NOV 10 1992

NOV 10 1992

PHARMACOLOGIST'S REVIEW

NDA 20,259

Date Submitted: 4-28-92 (N Document)
Date Assigned: 4-28-92
Date Review Completed: 11-3-92

Related INDs:

Sponsor: Burroughs Wellcome Co.
Research Triangle Park, N.C. 27709

Drug: atovaquone (566C80; 566C); 2-[trans-4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone

Formulation: micronized drug in oral tablets

Proposed Indication: Treatment of Pneumocystis carinii pneumonia

Abbreviations:

PCP	Pneumocystis carinii pneumonia
0.25% MC	0.25% w/v aqueous methylcelluose
IV	intravenous
po	oral or orally
566C	BW 566C80, A566C, atovaquone
HNQ	hydroxynaphthoquinone (HNQ)

INTRODUCTION

Atovaquone (566C; 566C80; A566C) is a novel 3-substituted, 2-hydroxynaphthoquinone that exerts broad spectrum antiprotozoal activity. It was developed originally as an antimalarial agent. Atovaquone showed activity for the treatment and prophylaxis of PCP in the immunosupressed rat model and for treatment in the mouse toxoplasmic encephalitis model.

PHARMACOLOGY

Atovaquone, like other hydroxynaphthoquinones, is a potent and selective inhibitor of the mitochondrial electron transport chain at the cytochrome bc1 complex in several parasitic protozoa. This leads to inhibition of pyrimidine synthesis in these organisms since dihydroorotate dehydrogenase, a key enzyme in the pyrimidine biosynthetic pathway, is linked to this transport chain via ubiquinone. Plasmodium cannot salvage preformed pyrimidines as can mammalian systems. In contrast, Pneumocystii and Toxoplasma can salvage preformed pyrimidines so the mechanism of action against these protozoa is unclear.

Comment: The specificity of atovaquone for inhibition of the mitochondrial bc1 complex in pneumocystis carinii relative to

inhibition of the mitochondrial bc1 complex in mammalian cells is not known. Related 1,4 hydroxynaphthoquinones have been shown to inhibit the bc1 complex of the mitochondrial respiratory chain of mammalian cells. This is discussed in greater detail on page 47 of this review.

PHARMACOKINETICS

The nonclinical pharmacokinetics of atovaquone following intravenous or oral (gavage or feed admixture) administration have been evaluated in single dose and multiple dose studies. Pharmacokinetic studies in the mouse, rat, and dog include an evaluation of the absorption, distribution, metabolism, excretion, absolute bioavailability, and dose proportionality. Reports verifying exposure to the drug in toxicology studies were included.

During the development of atovaquone, two validated analytical methods were used to determine the levels of atovaquone in biological fluids. A [redacted] method was used in early studies. This method had been designed to measure the levels of atovaquone required for a malaria indication and had an upper limit of quantitation of only approximately [redacted] To measure the higher levels of atovaquone in nonclinical studies and clinical studies for a PCP indication, a [redacted] method was developed. The pharmacokinetic studies conducted in the United Kingdom (UK) used a different version of the [redacted] method that was used in studies conducted in the Unites States (US). The submission contains studies showing that the [redacted] method and the two [redacted] methods were cross validated and shown to produce comparable results.

The pharmacokinetic studies included in this submission are listed below, grouped according to the species tested. In all oral studies, unless otherwise specified, atovaquone was administered as a micronized drug suspended in 0.25% aqueous methylcellulose. The list is followed by a discussion of the study results for each species and an end summary. The results of pharmacokinetic studies performed in conjunction with toxicology studies are presented in the toxicology section.

Mouse Pharmacokinetic Studies

1. An Experiment to Demonstrate the Presence of BW 566C80 in Mouse Plasma Following a Single Oral Dose of 100 mg/kg. Study BDAM/86/05.

2. The Disposition of 14C-566C80 in Male and Female CD-1 Mice Following Oral Administration of 14C-566C80 at the Dose Level of 100 mg/kg. Study BPAT/89/0101.

3. A Qualitative Whole Body Autoradiographic Study of the Distribution of Radioactivity in Male CD-1 Mice Following Oral Administration of = 566C80 at 100 mg/kg. BPAT/91/0064.

4. The Excretion and Metabolic Profiles of Total Radioactivity in CD-1 Mice Following a Single Dose of 14C-566C80. BPAT/91/0071.

5. The Excretion and Metabolic Profiles of Total Radioactivity in CD-1 Mice Following Multiple Oral Doses of 566C80 at 200 mg/kg and a Final Single Oral dose of 14C-566C80 at 200 mg/kg. BPAT/91/0072.

Rat Pharmacokinetic Studies

1. Plasma Concentrations of Compounds 58C80, 993C76, 568C80, 566C80, and 379C80 in Rats After a Single Dose. Study BDAM/81/01.

2. The Plasma Pharmacokinetics of A566C in the Male Wistar Rat Following a Single Oral or Intravenous Administration of (14C)-A566C at 5 mg.kg-1. Study BPAT 87/55.

3. The Pharmacokinetics of Total Radioactivity in the Male Wistar Rat Following Single Oral Administration of 14C-566C80 at 5, 20, 100, and 500 mg/kg. Study BPAL/86/19.

4. A Pharmacokinetic Study of 566C80 in Rats After Single Oral Doses of 20, 100, 500, and 1000 mg/kg (MB053). Study TBZZ/91/0036.

5. A Pharmacokinetic Study of 566C80 in Rats After Multiple Doses of 100 mg/kg/day (MB064). Study TBZZ/91/0037.

6. A Pharmacokinetic Study of 566C80 After a Single Intravenous Dose (20 mg/kg) to Male and Female Rats (PDM 0003). Study TBZZ/91/0039.

7. Qualitative Wholebody Autoradiographic Study of the Distribution of Radioactivity in Male and Female Albino and Male Pigmented Rats Following the Oral Administration of 14C-566C80 at 5 mg.kg-1. Study BPAT/87/35.

8. A Qualitative Whole Body Autoradiographic Study of the Distribution of Radioactivity in Pregnant Rats Following a Single Oral Administration of 14C-566C80 at 5 mg/kg. Study BPAT/89/40.

9. Tissue Distribution of 14C-A566C in the Male Wistar Rat Following Oral Administration at 5 mg/kg. BPAT/88/60.

10. Tissue Distribution Study of 14C-566C80 in the Sprague-Dawley Rat Following a Single Oral Administration of 500 mg/kg.

BPAT/90/0066.

11. The Tissue Distribution of 14C-A566C in the Male Wistar Rat Following Oral Administration at 5 mg/kg. BPAT/88/60.

12. Tissue Distribution Study of 14C-566C80 in the Sprague-Dawley Rat Following a Single Oral Administration of 500 mg/kg. BPAT/90/0066.

13. Tissue Distribution of 566C80 in the Rat Following Intravenous Administration of Medisperse Formulation. BDDM/90/0068.

14. The Milk Transfer of 14C-566C80 in the Rat Following a Single Oral Administration at Dose Levels of 10 mg/kg or 250 mg/kg. BPAT/91/0012.

15. The Placental Transfer of 14C-566C80 in the Rat Following a Single Oral Administration at 1000 mg/kg. BPAT/90/0068.

16. An Excretion Balance Study in Male and Female Wistar Rats Following a Single Oral Dose of 14C-566C80 at 5 mg/kg. Study BPAT 87/12.

17. An Excretion Balance Study in Male Wistar Rat Following Oral Administration of 14C-566C80 at 10 mg/kg and 250 mg/kg. BPAT/90/0025.

18. The Biliary Excretion of Total Radioactivity in the Wistar Rat Following Oral Administration of 14C-566C80 at a Dose Levels of 100 mg/kg. BPAT/91/0131.

Dog Pharmacokinetic Studies

1. A Pharmacokinetic Study of 566C After a Single Intravenous Dose to Beagle Dogs (PDM 001). Study TBZZ/91/0043.

2. The Rates and Routes of Elimination and Pharmacokinetics of (14C)-A566C by Male and Female Beagle Dogs Following a Single Oral or Intravenous Administration at 5 mg kg-1. Study BPAT/87/57.

3. Excretion Balance and Pharmacokinetic Study in the Beagle Dog Following Oral Administration of 14C-566C80 at 10 and 250 mg/kg. BPAT/90/0026.

4. The Comparative Bioavailability of 566C80 following Oral Administration as 3 Test Formulations to Beagle Dogs. BPAT/89/57.

5. The Exposure of 566C80 in the Dog Following Multiple Oral

Administration of 566C80 at 100 mg/kg/day for 14 days. BPAT/90/0065.

Rabbit Pharmacokinetic Studies

1. Excretion and Plasma Profile of Radioactivity in the Rabbit Following Oral Administration of 14C-566C80. BPAT/90/0067.

2. Report of a Qualitative Whole Body Autoradiographic Study of the Distribution of Radioactivity in Pregnant Dutch Rabbits Following a Single Oral Administration of 14C-566C80 at 300 mg/kg. BPAT/89/0058.

Monkey Pharmacokinetic Studies

1. A Qualitative Wholebody Autoradiographic Study of the Distribution of Radioactivity in Male Aotus Monkeys Following the Oral Administration of (14C)-566C80 at 10 mg.kg-1. Study BPAT/87/41.

In Vitro Studies

1. An Assessment of the In Vitro Plasma Protein Binding of 566C80. Study BPAL/87/12.

2. A Study of Human Plasma Protein Binding of Quinine in the Presence of Absence of 566C80. Study BPAT/88/0063.

3. Protein Binding of 566C80 in Human Plasma and Possible Binding Displacement Interaction with Phenytoin. TBZZ/90/0057.

4. In Vitro Metabolism of Hydroxynaphthoquinones by Hepatic Microsomes From Man, Cynomologus Monkey, and Mouse. Study BDAM/86/04.

5. A Study of Human Plasma Protein Binding of Quinine in the Presence or Absence of 566C80. BPAT/88/0063.

6. Protein Binding of 566C80 in Human Plasma and Possible Binding Displacement Interaction With Phenytoin. TBZZ/90/0057.

7. A Study to Investigate the Effects Upon Hepatic Drug Metabolizing Enzyme Activity in Male CD-1 Mice of Daily Oral Administration of 566C80 for 5 and 10 Consecutive Days at 50 and 200 mg/kg. Study BPAT/91/0066.

8. A Study to Investigate the Effects Upon Drug Metabolizing Enzyme Activity in Male Wistar Rats of Daily Oral Administration of 566C80 for 5 and 10 Consecutive Days at 10 and 100 mg/kg. Study BPAT/91/0002.

Additional Studies and Reports

1. An Analytical Method for the Quantification of 566C80 in Plasma. Study BDGC/87/0004.

2. Validation of the Plasma 566C80 Analytical Method. Study BDGC/89/0004.

3. Validation of a [redacted] Assay for the Measurement of 566C80 in Plasma. Study TBZZ/90/0036.

4. An Analytical Method for the Quantification of atovaquone in Plasma Using [redacted] and [redacted] Study BDCA/91/0004.

Mouse Pharmacokinetic Studies

An initial study was performed in male CD-1 (3/group) to verify absorption of atovaquone (100 mg/kg) following a single oral dose. The presence of atovaquone in plasma at up to 24 hours post-dose was verified using UV spectroscopy and co-chromatography. Plasma levels were not determined.

In a disposition study, CD-1 mice (33/sex) received oral doses of 14C-atovaquone (100 mg/kg). The study was conducted in accordance with GLP at [redacted] using [redacted]. Urine and feces were collected for the periods 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post-dose. Urine was also collected for the periods 0-6 and >6-24 hours post-dose. The major route of excretion of radioactivity was the feces, with urinary excretion accounting for less than 1% of the dose. In the 0-24 hour period, the mean percent dose recovered in the urine and feces was 0.35% and 69.92%, respectively. For the 0-168 hour period, the recovery in urine and feces was 0.58% and 88.89%, respectively.

In an autoradiographic study, male CD-1 mice received 14C-atovaquone (100 mg/kg) and were sacrificed at 2, 8, and 24 hours after dosing. At all time points, the highest levels of radioactivity were seen in the contents of the stomach and intestines. Radioactivity was also distributed to liver, heart blood, and brown fat; lower levels were seen in the kidney. At 8 and 24 hours, barely detectable levels were observed in the brain and spinal cord.

The excretion and metabolic profile of oral, intravenous, and intraperitoneal 14C-atovaquone (100, 5, and 5 mg/kg), respectively) was studied in male and female CD-1 mice. During the first 48 hours post-dose, percent of radiolabelled dose recovered in the feces from the oral, intravenous, and

intraperitoneal dose were [redacted] respectively; urinary recoveries were [redacted] respectively. Metabolic profiling showed 1 major radiolabeled peak that was identified as unchanged atovaquone. Three other minor metabolites were unidentified. These metabolites accounted for approximately 8%, 18% and 17% or an oral, intravenous, and intraperitoneal dose, respectively.

In another excretion and metabolic profile study, mice received atovaquone by gavage once daily for 10 days and 14C-atovaquone on the 11th day of the study. More than — of the radiolabelled dose was recovered in the feces within the first 48 hours. Radioactivity was excreted almost exclusively in the feces, with urinary excretion accounting for less than — of the dose. There were no apparent differences between the elimination profiles of male and female mice. Analysis of fecal samples showed a major peak, unchanged atovaquone, and three minor components that were not identified. The minor components accounted for 6.8% and 9.6% of the administered dose in male and female mice, respectively.

Rat Pharmacokinetic Studies

Sixteen rat pharmacokinetic studies were included in this submission. In a preliminary study, levels of several naphthoquinones, including atovaquone, were determined in rat plasma. Male COBS rats (3/group) received a single dose of either 58C80CA, atovaquone, 379C80CB, and 993C7WC (100 mg/kg) by gavage. Concentrations of drug in plasma at 1, 2, 4, 6, 12, 24, and 48 hours post-dose were determined by [redacted] analysis. The following values were determined for 566C: AUC0-inf= 820 ug/ml/hr; $t_{1/2}$ = 16 hr; k_a = 0.38; k_{el} = 0.042.

In another study, male Wistar COBS rats (4 groups; 3 rats/group) received a single oral dose of ^{14}C-atovaquone (0, 5, 20, 100, or 500 mg/kg) by gavage. Blood samples for plasma analysis were taken from the lateral tail vein at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours post-dose. Table 1 shows the calculated pharmacokinetic values. This table was taken from Volume 1.15, page 4658 of the submission.

Table 1. Pharmacokinetics of 14C-atovaquone following a single oral dose to male rats

Dose mg/kg	Tmax h	Cmax ug equivalents/ml	$T_{1/2}$ h	AUC ug/ml/hr	Cl/F ug/ml/h	no. rats
5	6-8	9.7	17.6	236	21.2	3
20	6-8	30.0	25.6	1178	17.0	3
100	6-8	56.9	15.2	1493	67.0	3
500	6-8	77.6	17.0	2238	223.4	2

Peak plasma concentration levels increased with increasing dose in a non-linear manner. A 100-fold increase in dose level resulted in only an 8-fold increase in Cmax and a 9-fold increase in AUC. This is thought to be due to a decrease in the fraction of dose absorbed at the higher doses. The data suggested the use of 500 mg/kg as the top level in the toxicology studies since higher doses may not significantly increase systemic exposure to this compound.

The pharmacokinetics of atovaquone (5 mg/kg) after i.v. and p.o. administration were compared in male Wistar COBS rats. The rats were fasted overnight before dosing and 3 hours after dosing; otherwise food and water were allowed ad libitum. Rats received a single dose of 14C-atovaquone (5 mg/kg) either intravenously (in glycofurol) or orally. Sample times were 15 and 10 minutes pre-dose; 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose. Plasma samples were analyzed for radioactivity by [redacted] and for unchanged drug by [redacted] The results are shown below in Table 2.

Table 2. Pharmacokinetics of atovaquone following administration of 5 mg/kg either IV or PO to male Wistar rats

PARAMETER	IV	PO
Dose	3.67	2.62
Vc $1.kg^{-1}$	0.08	-
Vd	-	0.20
Vd beta	0.20	-
AUC (0-inf)	556	85
Cl	0.11	0.11
$t_{1/2}$ k_a	-	2.28
t 1/2 alpha	0.39	-
t 1/2 beta	21.77	20.97
Cmax	-	2.15
Tmax	-	8.18
Absolute bioavailability		21%

Plasma levels of total radioactivity and unchanged drug produced almost identical profiles, suggesting the absence of metabolite(s) in plasma following either oral or intravenous administration. After the intravenous dose, pharmacokinetics followed a two-compartment model with the parameters in the above table. The initial distribution half-life was 0.39 hours, the volume of distribution during the elimination phase was limited (0.2 L/kg), total plasma clearance was slow (0.11 ml/min), and elimination was slow (t1/2=21.77h). After the oral dose, the pharmacokinetics followed an apparent one-compartment open model. Absorption was slow (absorption t 1/2 =2.28 hr) and incomplete. The distribution of unchanged drug was relatively low (0.2 L/kg) and the elimination half-life was slow (0.11 ml/min).

Male and female Sprague-Dawley rats each received a single dose of atovaquone (20 mg/kg in cosolvent formulation) by bolus injection into the tail vein. Blood samples for determination of plasma concentrations of 566C were obtained at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 23, 30, 48, 72, and 96 hours after dosing.

Plasma levels of atovaquone were detectable up to 96 hours after the dose. Twelve hours after dosing, a second peak was observed in the plasma concentrations of atovaquone in male and female rats (53.4±15.5 and 64.3±24.1 ug/ml, respectively). This second peak was observed in previous animal and human studies using single oral doses of atovaquone, and suggests enterohepatic recycling of atovaquone. The calculated pharmacokinetic parameters are shown in Table 3.

Table 3. Noncompartmental pharmacokinetics of atovaquone (20 mg/kg) IV administration to rats

Sex	Cmax* (ug/ml)	AUC(0-inf) (ugxhr/ml)	t1/2 (hr)	CL (L/hr)
Male	101	1592	27	0.013
Female	95	2394	31	0.008

* The Cmax represents the plasma concentration at 5 minutes after the injection, which was the first sampling time.

The tissue distribution of 14C-atovaquone in male and female albino Wistar COBS and male pigmented PVG/C rats following a single oral dose of 14C-atovaquone was studied using [redacted] Wistar COBS rats (8/sex) and PVG/C rats (4 males) received a single dose of 14C-atovaquone (5 mg/kg) by gavage; food and water were allowed ad libitum throughout the study. The rats were killed at 1, 7, 36, and 96 hours after dosing. A blood sample was taken from each rat prior to death for determination of radioactivity in blood. The highest blood levels of radioactivity were observed at 7 hours post-dose. Levels declined by 36 hours and were very low at 96 hours.

At 1 hour post-dose, the majority of the drug remained in the stomach and upper small intestine. The highest levels of absorbed drug were associated with the blood, with levels in liver and fat being comparable to but lower than the blood level. No distribution differences were observed between sexes. At 7 hours post-dose, residual drug remained in the stomach and small intestine but most was associated with the lumen of the large intestine and colon. Drug disposition was similar to that at 1 hour, with highest levels associated with blood, fat and liver, lower levels in kidney, and even lower levels elsewhere. At 36 hours, most radioactivity was in colon or fecal pellets, with systemic levels just greater than the detection limits of the X-ray film. At 96, radioactivity was discernible in the colon and fecal pellets and systemic levels were barely detectable.

Comment: In summary, the pattern of distribution for 14C-atovaquone showed limited absorption and restricted distribution. Little radioactivity associated with the kidney and high levels associated with the GI tract suggests that urinary excretion is insignificant and the major route of elimination of drug and/or metabolites is fecal. Systemic distribution appears restricted to liver, fat, and blood. Atovaquone and/or its metabolites do not bind to melanin and are not retained by any tissue or organ.

In another distribution study performed at [redacted], rats received 14C-atovaquone (5 mg/kg) by gavage. Levels of total radioactivity were determined in selected tissues at 1, 7, 36,

and 96 hours post-dose. Recovery of radioactivity ranged from [redacted] of the dose. The main route of excretion was the feces, with only [redacted] of the drug eliminated in the urine. A summary of the results is shown in the table below (page 5114, Vol. 1.17).

Table 4. Radioactivity Levels in Rat Tissues Following a Single Oral Dose of 14C-atovaquone

	Mean Percent of Dose			
Sample	1h	7h	36h	96h
Whole blood	1.94	3.70	0.88	0.21
Liver	0.77	1.14	0.50	0.10
Kidney	0.08	0.10	0.03	0.01
Brain	0.01	0.01	0.00	0.00

In another distribution study, Sprague-Dawley rats received a single dose of 14C-atovaquone (500 mg/kg) by gavage and were sacrificed at 6, 24, 72, and 168 hours post-dose.

The distribution of a single oral dose of 14C-atovaquone (5 mg/kg) in pregnant rats was studied using qualitative wholebody autoradiography. The rats were sacrificed at 7, 18, 24, and 72 hours after dosing. The results were similar to other distribution studies described above, with poor absorption and limited distribution of atovaquone. Radioactivity passed through the placenta and was distributed to the skin and fatty tissues of the fetus. Although the majority of the radiolabel was excreted from the maternal rat in the feces by 72 hours, the levels of radioactivity did not diminish in the fetus. This indicated poor elimination of the radiolabel from the fetus.

14C-Atovaquone (10 and 250 mg/kg in methylcellulose) was administered orally to lactating rats. Plasma and milk levels of radioactivity were determined at 1, 7, 30, and 96 hours post-dose. The kinetics of radioactivity in milk paralleled the kinetics in plasma at both doses. Milk levels of radioactivity were consistently lower than plasma levels, with plasma:milk ratios of 3-5:1 observed for most collection times.

The placental transfer of 14C-atovaquone (1000 mg/kg) following a single oral dose was studied in rats. The rats were dosed on days 11 or 17 of gestation and were sacrificed 24 hours after the dose. Rats dosed on gestation day 11 showed a mean plasma:conceptus radioactivity ratio of 5:1. The corresponding ratio for rats dosed on Day 17 was 2:1. Radioactivity levels in placenta and amniotic fluid were approximately 1/2 the levels in the fetus.

The rates and routes of elimination of 14C-atovaquone was studied in Wistar COBS rats. The rats (3/sex/group) each received a

single dose of 14C-atovaquone (5 mg/kg) by gavage. Water was allowed ad libitum throughout the study. Food was withdrawn overnight before dosing and for 4 hours after dosing; otherwise was ad libitum. Urine and feces were collected for 96 hours and expired air was collected up to 24 hours.

The mean total recovery of radioactivity of administered dose over 96 hours was ___ The major route of elimination was fecal with a total recovery of radioactivity equal to ___ over 96 hours. Although it is not clear from this experiment whether this is due to poor absorption and/or biliary excretion, other experiments show that biliary excretion is significant. Urinary excretion accounted for 0.21% of the dose and carcass residues for 1.4%. Radioactivity was not detectable in the expired air. Radioactive peaks of than unchanged atovaquone and additional peaks were detected in urine and fecal extracts but at present it is not certain whether these are metabolites or impurities. No sex-related differences were observed.

___ CD rats received intravenous doses of atovaquone (0, 5, 10 or 15 mg/kg/day in cosolvent formulation) for 5 consecutive days, then every other day for either a total of 10 doses (the 15 mg/kg/day group) or a total of 17 doses (the 5 and 10 mg/kg day groups). Originally, the 15 mg/kg group was scheduled to received 17 doses, but the animals were sacrificed after 10 doses due to tissue damage at the injection sites (protocol modification, Vol 5, p. 883). Satellite groups of rats (3/sex) treated with atovaquone were used for drug plasma determinations. Blood samples for pharmacokinetics analysis were obtained 24 and 48 hours after the fifth dose and 24 and 48 hours after the last dose. Plasma concentrations of atovaquone were determined using ___ analytical method. The data are shown below in Table 4. This table was taken from page 7 of the study report, Document #M(B2) of the NDA submission.

Table 4. Mean (±SD) Plasma Concentrations (ug/ml) of Atovaquone Following IV Administration to Sprague-Dawley Rats

Males

	Dose (mg/kg)		
Time	5	10	15
24 h after 5th dose	6.6±1.8	16.9±7.6	13.2±5.0
48 h after 5th dose	2.1±1.6	6.7±2.3	4.81±1.0
24 h after last dose	6.5±1.4	7.1±1.0	9.5±2.4
48 h after last dose	2.3±1.3	6.4±0.6	6.9±2.3

Females

	Dose (mg/kg)		
Time	5	10	15
24 h after 5th dose	7.9±0.2	14.2±4.8	21.5±7.6
48 h after 5th dose	2.6±1.5	3.4±1.8	11.8±2.9
24 h after last dose	8.2±4.2	11.3±4.7	16.2±3.5
48 h after last dose	4.36±10.8	9.9±3.5	13.5±0.9

The plasma concentrations in different rats within each dose group were variable. Average plasma concentration increased with increasing dose but the increase was not dose-proportional. The data provide evidence of exposure to atovaquone in the study.

In a study in male and female rats, the Cmax values of atovaquone (five minutes after a single IV bolus injection) were 101 and 95 ug/ml, respectively; and AUC(0-inf) values were 1592 and 2394 ugxhr/ml, respectively. The $t_{1/2}$ was long (27 and 31 hours for males and females, respectively). Plasma levels of atovaquone were detectable up to 96 hours after the single dose. A second peak plasma concentration was observed 12 hours after the dose. This second peak has been observed after single oral doses in previous animal and human studies and probably represents enterohepatic recycling of atovaquone.

The rate and routes of excretion and metabolic profile of atovaquone were studied in male Wistar rats following a single oral dose of 14C-atovaquone (10 or 250 mg/kg). At 10 mg/kg, — of the dose was recovered in the feces in the first 48 hours and — of the dose was recovered in the feces within 7 days. At 250 mg/kg, — of the dose was recovered in the feces by 48 hours post-dose and —, was recovered within 7 days. Urinary excretion over the 7 days accounted for less than 0.2% of the dose. Fecal samples contained one major radioactive peak that was identified as unchanged atovaquone. Four other peaks representing minor metabolites accounted for 14.9% and 12.2% of the dose excreted in the first 24 hours at 10 and 250 mg/kg, respectively. Although the minor metabolites have not been identified, it appears that one peak may represent a reduced form of atovaquone and another peak may represent a product of desaturation.

The excretion of total radioactivity in the bile was studied in rats (2/sex) having surgically implanted cannulae in the common bile duct. A single dose of 14C-atovaquone (100 mg/kg) was administered orally. Bile was collected continuously (for 3 hours periods) up to 48 hours post dose and analyzed for total radioactivity and unchanged drug. By 12 hours post-dose, only — of the dose was recovered in the bile. After 12 hours, the rate of excretion in the bile increased and — was recovered by 48 hours post-dose. There were no sex differences in the excretion of total radioactivity. Bile from male and female rats contained 0.35% and 0.95% of the dose as unchanged atovaquone. The remaining activity (2.43% in male and 1.59% in females) consisted of unidentified metabolites.

Dog Pharmacokinetic Studies

The pharmacokinetics of 14-atovaquone following single intravenous or oral administration to Beagle dogs were studied.

Beagle dogs (3/sex) received 14C-atovaquone (5 mg/kg) either orally by stomach tube or i.v. (in glycofurol) at an interval of approximately 2 weeks. Blood samples were taken at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 168, and 336 hours post-dose. Urine and feces were collected during the 72 hour metabolism study. The results are shown in Table 6.

Table 6. The Pharmacokinetics of 14C-atovaquone Following a Single IV or PO Dose to Beagle Dogs

PARAMETER	IV mean±SD	PO mean±SD
dose	5.7	5.2
AUC (ug/ml/hr)	191.7±63.6	80.9±70.1
$t_{1/2}$ alpha (hr)	1.5±0.4	-
$t_{1/2}$ beta (hr)	23.4±12.3	21.2±7.0
Cmax (ug/ml)	0.5-4.3	1.0-5.2
Tmax (hr)	4-72	1.5-72
Total apparent plasma clearance (ml/hr/kg)	32.6±10.4	36.0±11.7

apparent oral biavailability = 57.0±64.0% (range=9.6-168%)

Levels of unchanged drug in the plasma after intravenous or oral dose were lower than total radioactivity suggesting the presence of metabolite(s). Large individual variations that occurred for absorption and distribution data prevented meaningful pharmacokinetic modeling so results were model independent. Peak plasma levels of unchanged drug ranged from [redacted] ug/ml and occurred 4-72 hours after p.o. dose. Peak plasma levels of total radioactivity (concentration = [redacted] ug/ml) occurred 1.5 - 72 hours after p.o. dose. The AUC ranges were 13-196 ug/ml/hr (p.o.) and 129-244 ug/ml/hr (i.v.). Apparent bioavailability was 57.0 ± 64.0% (range = 9.6 - 168%). Elimination after p.o. dose was single phase with half-lives of 21.2 hr (unchanged drug) and 23.5 hours (total radioactivity). Elimination after i.v. dose was biphasic with a rapid early distribution phase. The distribution half-lives were 1.5 h (unchanged drug) and 1.6 hr (radioactivity). Elimination half-lives were 23.4 hr (unchanged drug) and 28.0 hr (radioactivity). Total plasma clearance rates were slow - 36.0±11.7 ml/hr/kg (p.o.) and 32.6±10.4 ml/hr/kg (i.v.).

Total radioactivity recovered after dosing was 88.1±23.6% (p.o.) and 68.5±23.6% (i.v.). The fecal elimination was the major route after p.o. and i.v. dose (87.5% and 68.1% respectively). Radiolabel was detected in the bile and 4 dogs showed biphasic plasma concentration/time profiles suggesting enterohepatic

recirculation, which may explain the bioavailability figure of >100%. Urinary excretion was low - 0.6% (p.o.) and 0.4% (i.v.). Fecal analysis showed 2 broad radioactive peaks in addition to unchanged drug, with one peak being two-fold higher in the orally dose dogs.

Three male Beagle dogs each received a single dose of atovaquone (2.5 mg/kg) as a rapid (approximately 45 second) injection into the foreleg vein. Blood samples for determination of plasma concentrations of atovaquone were obtained at pre-dose, at the end of the injection, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 23, 48, 72, 96, and 120 hours after the dose.

Plasma levels of atovaquone were detectable at 120 hours after the dose. The average pharmacokinetic parameters from the three dogs are shown in table 7.

Table 7. Noncompartmental Pharmacokinetic Parameters of Atovaquone (2.5 mg/kg) After Intravenous Administration to Beagle Dogs (N=3) (mean±SD)

Cmax [End of 45 sec infusion (ug/ml)]	AUC (0-inf) (ugxhr/ml)	AUC (0-24h) (ugxhr/ml)	t1/2 (hr)	CL (L/hr/kg)
30.1±5.2	145.0±7.2	62.3±16.4	54±7	0.24±0.04

In a 17-18 day toxicity study for the intravenous cosolvent formulation, Beagle dogs (5/sex/group) each received atovaquone (5, 10, or 20 mg/kg/day in cosolvent) by rapid (5 ml/min) intravenous infusion once daily. Blood samples for pharmacokinetic analysis were obtained 24 hours after the 5th and last doses. Blood samples were also obtained from 2 males and 2 females from each dose group 72 and 120 hours after the last dose. The plasma concentrations of atovaquone were determined using an —— analytical method. The results are shown in Table 8. This table was taken from page 7 of the report submitted in amendment M (B2) of the NDA submission.

Table 8. Mean (±SD) Plasma Concentrations (ug/ml) of Atovaquone After IV Administration to Beagle Dogs

Males

Time	Dose (mg/kg) 5	10	20
24 h after 5th dose[a]	7.2±2.6	13.0±3.1	23.3±10.7
24 h after last dose[a]	8.7±3.6	15.6±5.2	25.7±12.0
72 h after last dose[b]	2.8±2.3	3.7±3.9	11.7±13.1
120 h after last dose[b]	0.9±1.0	1.4±1.8	4.2±5.6

Females

Time	Dose (mg/kg) 5	10	20
24 h after 5th dose[a]	6.8±0.9	13.1±1.7	31.7±3.3
24 h after last dose[a]	9.3±2.1	18.2±6.3	37.6±12.2
72 h after last dose[b]	3.0±0.8	2.3±1.2	9.4±5.7
120 h after last dose[b]	0.8±0.0	0.8±0.6	2.3±1.8

[a] n=5; [b] n=2

Plasma concentrations of atovaquone among different dogs in the same dose group varied up to 5.5-fold. In most cases, a particular dog had either consistently high or low plasma concentrations of atovaquone relative to other dogs in the same group at steady state and subsequent to termination of treatment.

The pharmacokinetics and excretion balance of atovaquone were studied in the Beagle dog. The study was conducted according to GLP at [redacted] using unlabeled atovaquone (Batch No. Q8M) and 14C-atovaquone (Batch No. CI, Specific Activity 155 uCi/mg, 5.7 MBq/mg). Fasted Beagle dogs (3/sex) received single oral doses of 14C-atovaquone (10 or 250 mg/kg suspended in 0.5% aqueous methylcellulose). The pharmacokinetics are shown in Table 9 below (taken from page 4914 of Vol. 1.16). Urinary excretion accounted for less than 0.4% of the dose in male and female dogs at both doses. At 10 mg/kg, [redacted] and [redacted] of the dose were recovered in the first 48 hours and within 10 days, respectively. At 250 mg/kg, [redacted] and [redacted] of the dose were recovered in the first 48 hours and within 10 days, respectively. Metabolic profiling showed 1 major radioactive peak in fecal samples that was identified as unchanged atovaquone.

Table 9. The Pharmacokinetics of Atovaquone in the Beagle Dogs Following a Single Oral Dose at 10 mg/kg and 250 mg/kg

	Dose - 10mg.kg^{-1}						
	Male 1	Male 2	Male 3	Female 4	Female 5	Female 6	Mean
AUC_{∞}	192.6	39.2	21.7	188.0	167.1	134.0	123.8 ± 75.4
t½β	30.1	38.5	28.9	34.7	34.7	63.0	38.3 ± 12.6
Cmax	3.77	1.06	0.56	2.28	2.16	1.39	1.87 ± 1.14
Tmax	24	2	1 and 3	48	48	24	
Cl/F	0.05	0.26	0.46	0.05	0.06	0.07	0.16 ± 0.17
Vd/F	2.26	14.17	19.20	2.66	2.99	6.78	8.01 ± 7.09
	Dose 250mg.kg^{-1}						
	Male 1	Male 2	Male 3	Female 4	Female 5	Female 6	Mean
AUC_{∞}	474.1	150.0	112.2	1234.3	1056.0	1447.9	745.8 ± 575.9
t½β	24.8	40.8	30.1	43.3	46.2	115.5	50.1 ± 33.1
Cmax	9.69	3.41	2.82	12.46	10.51	7.66	7.76 ± 3.92
Tmax	24	1	1	48	48	48	
Cl/F	0.53	1.67	2.23	0.20	0.24	0.17	0.84 ± 0.89
Vd/F	18.83	98.04	96.88	12.66	15.78	28.78	45.16 ± 40.87

AUC_{∞} ($\mu g.ml^{-1}$ x h) - area under the plasma concentration-time curve
t½β - (h) - half-life of elimination
Cmax ($\mu g.ml^{-1}$) - maximum observed plasma concentration
Tmax (h) - time at which Cmax was observed.
Cl/F ($l.h^{-1}.kg^{-1}$) - apparent clearance
Vd/F ($l.kg^{-1}$) - apparent volume of distribution.

Rabbit Pharmacokinetic Studies

The pharmacokinetics and metabolic profile of atovaquone were studied in female rabbits (3/group). The study was conducted according to GLP at
The rabbits received single oral doses of 14C-atovaquone (300 or 1200 mg/kg). Plasma samples for pharmacokinetics were taken at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours post-dose. At 300 mg/kg, the mean±SD values for Cmax, Tmax, AUC_{0-inf} were 7.1±3.0 ug/ml, 15.8 hours, and 350.8±132.6 ug/ml. Most of the dose (— was recovered from the feces in the first 48 hours and — of the dose was recovered within 7 days. At 1200 mg/kg, the mean±SD values for Cmax, Tmax, and AUC_{0-inf} were 11.9±1.2

ug/ml. The main route of excretion was fecal, with [redacted] and [redacted] of the dose recovered in the first 48 hours and within 7 days, respectively. At both doses levels, urinary excretion accounted for less than 3% of the dose. The major metabolic peak contained unchanged atovaquone.

Monkey Pharmacokinetic Studies

The tissue distribution of 14C-atovaquone in male Aotus monkeys following administration of a single oral dose of 10 mg/kg was studied. Eight male Aotus monkeys were allowed food and water ad libitum throughout the study. Each received a single dose of (14C)-atovaquone (10 mg/kg) through a stomach tube. Monkeys were killed at 1, 7, 36, and 96 hours post-dose and wholebody autoradiography was performed.

At approximately 1 hour post-dose, most of the dose remains in the stomach, upper GI tract and esophagus. Very low levels are seen systemically, with the highest being associated with the liver and kidney (mostly in the renal cortex). No other organs or tissues are discernible. At 7 hours post-dose, the highest levels are in the lower large intestine and the gall bladder. Higher levels are now present systemically, again mostly in the liver and kidney. At 36 hours, levels are greatly reduced except for material in the gall bladder, liver and kidney. At 96 hours, barely detectable levels are seen in the gall bladder and kidney.

Comment: In summary, 14C-atovaquone shows poor absorption and limited distribution, mostly to the liver and kidney. There is evidence for prolonged biliary excretion and urinary excretion. (14C)-Atovaquone and or its metabolites are seen in the GI tract for several hours and are eliminated in the feces. There was no evidence of binding to melanin or accumulation in other tissues.

In a study in male dogs, the plasma concentration of atovaquone at the end of a 45 minute infusion was 30.1 ug/ml and the calculated AUC_{0-inf} value was 145.0 ugxhr/ml. The $t_{1/2}$ was 54 hours, and atovaquone levels were detectable up to 120 hours after the dose.

In Vitro Studies

The extent of binding of atovaquone to plasma proteins and possible drug interactions have been investigated in vitro. The extent of plasma protein binding of 14C-atovaquone in rat, dog, monkey, and human plasma at concentrations of 0.1, 2.0, and 11.0 ug/ml. Atovaquone was highly protein bound (>99%) to plasma proteins from rat, dog, and man from 0.1 to 90 ug/ml. No significant concentration dependent effect was observed at these

levels. Atovaquone (0.1, 1, and 5 ug/ml) did not affect the human plasma protein binding of quinine (1 and 10 ug/ml) or phenytoin (15 ug/ml).

The metabolism of atovaquone and other hydroxynaphthoquinones (58C80, 59C80, and 306C81) by hepatic microsomal preparations from man, mouse, and cynomologus monkey was investigated. Neither human, monkey nor mouse microsomes appeared to metabolize atovaquone to any detectable metabolite.

Mice receiving atovaquone (0, 50 or 200 mg/kg/day) orally for 5 or 10 consecutive days had increased liver to body weight ratios and total cytochrome P-450 levels. Atovaquone significantly induced 7-pentoxyresorufin-O-dealkylase (cytochrome P-450 IIB family) and glucuronyl transferase. In male Wistar rats, atovaquone (0, 10 or 100 mg/kg/day in 0.25% methylcellulose for 5 or 10 days) did not have an effect on these parameters.

SUMMARY OF RESULTS OF NONCLINICAL PHARMACOKINETIC STUDIES

Atovaquone is a novel hydroxynaphthoquinone that exerts broad spectrum antiprotozoal activity via inhibition of dihydroorotate dehydrogenase, a key enzyme in the pyrimidine biosynthetic pathway. Its solubility in water is so limited that pH and pKa measurements could not be determined. The oral dosing formulation for atovaquone in these studies was a suspension of micronized drug in aqueous 0.25% methylcellulose.

In all species tested, the absorption of atovaquone following oral administration is limited and slow. The absolute oral bioavailability in the rat at 5 mg/kg was approximately 21%. The proportionality of serum level to administered dose was not linear, probably due to a decreased fraction of drug absorbed at higher doses. It is highly bound (>99%) to rat, dog, monkey, and human plasma proteins. Wholebody autoradiographic studies in the mouse, rat, and monkey, showed that the distribution after a single dose is limited to stomach, intestines, liver, kidneys, fat, and blood. In all species tested, the major route of elimination is fecal with almost no urinary excretion. No metabolites of atovaquone were detected in <u>in vitro</u> studies using mouse, rat, dog, monkey, or human hepatic microsomes. In vivo studies in the mouse, rat, and dog showed that the most of the atovaquone dose is excreted unchanged in the feces; minor metabolites in the feces have not been identified.

Plasma concentration increases were not proportional to dose increases in the 28-day rat and dog toxicology studies. Increasing the dose from 200-500 mg/kg/day (i.e. 25X) in rats increased AUC by only 5X. Similar results were seen in dogs. Therefore, doses higher than 500 mg/kg may not significantly increase systemic exposure to atovaquone.

NON-CLINICAL PHARMACOLOGY STUDIES

1. General Pharmacological Studies on the Antimalarial Compound BW A566C Following Oral Administration To Conscious Mice. Study BPHP/87/46.

2. General Pharmacological Studies on the Antimalarial Compound BW A566C Following Oral Administration to Conscious Rats. Study BPHP/87/0047.

3. Cardiovascular Effects of High Dose Levels of BW A566C in the Anaesthetized Rat. Study BPHP/89/0027.

4. The Cardiovascular Evaluation of a Single Oral Dose of BW A566C in the Conscious Beagle Dog. Study BPHP/88/0011.

5. The Cardiovascular and General Behavioral Effects of 20 mg/kg 566C80 (cosolvent formulation) Administered Intravenously to the Conscious Beagle Dog. Study BPHP/91/0033.

6. The Cardiovascular, Autonomic Nervous, and Respiratory Effects of 566C80 (20 mg/kg) in a Co-solvent Formulation Administered Intravenously to Anaesthetized Cats. Study BPHP/91/0036.

7. Effects of IV Infusions of 566C80 on Cardiovascular, Respiratory, and Autonomic Function of Anaesthetized Beagle Dogs. Study TPZZ/91/0039.

8. The Cardiovascular and Autonomic Nervous and Respiratory Effects of 566C80 (20 mg/kg) in a Co-solvent Formulation Administered Intravenously to Anaesthetized Cats. Study BPHP/91/0036.

The effects of single oral doses of atovaquone were studied in female CD-1 mice. The mice were allowed food and water ad libitum during the study except for the time period ranging from 2.5 hours prior to and 2.5 hours following dosing.

Mice (5/group) were given 0, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, or 2000 mg/kg atovaquone were observed for behavioral changes and signs of toxicity at frequent intervals up to 5 hours post-dose, at 24 hours, and once or twice on days 1, 3, 6, 9, 12, and 14 post-dosing. Body weight and rectal temperature were recorded pre-dose on the first day of dosing, and during each behavioral assessment. No behavioral changes or signs of toxicity were observed at any dose level up to 14 days post-dose. No drug-induced effect on body weight or rectal temperature was seen.

Mice (6/group) received atovaquone (0, 20, 100, or 500 mg/kg) 1 hour before receiving a suspension of powdered charcoal:white

flour (1:2). The mice were killed 20 minutes later and the stomach and entire small intestine to the cecum were removed. The furthest travel of the charcoal mixture from the stomach was measured and expressed as a percentage of total length. Gastrointestinal propulsion was not modified at 20 or 100 mg/kg but was potentiated slightly (23.6% $p<0.05$) at 500 mg/kg.

Comment: This study design cannot be used to conclude that atovaquone has no effect on GI propulsion. Atovaquone is absorbed slowly following an oral dose. The charcoal administered 1 hour later could adsorb a large part of this dose. Therefore, it is not clear whether the lack of effect is due to inactivity of atovaquone or due to adsorption to the activated charcoal.

Groups of 6 mice each received atovaquone (0, 20, 100, or 500 mg/kg) 1 hour before receiving an i.v. injection of pentobarbitone to induce sleep. Mice were observed continuously until they were able to walk to the edge of the table. Elapsed sleeping time = Walk time - Injection time. Barbiturate sleeping time was not changed by atovaquone.

In a similar set of experiments, male Wistar rats were allowed food and water ad libitum except for the 18 hour period before dosing and 2.5 hour period after dosing. Atovaquone (batch Q3, suspended in aqueous 0.25% MC) was administered by gavage.

Rats (5/treatment group; 8/control group) received either 20, 50, 100, 200, 500, 1000, or 2000 mg/kg atovaquone. Animals were observed for behavioral changes and signs of toxicity at frequent intervals up to 5 hours post-dose, at 24 hours, and every 2 or 3 days for 14 days. Body weights and rectal temperatures were recorded prior to dosing and during each behavioral assessment on subsequent days. At 1 or 2 g/kg, 2 of 5 rats showed slightly depressed locomotor activity and 1 from each level showed slight breathing rate decrease; these effects occurred at 20-160 minutes post-dose after which the rats appeared normal. Lower doses were without any effect up to 14 days post-dosing. There was no significant effect on body weight gain or rectal temperature for up to 14 days post-dosing.

Rats (6/group) received 0, 10, 30, 100, or 300 mg/kg atovaquone orally 1 hour before receiving a suspension of powdered charcoal:white flour (1:2). The rats were killed 30 minutes later and the stomach and entire small intestine to the cecum was removed. The furthest travel of the charcoal mixture from the stomach was measured and expressed as a percentage of the total length. Gastrointestinal propulsion and duration of barbiturate-induced sleep were not significantly affected by atovaquone.

Comment: For the conclusion on the GI propulsion study, the comment is the same as that for the mouse study applies (see

above).

Rats (6/group) each received 0, 10, 30, 100, or 300 mg/kg atovaquone orally 1 hour before receiving an intravenous injection of pentobarbitone to induce sleep. Rats were observed continuously until righting reflex returned and some walking movements were discernible. Atovaquone did not affect the duration of duration of barbiturate sleep.

Female Wistar rats (8/group) received 0, 20, 100, or 500 mg/kg/day atovaquone by gavage once daily for 5 days. Approximately 3 hours after the last dose, the rats were anaesthetized with Inactin and arterial blood pressure and ECG were recorded for 60 minutes. At the end of this procedure, blood samples for determination of plasma concentrations of atovaquone were taken. The group mean (±SD) plasma concentrations for 20, 100, and 500 mg/kg/day were 34±2.7, 59±3.2, and 77±6.6 ug/ml, respectively; the time point after dosing is not clear. Both control and atovaquone-treated rats showed a gradual decline in arterial blood pressure and heart rate and an increase in the P-R interval over the 60 minute period. The profile of the atovaquone-treated rats was similar to that of vehicle controls.

Beagle dogs, 4 males (2 with carotid loops and 2 with a carotid cannula) and 8 females (4 carotid loops, 4 carotid cannula), 12-24 months old, 9.0-15.2 kg, fasted 15-17 hours prior to dose, received atovaquone orally (20 mg/kg). Observations included blood pressure, ECG recordings, general behavior, plasma drug levels (samples taken immediately pre-dose and at 5, 10, 15, 30, and 45 minutes, 1, 2, 3, 4, 6, 8, and 12 hours after dosing and 1, 3, 6, 10, and 13 days after), blood clinical chemistry and hematology (immediately pre-dose and at 1, 3, 6, 10, and 13 days post-dose.

The plasma concentration of atovaquone was biphasic, with one peak at 2-3 hours post-dose and a second peak between 12 and 24 hours post-dose, after which plasma concentrations declined to non-detectable levels by 13 days post-dose. No significant effect was seen on arterial blood pressure, heart rate, or myocardial conduction for up to 13 days post-dose. No autonomic or behavioral effects and no blood chemistry and hematology changes were observed.

Atovaquone in cosolvent (0 or 20 mg/kg) or 0.9% saline was administered to 4 conscious male Beagle dogs by 30 minute intravenous infusion. The dogs receiving atovaquone or cosolvent alone showed a slight rise in blood pressure and heart rate, prolongation of the ECG P-R interval, and a secondary bradycardia. Blood pH and acid/base showed a slight alkalosis. A diuretic effect was noted. The mean peak plasma level of atovaquone at the end of infusion was 80.3 ug/ml and the mean

AUC_{0-inf} was 1178 ugxhr/ml.

In another intravenous study, adult male Beagle dogs (3/group) were anaesthetized with allobarbital-urethane before receiving a 30 minute intravenous infusion of atovaquone (0 or 20 mg/kg in the cosolvent formulation). Cardiovascular, autonomic, and respiratory functions were monitored during the infusion and for 100 minutes after completion of the infusion. Blood samples for determination of plasma concentrations of atovaquone were obtained before dosing and at 30 and 110 minutes after completion of the infusion.

Minor changes (<10%) in mean arterial blood pressure and heart rate occurred during the study following the infusion of either 20 mg/kg atovaquone or the vehicle control. No arrhythmias were observed during this study. Mean respiratory rate was increased up to 31% and mean minute volume was increased up to 30% relative to baseline (pretreatment) values during the infusion of atovaquone. Increases in minute volume (up to 68%) occurred during the 100 minute period after the infusion. Atovaquone did not significantly alter autonomic function at this dose.

Anaesthetized cats received 20 mg/kg atovaquone in cosolvent formulation by 30 minutes intravenous infusion and the cardiovascular, autonomic, and respiratory effects were studied. The peak plasma concentration of atovaquone at the end of the infusion was . —— ug/ml. At 30 and 180 minutes after the end of the infusion, the mean (±SD) plasma levels declined to 90±19 and 39±4 ug/ml, respectively. Atovaquone caused a weak, slowly developing inhibition of vagal nerve-induced bradycardia, and an increase in respiratory tidal volume and minute volume.

NON-CLINICAL TOXICOLOGY STUDIES

Single and Multiple Dose Studies: Oral and Intravenous Routes

1. Report of an Acute Oral Toxicity Study of A566C in the CD-1 Mouse. Study BPAT/88/0007.

2. An Acute Intravenous Toxicity Study in the Mouse with 566C80. Study TTEP/91/0026.

3. Report of an Acute Oral Toxicity Study of A566C in the Wistar Rat. Study BPAT/88/0006.

4. An Acute Intravenous Toxicity Study in the Rat with 566C80. Study TTEP/91/0025.

5. A One-Day Intravenous Infusion Study in Beagle Dogs Given 566C80. Study TTEP/91/0039.

6. A Five-Day Infusion Toxicity Study in Beagle Dogs Given 566C80. Study TTEP/91/0040.

7. One-Month Oral Toxicity Study in the Rat with A566C. Study BPAT/87/0044.

8. Report of a One-Month Toxicity Study of Oral A566 in the Dog. Study BPAT/87/50.

9. A 30 Day Intravenous Toxicity Study in Rats Given 566C80. Study TTEP/91/0041.

10. A 17-18 Day Intravenous Toxicity Study in Beagle Dogs Given 566C80. TTEP/91/0038.

11. A 90 day Oral Dose-Range Finding Study in Mice Given 566C80 in the Diet. Study TTDR/91/0017.

12. A 90 Day Oral Dose-Range Finding Study in Rats Given 566C80 in the Diet. Study TTDR/91/0011.

13. Report of a 6 Month Oral Toxicity Study of 566C80 in the Wistar Rat. Study BPAT 88/0064.

14. A Six-month Oral Toxicity Study in Beagle Dogs Given 566C80. Study TTEP/90/0008.

Cardiovascular Toxicity in the Dog: Intravenous Infusion

1. A Single Intravenous Toxicity Study (One Hour Infusion) in Beagle Dogs Given 566C80. Study TTEP/92/0010.

Special Studies

1. Perivenous and Intramuscular Irritation Study in Beagle Dogs. TTEP/91/0037.

2. An In Vitro Hemolysis and Protein Flocculation Study. TTEP/91/0036.

Reproduction Studies

1. Reproduction/Fertility Study in Rats Given 566C80 by Gavage. Study TTEP/92/0009.

2. A Teratology Study in Rats Given 566C80 by Gavage. Study TTEP/90/0073.

3. Peri- and Postnatal Study in Rats Given 566C80 by Gavage. Study TTEP/92/0018.

4. A Teratology Study in Rabbits Given 566C80 by Gavage. Study

TTEP/90/0074.

Genotoxicity Studies

1. Evaluation of A566C for Mutagenicity using the Ames Salmonella/Microsome Incorporation Test. Study DBAT/88/0008.

2. L5178Y/TK +/- Mouse Lymphoma Mutagenesis Study with 566C80. Study TTEP/90/0060.

3. An In Vitro Cytogenetic Study in Cultured Human Lymphocytes with 566C80. Study TTEP/90/0063.

4. A Micronucleus Assay in Mice with 566C80. Study TTEP/90/0069.

SINGLE AND MULTIPLE DOSE TOXICOLOGY STUDIES

In the oral toxicology studies, atovaquone was administered as micronized drug suspended in 0.25% aqueous methylcellulose unless stated otherwise.

Toxicology Studies in the Mouse: Oral Dosing

CD-1 mice (5/sex/group) received either 0 or 1825 mg/kg atovaquone as a single dose by gavage. All animals were monitored for 14 days post-dosing for mortality, signs of treatment-related toxicity, and effects on body weight. At the completion of the study, all surviving mice were sacrificed by CO2 asphyxiation and autopsied.

No drug-related deaths occurred. One male mouse dosed with 1825 mg/kg atovaquone died on day 2 of the study due to a gavage accident. There were no treatment-related clinical signs, effects of body weight, or gross observations at autopsy.

In a dose-ranging study, male and female mice (16/sex/group) received atovaquone in the diet (0, 50, 200, or 800 mg/kg/day) for 90 days. Blood samples for determination of 566C plasma levels were obtained on study days 6 and 90. Necropsy was performed on all surviving animals at the end of the study.

On study days 2-4, 10/16 males and 11/16 females given 800 mg/kg/day atovaquone were found dead. The mice died overnight with no premonitory signs. The cause of death is uncertain. No treatment-related changes were observed for clinical signs, body weights, food consumption, or hematology. Drug and dose-related increases in absolute and relative liver weights were observed in all groups. Gross necropsy revealed a higher incidence of animals with no abdominal fat in the atovaquone treated groups relative to the control group. Histopathology revealed

treatment-related alterations in the liver. The alterations were characterized by diffuse mild to moderate periacinar hepatocellular hypertrophy, randomly distributed minimal to mild individual cell necrosis of hepatocytes, and minimal to mild focal areas of hepatocellular necrosis. Transmission electron microscopy of liver samples revealed excess of lipid and smooth endoplasmic reticulum in the 50 mg/kg/day group and large pools of smooth endoplasmic reticulum and lipid bodies in the 200 mg/kg/day group.

Toxicology Studies in the Mouse: Intravenous Dosing

In a single dose study, _____ CD-1 mice (5/sex/group) each received intravenous doses of atovaquone (20, 25, or 30 mg/kg in cosolvent) by slow bolus injection. Based upon body surface area conversions, these doses are approximately equivalent to human doses of 1.7, 2.1, and 2.5 mg/kg.

All deaths occurred on the day of dosing as shown below:

Dose of 566C (mg/kg)	Number dead/number dosed males	females
20	0/5	0/5
25	3/5	3/5
30	3/5	4/5

The median combined lethal dose was 26.3 mg/kg.

Clinical signs occurring immediately to 3 minutes after dosing and prior to death included clonic convulsions, gasping, opisthotonos, prostration, labored breathing, and/or pink exudate from the nose. Mottled and spongy lungs were noted at necropsy.

These signs were also noted in animals that survived the first day of dosing. Scabs on the tails were noted in all mice.

Toxicology Studies in the Rat: Oral Dosing

Wistar rats (5/sex/group) received either 0 or 1825 mg/kg atovaquone as a single dose by gavage. All rats were monitored for 14 days post-dosing for mortality, signs of treatment-related toxicity, and effects on body weight. At the completion of the study, all surviving rats were sacrificed by CO2 asphyxiation and autopsied.

There were no drug-related deaths. A slight decrease in weight gain on Study Day 4 was noted in the group of female rats dosed with 1825 mg/kg atovaquone. There were no treatment-related clinical signs, other effects on body weight, or gross observations at autopsy.

In a 28-day toxicology study, Wistar rats (15/sex/group) received either 0, 20, 100, or 500 mg/kg atovaquone by gavage once daily for 28 days. On the day following the final dose, the first 10 males and 10 females per group were sacrificed and autopsied. The remaining 5 males and 5 females per group were sacrificed and autopsied after an additional 14-day treatment-free period. An additional eight satellite groups of rats (3/sex/group) were dosed in conjunction with the main study to measure blood levels in plasma for pharmacokinetic data. Samples were taken at 1 and 6 hours post-dosing on days 1 and 24, pre-dose on days 2, 4, 8, and 25, and on days 31, 35, and 43.

There were no treatment-related effects on clinical signs, body weight, body/organ weight, food and water consumption, ophthalmoscopy, gross observations at autopsy or histopathology. There were no drug-related deaths. One male rat (100 mg/kg/day dose) was killed on day 9 reportedly because of emaciated condition from damaged teeth. One female rat (100 mg/kg/day dose) was found dead on day 18 and the cause of death could not be determined. The pathologist reported a focal keratitis with associated anterior synechia in this rat. There was a treatment-related incidence of pink-colored plasma reportedly related to presence of compound and/or metabolites and not due to hemolysis.

Increases in plasma concentration of atovaquone were not proportional to dose. Increasing the dose five-fold from 20 to 100 mg/kg/day gave a two-fold increase in Cmax and AUC; the increase from 100 to 500 mg/kg/day gave Cmax increases of 17% and 52% for males and females respectively with similar increases in AUC. Pre-dose plasma concentrations were constant for the first 8 days, but increased by day 25 relative to day 8 at 100 mg 500 mg/kg/day.

A six month toxicology study was conducted in accordance with GLP at The Wellcome Foundation, Ltd., Beckenham England. Wistar (COBS) rats (30/sex/group) each received atovaquone (0, 20, 100, or 500 mg/kg/day) orally once daily for 6 months. All surviving rats were necropsied at the end of the dosing period, with the exception of 10 rats/sex/group. These rats were assigned to a 28-day treatment-free recovery period and were necropsied. Satellite groups of 5 rats/sex were used for pharmacokinetic studies. These rats received the same doses as the rats in the main toxicology study. Blood samples for determination of the concentration of 566C in plasma were obtained on Study Day 2 (24 hours after the first dose) and 24 hours after the first dose in weeks 4, 13, and 26.

The plasma levels of atovaquone were not proportional to dose levels. On day 2 (24 hours after the first dose), the plasma levels were 15, 32, and 56 ug/ml at 20, 100, and 500 mg/kg, respectively. These data show that, although the administered dose was increased by 25 times, the plasma levels only increased

approximately fourfold. There was a progressive increase in plasma trough levels over the dosing period. Between week 4 and week 26, there was a twofold increase in plasma levels at the lowest dose; the increase was less pronounced at the mid and high doses. Plasma levels were higher in females than in males at the same dose level throughout the study. In week 13 samples, some control animals appear to have trace plasma levels of atovaquone (up to); this was not seen at other sampling times.

Thirteen rats died during this study. Five deaths were due to dosing errors. The cause of 2 deaths could not be determined because of autolysis. One rat was sacrificed for humane purposes because it was suspected of having a broken leg. The sponsor states that neither microscopic examination at autopsy nor histological examination could account for the reason for death of the remaining 5 rats.

The only treatment-related clinical sign was yellow fecal pellets at the 500 mg/kg/day dose, probably due to presence of atovaquone and/or its metabolites in the feces. There was no drug-related effect on body weight changes, food and water intake, or ophthalmoscopy.

At week 5, male and female rats receiving 100 or 500 mg/kg/day had slightly decreased red blood cell counts and packed cell volumes. Male rats at 500 mg/kg/day and female rats at 100 and 500 mg/kg/day had increased reticulocyte counts. Female rats had a slightly increased mean cell hemoglobin and a dose-related mean cell hemoglobin concentration. At week 14, male and female rats receiving 100 or 500 mg/kg/day still had slightly reduced red cell counts, although the packed cell volumes were comparable to controls. All treated rats had decreased mean cell volumes and mean cell hemoglobin. At 26 weeks, female rats at 20 mg/kg/day as well as males and females at 100 and 500 mg/kg/day showed slightly decreased red cell counts and increased mean cell hemoglobin. Female rats showed a dose-related increase in total white cell counts at all dose levels; male rats showed this increase at 100 and 500 mg/kg/day. These hematological effects reversed by the end of the 4 week recovery period.

Plasma samples showed a dose-related pink coloration at all dose levels.

Histopathologic examination of tissues showed hemosiderin deposition in the red pulp of the spleen and focal liver necrosis in male and female animals receiving atovaquone. The necrosis consisted of random foci of coagulative necrosis that were negative for bacterial pathogens. Male rats showed focal mononuclear cell infiltration in the prostate. After the recovery period, the liver and prostate changes reversed but the spleen changes persisted.

In a range-finding study, rats (13/sex/group) received atovaquone (0, 50, 200, or 800 mg/kg/day) in the diet for 90 days. Blood for determination of atovaquone plasma levels were obtained from three rats/group on study days 6 and 85. Necropsy was performed on all surviving rats on post-dose day 1.

Except for pink tinged fur in the atovaquone treated groups, no treatment-related clinical signs were observed. There were no treatment-related effects on body weights, food consumption, clinical chemistry, or gross necropsy. At the end of dosing, a slight decrease in hematocrit, hemoglobin, and red blood cells counts was noted in males and females in the 800 mg/kg/day group. The rats in the 800 mg/kg/day group showed slight increases in absolute and relative livers weights. Although no histopathologic changes were noted in the liver, [redacted] revealed an abundance of smooth endoplasmic reticulum in the liver samples (Appendix 1, Memorandum from M.J. Dykstra to K. Ayers, 2-27-91).

Toxicology Studies in the Rat: Intravenous Dosing

In a single dose study, [redacted] CD rats (5/sex/group) each received atovaquone (25, 35, or 45 mg/kg in cosolvent formulation) by slow bolus intravenous injection. Based upon body surface area, these doses are approximately equivalent to 3.6, 5, and 6.4 mg/kg in humans. Animals found dead were examined grossly and discarded. Surviving rats were sacrificed 14 days after dosing and gross necropsies were performed.

All deaths occurred within 2-7 minutes following the dosing as summarized below:

Dose of 566C (mg/kg)	Number dead/number dosed Male	Female
25	0/5	0/5
35	2/5	1/5
45	5/5	5/5

The median combined lethal dose for male and female rats was 35.7 mg/kg.

The clinical signs noted before deaths of rats receiving 35 mg/kg or 45 mg/kg included clonic convulsions (immediate to 2 minute onset), gasping, prostration, Straub tail, labored breathing, ataxia, paddling of hindlimbs, and white or pink exudate from the nose. Gross necropsy revealed mottled and spongy lungs in all rats that died.

Many of the signs described above, including ataxia, labored breathing, decreased activity, prostration, clonic convulsions, and red urine, were noted in animals that survived the study.

Starting from post-dose day 3, scabs were noted on the tails of some rats that received 35 mg/kg. No gross findings were observed at the 14-day necropsy.

In a one month study, [redacted] CD rats received intravenous doses of atovaquone (0, 5, 10 or 15 mg/kg/day in cosolvent formulation) for 5 consecutive days, then every other day for either a total of 10 doses (the 15 mg/kg/day group) or a total of 17 doses (the 5 and 10 mg/kg day groups). On the basis of body surface area, this corresponds to approximately 0.7, 1.4, and 2.1 mg/kg in humans. Originally, the 15 mg/kg group was scheduled to received 17 doses, but the animals were sacrificed after 10 doses due to tissue damage at the injection sites (protocol modification, Vol 5, p.883). Immediately after injection of atovaquone into the tail vein, the site was flushed with saline to reduce the potential irritation from the atovaquone formulation. The cosolvent vehicle control group consisted of 22 rats/sex. The saline control and 566C-treated groups contained 14 rats/sex. Satellite groups of rats (6/sex) treated with atovaquone were used for drug plasma determinations on study days 6 or 7. The post-dose period for cosolvent vehicle and saline controls, and atovaquone (5 and 10 mg/kg) was 99 days; the post-dose period for the group receiving atovaquone (15 mg/kg) was 113. The study was conducted according to GLP at Burroughs Wellcome Co, RTP using Lot No. PDL/BMR/4088.

Two of five rats that received 20 mg/kg on the first day of study died and were replaced. The rats that survived and the new rats received 15 mg/kg/day for the rest of the study.

On the first day of dosing, deaths occurred in 2/5 male rats receiving 20 mg/kg atovaquone. Because of these deaths and clinical signs noted in 3 surviving male rats, the high dose was lowered to 15 mg/kg. Deaths also occurred on the first day of dosing in 1/17 males and 2/20 females receiving 15 mg/kg atovaquone. No clinical signs were noted prior to death of males rats receiving 20 mg/kg atovaquone because the animals were found dead. Clinical signs noted before death in rats receiving 15 mg/kg 566C included labored breathing and/or gasping, forelimb extension, and prostration.

Other deaths occurred during the remainder of the study. At 10 mg/kg, one female died during the recovery period (24 days after the last dose). At 15 mg/kg, one female died on day 4 and one on study day 13. One male and one female showed gasping, labored breathing, rapid breathing or decreased activity after injection of the cosolvent vehicle.

Other clinical signs related to injection of cosolvent vehicle or atovaquone in cosolvent were: black and/or purple and/or red discoloration, scabs, raw areas, swelling, clear and/or brown exudate and loss of distal portions of the tail. These findings

were more severe in atovaquone-treated animals.

Body weights of male rats receiving atovaquone (10 or 15 mg/kg) were slightly decreased relative to the saline control group during the dosing period. These changes reversed by recovery day 36.

A slight to moderate dose-related decrease was observed in red blood cell count, hematocrit, and hemoglobin in all groups receiving atovaquone cosolvent. At 10 and 15 mg/kg (males and/or females) and 5 mg/kg (females), there were slight to moderate increases in mean corpuscular volume, red cell distribution width, and absolute and relative reticulocyte counts. Two male rats, one given 10 mg/kg and one given 15 mg/kg were evaluated during a 49 day post-dose period to assess reversibility. Hematological effects were either reversed or returning to normal by day 49.

There were no treatment-related changes in clinical chemistry, or urinalysis. Ophthalmoscopy showed pale retinal choroidal vessels in one male at 15 mg/kg and a pale fundus in one female at 10 mg/kg. The relationship of these findings to treatment with atovaquone is not clear.

At the end of the treatment period, absolute and relative spleen weights were increased in male and female rats receiving 10 or 15 mg/kg atovaquone. Absolute and relative kidney weights were increased in male and female rats receiving 15 mg/kg. Absolute and relative adrenal weights were increased in rats receiving cosolvent vehicle control and atovaquone (5, 10 and 15 mg/kg). All changes in absolute and relative organ weights were non-dose related and reversible.

Gross pathology changes were limited to the injection site. These are described above under clinical signs.

Histopathological examination of the injection site showed treatment-related irritation in rats receiving cosolvent vehicle alone or atovaquone in cosolvent. Findings were phlebitis, thrombus, cellulitis, ulcerative dermatitis, and hemorrhage. The incidence of changes was higher in rats receiving atovaquone compared to those receiving cosolvent vehicle alone. At the end of the dosing period, a mild nephropathy was noted in 2 male rats receiving 15 mg/kg. The nephropathy was characterized by tubular dilatation and regeneration of proteinaceous casts. Minimal interstitial inflammation was observed. Nephropathy was not observed in recovery animals. A minimal to mild focal vacuolar encephalopathy was observed in two male rats receiving 15 mg/kg atovaquone. The encephalopathy was highly localized and asymmetrical and involved different areas of the brain in the two animals. The relationship of the nephropathy and encephalopathy findings to treatment with atovaquone is not clear.

Toxicology Studies in the Dog: Oral Dosing

In a 28-day study, Beagle dogs (5-6 months old) received atovaquone by gavage once daily at either 0, (5/sex/group), 20 (3/sex/group), 100 (3/sex/group), or 500 mg/kg/day (5/sex/group). At the end of the dosing phase, 3 dogs of each sex were sacrificed and autopsied. The remaining 2 dogs of each sex in the control and high dose group were allowed a 14-day treatment-free period and were sacrificed and autopsied.

For pharmacokinetic data, blood samples were collected on day 12 prior to the initiation of study, study day 1 (1 and 6 hours post-dose), pre-dose on days 2, 4, 8, day 17 (1 and 6 hours post-dose), and pre-dose on days 18 and 28. Samples were collected from recovery animals on days 31, 35, 43.

Two female dogs in the 500 mg/kg/day group died from 1) premature sacrifice on study Day 8 and 2) unscheduled death on day 20. Both deaths were due to accidental inhalation/instillation of dosing material into the lung. No other deaths occurred. There was yellow discoloration of feces in all male and female dogs at 500 mg/kg/day and in one male dog at 20 mg/kg/day, reportedly related to unabsorbed drug. No other treatment-related clinical signs were reported. Plasma samples obtained on Study Day 8 and 24 at all dose levels of atovaquone had a pink color that appeared to be dose-related and was not associated with hemolysis, but reportedly related to the presence of atovaquone and/or its metabolites. No treatment-related effects were noted on body weight, absolute or relative (percent of body weight) organ weights, electrocardiography, or ophthalmoscopy, or histopathology.

Oral absorption was variable at all doses. Plasma concentration increases were not proportional to dose increases; A five-fold dose increase of 20-100 mg/kg/day gave 2.5 fold increase in AUC and increase from 100-500 mg/kg/day gave 1.9 fold increase in AUC.

In a 6-month study, Beagle dogs (6/sex/group, aged 11-14 months at the start of the study), received atovaquone (0, 20, 100, or 500 mg/kg/day) orally once daily for 6 months. Micronized atovaquone was administered in gelatin capsules. Each dog received 350 gm Dog Diet daily, 2-3 hours after dosing with atovaquone. Tap water was available ad libitum. At the end of the dosing period, 4 dogs/sex/group were sacrificed and necropsied. The remaining 2 dogs/sex/group were observed for a 30-day post-dosing period before being sacrificed and necropsied.

Clinical observations were made daily throughout the study. Body weights were recorded once pre-test, once weekly for the first three months, every 4 weeks, then weekly during the post-

dosing observation period. Food consumption was recorded once weekly for the first three months, every 4 weeks thereafter, and weekly post-dosing. Clinical pathology, ophthalmology, and urinalysis were performed once pre-test, at dosing days 30, 90, and 180, and once at the end of the post-dosing period. Electrocardiographic examinations were performed twice pre-test, at dosing days 30, 90, and 180, and once at the end of the post-dosing period.

At necropsy, weights were obtained for selected organs. Gross examination and histopathology were performed on tissues from dogs in all groups.

Plasma samples for pharmacokinetic studies were obtained 1) pre-dose on days 5, 40, 97, 173 and 2) on days 202 and 215 (14 and 27 days after the last dose). Concentrations of atovaquone were determined using an ——— method. The limit of detection for this assay is ———

As in the one-month study, there was considerable variation in atovaquone plasma concentrations between individual dogs at each dose level.

Comment: The variability is absorption is likely due to the administration of 566C on an empty stomach rather than with food.

The overall mean plasma concentrations for each dose level shown below were determined by combining plasma values for all dogs (male and female) on days 5, 40, 97, and 173.

Atovaquone (mg/kg/day)	Overall mean plasma concentration (mcg/ml)
20	4.48 ± 3.07 (range = 0.73-12.01)
100	6.22 ± 5.42 (range = 0.76-21.57)
500	14.02 ± 8.52 (range = 2.10-35.59)

Mucoid and soft, watery feces with yellow coloring (probably due to test material) were observed. There were no drug-related effects on appearance, behavior, body weights, food consumption, hematologic or clinical chemistry parameters, urinalysis, or the ophthalmic and electrocardiographic examinations. No significant findings were observed at necropsy, in organ weights, or in histopathologic examinations.

Toxicology Studies in the Dog: Intravenous Dosing

In a vascular irritation study, Beagle dogs (3/sex/group) each received atovaquone (20 mg/kg in cosolvent formulation) or 0.9% saline in opposite forelimbs by intravenous infusion over a 30 minute period using an intravenous infusion pump. On the basis

of body surface area, this dose corresponds to approximately 10 mg/kg in humans. Prior to the infusion, the dogs were anaesthetized by an intramuscular injection of ketamine.

No treatment-related clinical signs or effects on body weights were noted. Gross observation showed thickened injection sites in 2/3 male and 1/3 female dogs. Histopathology at the injection site showed 1) focal hemorrhage in 1/3 female dogs and 2) minimal to mild phlebitis and recently formed thrombi in 2/3 male and 2/3 female dogs.

Comment: The stated objective for the conduct of this study was to determine the vascular irritation potential of the atovaquone cosolvent formulation. In order to meet this objective, the study should be designed to infuse atovaquone only. In the study reported here, the vein was flushed with normal saline after the atovaquone infusion. Therefore, this study was not designed to fully explore the potential irritation that can be caused by the atovaquone in cosolvent itself. The results reported here represent the irritation for this injection followed by a saline flush rather than the irritation from atovaquone cosolvent alone, which is likely much worse.

Another vascular irritation study was conducted to determine the vascular irritation potential of the atovaquone cosolvent formulation following intravenous infusion into the same vein for 4-5 days.

Before and after the infusion with atovaquone, the indwelling catheter was flushed with approximately 10 ml. of 0.9% saline Before each infusion, the dogs were anaesthetized with an intramuscular injection of ketamine. Beagle dogs (4/sex) each received either atovaquone (20 mg/kg/day in cosolvent formulation) or 0.9% saline in opposite forelimbs by intravenous infusion over a 30 minute period using an infusion pump. Two dogs/sex were necropsied on post-dose day 1, and the other two dogs/sex were necropsied on post-dose day 15.

Swelling of the forelimb infused with atovaquone was noted in 5/8 dogs. This swelling reversed by post-dose day 11. Soft or mucoid stools were noted in 3/8 dogs. Emesis was noted in one female dog on post-dose day 3. Treatment-related local hemorrhage was noted in 1/4 male and 2/4 female dogs and local thickening was noted in 2/4 males and 1/4 females. Histopathology showed one or more of the following changes in all dogs: mild to moderate phlebitis, thrombi, mild to moderate perivasculitis, and perivascular hemorrhage. The perivascular hemorrhage and focal perivasculitis was also observed at the saline injection site. After the recovery period, 1 female dogs showed a recanalizing thrombus and 1 male and 1 female showed minimal to mild phlebitis with organizing, recanalizing thrombi.

Comment: These findings represent what happens when the vein is flushed with saline after injection, not the irritation potential of atovaquone in cosolvent itself. This is the minimum irritation that would be expected. Same comments as #3 above.

Beagle dogs (5/sex/group) each received intravenous injections of either cosolvent vehicle, 0.9% saline, or atovaquone in cosolvent (5, 10, or 20 mg/kg/day) for 17-18 days. On the basis of body surface area, this corresponds to approximately 2.5, 5, and 10 mg/kg in humans. The intravenous injections were administered by infusion pumps at a flow rate of 5 ml/min. After the cosolvent and atovaquone injections, the site was flushed with 5-10 ml 0.9% saline. Three dogs/sex/group were sacrificed at the end of the treatment period. The remaining 2 dogs/sex/group were assigned to a 29-30 day recovery period and were necropsied at the end of that period.

All dogs survived the study. The atovaquone and cosolvent injected forelegs swelled so much during the study that venipuncture became more difficult as the study progressed. As a result, some dogs received only partial doses or no doses on certain days. The swelling lasted up to 8 days post-dose for males and 29 days pots-dose for females. Other observed signs included raw areas, bruising, and limping.

No treatment-related changes were noted in body weights, food consumption, ophthalmoscopy, electrocardiographic examination, clinical chemistry, or organ weights.

Dogs receiving atovaquone showed slight to moderately decreased values for hemoglobin, hematocrit, and red blood cell counts. These changes were accompanied by slightly increased values for relative and absolute reticulocyte counts in females receiving 10 mg/kg atovaquone and in males and females receiving 20 mg/kg/day. These changes had reversed by day 23 of the recovery period.

Dogs receiving 20 mg/kg/day atovaquone showed slight increases in the quantity of urine with a corresponding decrease in specific gravity. These findings reversed during the recovery period. There were no remarkable histological findings in the kidneys of these dogs or in the other dogs in this study.

Treatment-related gross changes were noted only at the injection sites. The principal alteration was hemorrhage. The only drug-related histopathological changes that were noted occurred at the injection site. Histopathological changes at the injection site included phlebitis, thrombi (recent, organizing, or organized and recanalizing), perivasculitis (focal to diffuse, minimal to moderate, subacute), and perivascular hemorrhage. Some reversal was observed at the end of the recovery period.

Comments: The sponsor stated that no difference of severity was

noted among the atovaquone groups for phlebitis and perivasculitis from the intravenous injection. Since all the sites were flushed with saline after the injection, one would not expect to see a dose-related difference in severity of these findings.

Cardiovascular Toxicity in the Dog: Intravenous Infusion

This study was conducted in accordance with GLP at Burroughs Wellcome Co., Research Triangle Park, NC using lot #PDL/BMR/4090. The date of the final study report is April 27, 1992.

Purpose: The study was performed to investigate the cardiotoxic potential of intravenously administered atovaquone in cosolvent formulation. The formulation consists of atovaquone (5 mg/ml) in a cosolvent vehicle composed of

Method: Eight groups of male and female Beagle dogs (1/sex/group) were used in this study. The dogs were 11-13 months old. The study had a crossover design so that, on separate occasions, the same dogs received atovaquone or vehicle control. The dogs received either saline (12 mg/kg), cosolvent alone (4, 8, or 12 ml/kg), or atovaquone (20, 40, 60 mg/kg). Another group received atovaquone (10 mg/kg). All doses were administered in the left or right cephalic vein by intravenous infusion over 60 minutes using catheters and an infusion pump. Approximately 0.5 ml. of a 1:10 solution of heparinized saline was flushed through the catheter to prevent blood clotting in the catheter. Electrocardiographic (ECG) examinations were performed using an . ECG examinations were performed in the standing position with the dog restrained in a sling immediately prior to infusion, 15, 30, 45, and 60 minutes after the start of the infusion, and 1, 2, 3, 4, 5, and 24 hours after completion of the infusion.

Blood samples for determination of plasma concentrations of atovaquone were taken at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, and 25 hours after the start of the infusion.

Results: The following clinical signs occurred at each dose:

Test Substance	Dose (mg/kg)	Dose volume (ml/kg)	Treatment-Related Clinical Signs
Saline	-	12	none
Cosolvent	-	2	none
Cosolvent	-	4	decreased activity
Cosolvent	-	8	decreased activity, involuntary urination, pale mucous membranes
Cosolvent	-	12	ataxia, decreased activity, excessive licking, involuntary urination, irregular breathing, and salivation
atovaquone	10	2	none
atovaquone	20	4	decreased activity
atovaquone	40	8	ataxia, decreased activity, involuntary urination, irregular breathing, pale gingiva, and retching/emesis
atovaquone	60	12	ataxia, jerking body movements, rigid limbs, opisthonos, Straub tail, lateral recumbency, prostration, convulsions, decreased activity, dilated pupils, excessive licking, retching/emesis, involuntary urination/defecation, irregular breathing, relaxed nictitating membranes, salivation, soft feces, uncontrolled eye blinking, upward eye rotation, and vocalization.

At 60 mg/kg atovaquone, one female dog (#92-360) died prior to completion of the infusion. Clinical signs started 56 minutes after the start of the infusion and the dog died approximately 1 minute later. The clinical signs were prostration followed by convulsions, paddling limb movements, jerking body movements, pupillary dilation and cessation of breathing. The dog's plasma concentrations of atovaquone at 0.25, 0.5, 0.75, and approximately 1 hour after the start of the infusion were _____ respectively. Neither gross examination nor histopathology revealed any treatment-related changes. Death was attributed to the convulsion.

The other female dog receiving 60 mg/kg atovaquone convulsed at 57 and 68 minutes after the start of the injection but survived the convulsive episodes. One of the two male dogs receiving 60 mg/kg atovaquone showed only intermittent jerking body movements. Decreased activity was noted in dogs starting approximately 19 minutes after the start of the infusion. Other treatment-related

signs started approximately 35 minutes after the start of the infusion.

Electrocardiographic examination showed atrial T wave exaggeration and primary and secondary as summarized below:

Atrial T wave exaggeration:

saline control - no effect
cosolvent - females at 2 ml/kg; males and females at 4, 8, and 12 ml/kg
atovaquone in cosolvent - females at 10 mg/kg; males and females at 20, 40, and 60 mg/kg

1° AV block:
saline control - no effect
cosolvent vehicle - males and females at 12 ml/kg
atovaquone in cosolvent - males and females at 60 mg/kg

2° AV block:
saline control - no effect
cosolvent vehicle - males and females at 4 and 12 ml/kg; females at 8 ml/kg
atovaquone in cosolvent - females at 10 and 40 mg/kg; males and females at 20 and 60 mg/kg

Additional comments with respect to cardiotoxicity can be found in the memo by K.M. Wu, Ph.D. that is attached to this review as an addendum.

No treatment-related hematological changes were noted.

At the end of the infusion, plasma concentrations of atovaquone at 10, 20, 40, and 60 mg/kg were 32.6±0.88, 65.68±4.92, 121.21±21.46 and 164.12±17.42 ug/ml, respectively.

Comment: Both cosolvent and atovaquone in cosolvent caused second degree AV block. It is not clear whether convulsions preceded cardiac arrhythmia in the female dog that died because the dog was not being monitored by ECG at the time of the convulsion. Any conclusion about a lack of cardiac findings before the convulsion occurred without stating this fact is misleading.

There appears to be interindividual variation in the susceptibility to acute toxicity in this study because, although 3/4 dogs showed the same clinical signs and had similar plasma levels of drug, only one dog died. This clinical observation is similar to that seen in mouse and rat studies submitted to the original IND. In those studies, animals either survived or died, even though they showed similar signs after injection.

At the two lower doses, toxicity appears to be due to the cosolvent vehicle because the signs of toxicity are similar between cosolvent vehicle and atovaquone treated groups. At higher doses, however, additional clinical signs are observed in the atovaquone treated groups, suggesting that these signs might be due to the toxicity of atovaquone itself.

Reproduction Studies in the Rat

A rat Segment I reproduction and fertility study was conducted in accordance with GLP at Burroughs Wellcome laboratories in Research Triangle Park, NC. The F0 parental generation rats received atovaquone (0, 100, 300, or 1000 mg/kg/day) once daily by gavage. The F0 males were dosed 73 days prior to mating, throughout the mating period, and until completion of the cesarean section of female rats. The F0 females were dosed 14 days prior to mating and until gestation day 19 (teratology portion) or day 20 postpartum (littering portion).

No toxicity was observed in the F0 males at any dose. No effects on F0 generation survival, gestation length and parturition or fetal morphological development or survival. At 300 mg/kg/day, a decrease in fertility index (number pregnant females/number of females paired with males) was noted. This decrease was not noted at 1000 mg/kg/day. At 1000 mg/kg/day, the F1 generation male rats took longer than controls to escape the swimming M-maze on the first day of testing. The significance of this finding relative to potential human toxicity is not clear.

A rat Segment II teratology study (also called TOX 516) was conducted in accordance with GLP at Burroughs-Wellcome in Research Triangle Park, North Carolina. Female Sprague-Dawley rats (30/group) each received total daily doses of atovaquone (0, 250, 500, or 1000 mg/kg/day). The doses were administered as divided doses, twice daily, with the afternoon dose being administered approximately 6 hours after the morning dose. The rats were dosed on gestation days 6 through 15. The day of mating was designated as day 0 of gestation. Satellite groups of pregnant dams (6/group) were used for drug plasma determinations. Plasma samples were collected on day 16, 24 hours after the morning dose on day 15.

No drug-induced changes were noted for clinical signs, body weights, or food intake. There were no treatment-related effects on maternal pregnancy rate, gross findings, or gravid uterine weights. No adverse effects were observed in the number of live and dead fetuses, early and late resorption, implantation sites, corpora lutea, or fetal weights and lengths. The number of visceral and skeletal variations or malformations was not affected by treatment with atovaquone. At 1000 mg/kg/day, there was a statistically significant decrease in the number of litters

having malaligned sternebra, a minor skeletal variation. This observation is not toxicologically significant.

On day 16, the mean trough plasma concentrations of atovaquone in the pregnant rats were 59.35 (31-94), 59.00 (47-71), and 76.20 (65-99) ug/ml for 250, 500 and 1000 mg/kg/day, respectively. Trace levels of atovaquone were noted in the plasma samples of the control rats.

A rat Segment III peri- and postnatal study was performed according to GLP at Burroughs Wellcome Laboratories in Research Triangle Park, NC. Female rats received atovaquone (0, 250, 500, or 1000 mg/kg/day) by gavage once daily from gestation day 15 through delivery and until the 20th day of lactation (postpartum day 20). On lactation day 21, the dams and their pups were sacrificed and examined grossly. Observations and measurement included clinical signs and mortalities, body weights, and food consumption of dams.

One of 30 female rats (#91-7017) receiving atovaquone (1000 mg/kg/day) died on gestation day 20. The cause of death was unknown. No treatment-related clinical signs were noted in the dams or pups. There were no treatment-related changes in F0 dams' body weights or body weight gains or food consumption during gestation. No treatment-related effects were noted for pregnancy and litter dates or in the F1 generation dead pups external, visceral or skeletal findings. In summary, in this study, atovaquone did not affect gestation length, parturition, or survival of the F1 generation.

Reproduction Studies in the Rabbit

A rabbit teratology study was conducted in accordance with GLP at Burroughs-Wellcome Co., Research Triangle Park, North Carolina. Female New Zealand white rabbits (25/group*) each received atovaquone total daily doses of atovaquone (0, 300, 600, or 1200 mg/kg/day) orally by gavage on gestation days 6 though 18. The total daily dose was administered as two divided doses, with the afternoon dose being administered approximately six hours after the morning dose. The day of artificial insemination was designated as day 0 of gestation. All surviving rabbits were sacrificed for cesarean section on day 29. Satellite groups of rabbits were used for maternal plasma and fetal drug level determinations. Maternal blood and fetuses were collected on day 19, 24 hours after the morning dose on day 18.

* The original number of rabbits/group was 17. Due to a number of dosing accidents in these rabbits, 8 additional rabbits were added to make a total of 25 rabbits/group. In the final study report, the original 17 rabbits/group are referred to as the first replicate and the 8/group that were added are called the

second replicate in the final study report.

Results of First Replicate: A total of 17 rabbits died. Eleven of these rabbits died from dosing accidents; the cause of death for the remaining 6 rabbits was not determined. Three rabbits receiving 1200 mg/kg/day aborted. Two of these rabbits were sacrificed but showed no remarkable gross lesions. The third rabbit died after aborting and the cause of death was not determined. Bright yellow feces was noted in 5/7 rabbits in the group receiving 1200 mg/kg/day.

Rabbits receiving 300 mg/kg/day had decreased food consumption relative to the control group but the decrease was not statistically significant. Rabbits receiving 600 mg/kg/day had significantly decreased food consumption relative to the controls on gestation days 6-12. Rabbits receiving 1200 mg/kg/day had 1) significant decreases in mean body weights on gestation days 18 and 19, 2) significant reductions in mean body weight gain over gestation days 6-9, 6-18, and 15-18, and 3) significant decreases in food consumption on gestation days 6-12, 12-19, and 19-24.

In the group receiving 1200 mg/kg/day, mean fetal body length was significantly decreased. Mean fetal weights in this group were marginally lower than control values but the difference was not statistically significant. There were no statistically significant effects or dose-related effects on the numbers of implantations, viable fetuses or resorption per dam or on the incidences of fetal malformations and variations.

Results of Second Replicate: Seven of the 32 additional rabbits died during the study. Six deaths were due to gavage accidents; the cause of one death was not determined. Two rabbits receiving 1200 mg/kg/day aborted. One of these rabbits had an enlarged gallbladder, the other rabbit did not have remarkable findings. Five of 8 rabbits receiving 600 mg/kg/day and 8/8 rabbits receiving 1200 mg/kg/day had bright yellow feces.

Rabbits receiving 300 mg/kg/day showed marginal weight loss over gestation days 6-18 and slightly (but not significantly) decreased food consumption. Rabbits receiving 600 mg/kg/day showed body weight loss over days 6-18, significantly reduced body weight changes on days on days 12-15, and slightly (but not significantly) decreased food consumption. Rabbits receiving 1200 mg/kg/day showed body weight loss over days 6-18, significantly reduced boy weight changes on days 6-18, and significantly reduced food intake on days 6-12.

No adverse effects on fetal lengths or weights were observed in the second replicate. The mean numbers of early resorption and post-implantation loss per dam were higher in rabbits receiving 600 or 1200 mg/kg/day, but the differences were not statistically significant. There were no significant effects on external,

visceral, or skeletal malformations and variations.

Comments: As in previous studies, the yellow feces is probably due to the presence of atovaquone and/or its metabolites. Maternal toxicity is evident at 1200 mg/kg/day and marginal at 600 mg/kg/day. It cannot be determined whether the decreases in mean fetal body lengths and weights were due to atovaquone itself or were secondary to maternal toxicity.

The mean (±SD) maternal plasma and fetal concentrations of atovaquone on day 19 (24 hours after the dose on day 18) and the fetal/maternal concentration ratios are shown in Tables 1 and 2, respectively. The data is taken from Table 2 in Appendix 3 of the study report.

Table 1. Mean ±SD concentrations of atovaquone

Dose of atovaquone (mg/kg/day)	300	600	1200
Maternal plasma (ug/ml)	7.76±2.12	11.64±5.23	15.94±5.70
Fetal tissue (ug/g)	2.83±0.34	2.95±0.20	2.86±0.55

Table 2. Fetal tissue/maternal plasma concentration ratio

Dose of atovaquone mg/kg/day	Fetal/maternal ratio (mean±SD)
0	-
300	0.38±0.08
600	0.28±0.11
1200	0.20±0.11

Mutagenicity Studies

Atovaquone was tested for mutagenicity in Salmonella typhimurium strains TAI535, 1537, 1538, 97, 98, and 100 with and without S9 liver microsomal fraction according to the methods of Ames and the Yahagi Modification. Atovaquone was non-mutagenic in the Ames test at up to 79 ug/plate (29.3 ug/ml) and the Yahagi modification up to 32 ug/plate (11.9 ug/ml) with and without the S9 fraction. The compound could not be evaluated above these concentrations because of precipitation.

Atovaquone was negative in the mouse lymphoma assay and in the cultured human lymphocyte assay in the absence and presence of metabolic activation with S9. Atovaquone caused a statistically significant increase in the number of MN-PCEs at 72 hours after oral dosing with 5000 mg/kg. The approximate range of plasma levels of atovaquone in human studies is 5-35 ug/ml. The relationship of this finding to potential human toxicity is

unclear.

Atovaquone was tested for its potential to induce mutations at the TK+/- locus in the mouse lymphoma assay. At concentrations up to 50 ug/ml (the limit of solubility), atovaquone was negative in both the absence and presence of metabolic activation with S9.

Atovaquone was evaluated for its potential to produce chromosome aberrations in cultured human lymphocytes in the absence and presence of metabolic activation with S9. In the absence of S9, atovaquone was tested at concentrations from 2.5-25 ug/ml. In the presence of S9, atovaquone was tested at concentrations from 15-100 ug/ml. No dose-related increases in structural chromosomal damage and no significant increases in aneuploidy or polyploidy were observed.

The ability of a single oral dose of atovaquone to cause chromosome breaks and/or spindle malformation in mouse bone marrow cells was studied. CD-1 mice (5/sex/group) each received a single oral dose of atovaquone (0, 1000, 2000, 3000, 4000, or 5000 mg/kg in 0.25% methylcellulose). An additional positive control group received a single intraperitoneal dose of cyclophosphamide (60 mg/kg). An additional four groups of mice (9/sex) were used for plasma level determinations; these mice each received either 0, 1000, 3000, or 5000 mg/kg atovaquone orally in 0.25% methylcellulose. Blood samples determination of atovaquone plasma levels were obtained 3, 24, 48, and 72 hours after dosing.

The mean±SD plasma levels 3 hours after a dose of 1000, 3000, and 5000 mg/kg in male mice were 56.8±12.4, 63.8±10.4 and 59.7±16.0 ug/ml, respectively; the levels in female mice were 47.5±10.0, 64.9±70.6, and 63.6±14.5, respectively. Analysis by 1-way ANOVA showed for male mice that the plasma concentrations in the three dose levels were not significantly different from one another. However, for female mice, the 3-hour plasma concentrations in the low dose group were statistically significant from the mid- and high dose groups. The plasma levels for other time points are shown below.

	Dose of atovaquone (mg/kg)		
	1000	3000	5000
Time	Average plasma levels (ug/ml) in male mice		
24 hr:	no sample	51.3	40.7
48 hr:	8.5	11.9	20.3
72 hr:	1.7	3.7	7.1
	Average plasma levels (ug/ml) in female mice		
24 hr:	no sample	47.6	42.5

48 hr:	14.6	12.9	23.4
72 hr:	5.1	9.3	17.6

Atovaquone was negative in this study.

Special Studies

Male and female Beagle dogs (2/sex) received perivenous and intramuscular doses of either atovaquone (5 mg/ml in cosolvent formulation) or ampicillin (100 mg/ml), which served as a positive control. One-half ml of solution was injected at each site. One dog/sex was sacrificed 24 hours and 7 days after dosing.

One male dog showed signs of pain and discomfort after injection of atovaquone and was administered butorphanol tartrate, an analgesic, for 7 days. One dog/sex showed swelling of the injected forelimb starting at 140-165 minutes post-dose and lasting for approximately 2.5 hours. At the 24-hour post-dose necropsy, red and yellow discoloration was observed at atovaquone injection sites; ampicillin injection sites showed red hemorrhage. Seven days post-dose, atovaquone sites showed red and brown discoloration; ampicillin injection sites were not remarkable. Histological examination of tissue samples taken 24 hours post-dose showed perivascular hemorrhage and cellulitis in the atovaquone and ampicillin treated dogs. Seven days post-dose, cellulitis and myositis was observed in atovaquone treated dogs only.

The results showed that perivenous and intramuscular injection of a single dose of atovaquone, (to simulate misdosing), caused minimal to mild perivenous and intramuscular injection that persisted up to 7 days post-dose.

This study was conducted to determine the potential for atovaquone to cause hemolysis or protein flocculation after IV administration. For the hemolysis assay, either atovaquone in cosolvent, cosolvent alone, or 0.9% saline were added to either 1) a 50% suspension of washed red blood cells in 0.9% saline or 2) whole blood. The mixture was incubated at 37°C for 30 minutes, centrifuged, and hemoglobin levels were determined spectrophotometrically.

For the protein flocculation assay, either atovaquone in cosolvent, cosolvent alone, or 0.9% saline were added to plasma. The mixtures were incubated for 30 minutes at 37°C. Results were assessed visually when the test solutions were added. Turbidity measurements were determined after 30 minutes using a nephelometer.

Comment: The sponsor concludes that atovaquone produced no protein flocculation and insignificant amounts of free hemoglobin from whole blood in this study. This conclusion is problematic in light of the known aqueous insolubility of atovaquone. In order for atovaquone to interact with plasma proteins or red blood cells in these assays, the atovaquone would have to be in solution. According to the CMC section of this submission, atovaquone precipitates from solution when the injection formulation is added to normal saline. Therefore, the addition of atovaquone in the cosolvent formulation to either the red blood cells in normal saline, whole blood, or plasma would result in the precipitation of atovaquone from solution. The precipitated atovaquone would not be available to interact with red cells or plasma protein and lead to negative results. Rather than indicating a lack of potential hematolytic or protein flocculation activity, the negative results probably indicate a lack of physical interaction between atovaquone and the cells or plasma components under the condition of these assays.

DISCUSSION

The purpose of the nonclinical toxicology studies is to establish the full spectrum of potential adverse effects of a drug and to provide a meaningful basis for comparison between findings in animal studies and potential human toxicity. The nonclinical studies were reviewed and interpreted in light of these purposes.

The methods and results of the nonclinical studies are discussed in detail in the preceeding sections of this review. This discussion of the nonclinical data will focus on selected aspects of the toxicity studies: interpretation of the results based upon mechanism of action and pharmacokinetic parameters, dose/response relationships, and time dependency of toxicity. Comments will be provided for reproduction, mutagenicity, and carcinogenicity studies and unexplained deaths in animals following oral and intravenous dosing. The relationship of the nonclinical findings to potential human toxicity and conclusions will be presented.

In all species tested, atovaquone demonstrated poor absorption, limited distribution, high protein binding, and a long half-life. Atovavone does not appear to be metabolized in any of the species tested. Because of poor absorption following an oral dose, the results of the nonclinical pharmacology and toxicology studies must be interpreted in the context of the pharmacokinetic data. Because the pharmacokinetics of atovaquone in the nonclinical species is similar to the pharmacokinetics in man, the exposure relationships can be compared on the basis of mean areas under the curve or AUCs.

The relationship between the estimated exposure to atovaquone in rats at doses used in the toxicology studies is compared to the

estimated exposure to atovaquone in humans at steady state at a dose of 50 mg/kg/day. The clinically determined AUC_{0-24} value at an oral dose of 750 mg four times daily is taken from pharmacokinetic data in the NDA submission. This dose was chosen for comparison because it approximates the human exposure at the dose recommended in the labeling. It must be noted that the human AUC and Cmax levels used for purposes of comparison reflect the higher levels achieved in patients who were not clinically ill. Patients who were clinically ill had levels that were approximately 1/2 of these values, so the ratio of animal:human exposure would be double for such patients.

Rats received oral doses of 20, 100, or 500 mg/kg/day during the one and six month toxicology studies. In other studies, doses up to 2000 mg/kg were administered. It must be noted that pharmacokinetic data in the submission demonstrated that, because of poor absorption, exposure to atovaquone is not appreciably greater at oral doses higher than 500 mg/kg. The calculated AUC_{0-inf} values for each dose were reported in the submission. The AUC values are nonlinear with dose. For example, a 25-fold increase in dose from 20 to 500 mg/kg resulted in an approximate 2-fold increase in AUC.

On the basis of mean AUC, the oral exposure in rats at 20 mg/kg atovaquone was approximately 1.3 times the human exposure at 50 mg/kg/day in divided doses. At 100 mg/kg and 500 mg/kg, the estimated exposure was approximately 1.8 and 2.2 times the human exposure, respectively.

A comparison of mean maximum plasma levels, or Cmax, reveals a similar relationship between rat and human exposure. An oral dose of 20 mg/kg provides an exposure that is slightly less that achieved in humans. Doses of 100 and 500 mg/kg provide exposures that are approximately 1 and 1.4 times the estimated human exposure, respectively.

The AUC values were variable in the dog. Oral doses between 20 and 500 mg/kg/day provided an exposure less than or approximately equal to the human exposure during the clinical trials.

Oral doses of atovaquone ranging from 20-500 mg/kg/day did not cause anaemia in rats during a one month study. However, the same doses did cause a slight anaemia with hemosiderin deposition in the spleen in rats during six month studies. Anaemia was observed at week five of dosing. After a 30-day drug free recovery period, the anaemia reversed but the hemosiderin deposition persisted. Atovaquone did not cause anaemia in dogs after either one or six months of oral dosing.

Because of limited absorption, atovaquone's full spectrum of toxicity of atovaquone could not explored with oral dosing. The

higher exposures achieved in the intravenous studies did provide a more complete picture of atovaquone's potential toxicity. Intravenous atovaquone produced a moderate anaemia in rats after 1-2 days of dosing during a one month study; no hemosiderin deposition was observed in the spleen. Slight to moderate anaemia became apparent in dogs by day 8 of intravenous dosing.

Sudden, unexplained deaths occurred in mice and rats during the intravenous studies. In rats, the deaths occurred at plasma levels greater than 100 ug/ml. Plasma levels were not available for the mice. The dose-response curve for mortality was steep and the mechanism of toxicity appears to be distinct from the mechanism causing the anaemia.

The slight to moderate hematologic findings and the histopathologic findings for atovaquone were consistent with findings for related 1,4 hydroxynaphthoquinones. Atovaquone is structurally similar to dichloroallyl lawsone (DCL) and lapachol. These compounds were investigated as potential anti-cancer agents by the National Cancer Institute in the 1970's. Like atovaquone, both DCL and lapachol caused anaemia and hemosiderin deposition in the spleen in animals. Also like atovaquone, they act by inhibiting the bc1 complex of the mitochondrial respiratory chain. Because of its high rate of energy utilization, the heart could be a potentially sensitive target organ of toxicity for such compounds.

In monkeys, DCL caused an acute cardiotoxicity that was related to maximum plasma levels of 130 ug/ml. In dogs, DCL caused convulsions and cardiac arrest during 1 hour intravenous infusions at doses of 62.5 to 125 mg/kg.

Findings similar to those for DCL were observed in dogs during and after a 1 hour intravenous infusion of atovaquone at 60 mg/kg. Two of 2 female dogs convulsed and 1 of the two died. One of 2 male dogs showed intermittent jerking body movements. The mean plasma concentration of atovaquone at the end of the 60 minute infusion was 164 ug/ml, or approximately 3.6 times the average peak plasma levels that occurred after oral doses during clinical trials. Because of limitations in the study design, no definitive conclusions can be made with respect to cardiotoxicity in this study. An additional study is planned to explore this issue further.

Unexplained deaths also occurred during dose ranging studies in which mice received 800 mg/kg/day atovaquone in the feed. On study days 2-4, 10/16 male and 11/16 female mice died overnight. Unexplained deaths also occurred in 8/100 rabbits in the teratology study. The relationship of these findings to potential human toxicity is not clear.

In the rat, a Segment I reproduction and fertility study, a

Segment II teratology study, and a Segment III peri-and post-natal study were performed. The rats received doses that ranged from 100 to 1000 mg/kg/day. There were no remarkable findings in these studies.

A Segment II teratology study was performed in the rabbit at doses of 300, 600, and 1200 mg/kg/day. Maternal toxicity was marginal at 600 and was evident at 1200 mg/kg/day. At the high dose, five rabbits aborted. Mean fetal body lengths and mean fetal weights were decreased and there were higher numbers of early resorption and post-implantation loss per dam. It is not clear whether the effects were due to atovaquone or were secondary to maternal toxicity.

Because of these results, Pregnancy Category C labeling is proposed. This category is assigned if 1) animal reproduction studies have shown an adverse effect on the fetus, 2) there are no well-controlled studies in humans, and 3) the benefit from the use of the drug in pregnant women may be acceptable despite its potential risks.

Rat studies showed that atovaquone is transferred across the placenta to the fetus and is excreted into the milk. This information will be reflected in the appropriate sections of the labeling.

Atovaquone was negative in the Ames Salmonella assay, the mouse lymphoma assay, and the cultured human lymphocyte assay in the absence and presence of metabolic activation. It was also negative in the mouse micronucleus assay.

Carcinogenicity studies in mice and rats are in progress. The final report for the rat carcinogenicity study is scheduled for submission during the third quarter of 1993. The final report mouse study is scheduled for submission during the fourth quarter of 1994.

SUMMARY

1. Pharmacokinetic parameters, particularly variability in absorption following an oral dose, were similar between animals and humans.

2. In agreement with human clinical findings, anaemia did not occur in dogs after oral dosing with atovaquone because the exposure in these studies was less than or equal to the exposure in humans.

3. During chronic oral and intravenous studies, anaemia occurred in rats at exposures that were approximately twice the human exposures in clinical trials. This suggests that anaemia may

become apparent after chronic dosing in humans if higher plasma levels of atovaquone are achieved through the use of a different oral formulation.

4. Atovaquone appears to demonstrate both an acute and a chronic toxicity in nonclinical studies

5. The acute toxicity, sudden death, may be related to a mechanism that is different from the mechanism for anaemia, appears to be related to Cmax values 100 ug/ml in the rat and 164 ug/ml in the dog).

6. The chronic toxicity, anaemia, may be related to the oxidative properties of atovaquone, and appears to be related to Cmax values greater than or equal to 45 ug/ml.

7. Because of poor oral absorption, the doses in the rat and dog toxicology studies resulted in exposures that ranged from less than to slightly greater than the human exposure during the clinical trials.

8. Reproduction studies have been conducted in rats and rabbits. Because of toxicity in rabbits, Pregnancy Category C is proposed. Atovaquone is transferred across the placenta to the fetus and is excreted in the milk of lactating rats.

9. Atovaquone was negative in mutagenicity studies.

10. Carcinogenicity studies have not been submitted.

CONCLUSIONS

From the standpoint of pharmacology/toxicology, NDA 20,259 is approvable. Several requests are made with respect to the labelling, and these are outlined below.

REQUESTS

The following revised text should be considered for incorporation into the label by the sponsor. These revisions are based upon labeling specified in 21 CFR 201.57. The appropriate text is proposed.

1.

2. Pregnancy:

[redacted]

ma

l

3. Nursing mothers: It is not known whether atovaquone is excreted into human milk.

[redacted]

4. Animal toxicology:

[redacted]

151

Frances A. Mielach, Ph.D.
Reviewing Pharmacologist, DAVDP

Concurrences:
HFD-530/Pre-Clin Dep/LRosenstein
HFD-530/SPharm/MDGreen
Mielach/Pharm/11-3-92

cc:
HFD-530 Original IND/NDA
HFD-530 Division File
HFD-530/LRosenstein
HFD-340
HFD-530/CSO
HFD-530/Pharm/Mielach
HFD-530/MO
HFD-530/Chem
HFD-530/Micro
HFD-345/GJames
HFD-502

REFERENCES

1. McKelvey, Eugene M., M. Lomedico, K.Lu, M. Chadwick, and T.L. Loo. 1979. Dichloroallyl lawsone. Clin. Pharmacol. Ther. 25(5,part 1): 586-590.

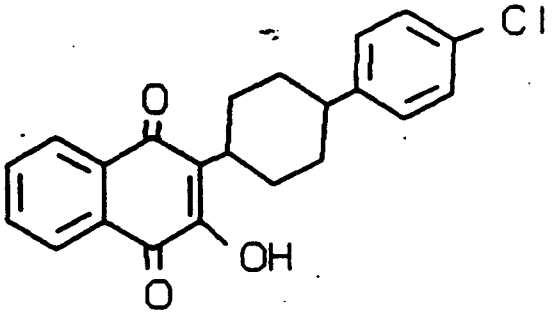
2. Initial Pharmacologist Review of IND 11,476. 1975. Doo Y. Lee-Ham, HFD-150.

3. Morrison, R.K., D.E. Brown, J.J. Oleson, and D.A. Cooney. 1970. Oral toxicology studies with lapachol. Toxicology and Applied Pharmacology 17, 1-11.

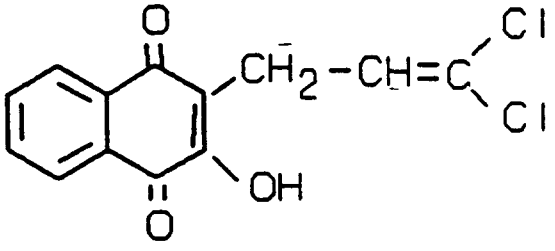
APPENDIX 1

Chemical Structures of Atovaquone and Related Hydroxynaphthoquinones

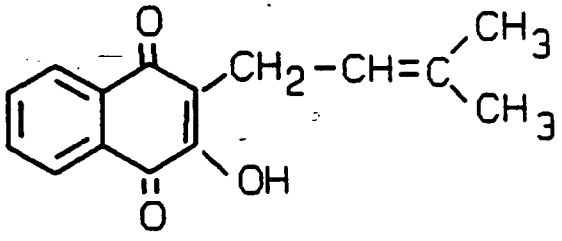
Atovaquone



Dichloroallyl lawsone (DCL)



Lapachol



APPENDIX 2

Atovaquone Lot Numbers Used In GLP Toxicology Studies

SPECIES	STUDY TITLE	DOC NO	566C80 LOT NO
CD-1 Mouse	An Acute Oral Toxicity Study in the Mouse	BPAT/88/7	17937
CD-1 Mouse	An Acute Intravenous Toxicity Study (Limit Test) in the CD-1 Mouse	TTEP/91/0026	36991
Wistar Rat	An Acute Oral Toxicity Study in the Rat	BPAT/88/6	17937
CD Rat	An Acute Intravenous Toxicity Study (Limit Test) in the CD Rat	TTEP/91/0025	36991
Beagle Dog	A One Day Intravenous Infusion Toxicity Study in Beagle Dogs	TTEP/91/0039	36991
CD-1 Mouse	A 14-Day Oral Toxicity Study in Mouse	BPAL/85/10	Lot No. Not Assigned
Wistar Rat	One Month Oral Toxicity Study in the Rat	BPAT/87/44	17937
CD-1 Mouse	A 90-Day Oral Toxicity Study in Mice	TTDR/91/017	29034
CD Rat	A 90-Day Oral Toxicity Study in Rat	TTDR/91/0011	29034
Wistar Rat	A Six Month Oral Toxicity Study in Rat	BPAT/88/0064	23031
CD Rat	A 30-Day Intravenous Toxicity Study in Rats	TTEP/91/0041	36991
Beagle Dog	One Month Oral Toxicity Study in Dog	BPAT/87/50	29034
Beagle Dog	A Six Month Oral Toxicity Study in Beagle Dogs	TTEP/91/0008	23030
Beagle Dog	A Five-Day Intravenous Infusion Toxicity Study in Beagle Dogs	TTEP/91/0040	36991
Beagle Dog	A 17-18 Day Intravenous Toxicity Study in Beagle Dogs	TTEP/91/0038	36991

APPENDIX 2 (CONTINUED)

Atovaquone Lot Numbers Used in GLP Toxicology Studies

SPECIES	STUDY TITLE	DOC. NO.	566C80 LOT NO.
Beagle Dog	Perivenous and Intramuscular Irritation Study in Beagle Dogs	TTEP/91/0037	36991
Man	An In Vitro Hemolysis and Protein Flocculation Study	TTEP/91/0036	36991
CD Rat	A Teratology Study in Rats	TTEP/90/0073	23030
NZW	A Teratology Study in Rabbits	TTEP/90/0074	23030/23031
Salmonella Typhimurium	Ames Test	DBAT/88/E	23030
Mouse	A Micronucleus Assay in Mice with 566C80	TTEP/90/0069	23030
Mouse Lymphoma	L5178Y/tk ± Mouse Lymphoma Mutagenesis Study	TTEP/90/0060	23030
Human Lymphocytes	An In Vitro Cytogenic Study in Cultured Human Lymphocytes	TTEP/90/0063	23030