compound A were similar following dosing at 0.02 mg/kg as at 1 mg/kg, indicating that in the case of Compound A, the kinetics of absorption, distribution and elimination in the dog appear to be linear across this 50-fold dose range. Moreover, the exceptional sensitivity of AMS provided a pharmacokinetic profile of Compound A, even following a microdose, which revealed aspects of the disposition of this agent that were inaccessible by conventional techniques.

The applications of accelerator mass spectrometry (AMS) are broad ranging and vary from studying environmental and ecological issues such as the isotopic composition of the atmosphere, soil and water (Hughen et al., 2000; Beck et al., 2001; Keith-Roach et al., 2001; Mironov et al., 2002), to archaeology and volcanology (Stafford et al., 1984; Vogel et al., 1990; Smith et al., 1999) to its use as a bioanalytical tool for nutritional research (Buchholz et al., 1999; Deuker et al., 2000; Weaver and Liebman, 2002). Biomedical applications of AMS and its use in the arena of pharmaceutical research also have been detailed in review articles (Barker and Garner, 1999; Garner, 2000; Turteltaub and Vogel, 2000). To date, most studies on the metabolism and disposition of xenobiotics by AMS have focused on how carcinogens bind to DNA and proteins to form adducts (Turteltaub et al., 1990, 1997; Frantz et al., 1995; Dingley et al., 1999; Li et al., 2003). Its application to the field of pharmaceutical sciences has been limited to a few studies (Kaye et al., 1997; Young et al., 2001; Garner et al., 2002). However, the pharmaceutical industry is becoming increasingly aware of the potential benefits that may accrue from the ultra high sensitivity afforded by AMS in terms of evaluating the pharmacokinetics of lead drug candidates in early development. Specifically, AMS allows administration of sub-pharmacological doses (microdoses) of carbon-14 or tritium-labeled investigational drugs to animals or humans at radiologically insignificant levels with the goal of obtaining preliminary information regarding the absorption, distribution, metabolism, and excretion of test compounds (Turteltaub and Vogel, 2000). An unresolved issue, however, is whether the pharmacokinetics determined following a microdose are representative of those following a conventional (pharmacological) dose (Lappin and Garner, 2003).

This paper examines the linearity of kinetics of an antiviral nucleoside analogue, Compound A, across sub-pharmacological and pharmacological dose ranges in the dog prior to initiation of a human microdose study.

The specific objectives of this study, therefore, were 1) to assess the pharmacokinetics of Compound A in dogs by a conventional dosing approach utilising LC-MS/MS for sample analysis, 2) to assess the pharmacokinetics of Compound A in dogs by the microdose approach utilising AMS for sample analysis, 3) to compare the pharmacokinetics of Compound A at a microdose versus a pharmacological dose, and 4) to validate AMS for this application and to compare the sensitivity of AMS to that of LC-MS/MS.

MATERIALS AND METHODS

Chemicals. [^{14}C]Compound A (Fig. 1) was prepared by the Labeled Compound Synthesis Group (Merck Research Laboratories, Rahway, NJ). The specific activity of the material was 183.5 μCi/mg and the radiochemical purity was >98%. Unlabeled Compound A was synthesized by the Department of Process Research (Merck Research Laboratories, Rahway, NJ). Internal standard was obtained from Sigma-Aldrich (St. Louis, MO). All other solvents and reagents were of either HPLC or analytical grade. All dosing solutions were prepared based on the molecular weight of the free base.

Animal Facility for the AMS study. A room in the Merck Research Laboratories Drug Metabolism animal facility was designated for the purpose of conducting this AMS study in dogs. The room had not been used for any studies involving radiolabeled compounds in the past 6 years. The walls and dog cages were cleaned with detergent numerous times to remove any traces of ^{14}C from previous uses. Small glass fiber filters measuring 2.4 mm in diameter (Fisher Scientific, Pittsburgh, PA) were wet with alcohol and swiped over small areas of interest for monitoring background ^{14}C. Several such swipes were taken of the dog cages, food bowls, water supply, fume hood, air vents, door handles, and bench top surfaces. These were sent to Lawrence Livermore National Laboratory (Livermore, CA) for analysis to certify the absence of background ^{14}C contamination in the room and the environment in which the AMS study was to be performed. The results from these swipe test samples indicated that the environment was suitable for the conduct of an AMS study. No significant areas of contamination were identified.

All procedures for the study were carried out with utmost care to avoid contamination of samples with external sources of ^{14}C. Disposable supplies were used

for all sample preparation and handling steps. In general, extreme care was exercised in setting up this facility in order to assure the successful conduct of the AMS component of the study (Buchholz et al. 2000). Moreover, access to this facility was limited to those researchers directly involved in the study.

Pharmacokinetic Studies. All animal studies described in this paper were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Male Beagle dogs from Marshall Farms (North Rose, NY) weighing 8.3-11.2 kg were used for the pharmacokinetic studies. Dogs were fasted overnight prior to drug administration and fed 4 hr post dose. The oral (p.o.) dose solutions were prepared in 0.5% aqueous methylcellulose and the intravenous (i.v.) dose solutions were formulated in saline. Administration of the i.v. dose was via the cephalic vein as a slow bolus over 30 sec at 0.1 mL/kg and that of the oral dose solution was by gavage. Three groups of dogs (n=2/group) were dosed with [^{14}C]Compound A. The first group of dogs was administered a 1 mg/kg oral dose (specific activity 0.017 μCi/mg). The second group of dogs was administered a 0.02 mg/kg oral dose (specific activity 0.546 μCi/mg) and the third group of dogs received a 0.02 mg/kg intravenous dose (specific activity 0.428 μCi/mg). Blood samples were collected from the jugular vein at predose and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 28, 32, 36, 48, 56, 72, and 80 hr following drug administration. An additional sample at 0.083 hr (5 min) was collected following i.v. administration of the compound**.**

In a separate pharmacokinetic study, dogs (n=4, cross-over study design) were dosed with unlabeled Compound A. For the first leg of this study, the dogs were administered a 0.4 mg/kg i.v. dose. Two weeks later, the dogs were administered a 1 mg/kg oral dose. Blood samples were collected from the jugular vein at predose and at 0.25, 0.5, 1, 2, 4, 6, 8,

12, 24, 28, 32, 48, and 72 hr following drug administration. An additional sample was collected at 0.083 hr (5 min) following i.v. administration**.** All blood samples were collected in EDTA tubes and plasma was harvested by centrifugation. All samples were stored at -20°C or -70°C until analysis.

LC-MS/MS of Compound A in Plasma. To 50 μL of plasma samples was added 50 μL of methanol:water (50:50; v/v), 25 μL of working internal standard, and 1 mL of 0.1 M sodium phosphate solution (pH 2.0). The contents were briefly vortexed and the analytes (Compound A and internal standard) were extracted from plasma samples using an Oasis MCX (30 mg) extraction plate (Waters Corp., Milford, MA, USA). The plate was rinsed with 0.5% formic acid (0.5 mL) followed by acetonitrile (0.5 mL), and the analytes were eluted with 300 μL acetonitrile:water:ammonium hydroxide (90:8:2; v/v/v). The eluent was neutralized with acetic acid and aliqouts (20 μL) injected onto the HPLC-MS/MS system. Concentrations of parent (Compound A) were determined from a standard curve prepared daily by adding stock working standards of drug and internal standard into 50 μL of drug-free dog plasma. The validated assay demonstrated good linearity and reproducibility for Compound A over the concentration range 2 to 2000 ng/mL. The limit of quantitation was 2 ng/mL. The values for assay accuracy and precision (%CV) ranged from 95.5 to 102.3% and 2.1 to 7.2%, respectively. Compound A was shown to be stable in dog plasma during the time required for storage and analysis of the samples and to up to three freeze-thaw cycles (freezing at – 20ºC and thawing at room temperature).

The HPLC system consisted of a Perkin Elmer LC200 pump (Norwalk, CT) and a Varian Prostar 430 autosampler (Walnut Creek, CA). The mass spectrometer was a PE Sciex API 4000 triple quadrupole system (Foster City, CA, USA) equipped with a heated

nebulizer interface (500ºC) operated in the positive ion electrospray mode. Multiple reaction monitoring of the precursor-to-product ion pairs *m/z* for Compound A and *m/z* for internal standard was used for quantitation. PE Sciex Analyst Version 1.1 software was used to collect and process the data. The mobile phase consisted of acetonitrile:10 mM ammonium acetate (9:1; v/v), adjusted to pH 4.0 with acetic acid, at a flow rate of 400 μL/min. Chromatography was performed on a 2.0 x 100 mm, 5 μm TOSOH BIOSEP TSKgel Amide-80 column with a 2.0 x 10 mm guard cartridge of the same packing (Montgomeryville, PA).

AMS Measurement of Total ^{14}C in Plasma. All biological samples were shipped from Merck Research Laboratories to the Lawrence Livermore National Laboratory on dry ice. Upon receipt, samples were unpacked, inspected, and stored frozen (-20°C) pending analysis. Samples were converted to elemental carbon for AMS quantitation of ^{14}C in a two-stage process. First, samples were oxidised to CO_2 in individual sealed tubes containing cupric oxide, followed by reduction onto approximately 10 mg iron catalyst in a second tube over zinc and titanium hydride (Vogel, 1992, Ognibene et al., 2003). This process requires approximately 400 μg of carbon, corresponding to the amount available in approximately 10 μL of plasma. Aliquots were obtained from the defrosted plasma samples and placed in quartz combustion tubes. The exact sample mass in this process is not important, as AMS measures an isotope ratio that is converted to equivalent amounts of drug per mL or mg of sample using the known isotopic content of the labeled compound and the average or individual carbon content of each sample (Vogel et al., 2001; Vogel and Love, 2003).

Reduced samples were pressed into aluminum holders, mounted in a wheel

containing up to 58 samples of which six represented standards with well-defined isotopic compositions, and analysed by the 1 megaVolt AMS spectrometer at Lawrence Livermore National Laboratory (Ognibene et al., 2002). Each sample was measured to > 15,000 ^{14}C counts at least 3 times and, if necessary, up to 7 times such that the last 3 measurements agreed to within 3% of each other without monotonic trend. The reported isotope ratio was derived by comparison of the measured isotope ratio to that of the known standards. Results were reported in the unit "Modern", which is equivalent to 97.8 attomole of ^{14}C per mg carbon or 6.11 fCi of ^{14}C per mg carbon or 0.0136 dpm per mg carbon. "Modern" represents the expected natural level of ^{14}C in the biosphere and is a NIST-traceable quantity (NIST standard reference material 4990). AMS-derived ^{14}C concentrations, which include parent compound as well as metabolites, were converted to nanogram equivalents of Compound A per mL (ng eq./mL) using the specific activity of the dosed compound.

Pharmacokinetic analysis. Plasma concentrations of Compound A (measured by LC-MS/MS) and total ^{14}C levels (measured by AMS) were used to estimate pharmacokinetic parameters for each treatment group and subject. The areas under the plasma concentration-time curve (AUC_{0-t}) were calculated from the first time point up to the last time point with measurable drug concentration using the linear trapezoidal or linear/log-linear trapezoidal rule. The remaining area under the plasma concentration-time curve (AUC_{t-inf}) was estimated by dividing the observed concentration at the last time point by the elimination rate constant, and this value was used to estimate the AUC_{0-inf}. The percentage AUC extrapolated (% AUC Extrap) was a function of $(AUC_{0-inf} - AUC_{0-t}) \cdot 100/AUC_{0-inf}$. The i.v. plasma clearance was calculated by dividing the dose by AUC_{0-inf}.

The alpha, beta and gamma half-lives ($T_{1/2\_}$, $T_{1/2\_}$, and $T_{1/2\_}$ respectively) were determined by fitting the plasma concentration-time data to a triexponential decay model with intravenous bolus input and linear first-order elimination from the central compartment using iterative unweighted nonlinear least squares regression analysis. The regression lines through the plots of observed versus predicted concentrations did not differ from the line of identity, and no bias was observed. The volume of distribution at steady state (Vd_{ss}) was obtained from the product of the plasma clearance and the mean residence time. The maximum plasma concentration (C_{max}) and the time at which maximum concentration occurred (T_{max}) were obtained by inspection of the plasma concentration-time data. Absolute bioavailability was determined from dose-adjusted ($AUC_{0\text{-}inf}$) ratios.

RESULTS

The pharmacokinetics of Compound A in dogs based on total ^{14}C levels determined by AMS were obtained following both intravenous and oral administration. For comparison, the mean pharmacokinetic parameters of Compound A as determined by a specific LC-MS/MS assay were derived from a separate study in which unlabeled compound was administered to dogs, and are presented in Table 1. The average pharmacokinetic parameters derived from the AMS study are summarized in Table 2. Compound A exhibited low plasma clearance (CLp) (average 4.4 mL/min/kg) and multiphasic kinetics with a long terminal elimination half-life ($T_{1/2\_}$) (average 19.4 hr). The volume of distribution at steady state (Vd_{ss}) was large (8.2 L/kg) and exceeded total body water. Following oral dosing, the absolute bioavailability of Compound A was high (average 82%) with a T_{max} and C_{max} of 0.38 hr and 12.7 ng equiv./mL, respectively. These AMS-derived parameters were comparable to the pharmacokinetic properties obtained for Compound A following analysis by LC-MS/MS (see Table 1).

Plasma concentration versus time profiles for Compound A after a single 0.02 mg/kg i.v. bolus or oral dose were determined by AMS as well as by LC-MS/MS and are presented in Figs. 2 and 3. It should be noted that due to the small dose administered to dogs, it was not possible to quantify plasma levels beyond 2 hr post dose by LC-MS/MS as the levels had dropped below the lower limit of quantitation (2 ng/mL). The much more sensitive technique of AMS permitted monitoring of plasma concentrations of Compound A down to 0.2 ng/mL from which a pharmacokinetic profile at the 0.02 mg/kg microdose was obtained.

The plasma concentration-time profiles of total ^{14}C versus unchanged Compound A

following a single 1 mg/kg oral dose are presented in Fig. 4 and the mean pharmacokinetic parameters are summarised in Table 3 along with those of the 0.02 mg/kg oral dose for comparison. The $AUC_{0\text{-}t}$ and C_{max} ratios of parent Compound A to total ^{14}C levels were 0.77 and 0.76, respectively, indicating that the parent compound accounted for the majority of the ^{14}C in plasma. The $AUC_{0\text{-}t}$ and C_{max} at 1 mg/kg were 2846 ng equiv. · hr/mL and 659 ng equiv./mL respectively. At the 50-fold lower dose of 0.02 mg/kg, the $AUC_{0\text{-}t}$ and C_{max} were proportionately lower (54.5 ng equiv. · hr/mL and 12.7 ng eq./mL, respectively) i.e., 2725 ng equiv. · hr/mL and 635 ng eq./mL, respectively when dose normalized for a dose of 1 mg/kg.

DISCUSSION

Low or microdose studies in humans potentially have an important place in the drug development process in as much as they can offer an early determination of the pharmacokinetic properties of a compound and thus assist in the selection of those drug candidates that possess optimal disposition properties for further evaluation in the clinic (Lappin and Garner, 2003). However, an underlying assumption with this approach is that the PK at the microdose reflect the kinetics at the pharmacological dose. This is especially important for compounds that are retained in a target tissue, such as anti-osteoporotics in the bone (Stepensky et al., 2003). In situations where the target tissue cannot be directly sampled, disposition properties of compounds can be estimated from the long-term kinetics of elimination through plasma or excreta (Gregory et al., 1998). Pharmacokinetic studies at sub-pharmacological doses, therefore, need to utilise analytical procedures that can define not only the initial absorption and distribution phases accurately, but also any slow elimination phase during which analyte concentrations may be very low. AMS has extremely high sensitivity for tracing isotopically labeled compounds, utilizes small sample sizes (5-10 μL) and can provide detailed kinetic profiles at low drug concentrations.

Currently there are no published comparisons of the kinetics of a pharmaceutical compound at pharmacological versus sub-pharmacological doses employing microdosing strategies. The present study thus provides the first description of the pharmacokinetics of a development candidate assessed under these two dosing regimens and demonstrated that, in the case of Compound A, the kinetics of absorption, distribution and elimination in the dog appear to be linear over a 50-fold dose range. While there is no guarantee that

this finding will replicate in human subjects, these pilot studies in dogs nevertheless provide some reassurance that microdose studies with Compound A in humans would provide meaningful predictions of kinetics at higher (pharmacological) doses. From a methodological standpoint, microdose/AMS studies are labour-intensive with respect to sample collection and handling due to the need to prevent contamination by extraneous sources of ^{14}C. However, when appropriate procedures are put in place, excellent concordance can be obtained between measurements conducted by AMS and LSC (Kaye et al., 1997; Garner et al., 2000; Young et al., 2001). In the present study, plasma levels of unchanged Compound A following a 1 mg/kg oral dose, measured by a specific LC-MS/MS assay, proved to be closely similar to those of total ^{14}C, assessed by AMS. Therefore, it could be concluded that Compound A accounted for virtually all of the drug-related material in the circulation of dogs given a pharmacological dose. However, when the animals were treated with Compound A at a microdose (0.02 mg/kg), either orally or by the intravenous route, the limited sensitivity of the LC-MS/MS assay did not allow plasma concentrations of parent drug to be followed beyond the 2 hr time point, whereas levels of total ^{14}C remained well above limits of detection by AMS through the final (80 hr) time point. More importantly, the greatly enhanced sensitivity of AMS revealed the multiphasic kinetic profile of Compound A, which was not evident in the corresponding dataset from the LC-MS/MS analyses, thereby reinforcing the need for AMS in microdose studies.

Previous reports on the use of AMS strategies in support of early drug development have focussed largely on the benefit associated with exposure of human subjects to very low levels of radiation (Young et al., 2001; Garner et al., 2002.). The dose

of radioactivity used in the present dog study was at least 500 to 1000-fold lower than would be employed for a conventional high dose radiotracer study in which LSC was to be used for monitoring drug-related material in plasma and excreta. According to European regulations, exposure to < 120 nCi of radioactive test material does not require special regulatory approval since the level of exposure to ionizing radiation from such small doses is lower than that expected from the background environment. In the U.S., radiological materials with specific activities less than 50 nCi/g can be disposed off by licensed radioactivity users as non-radioactive waste to a limit of 1 μCi per year (as stipulated in the Code of Federal Regulations 10-CFR-20.2005). Currently, there are two published examples of clinical AMS studies where the disposition of pharmaceuticals was defined in humans following administration of low levels of ^{14}C-labeled drugs (Young et al., 2001; Garner et al., 2002). The study reported in the present communication highlights a further benefit of AMS for pharmaceutical applications, namely the definition of the full pharmacokinetic profile of a test compound, even following a microdose, that would not have been possible by conventional techniques due to limitations of sensitivity. Human PK measurements have also been determined over long time periods using AMS to quantify low levels of nutrients in a general population including healthy young adults (Dueker et al. 2000; Lemke et al., 2003).

In summary, the current study successfully validated the technique of accelerator mass spectrometry by investigating the disposition of Compound A in dogs which afforded pharmacokinetic data in excellent agreement with those obtained via conventional techniques. The study also validated for the first time the use of a microdose strategy to assess the pharmacokinetic properties of a drug candidate by

demonstrating linearity across a 50-fold dose range. Investigators of this type in animal species should prove valuable in assessing the suitability of microdose/AMS approaches for exploratory pharmacokinetic studies in human subjects.

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FOOTNOTES

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[1]Abbreviations

FIGURE LEGENDS

FIG. 1. Structure of [^{14}C] Compound A and internal standard. The asterisk in the structure of Compound A denotes the position of the ^{14}C radiolabel.

FIG. 2. Mean plasma concentration-time profiles for Compound A (ng/mL; _) and total ^{14}C levels (ng equiv./mL; _) on a linear scale (A) and semi-log scale (B) following intravenous administration of 0.02 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n=2).

FIG. 3. Mean plasma concentration-time profiles of Compound A (ng/mL; _) and total ^{14}C levels (ng equiv./mL; _) following oral administration of 0.02 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n=2).

FIG. 4. Mean plasma concentration-time profiles of Compound A (ng/mL; _) and total ^{14}C levels (ng equiv./mL; _) following oral administration of 1 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n=2).

TABLE 1

Mean Pharmacokinetic Parameters of Compound A in Dogs Following Administration of a Pharmacological Dose as Determined by LC-MS/MS analyses

Parameter	**Intravenous Dose**	**Oral Dose**
Dose (mg/kg)	0.4	1.0
$AUC_{0\text{-}t}$ (ng · hr/mL)	1056.7 ± 168.2	2727.3 ± 400.8
$AUC_{0\text{-}inf}$ (ng · hr/mL)	1166.0 ± 154.2	3016.0 ± 395.2
% AUC extrapolated	9.8 ± 2.7	9.6 ± 3.4
CLp (mL/min/kg)	5.8 ± 0.7	-
$T_{1/2\_}$ (hr)	0.1 ± 0.1	-
$T_{1/2\_}$ (hr)	0.8 ± 0.2	-
$T_{1/2\_}$ (hr)	17.5 ± 0.7	-
Vd_{ss} (L/kg)	8.6 ± 2.4	-
C_{max} (ng/mL)	-	849.3 ± 168.2
T_{max} (hr)	-	0.44 ± 0.38
Oral bioavailability (%)	-	103 ± 5

The $AUC_{0\text{-}t}$ interval was from 0-72 hr. Data are presented as Mean ± SD (n=4).

TABLE 2

Pharmacokinetic Parameters of Compound A in Dogs (n=2) Following Administration of a Microdose as Determined by Accelerator Mass Spectrometry

Parameter	**Intravenous Dose**	**Oral Dose**
Dose (mg/kg)	0.02	0.02
$AUC_{0\text{-}t}$ (ng equiv. · hr/mL)	66.5; 66.7	59.4; 49.5
$AUC_{0\text{-}inf}$ (ng equiv. · hr/mL)	78.4; 73.0	66.0; 57.3
% AUC extrapolated	15.0; 8.4	10.0; 14.0
CLp (mL/min/kg)	4.3; 4.6	-
$T_{1/2\_}$ (hr)	0.13; 0.05	-
$T_{1/2\_}$ (hr)	0.71; 0.71	-
$T_{1/2\_}$ (hr)	22.7; 16.0	-
Vd_{ss} (L/kg)	9.4; 6.9	-
C_{max} (ng equiv./mL)	-	12.3; 13.0
T_{max} (hr)	-	0.5; 0.25
Oral bioavailability (%)	-	84; 78

The $AUC_{0\text{-}t}$ interval was from 0-80 hr.

TABLE 3

Average Oral Pharmacokinetic Parameters of Compound A in Dogs Over a 50-Fold Dose Range as Determined by Accelerator Mass Spectrometry Following Administration of a Pharmacological Dose verus a Microdose

Parameter	**Pharmacological Dose**	**Microdose**
$AUC_{0\text{-}t}$ (ng equiv. · hr/mL)	2846	2725
$AUC_{0\text{-}inf}$ (ng equiv. · hr/mL)	3113	3085
% AUC extrapolated	8.3	12
C_{max} (ng equiv./mL)	659	635
T_{max} (hr)	0.5	0.38

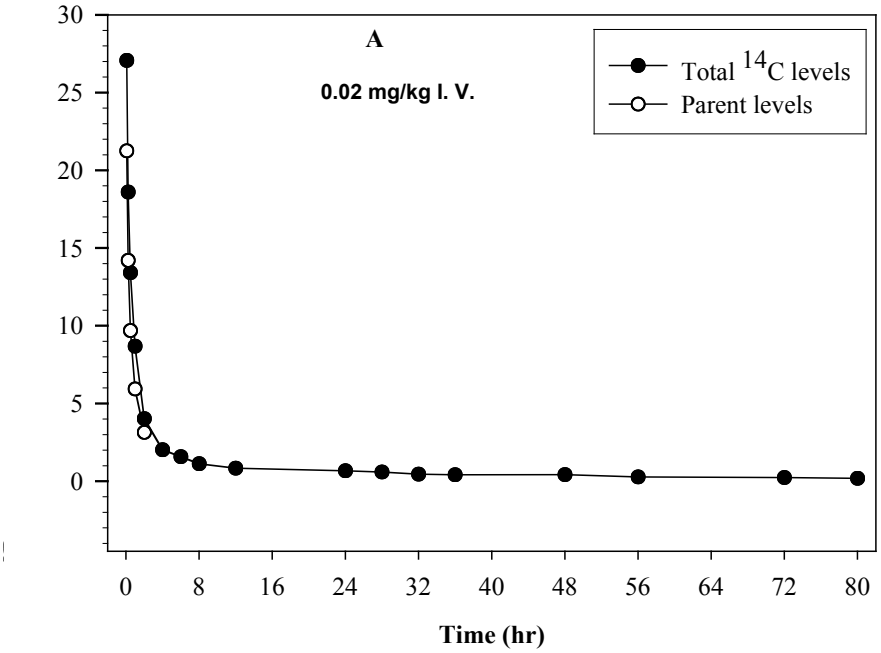
The $AUC_{0\text{-}t}$ interval was from 0-80 hr. Data are presented as an average of 2 determinations. The dose administered to dogs was 1 mg/kg for the pharmacological and 0.02 mg/kg for the microdose study. The microdose parameters in the table are presented as dose-normalized for a dose of 1 mg/kg.

FIG.1

Compound A

Internal standard

FIG. 2


A
0.02 mg/kg I. V.
Total ^{14}C levels
Parent levels
30
25
20
15
10
5
0
0
8
16
24
32
40
48
56
64
72
80
Time (hr)

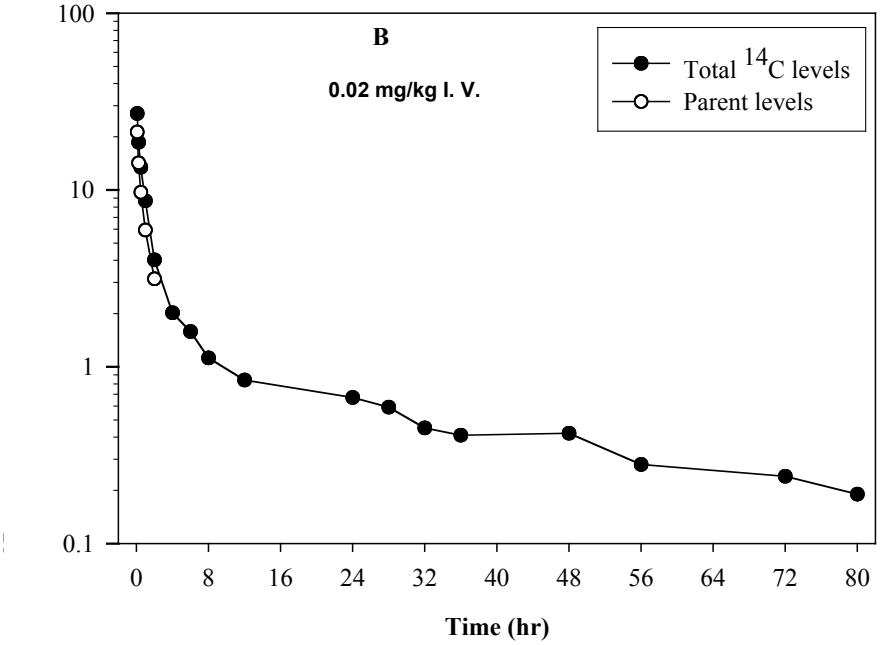

B
0.02 mg/kg I. V.
Total ^{14}C levels
Parent levels
100
10
1
0.1
0
8
16
24
32
40
48
56
64
72
80
Time (hr)

FIG. 3

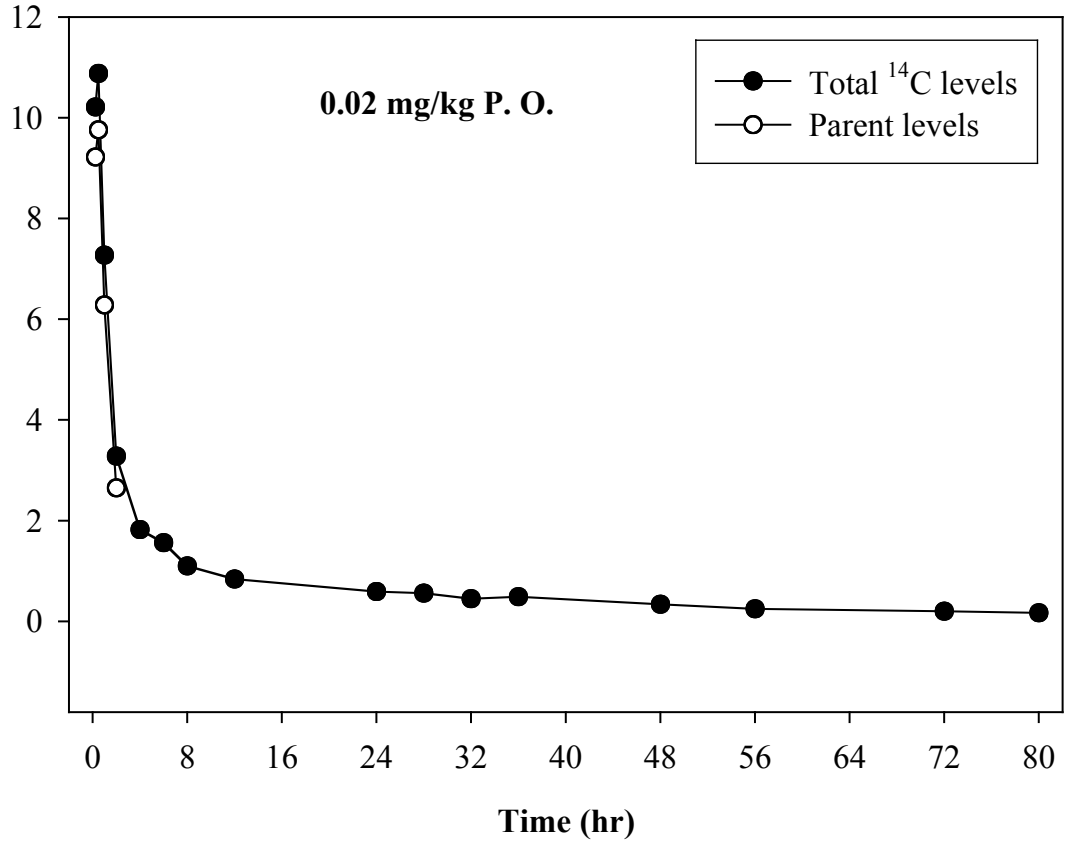
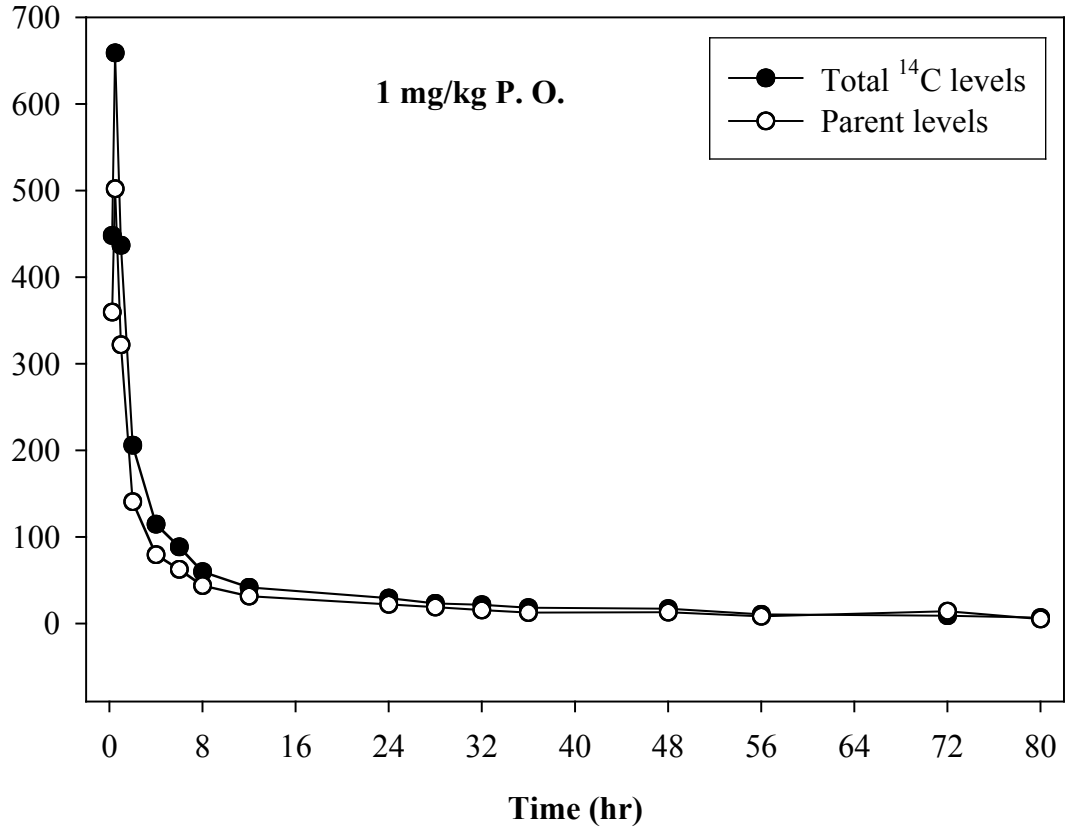

0.02 mg/kg P. O.
Total ^{14}C levels
Parent levels
12
10
8
6
4
2
0
0
8
16
24
32
40
48
56
64
72
80
Time (hr)

FIG.4


700
600
500
400
300
200
100
0
1 mg/kg P. O.
Total ^{14}C levels
Parent levels
0
8
16
24
32
40
48
56
64
72
80
Time (hr)